

**EDITOR-IN-CHIEF
MEYER R. ROSEN**

**HARRY'S
COSMETICOLOGY
VOLUME ONE**

**NINTH
EDITION**

NINTH EDITION

Harry's Cosmeticology

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Meyer R. Rosen

CChem, CPC, CChE, CFEI, DABFE, DABFET, FAIC

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I give special thanks to my Editors who took my thoughts and ideas and worked with me to find authors who knew their subjects and could organize and combine the thinking of-the- many to produce a unified-whole. Special thanks also to Navin Geria, Howard Epstein, Chia Chen, Bruce Victor, Bozena Michniak-Kohn, Ruud Overbeek, Manuel Gamez-Garcia, Michael Prencipe, Chuck Warren, Lee Stapleton, Adam Friedman, M.D., Ray Rigoletto, Roger McMullen, Randy Wickett, Martha Tate and so many others who have contributed to this book.

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encourage and support the IFSCC in its mission to bring beauty and health to the many through his clear thinking, close, objective, ever-questioning and challenging examination of the “science” associated with cosmetics and personal care. Once upon a time, he told me that while travelling the world, he always stayed in the same type of room of his favorite hotel chain because “it always made him feel like he was at home.”

A manager I once had told me that if “I had lemons, I should make lemonade”. And so it was with Johann, who turned the enormous amount of time he spent in travelling to producing an incredible volume of questioning, challenging and probing scientific papers for us to read and think about for years to come.

Wherever you are, Johann, I want you to know that your work and critical thinking approach to cosmetic science has impacted us all-and we miss you greatly.

Dedication

This book is dedicated to my wife Selma, my Soulmate, Committed Listener and Partner in the Journey-of-Life.

She who knows, and reminds me, to put the past in the past in order to open the doorway to the creation of new possibilities and generate new directions for growth in areas we do not know that we do not know.

How remarkable it is When mere words on paper Grow together
Beyond themselves.

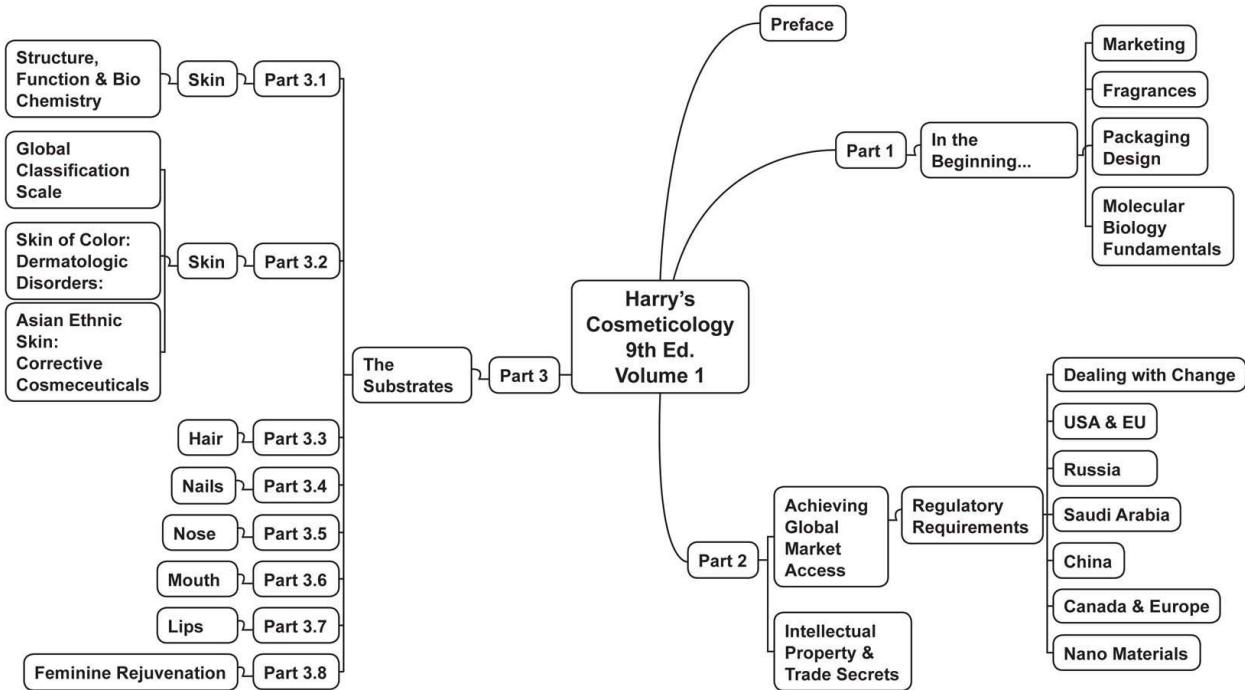
Such words as these Are tracks in time.

Memories of a mind Focused
A heart and soul
Ensconced.

—Meyer R. Rosen July 4, 2014

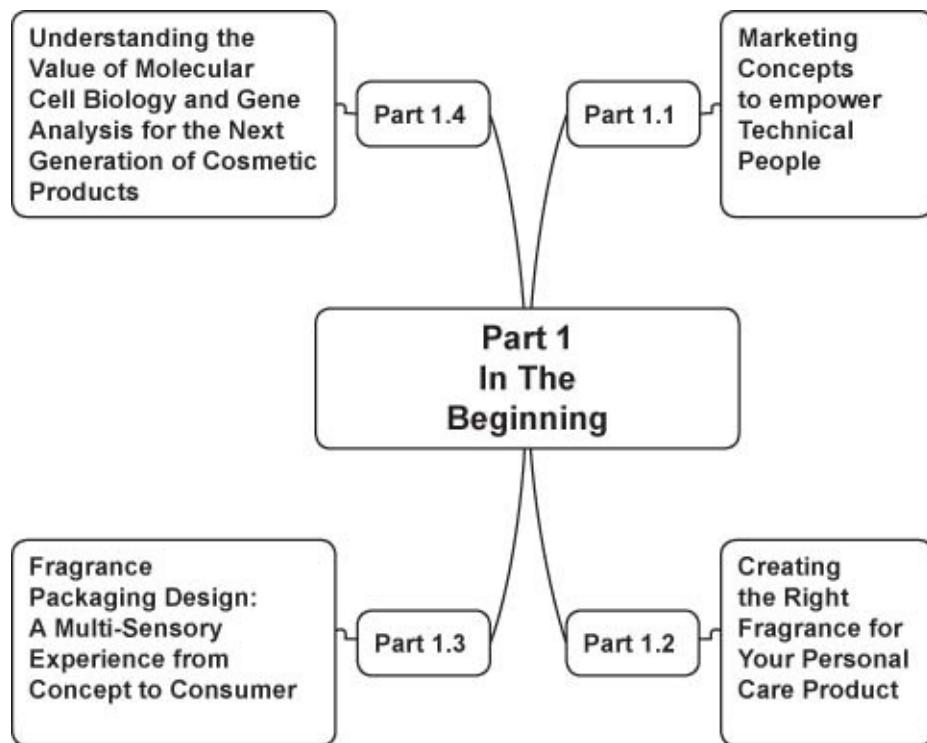
HARRY'S COSMETICOLOGY 9TH EDITION

VOLUME 1
IN THE BEGINNING REGULATORY
REQUIREMENTS THE SUBSTRATES



PART 1

IN THE BEGINNING



MARKETING CONCEPTS TO EMPOWER TECHNICAL PEOPLE

By

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ABSTRACT

Marketing is everywhere. Sometimes it's a good thing, since marketing is what drives revenue for the organization. But sometimes it's not so good. Skepticism aside, it's important to understand that marketing is not merely advertising or selling, but the execution of a carefully planned strategy. Marketing is much more than most people think, and much more than just a great story. In today's hypercompetitive, globalized marketplace, product development is largely a function of assessing market needs and delivering goods and services to meet those needs.

Innovation and R&D are very important, especially in the area of cosmetics and personal products. Ultimately, the marketer is the individual responsible for what will and what will not generate revenue for the organization. Thus, underlying consumer needs and the creativity of ingredient innovators, along with pricing, distribution, regulatory issues, and the promotion of products, is a phenomenon known as the *marketing concept*. The vast majority of new products fail, and so the marketer, at the very least, must ensure that the product looks, feels, smells, and works optimally while also generating the right combination of additional marketing-mix decisions. This chapter explores the methods to the “perceived madness” that often vexes even the most experienced product-development professional. It is intended as a guide to the reasoning behind why marketers do what they do.

We note, in passing, that it may seem odd for a technical book like this to begin with a chapter on the “marketing concept.” However, as we open the

panorama of a very detailed description of the “ingredients” that lead to successful cosmetic and personal care products, we must first look at the real world where, inevitably, there’s both a marketing and technology push competing with the ever-expanding knowledgeable consumer pull for products that work.

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1.1.1 THE MAGIC AND MYTHOLOGY OF MARKETING

Marketing is indeed everywhere, and depending on one’s role within a particular organization, marketing can represent different things to different people. This can become particularly problematic with regard to product development. What exactly is marketing’s role in the development of cosmetic products? The purpose of this chapter is to cut through the confusion and to clarify marketing’s role in personal care/cosmetics. First, let’s mention a few common myths.

1. “Marketing is selling and advertising.”—The first thing a student of marketing should learn is just how untrue this assertion is. Marketing is much, much more than selling and advertising, as it involves product, pricing, distribution, and promotional strategies.
2. “The marketer’s job is to sell whatever R&D develops.”—The second thing a student of marketing learns is that goods and services, in this age of hypercompetition, must be driven by assessing and meeting consumer needs. Goods and services that are not need-based seldom succeed in the marketplace.
3. “My new product has a great chance of succeeding.”—Actually, the vast majority of new products (about 90%) fail to meet expectations and are deleted from the company’s product mix within a few years.

4. “Word of mouth is a marketing activity.”—It is not a marketing activity, but rather a result of our communications efforts. When someone says that most of their marketing is word-of-mouth-based, especially in the age of Internet, be very concerned.
5. “Marketing is about telling consumers what they want to hear.”—A marketer must never mislead the consumer. If we overpromise and underdeliver we ultimately lose customers, who are very expensive to acquire. These unhappy people then tweet, blog, Yelp!, and otherwise report on the company’s malfeasance. This is not to underestimate the power that media, NGOs, government, competitors, supply-chain members, and other stakeholders have in keeping companies honest.

Indeed these are only a few of the most common misperceptions that both newbies and experienced product developers have when they think of marketing. So what is the true function of marketing?

All organizations have multiple functions within the company’s hierarchy. General management, HR, finance, accounting, production, operations, R&D, and IT are just some of these functions. Each has its own primary purpose. Finance manages cash flow and investments, accounting keeps records, HR manages personnel, R&D develops what might be the next big thing, etc. The primary function of marketing then, if we must boil it all down to one thing, is Revenue. Marketers must drive revenue or else the organization cannot exist, and to do so effectively, we must first discover what it is that consumers want and need. In some cases, a concept may be so new that consumers may not even be aware they have a need for it.

1.1.2 THE MARKETING CONCEPT

Academic professionals, in their never-ending quest for professional credibility, will often argue over things that seem silly to the rest of us. One of these arguments concerns the difference, if one actually exists, between a “want” and a “need.” For our purposes this argument is spurious. Different groups of people have different priorities, and so the idea of what someone wants versus what someone really needs can be debated until the cows come home. The point of the “Marketing Concept” is that we develop no new products unless we can prove that a large enough group of people will buy them. It’s as simple as that, but this means that product development is most often driven by marketing analysis and not by innovation, an assertion that does not traditionally sit very well with the

science crowd.

The reality is that the world of marketing is not at all black and white, and that product development is really a carefully planned and effectively implemented process that involves interplay between the folks whose job it is to study consumers (marketing) and the technical folks whose job it is to make a highly effective hair or skin care product. So let us now delve into the new product-development process with a particular focus on developing a strategic marketing plan.

1.1.3 ASSESSING THE MARKETING ENVIRONMENT

New product development begins with an idea. Ideas can come from many places—customers, competitors, employees, supply chain members, universities, and of course the research and development efforts of our cosmetic chemists. In many cases, ideas come from industries that have nothing to do with cosmetics or personal care. An interesting example is the use of information from the carpet industry by hair care product developers (fibers are fibers, after all). The concept of Open Innovation, described elsewhere in this book under Sustainability and EcoResponsibility, describes this process of ideation.

Many ideas are derived from formal brainstorming sessions, usually led by a high-level marketer (i.e., a Technical Marketing Expert) and involves members of all of these groups. Ideas that are initially thought to be feasible undergo “proof of concept” testing wherein relevant parties are interviewed, answer surveys, or participate in focus groups. Here we must prove that the concept *might* work *before* we conduct further analysis; and assessing consumer needs are a huge part of this process.

The next step is to conduct an analysis of the current business environment. This consists of both internal factors, which are controllable by the organization, as well as external factors, which are out of the marketer’s control. The resulting situation analysis (sometimes called business analysis), gives us all of the information that we need to develop a SWOT summary use in decision-making and risk reduction. The analysis itself is a very objective exercise, and as such, opinions are not needed until these can all be summarized as a series of Strengths, Weaknesses, Opportunities, and Threats. The first two areas are derived from the internal factors and the latter two from the external factors.

Internal factors include a full analysis of all the functional areas within an organization that could affect marketing strategy. This includes pretty much

everyone, and a good marketing plan is a cross-functional effort. Involving all relevant aspects of the organization makes for a better-quality plan, and more buy-in among key players as to how the organization is going to achieve its objectives. It all begins with the organizational mission, a statement of scope and purpose, and includes everything that is under the organization's internal control. Finance, IT, current marketing efforts, and R&D are among these, and strengths and weaknesses are what emerges from such analysis.

External factors include the areas that might present opportunities and/or threats to the organization and its attempts to develop and market products that people actually need. An analysis of the industries, consumers, and other factors like the economy and competition is an absolutely crucial part of making the right stuff for the right people. Market segmentation analysis is used—a process whereby a market is broken down into individual consumer segments, each of whose members have many important characteristics in common. Demographics such as age, gender, and income are combined with psychographics (behaviors and attitudes derived from surveys and focus groups) and geographics to form well-defined market segments. Once marketers can understand the people who comprise a particular segment, they are better able to meet their needs through tailoring the right combination of what we call the Four P's of Marketing or the Marketing Mix. This area may require exhaustive marketing research in the form of surveys, focus groups, and other methodologies.

TABLE 1 (The Green Marketing Model for Strategic Marketing Planning)

Internal Analysis

Mission Statement

Organizational Overview/Demographics

Marketing (Product, Price, Place, and Promotion Strategies)

Information Technology

Finance

Operations/Production

R&D

External Analysis

Industry Analysis

Competitive Analysis

Market Segment Data

Social Trends

Technological Trends

Economic Trends

Legal and Regulatory Environment

Political Environment

Natural Environment

SWOT

Objectives and Budget

Marketing Strategy and Program

Control and Evaluation

1.1.4 THE FOUR P'S

Although a few P's have been added by overzealous academics over the years, it is really only necessary to understand the "Original Four." Once the marketer has conducted a situation analysis and its companion SWOT summary, it is time to develop the marketing program, the foundation of which is Product, Price, Place, and Promotion strategies.

Product strategy is where the real work between marketing and R&D comes into play. The decision to use certified organic or natural ingredients instead of synthetic ingredients, for example, might be driven by marketing, but the reality of working with these ingredients and still being able to manufacture an excellent personal care product is another story. Product-development chemists and the folks working on the manufacturing end of things might have constraints that the marketer may not fully understand. However, it is the marketer whose job it is to monitor the marketplace and assess market needs. Teamwork and cooperation here are of paramount importance, and this is often where new products fail when cooperation falls below the bar-of-success. The wrong product is made for the wrong target market oftentimes because marketing and other organizational entities won't cooperate, and thus function too independently. Product strategy is the foundation of marketing. The team must decide on the ingredients, the positioning (different from competitors), packaging, branding, and other strategic considerations before pricing.

Pricing the product depends on several factors. First, the cost must be considered, including variable costs (cost of goods sold), as well as fixed costs such as general/administrative and sales/marketing expenses. These costs must be covered, but that's not all. On the demand side of the equation, the marketer must look at the prices of competitive and substitute offerings as well as what prices consumers might be willing to pay. A competitive product and market analysis can run in the several hundreds of pages and such reports may cost tens of thousands of dollars. Unless the marketer decides to sell the product directly to the consumer through the Internet, TV, direct mail, or some other channel, any pricing strategy must also consider both wholesalers (distributors) and retailers. The former intermediary often takes 10% or more of the retail price and the retailer can take as much as 50%. What is left over after cost and channel considerations is profit. This is a lot to consider, and the marketer has dozens of pricing strategies from which to choose.

Placing the product involves distribution decisions and choosing a mix of retail channels to sell it. Should the product be distributed exclusively, selectively, or intensively? Should it be found in higher-end natural products stores, or should it be sold in Walmart and through the grocery channel? Such decisions should not be taken lightly, and they depend fully on both the product and pricing strategies.

Promotion is what most people think of as “marketing communication.” Marketers have a myriad of promotional strategies and tactics to choose from and must find an acceptable mix under the marketing budget. Advertising, public relations, direct marketing, sales promotion, and personal selling are all important elements of marketing communication. Much of this communication is moving toward the digital medium, but it is important to realize that the majority of marketing is conducted through what we would call more traditional media such as TV, radio, magazines, newspapers, outdoor and indoor signage, etc. The advent of social media and the proliferation of digital advertising have made the marketer’s job far more difficult in recent times; but, on the bright side, they have made the control, evaluation, and quantification of marketing activities much easier. Marketers must engage in both “push” marketing to encourage distributors and retailers to carry the product, as well as “pull” marketing to inform consumers what the product is, what it can do (claims), and where it is available. This can be rather expensive as you might imagine, but without it, the product has no chance whatsoever of succeeding.

Before we look at the remaining steps in the product-development process,

something should be said about product claims. Historically the marketing of ingredients with great stories behind them (traditional use, exotic derivation, etc.) has forced marketers to make product claims for what the product is supposed to do, and unfortunately many product developers fail to include an efficacious amount of the ingredient, thus rendering the product ineffective—a phenomenon known as “dusting.” The FDA is watching ever more closely, and manufacturers are under increasing pressure to make products that really work. Not only this, but the nature of claims (especially with the FDA’s mandate to eliminate false or misleading structure-function claims) is of critical importance to providing proper information to the consumer looking for a solution to a problem. The natural and organic ingredients business has been plagued by this, but mainstream cosmetic and personal care also suffer from this syndrome. When products fail to meet expectations and marketers make promises that product developers cannot keep, the brand and ultimately the entire organization suffer.

1.1.5 DEVELOPMENT, PROTOTYPES, TESTING, AND COMMERCIALIZATION

Once a business analysis has been conducted, preferably in the form of a written strategic marketing plan, it is important for the product-development team to develop a prototype, or in the case of personal care, a final formula for testing purposes. Obviously shelf aging stability, bacterial contamination, and other testing are conducted until the results are satisfactory and any necessary changes are made to the formula to make it robust under foreseeable conditions. Even if the actives have been clinically tested, it is a good idea to test the product itself so that any claims made can be substantiated. Additional market research may also be conducted at this stage if the picture is still incomplete. Once the formula has been “finalized,” labels, containers, applicators, and all other considerations should be considered since packaging is such an important part of the consumer experience in this industry. In this regard, Part 1 of this book also includes a detailed description of how attractive packaging is designed. When several mockups have been made with finished product inside, it’s time for employees, suppliers, friends, family, and others who are closest to the organization to get an initial feel for the finished product.

In many industries, a product is market tested before it is released to the broader population. This is often done in specific stores or regions of the

country. Such test marketing can tip off the competition, but it may be important to reduce the risk of utter failure by doing a bit of test marketing. The personal care industry admittedly doesn't do much of this, but it is an important step to mention in any interpretation of the new product-development process. Hopefully, the organization's finance function has liberated enough cash flow to produce large volumes of the product and support it with the proper marketing efforts. Since marketing planning is always time-bound, the period of evaluation will culminate in an actual evaluation of what happened. Did the product introduction work? Why or why not? What should we have done differently?

Table 2 The Green Marketing New Product-Development Process

- Step 1 Idea Generation*
- Step 2 Concept Testing*
- Step 3 Business Analysis/Marketing Planning*
- Step 4 Product Development*
- Step 5 Market Testing*
- Step 6 Commercialization*
- Step 7 Evaluation of Market Results*

1.1.6 THE TRUTH ABOUT INNOVATION

Here is an important question to ponder. Just because an ingredient or a product is “new,” does that make it “innovative”? It seems that this word has been thrown around quite a bit and has lost much of its meaning, similar to the way that social media, and Facebook in particular, have somewhat diluted the word “friend.” And this begs the question: Are ingredients truly innovative, or is this just a bedtime story that product developers tell themselves so that they have a better reason to rise and shine in the morning? Just because a new botanical or ingredient blend is new or popular doesn't necessarily mean that it is innovative in any real sense. Let's explore this rather controversial assertion further.

The term “innovation” has been used to describe all kinds of underwhelming product developments, and the popular understanding of the term is that it means “new.” I think we can all agree that the telegraph, automobile, light bulb, radio, television, computer, and smartphone were all groundbreaking innovations; but what about each iteration of the iPhone? Is a new emollient truly innovative? What about a new natural preservative? Or, a new, naturally derived active ingredient?

Innovation and Product Development

Product Development is one of the author's favorite university courses to teach, and in this important class we learn that there are three forms of innovation.

1. A **continuous innovation** describes a new product that requires almost no change in consumer behavior, and so it represents very minor changes in an existing product type. An example of this would be an existing hand lotion product that features a new nonactive ingredient, or if you prefer a technological example, each and every iteration of the iPhone would be continuous. Many experts do not consider a continuous innovation to be very innovative at all.
2. A **dynamically continuous innovation** involves a major change in a minor behavior or a minor change in a major behavior. An example would be a hand lotion (either an existing product or a new one) that features an entirely new way of dispensing the liquid, such as moving from a jar to a pump. Or technologically speaking, it's the difference between a tablet and a laptop.
3. A **discontinuous innovation** can be very disruptive to the industry. An example in personal care would be a product (or a newly discovered ingredient) that has an entirely new market application, such as hair regrowth. The decade-old advent of skin and hair care products in the form of ingestible nutritional supplements may have been the last true discontinuous innovation in our sector. In tech terms, the inventions mentioned earlier in the article "Which Ones?" would qualify as discontinuous innovations.

In most cases, it's not really the ingredients that are innovative, but the finished product. And in every case, it's always the degree of consumer behavioral change required, and not the ingredient/product itself, that determines the type of innovation. It may sound academic, but what happens is that people in general become desensitized to words they hear all the time such as "innovative," and when they are underwhelmed by what they see, it serves to further numb the target market to the marketing message. I think we can all agree now that the vast majority of new products are continuous innovations. The really groundbreaking product, featuring an entirely new ingredient with an entirely new application, can only be introduced once. After that, successive products will be dynamically continuous or continuous innovations. As such, in almost all cases, "hot" ingredients are merely popular, and not really innovative.

The Patent Process

Now let's look at the patent process. In order to qualify for a patent in the U.S., an invention must be novel, involve an inventive step not obvious to a skilled person, and have an industrial application. If approved, the patent is exclusive for 20 years. Patents are awarded in cosmetic and personal care products all of the time, mostly in the form of process and composition of matter inventions. The vast majority of these do not require changes in consumer behavior, and so they cannot be considered innovations in any respect, and in too many cases the changes are things the consumer doesn't even notice. It's no small wonder then that many observers, including the author, believe that patents are far too easy to obtain and that this is actually very bad for innovation.

Unfortunately, this is often lost on most industry people (who have never taken business classes), but it is very basic science to those of us who have. We learn that new products (which fail 80–90% of the time) should be need-driven in almost all cases (excepting the area of needs that consumers didn't know they had in the first place, which is rare). The firm's job is to address, and in many cases exceed, expectations in meeting the market need while differentiating their offerings from competitive and substitute products. This attempt at differentiation generally results in a whole heck of a lot of noninnovation, some continuous innovation, and an occasional dynamically continuous innovation, but rarely does it result in something that disrupts the industry.

Nevertheless, marketers and media alike will continue to push “the next big thing” as manufacturers look for the next incredible ingredient and retailers tailor their product mix to meet the demands of their customer base. The author will take the road less traveled here and remind everyone that *new market applications* are what spawns innovation in the personal care world, and not necessarily a new ingredient that meets an existing need. So let's dispense with the hype and begin talking about ingredients and products in terms of how consumers view them rather than “innovations” that do not matter to the marketplace. Indeed market applications are crucial to justifying the use of certain ingredients as well as the development and commercialization of the products themselves.

1.1.7 THE MISSING LINKS

Our discussion has obviously been rather academic. The reality is that many product-development teams, not unlike groups of all kinds, are very dysfunctional. Obviously, we know that this perfect world exists only in the

minds of academics, and that the reality of product management is rather messy. Yet, as marketing professionals we must agree on a foundation of theoretical concepts and best practices so that we know how to approach each and every unique situation. Indeed the “science of marketing” must be applied as a basic framework for achieving organizational objectives.

In today’s hypercompetitive marketing environment, three areas in particular must be better addressed by corporate leaders if organizations are to increase the chances of their products’ success:

1. More cooperation is needed among all functional areas of product development, including marketing, R&D, and manufacturing, for a more seamless product introduction. Today’s company needs an interdepartmental understanding that products must be based on needs; that innovations come from R&D and can be incorporated into this need set, or in some rare instances, consumers might discover needs they didn’t know they had; and that all of this has to be feasible for manufacturing.
2. More cross-functional employees with multiple degrees would help matters vastly. It is entirely conceivable that members of the staff can have both marketing (business) degrees and chemistry (science) degrees. Dual-degree programs are rather common in today’s university environment and a second degree might only require one extra year of education. In addition, a person who has a BS-Chemistry can easily get an MBA or an MS-Marketing with an additional one or two years of education. Why must it always be one or the other? It is our observation that individuals who rise in companies the best are those who have both marketing and technical experience and background.
3. Over the past decades, companies have vastly reduced the amount of money they spend training existing employees, hoping that the universities have done their job. Don’t bet on it! Universities are great with theory, but application of theoretical concepts leaves a lot to be desired, and some of this burden must be reappropriated by the organizations themselves. Hire people with great aptitude and basic knowledge, and then train the heck out of them.

Marketing is really everyone’s responsibility. Inadequate efforts led by a failure to understand and apply the principles of marketing will almost guarantee product failure in an environment that sees 80–90% of new products languish on the shelves, slated for eventual deletion. In-depth business analysis and market testing, anchored by intensive market research, will greatly reduce a marketer’s

risk and will give the scientist more confidence that his/her creation will be a hit with consumers. Flying blind in an age of radar and GPS technology is ill advised even though it may be an exhilarating experience. *A failure to recognize and apply the Marketing Concept at all levels of the organization is akin to flying blind, and there is no glory in product failure.*

PART 1.2

CREATING THE RIGHT FRAGRANCE FOR YOUR PERSONAL CARE PRODUCT

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ABSTRACT

It has been said that no matter how good the technology in the bottle, when the consumer opens it, if it doesn't smell good, look good, feel good, and rub in well, then they won't buy it. As such, fragrances play an incredibly important role in the presentation of a cosmetic or personal care product.

Fragrances are a complex mixture of natural and synthetic materials designed to cover the malodor of a product base, make a cosmetic and personal care product smell appealing, and most importantly, reinforce the product's benefits to the consumer. They are used in nearly every product type and category!

The overall performance of a fragrance in a product is therefore critical to its commercial success. While products are typically developed with technology that works and claims are made as to what to expect, it is common practice for product developers to approach fragrance houses for advice as well as for recommendations as to what to purchase that will enhance the overall presentation of the product and achieve the goals of the brand.

This unique chapter is essentially divided into two parts. The first provides guidance and a general understanding of what, *and how*, to request product and services from a fragrance house. It also describes what can and should be expected of the fragrance house.

The chapter then goes into detail about the composition of fragrances and introduces the reader to the detailed thinking and tools that describe how a fragrance is composed by the Perfumer, and the best way to evaluate the performance of the fragrance in a product. This approach is key to choosing a successful fragrance. The chapter also contains discussion of the impact of EU (European Union) regulations on fragrance formulating and labeling relative to alleged allergens. Finally, it concludes with a focus on three important points: 1) a comprehensive discussion of the many different types of fragrances; 2) insight into making the best choice of asking about what the product formulator and marketer want to achieve; and 3) tips on how to best label these emotive actives.

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1.2.8 Cost Structure Of Fragrances

- a. Carriers
- b. Concentration-Cost Considerations for Fragrances

1.2.9 Troubleshooting Fragrances

- a. Color Changes
- b. Physical Product Stability
- c. Odor

1.2.10 Fragrance Types Defined

- a. Traditional Fragrances
- b. EU Allergen Label Free or Allergen Free
- c. Water-Soluble Fragrances
- d. Water-Dispersible Fragrances
- e. INCI Blends
 - 1. Natural INCI Blends
 - 2. Allergen-Label-Free INCI Blend
 - 3. Traditional INCI Blend
 - 4. “Unscented” INCI Blend
- f. Natural Fragrances
 - 1. Traditional Natural Blends
 - 2. Essential Oil Natural Blends
- g. GRAS Fragrances (Generally Recognized as Safe)

Conclusion

References

1.2.1 THE FRAGRANCE HOUSE

AS the phrase implies, a fragrance house specializes *solely* in the creation of fragrances. Most fragrance houses are structured in a similar way with respect to personnel and project flow. Usually, a Salesperson interacts with the cosmetic chemist to obtain information about the potential project. The Salesperson may ask the cosmetic chemist a variety of questions to help define potential fragrances for their products. For example, questions would be asked about the type of products in which the fragrance will be used; the type of fragrance or odor character desired; and any benchmarks or olfactory targets to consider; information would be required about the brand and target market, the desired price point of the fragrance, or the dosage of fragrance in the product. Other areas of interest will be the price per unit, regulatory considerations, and the expected volume of the fragrance to be sold per year if the project launches. This information is provided to the **Sales Management team** in the fragrance house to determine whether enough information has been given to assign a priority to the project.

Overall decisions are typically made using the financials of the project and the relationship of the fragrance house with the requesting company. If the project passes review, it is passed to the **Evaluation Department** to determine whether the fragrance requested already exists in the “library” (fragrances already created and approved by an Evaluator, but not sold to any company) at the fragrance house, or whether the request needs an investment of a perfumer to create a fragrance to fulfill the request. If suitable types of fragrances exist in the fragrance house's library, these will be chosen by the Evaluator, compounded, evaluated in a product base, and sampled to the cosmetic chemist for review. If no suitable fragrance exists internally, a Perfumer or Perfumers will have to create a fragrance for the request. The new fragrance creations will be reviewed by an Evaluator iteratively in accordance with the protocol described above, until they meet the requirements of the request.

The Perfumer–Evaluator team is a very important part of the fragrance house. An Evaluator is much like a food critic, or a golf caddy, whereas the Perfumer is much like a chef, or a pro golfer. Part of the role of an Evaluator is to understand the cosmetic chemist's request and extrapolate the request into an

olfactive structure that a Perfumer can create against. Evaluators also have a good understanding of current olfactive trends in their specialized product areas, and will use this information to guide the Perfumer to ensure the result is “on-trend” in odor, but is also pleasing overall. Evaluators don't tell Perfumers which specific materials to use, but rather what odor impressions or characters should exist in a creation and whether they are on-trend as per the Evaluator's understanding of their specific area of expertise. Evaluators provide a sounding board for the Perfumer to discuss olfactive directions and act as an impartial critique of a creation, much like a caddy provides a technical view of the shot at hand. Using the feedback and input of the Evaluators, Perfumers are free to use the materials that would be appropriate for the product type but also meet the regulatory restrictions of the particular geographical targets for the product. Due to the broad knowledge of the olfactive market involved, Evaluators generally specialize in fields such as Air Care, Household Care, or Personal Care. In the case of cosmetics and personal care we are primarily concerned with the evaluation points of personal care products that are used on skin and hair.

The Perfumer is a very specialized position. Most Perfumers have been trained by experienced Perfumers in an apprenticeship-type job training that takes from five to ten years. Many start as Fragrance Compounders and then move up to Perfumer's Assistant to Junior Perfumer and then Perfumer. During this time, a potential Perfumer studies and understands the general structures of classic and current fragrances as well as developing their own new creations. A Perfumer has committed to “olfactive-memory” up to 3,000 individual fragrance raw materials during their training. The majority of the required talent and creativity of the perfumer is in understanding how combinations, or “recipes” or “accords” of these materials interact with each other in a product base to form beautiful fragrance compositions that will meet the requirements of a request. The Perfumer is also expected to understand the current regulatory landscape and to know general stability parameters of the individual fragrance components in a variety of product bases such as shampoo, bodywash, conditioners, lotions, and styling gels so that the fragrances will be likely to perform as intended. Additional support is given to the Perfumer–Evaluator teams by the fragrance house's Applications department, which incorporates fragrances into product bases. The bases may be those supplied by the requesting company for a specific project, or may be a “house-base” developed by cosmetic chemists within the fragrance house.

An additional part of the Applications department's responsibility is to handle

any requests for stability testing and to create various product prototypes needed for Sales, Evaluation, and Perfumery. Other key teams that support Perfumery and Evaluation but have different duties include Marketing, which interacts heavily with Evaluation for defining overall trends and providing other marketing support.

The **Analytical Department**, extracts and analyzes the composition of specific fragrances of interest on the market that have not been created by the fragrance house in order to provide current “competitive” information to the Perfumery Department. The **Regulatory Department** provides the final determination as to whether a submitted fragrance meets all the requirements necessary for the request, and their information is invaluable not only to the Perfumer for a creative project, but also to the Evaluation department in the case of a fragrance chosen for a project from the library.

The actual creation of a new fragrance by a Perfumer generally occurs in front of a computer with a specialized software system that has a list of materials available to a Perfumer, and the final formula based on a weight or volume percent is quite like a recipe for baking or cooking. As the formulas are optimized over the Evaluation process, they are streamlined to include only the necessary materials at the proportions required for the performance of the fragrance. The Perfumer can recall the odor of several thousand materials and will first develop the beginnings of the fragrance formula in their “mind's-nose,” or they will start with a part of a formula that they have worked on in the past. The Perfumer may start a new project by putting together a fragrance formula that is about 85–90% complete and use the materials they are sure they want to use, in a ratio that they know, or can reasonably be sure will be appropriate for the request at hand. This methodology is highly useful in order to streamline the required iterative creative process. This “partial fragrance” is compounded by the Fragrance Compounder and returned to the Perfumer. If it is what the Perfumer was expecting, the Perfumer may ask the Applications department to apply the fragrance to a product base.

After evaluation in product base by the Perfumer and/or the Evaluator, the Perfumer may create several trials to complete the last 10–15% of the fragrance composition. Each trial is a specific twist on the fragrance that uses materials whose effects are more difficult to predict, or some untried out-of-the-box ideas that may or may not work well. These trials are also applied to the choice of product base, and these choices are evaluated and compared to each other and to the cosmetic chemist's request by the Perfumer and Evaluator. One or more of

the trials may go forward, or the Evaluator may ask the Perfumer to continue to further develop the concept and create more trials. This process is repeated until there are several fragrances ready to submit to the cosmetic chemist.

Usually from five to 15 trials are needed by a Perfumer to create a new fragrance that is deemed worthy to show to a cosmetic chemist. Depending on the project, several Perfumers may be assigned and then the Evaluator will make a selection of the chosen fragrances across multiple perfumers to give to the Salesperson to show to the cosmetic chemist in order to ensure each submitted fragrance is different enough to stand on its own. The cosmetic chemist will also be informed that the entire chosen range of fragrances is appropriate for his/her request. The fragrances that are completed, technically acceptable, and olfactively sound *but are not chosen by the Evaluator* are placed into the fragrance house's library for possible future use.

As a result of this process, a Fragrance House's library may have tens of thousands of finished formulas available for internal use. This is why most creative fragrance houses do not offer a "catalog" of fragrances . . . there are simply too many to catalog, they are too difficult to describe thoroughly, and since they have to be designed for a specific product type (for both regulatory and technical reasons described elsewhere in this chapter), it would not be a useful exercise.

If a Perfumer is unable to smell due to a cold or other reason, creative work continues since the formulations created by the Perfumer on the computer can be compounded and then smelled by the Evaluators or other Perfumers in a group setting. As the cosmetic chemist's request is always used as the criterion for being chosen, the team members can provide feedback to the Perfumers to help them improve the fragrance until they are able to smell again. When smelling for a living, the Perfumers and Evaluators learn to take very shallow breaths and to clear their airways in between evaluations. Most professional Perfumers and Evaluators simply space their smelling sessions strategically throughout the day rather than pollute their noses with strong-smelling materials such as coffee beans. After the Evaluator and Perfumer decide on the fragrance selection to be offered, the Salesperson shows the chosen fragrances to the cosmetic chemist. After some form of evaluation, the chemist should give feedback to the Salesperson on each of the submissions. This feedback goes to the Evaluator–Perfumer team and helps them for future requests, or helps to optimize a specific fragrance for a current request. The feedback can be about the fragrance's performance in the product base, the overall odor character, or any comment

about how well the fragrance fulfilled the request. All feedback is helpful to the fragrance house to understand their customer (the cosmetic chemist).

Once a fragrance is chosen by a cosmetic chemist and the decision is made to purchase the fragrance from the fragrance house, the Perfumer's formula is activated into the fragrance house's Production system, and is removed from the fragrance house's library so that it will not be used again for other customers. The Perfumery department sends a sample of the fragrance to be purchased to the Production's QC Department to be used as a standard. The flash point, refractive index, general color range, and specific gravity are already known from the trial samples sent to the cosmetic chemist. The QC chemists are trained to compare the standard to their productions via a "triangle test" to determine if the odor is comparable to the standard. (Each panelist is presented with two samples of the control and one sample of the test material, or two samples of the test material and one sample of the control, and they are asked to locate the "odd" sample; the results are statistically analyzed to determine whether the samples are different or not.) In addition, the refractive index, specific gravity, and color specs are compared to those that exist for each formula.

1.2.2 FRAGRANCE MATERIALS

Individual fragrance raw materials may be natural or synthetic. Each of these materials is carefully characterized by RIFM (Research Institute for Fragrance Materials—<http://www.RIFM.org>) and is regulated by IFRA (International Fragrance Association—<http://www.ifrana.org>). RIFM conducts safety and environmental studies on the fragrance raw materials and maintains the "master list" of materials that are permitted as fragrance materials. IFRA regulates the amount of each material that can be used in a fragrance and ultimately in a finished cosmetic product, for specific product categories, which is delineated in the QRA (Quantitative Risk Assessment) document. There are currently 11 product categories addressed in the IFRA regulations. They range from lip care to shampoos to wipes to candles. These categories are based on the expected dosage of a fragrance material that the public is expected to be exposed to and depends on the type of product used. It is useful for the cosmetic chemist to know in which of the IFRA categories their product falls, particularly when requesting paperwork from the fragrance house. IFRA actively educates the public as well as the chemist about the safety, history, production, creation, and use of fragrances via video and statements on their website and through social

media.

Natural fragrance materials are most often categorized as *essential oils*, *absolutes*, *concretes*, or *natural isolates*. **Essential oils** are usually obtained by steam distillation of the part of the plant that contains the essential oil. During the distillation process, the essential oil floats on the water. It is then separated from the water and any remaining water is removed. The odor of the essential oil is compared to standards for composition and overall odor. In the case of citrus oils, the essential oil is obtained by squeezing the peels of the fruits. As with other plants, the citrus oil is removed from any water that may be present after squeezing. Essential oils generally are relatively expensive versus a synthetic material (except, depending on the year, citrus oils—since the citrus industry produces a large amount of food products, and the peels are byproducts that can be used for the fragrance industry).

Essential oils are normally used in a standard fragrance (one that is not designed to be all natural) at levels from ~1 to 20%, while the remainder of the fragrance is synthetic. Essential oils add complexity and a certain “naturalness” to a fragrance, but they also add cost, as their pricing is dependent upon supply. These oils can fluctuate in cost dramatically depending on yield, political climate of the producing country, and even time of year. Weather disasters in citrus regions can create serious supply issues.

Concretes are obtained by the extraction of a plant using a hydrophobic solvent. This approach yields a product that is an essential oil mixed with waxes and any other hydrophobic material soluble in the solvent employed. A hydrophobic solvent is removed to yield the **concrete**. Further extraction of the concrete with ethyl alcohol and removal of the alcohol yields an **absolute**, which is the essential oil that contains any other materials soluble in ethyl alcohol. The waxes and other ethyl alcohol-insoluble materials are then removed, yielding a product that is completely soluble in ethyl alcohol. Absolutes of extremely faithful odor quality may also be obtained by supercritical carbon dioxide extractions of the plant as well. Concretes and absolutes are very expensive and are used as “touch” ingredients in fragrances. This means that in a fragrance they may each range in concentration from 0.001 to 0.100%. Even at these low levels they add significant cost, but they also add significant “odor value.” If they are removed their removal is quite noticeable, as the fragrance would smell less “rich” and less “extravagant.” Most of the time, these materials are only used in expensive, fine fragrances and very high-end creams and lotions.

Natural isolates are obtained by varying physical means from essential oils by

crystallization (such as crystallizing menthol out of peppermint oil), or fractional distillation, where an essential oil is distilled using heat and the various raw materials are isolated by means of separation, employing their differing boiling points. There are no chemical transformations made on natural isolates—they are merely components of essential oils that have been removed from the intact essential oil. These materials are generally also expensive since one must first obtain the essential oil, then put in some additional amount of work to obtain the isolate (usually by distillation). The use of these materials is discussed later in this chapter.

Synthetic fragrance materials are made by chemical reactions on starting materials. Many synthetic materials are derived from crude sulfate turpentine chemistry (as an example, see <http://www.renessenz.com/site/production>). Some are derived from petroleum products, and yet others are made starting from complex molecules isolated from plants. The majority of the commonly used fragrance materials are synthetic and form the framework of the fragrance. Advantages of synthetic materials are that they are typically very completely characterized by the supplier, are in plentiful supply, are relatively stable in cost, and are not seasonally dependent. There are more varied odor characters available in synthetic ingredients, and they are currently what consumers are used to smelling in their products.

Botanic materials are different than fragrance materials in that they are typically not used to impart an odor to the finished product but are used for marketing or performance reasons. These materials may be derived from any part of the plant, even those parts of individual plants that are not used to provide an essential oil (which vary per plant), with almost any appropriate solvent. Botanics are regulated on the cosmetic product's label by the PCPC (Personal Care Product Council, formerly the CTFA—<http://www.personalcarecouncil.org/>).

1.2.3 WHAT IS A FRAGRANCE COMPOSITION?

As we have stated earlier, fragrances are complex, well-balanced mixtures of individual natural and synthetic fragrance materials. A fragrance is perceived as an entity with its own aesthetic identity. The unique characteristics of each of the individual fragrance ingredients stay in the background and contribute to the fragrance as a whole without being suppressed.

The identity of the ingredients used in fragrances varies depending on the

desired odor profile, final product category, and form. For example, this would include but not be limited to oil-in-water emulsions or a transparent micellar solution. Fragrance houses and perfumers study which individual fragrance materials perform the best in each product type. They use this experience to combine the ingredients to create complex fragrances that should perform well in that product type. *Since the performance is primarily a qualitative assessment, consultation with an experienced fragrance house is essential if product developers are committed to producing a final acceptable product presentation.* It is also of importance to recognize early on that each product has a number of key points of evaluation in the use-cycle and that these must be determined and evaluated for optimal overall use results.

The creation of a fragrance also differs based on the desired effect upon the consumer. For example, technology is available to provide a refreshing burst of fragrance in use, or long-lasting soothing type or even multiple fragrance notes, delivered over time as the consumer wears it. Perfumers also understand which materials and overall odor characters can create an emotional response such as refreshment or soothing, as well as many other types of emotions and product properties. They are skilled at creating their fragrances to optimize the multiple connotations of these effects. Much work has been done in this area, as the impingement of fragrance molecules on the sensors in the nose is directly transmitted to the brain. The mechanisms of the relationship between pleasant and unpleasant, stimulating or calming, and other effects, have been well studied and will be presented in the Nose section below.

In view of the above discussion, one can see, for example, the actual fragrance materials and the balance of those materials used in a fresh green rosy musky fragrance (intended for use in a soft deluxe facial crème) will probably differ dramatically from a fresh green rosy musky fragrance designed for use in a shampoo, but they actually may smell quite similar.

1.2.4 CREATION / CONSTRUCTION OF FRAGRANCES

a. Top, Middle, and Base Notes

Fragrances are composed of a blend of top notes, middle notes, and base notes ("notes" may be used interchangeably with "character"), a blend that is often represented as a pyramid as seen in [Figure 1](#). A "note" or "accord" is defined as a type of odor character —almost like a mini-fragrance in itself. For example, a

"fresh rose" (perhaps versus a "dark red rose") will likely be composed of two to 20 individual fragrance materials, each of which will contribute a facet to the overall "note." For example, some of the fragrance materials will be grassy green in character and provide freshness, while other materials will be rosy for the overall character. Yet others may add a deep wet or dewy effect to that accord.

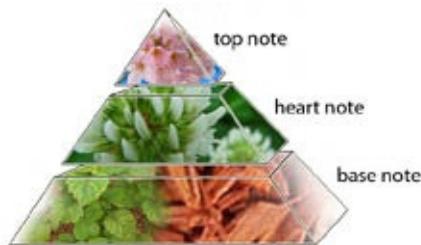


Figure 1 Fragrance Pyramid

To make the fragrance, several accords or character notes are combined in different ratios. Fragrance houses will provide on request the "breakdown" of the identity of the top, middle, and base-note characters using an industry standard known as a pyramid-type diagram or something equivalent. It should be emphasized that listed notes or characters are not usually single materials, but are the qualitative perceivable odor characters that the Perfumers and Evaluators have used to provide the overall effect. While artificial "mechanical noses" are being experimented with, at this time the perfumer's process remains a creative one. Such artificial noses are being used as a limited quality-control method in a production setting.

Top Notes are materials and olfactory characters that are immediately perceptible and tend to be the most volatile (volatility is explained in detail in the next section) materials in the composition. Citrus, green, marine, ozone, and some lighter fruity notes are often in the top notes of a fragrance.

The **Middle Notes** are the "Coeur"—the French word for "heart"—of the fragrance. These notes are often the most characteristic and recognizable part of the entire composition. Some florals, sweet, gourmand (edible), and some deeper fruity notes and lighter woody notes can be middle notes.

The formulation of a fragrance is truly a creative process. There is no quality control on fragrance notes during the process. The Evaluators have their own vocabulary, and that is what is taken to be the "standard." This subjective approach may not be quantitative, but *creativity demands a right-brain approach.*

The **Base Notes** of a fragrance are the longest-lasting part of the fragrance and tend to be the least volatile (i.e., have the lowest vapor pressure) of the notes in a composition. They act to support, round, and extend the richness of a fragrance. The base notes tend to be the most expensive part of a fragrance, so having them is important in order to ensure that a fragrance does not smell “cheap” or “thin.” Musks, woody notes, amber (rich sweet nonedible) notes, and deep heavy floral notes tend to be in the base note.

b. Fragrance Characters

Fragrances are designed by a Perfumer to have a specific odor character overall and to perform well with a defined character at discrete times in the product-use cycle. We use the term “character” as a term of art in this context and distinguish the term “characteristics” as being more consistent with measurable physical properties. By character, we mean the individual odor components involved in making the fragrance. The variation of the intended characters in different product types is what makes perfumery such a high art—extremely difficult and hard to predict. However, understanding the general processes at play can help a cosmetic chemist and marketing manager decide if a fragrance is appropriate for a product or not.

1.2.5 INTERACTION OF FRAGRANCE MATERIALS WITH PRODUCT BASES

A fragrance may smell one way out of the bottle but after being added to the product base it may smell differently in unexpected ways. Product bases in this chapter are generally those that are applied to skin or hair. For example, a facial crème generally is an oil-in-water emulsion, while a shampoo or body wash is a micellar solution and a bath oil is a solution of fragrance in oils with additives.

A review of how fragrances are able to be perceived is useful to help understand changes in fragrance character that may be seen after a fragrance is applied to a product.

There are three major hurdles that have to be surmounted for a fragrance material to be smelled, and therefore for a fragrance to be perceived as intended by the Perfumer. Each hurdle will be discussed in detail and summarized below.

First, Fragrance components need to be *volatilized* into the air in order to enter the nose and interact with the nasal sensors and be perceived by the brain. This is dependent on the intrinsic or inherent volatility of each component, or, how easy

it is to vaporize the component. A good measure of this parameter is the material's boiling point and vapor pressure. A low boiling point (high vapor pressure) means that it is easy to vaporize (out of the "neat" state, without other interactions), and a high boiling point (low vapor pressure) means that it is more difficult to vaporize at the standard conditions of use (i.e., temperature and pressure).

The ability of a fragrance component to be volatilized may change versus its behavior from the neat state due to its solubility in, or interaction with, a product matrix (Raoult's Law). This phenomenon is often due to its hydrophobicity or hydrophilicity. This variation of solubility character is one that can be quantified and indexed for comparison purposes as the ability of a material to partition between water and octanol. This measurement is represented as the $\log K_{ow}$ (Octanol-Water Partition Coefficient) of a material.

Each fragrance component has a *threshold concentration* that must be reached in the air in order for it to be perceived by a working nose. This is called the odor detection threshold. In other words, enough of the vapor has to be present at the nasal sensors to be able to perceive it, but that amount differs dramatically between fragrance materials and people.

a. Volatility / Boiling Point

As we mentioned earlier, the relative volatility of a fragrance material can be indicated by its boiling point. The higher the boiling point, the more energy it takes to vaporize a material, which means it is relatively less volatile. Conversely, the lower the boiling point of a material, the higher the volatility. Broadly speaking, materials that give the grassy green odor characters, citrus notes, and some fruity and lighter floral notes are the most volatile, while the heavier florals, sweet, woody, and musky odor characters are the least volatile. The pyramid chart of [Figure 1](#) can be enhanced to show general fragrance character and volatility relationships in [Figure 2](#).

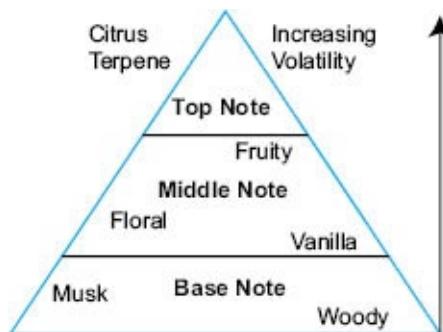


Figure 2 Volatility vs. Character

b. Hydrophobicity / Hydrophilicity (Octanol-Water Partition Coefficient, $\log K_{ow}$)

The solubility of a fragrance material in a homogeneous product base may influence the driving force for the material to vaporize in order to be smelled. Absolute water solubility is not an ideal measurement for how a fragrance material will perform in a product matrix. Of more importance is an understanding of the partitioning of a material between a water phase and an oilier phase (such as octanol). This view provides great insight into how a fragrance material will interact with the base.

A useful, simple description one learns early on is the saying “like dissolves like.” This means that hydrophilic materials are soluble in water, and hydrophobic materials are soluble in oil. Dissolving the hydrophilic sugar sucrose in water is easy, while dissolving sucrose in oil is difficult if not impossible. It is probable that some very small amount of sucrose is dissolving in the oil, but the ratio of concentration of sucrose dissolved in the oil to the amount of sucrose dissolved in the water is very small. Conversely, mixing kerosene with gasoline is easy, but mixing kerosene with water does not yield a homogeneous mixture. Again, there may be a very small amount of kerosene dissolved in the water, but the ratio of kerosene dissolved in the oil to the kerosene dissolved in the water will be extremely large.

It is easy to see in [Figure 3](#) what this conceptually would look like. For the mathematically minded chemist the equation is shown in [Figure 4](#), but the important idea is to understand that given a choice of oily phase or water phase, for every 1 unit of $\log K_{ow}$, 1,000 times more material prefers to be in the oily phase as opposed to being in the water phase.

$$\text{Log } K_{\text{ow}} \quad \log P_{\text{oct/wat}} = \log \left(\frac{[\text{solute}]_{\text{octanol}}}{[\text{solute}]_{\text{water}}^{\text{un-ionized}}} \right)$$

concentration of material in the octanol phase divided by concentration of material in the water phase (at equilibrium)

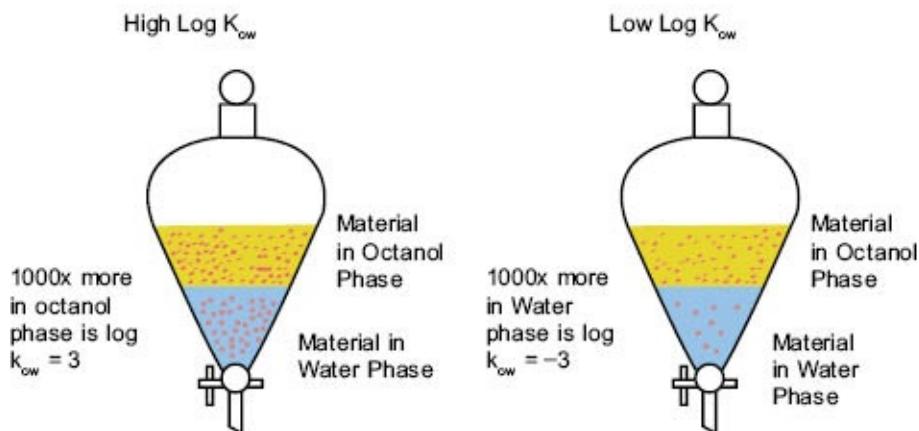


Figure 3 Octanol – Water Partition Coefficient

$$\text{Log } K_{\text{ow}} = \log P_{\text{oct/wat}} = \log \left(\frac{[\text{solute}]_{\text{octanol}}}{[\text{solute}]_{\text{water}}^{\text{un-ionized}}} \right)$$

concentration of material in the octanol phase divided by concentration of material in the water phase (at equilibrium)

Figure 4 Octanol – Water Partition Coefficient

Abbreviation: K_{ow} , Octanol-water partition coefficient

The same ideas are at play when fragrance materials are introduced into cosmetic products. Overall, fragrance materials are hydrophobic. Only a select few materials are truly hydrophilic and will dissolve to an appreciable amount in a water phase, but there is a range of solubilities and K_{ow} 's across fragrance materials and therefore to some extent across fragrance characters. Once again we must emphasize that fragrance materials are individual components while fragrance or odor characters are how they smell. A single fragrance material can have several odor characters and can be comprised of several fragrance raw materials. This is very generally described in [Figure 5](#).

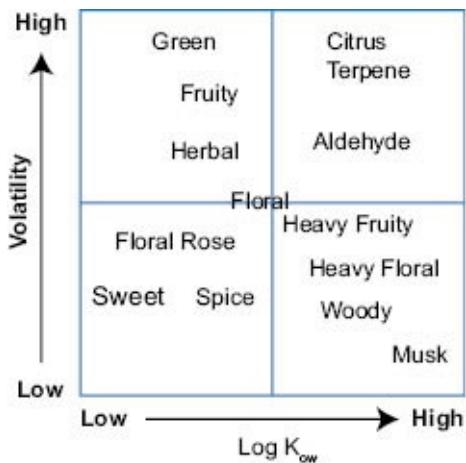


Figure 5 Volatility & Log K_{ow} vs. Character

c. Odor Detection Thresholds

Each fragrance material has a concentration at which it just becomes perceivable. From there, increasing the concentration results in increasing the perceived intensity of the material up to some maximum level—after which no further intensity increases are detectable. This is described in [Figure 6](#). The maximum perceptible intensity of each material is different, as is its odor detection threshold. It is more difficult to broadly assign fragrance characters to high threshold materials (need a lot to be able to smell them) and lower threshold materials (very low concentrations are able to be smelled). However, it is not surprising that characters related to ripe fruits and other foods have low thresholds to humans so that we are attracted to them. Also, characters related to fecal matter and decay also have very low thresholds, and result in humans being repelled by them. Characters like flowers, woods, and other “survival-neutral” types have higher detection thresholds. This is represented in [Figure 7](#).

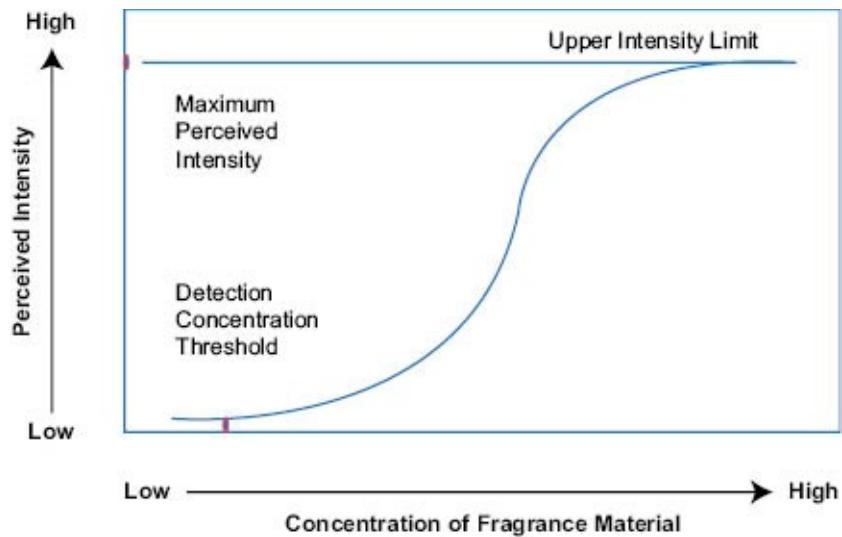


Figure 6 Fragrance Ingredient Perception

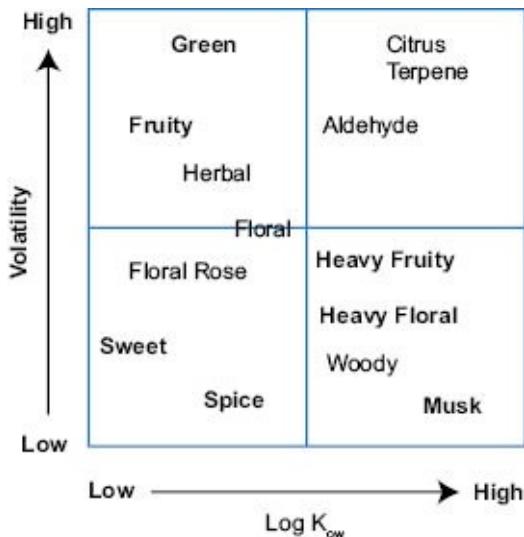


Figure 7 Volatility & K_{ow} & Threshold vs. Character

When Perfumers design a fragrance, these thresholds and intensities are optimally incorporated into a formula that smells “balanced” such that no single material is immediately identifiable. [Figure 8](#) The whole mix smells harmonious, and the entire mixture performs across many evaluation or product usage points such as out of the bottle. [Figure 9](#) As products are used, the odor profile of the fragrance changes due to the changes in concentration of the fragrance and the product. For example, when using a shampoo, the fragrance is at its highest dosage while it is in the bottle and applied to the hands. Then, when water is added and the product (and fragrance) is diluted, concentrations of each

fragrance material will eventually fall below their detection threshold and disappear from the odor profile. This shift is seen in [Figure 10](#).

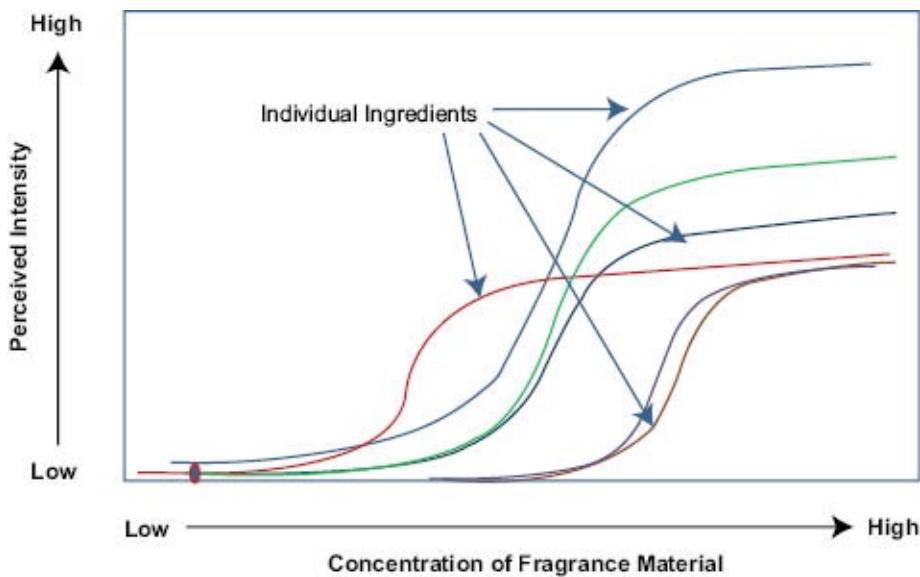


Figure 8 Fragrance Ingredient Mixtures

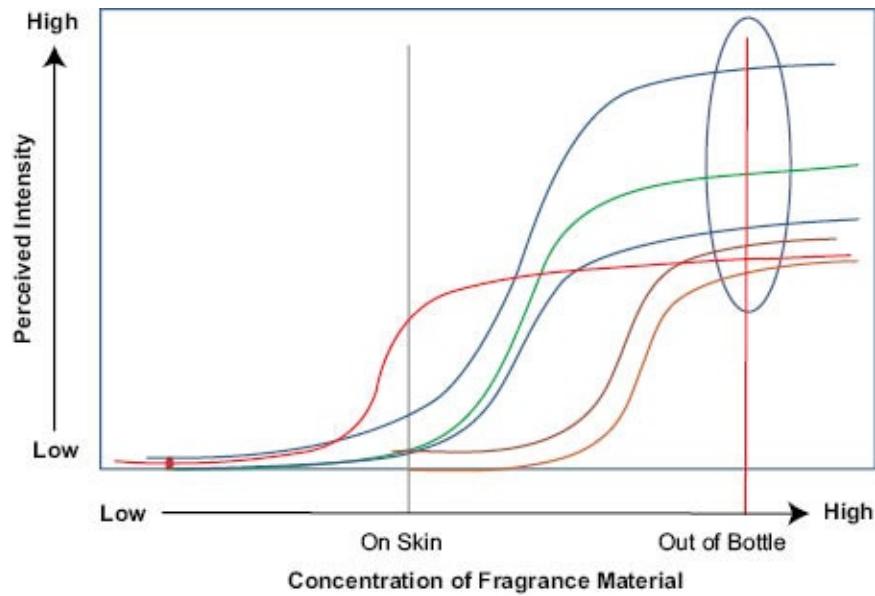


Figure 9 Fragrance Perception – Concentration

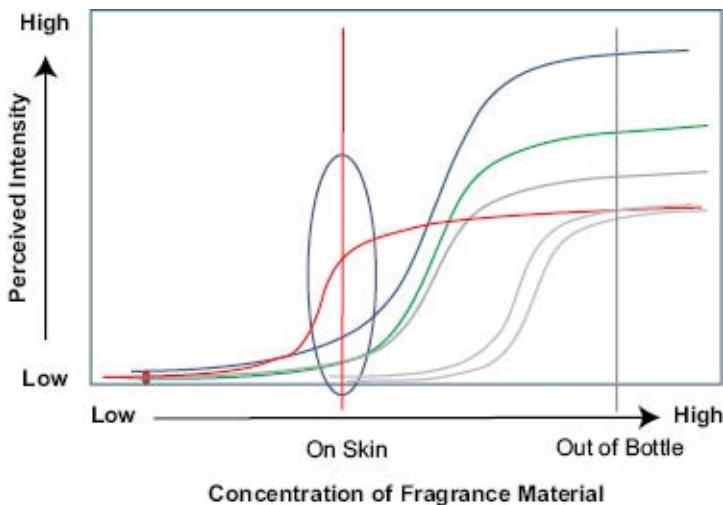


Figure 10 Fragrance Perception – Concentration

There are a few other phenomena that are sometimes mentioned with respect to detection threshold. “Anosmia” occurs when a specific person is unable to smell a specific material no matter the concentration. It has been well documented that many people are genetically unable to smell a certain type of musk, sandalwood, rotten fruit, or other very specific types of odorants. Total anosmia to all odorants is rare, but does occur; however, it is more likely to occur from injury, medications, or other trauma than genetic factors.

“Saturation” occurs when one has smelled a strong material at a high concentration that temporarily blocks any receptors from other odors, presumably due to saturation and overload of the receptor. This phenomenon is reversible and after a period of fresh air or overnight exposure the inability to smell will reverse itself. This may occur when smelling material with very low detection thresholds.

“Adaptation” occurs when an odor is smelled so often that the brain doesn't recognize it any longer. For example, when entering the monkey house at the zoo, the stench is often unbearable, but after a few minutes one doesn't really notice the odor. Upon exit and reentry, the process repeats itself—which could be adaptation and saturation presenting at the same time.

It is also useful to mention that some odorants show themselves not only by smell but by triggering a trigeminal nerve response. For example, ammonia has a characteristic odor but this odor may not be able to be covered as it also has a significant trigeminal response, which is an entirely separate neurological pathway from fragrance detection. It is for this reason that the smell of ammonia is extremely difficult or impossible to neutralize by fragrance alone.

1.2.6 EVALUATION OF FRAGRANCES

Fragrances used in personal care products are judged by the consumer at several stages in a product's use cycle. These use cycles are quite different among products and can have a significant impact on how Perfumers will create a fragrance for a specific product. For this reason it is important to explain to the fragrance house the intended nature of the product as well as its use-cycle in order to facilitate selection of the fragrance(s) that will be recommended. Each major event in a product-use profile has the possibility of being an evaluation point for the consumer, and should also be a point of evaluation for the chemist. Usually one evaluation point is more important than all the others, and it is highly useful to identify that evaluation point and ensure that the fragrance and product perform very well at that key point.

During an evaluation, the consumer (or the Evaluator) will consider the fragrance's character as applied to the product base, and the fragrance intensity. At times, the fragrance can be perfect with respect to character, but one must choose an intensity that is consistent with the product's use to make a perfect product. For example, the fragrance intensity of a high-end facial crème is usually much less than the fragrance intensity of a mass-market body crème. An Evaluator will be well aware of this difference and help recommend a level that is typical and appropriate for a product, while a Perfumer may be more likely to want to push the creative envelope.

a. Product-Use Cycle

The product-use cycle may start at the point of purchase. Many packages can be opened and the consumer can smell the fragrance of the product. On this basis alone, consumers can, may, and do make a decision as to whether the product will be purchased. It is also of great importance that the container for the product (and the fragrance) as well as the final packaging can actually be opened. Fragrance packaging design has been covered elsewhere in this book.

The fragrance house will focus strongly on this evaluation point. Normally in the fragrance house, all the potential submissions of the fragranced product will be evaluated against a control or benchmark if applicable. Products are usually compared to each other in 1–2 ounce wide-mouthed bottles with approximately 50% headspace to standardize the comparison conditions. From that selection, the fragrance house's Evaluator will choose a few to submit to the cosmetic chemist and marketing expert. Normally at least two to three times the amount of

final submissions will be compared internally and the best of these will be submitted. The remainder are put into the fragrance house's library for future use as mentioned previously in this chapter.

For packaging that cannot be opened, the consumer product-use cycle starts at the point where the consumer dispenses the product. This generally involves the dispensing and manipulation of a certain amount of product on the hands, and the evaluation point is then on the hands themselves. This is a difficult point to standardize and is considered by the fragrance house in the overall evaluation process—but not as a standalone evaluation point like many of the key evaluation points.

For rinse-off product categories, generally the next step in the product-use cycle is the addition of water to the product/fragrance in the hands or the application of the product to wet skin or hair. At this stage the “bloom” of the fragrance is very important, as usually the consumer is looking for a *burst* of pleasant fragrance to reinforce the product's claims. This is often the key evaluation point for the rinse-off type categories.

Then the product is rinsed out, leaving a certain amount of fragrance on the wet skin or hair. This is an evaluation point as well, but generally is not considered a key point for cosmetic products as opposed to laundry detergents or fabric softeners, where it may be the significant in-use evaluation point.

Finally, the skin or hair is dried, and there are further opportunities for consumers to evaluate the product's smell. Again this is normally not a key point of evaluation for consumers unless there are some type claims at this step, but the fragrance of the product and what is left after the whole use process should be pleasant.

For **lotion-type products or leave-on products** the point of evaluation after application to the hands is the application step where the consumer will rub or apply the product to the hair or skin in a manner consistent with the product category. The product's fragrance character and intensity at this step is very important to help reinforce the attributes of the product, and this can be very much a key evaluation point for products like facial lotion or hair gel.

For **leave-on products**, the evaluation stage continues for the remainder of the intended product effectiveness time frame with varying importance across product categories. For example, in a body lotion, the consumer may wish to be reassured the moisturizing effect has continued for several hours after application, making this perhaps a key evaluation point. However, for a hair gel,

the fragrance intensity and character after several hours of application is fairly irrelevant as long as it is not unpleasant and the product is still holding the hair firmly. All this is in contrast to a fine fragrance where every single moment from the moment of application to the moment in which the fragrance is washed away could be considered a key evaluation point.

b. Fragrance Complexity

Perfumers must create fragrances to be pleasant and appropriate at each of the key evaluation points in the use process. Multiple evaluation points usually indicate the fragrance will need to be more complex and take more time to create and evaluate within the fragrance house. As such, the fragrance created for this type of situation is usually more sophisticated and will be on the higher end of a price point to make a fragrance that will really “wow” a consumer. Certainly less expensive fragrances can be acceptable, but will compromise on certain evaluation points. It may be up to the fragrance decision-maker to determine if the compromises are within the scope of the project and its intended market. Any unusual information about the relative importance of evaluation points should be communicated to the fragrance house; otherwise, the fragrance house will use what seems to be the most appropriate for the product type.

1.2.7 LIMITATIONS OF FRAGRANCES

Fragrances are used to impart a pleasant odor to a product and cover the usual malodor of the product base, in addition to helping reinforce the product's performance benefits. Unfortunately there are some odors that occur in a product base that are unable to be completely masked by fragrance. Highly volatile (gaseous) and strong odors such as ammonia or sulfur dioxide from bisulfite-containing products can be mitigated to some extent, but because fragrances are made from materials with lower vapor pressures than these gases, there is very little hope that a fragrance will vaporize and reach the nose more quickly than these types of odors. Fortunately, concentrations of bisulfites in products tend to be low enough to allow fragrances to have a positive effect on this odor, but ammonia in products such as hair color is at a concentration such that only a little effect may be noticed.

1.2.8 COST STRUCTURE OF FRAGRANCES

Fragrances are constructed of the odiferous natural and synthetic fragrance raw materials described above and, usually, approximately 5 to 20% an odorless carrier that is technically necessary for the fragrance's physical stability, or is introduced as a processing aid for one or more raw materials. The major cost associated with the fragrance is in the actual odiferous fragrance materials. The carrier materials are normally relatively less expensive, but they still add a certain cost to the fragrance formula as demonstrated below.

Fragrances often contribute a proportionally larger cost to a final product than many of the other materials, or are much more expensive per pound or kilo than many of the other materials in the product. However, it is not a good idea to ask the fragrance house for a diluted version of a fragrance just to reduce the purchase price per pound of fragrance. It is best to calculate the contribution of the fragrance to the final product and use that as a guide to whether the fragrance is affordable or not for that product.

a. Carriers

Fragrance houses often ask which type of product the fragrance will be used for. This information is important for the Perfumers to decide which carrier will be used in the fragrance. Carriers such as dipropylene glycol have little solubility in products such as bath oils or some silicones, while very hydrophobic carriers such as isopropyl myristate that may work in oils and silicones may not be appropriate for a sulfate-free shampoo. Using past experience, the fragrance house will match an appropriate carrier with the product type. If an unfragranced product base can be provided to the fragrance house, they will often make tests to ensure that the fragrance is appropriate for the product and will, generally, be able to provide a recommended use range for the fragrance.

b. Concentration-Cost Considerations for Fragrances

Buying a fragrance that is as concentrated as much as possible is the most cost-effective approach to using a fragrance. An easy way to see the advantage of using a concentrated fragrance is to consider a fragrance that costs \$40/kg. If the fragrance is formulated into a product at 0.5%, the cost contribution of the fragrance is \$0.20 per kilo of finished product. If the fragrance house is told to reduce the purchase price of the fragrance to \$20/kg, and it is just diluted with additional carrier costing \$7.50/kg, then the concentrated “smelling” part of the fragrance is now not 50% of the formula. However, due to the cost of the carrier, it will be only approximately 38% of the diluted formula. [Figure 11](#)

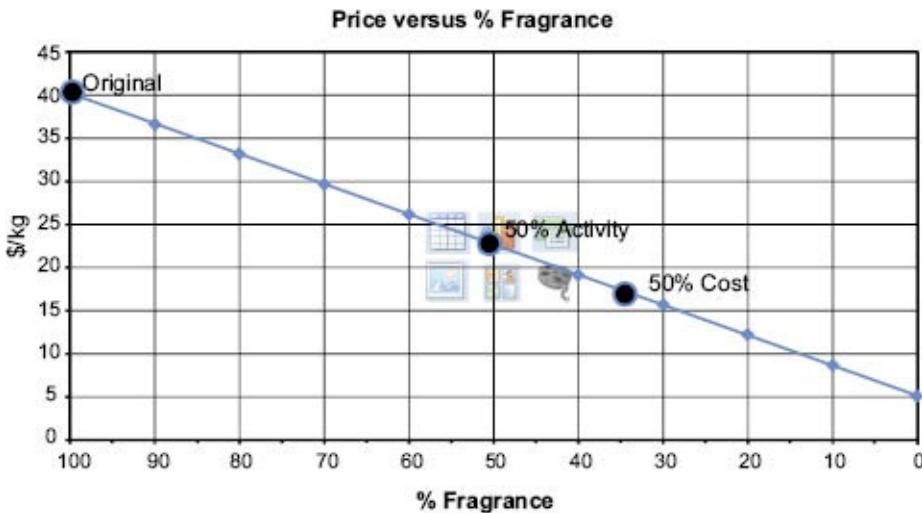


Figure 11 Dilution Reduces Fragrance Activity

To provide an equivalent olfactory effect (such as achieving the same intensity) as the original fragrance, more of the diluted fragrance would have to be used, so in this example the dosage needed of the dilute fragrance would be 1.3% in the final product (versus the 0.5% of the original concentrated fragrance). The 1.3% of fragrance used in the same product contributes \$0.26/kg to the finished product. It is \$0.06/kg more expensive to use more of a dilute fragrance in a finished product than it would be to use a lower dosage of a more concentrated fragrance, even though the purchase price of that fragrance as a single ingredient is higher. Aside from cost consideration, the additional carrier may affect the physical properties of the finished product or cause other issues. The bottom line is that it is preferable, when possible, to use as concentrated a fragrance as is reasonable.

1.2.9 TROUBLESHOOTING FRAGRANCES

There are times when fragrances can cause a product base to have a shift in color or odor, or cause separation. Color or odor stability issues may be particularly evident in a rapid-aging testing technique or exposure of the fragrance to UV or fluorescent lighting conditions. Phase stability (separation) issues may present themselves as part of a freeze-thaw process—where the product will turn into distinct layers; or if there are pearlizers used in a product, one may see a separation of the pearlizers upon heating that is not reversed upon cooling. Before approaching a fragrance house to solve an issue, it is imperative to ensure that the unfragranced base under the identical conditions did not have the same

issues as was seen in a fragranced sample. If the unfragranced base turned the same color or phase split under the same conditions, then it is probably not the fragrance alone that is causing the issue.

Once it is ascertained that the fragrance is causing or exacerbating such issues, document the condition(s) in which the issue(s) was noted, the dosage of the fragrance, the packaging in which the condition was noticed, how long it took to occur, and any other details that will help the fragrance house reproduce the problem. Normally, the fragrance house will try to reproduce the change in the actual product base, and have a Perfumer rework the fragrance formula to help alleviate the issue. Much of the time the odor profile of the fragrance will be intact, but there are times that a shift must occur. It is best to provide the fragrance house with an unfragranced base to test variations of the fragrance and to allow enough time for a few cycles of variations to occur.

a. Color Changes

Shifts in color of a finished product are sometimes caused by fragrances. A very sweet, gourmand fragrance containing a lot of vanilla or spun-sugar notes will often turn a product yellowish or sometimes light pink. Many times there is not a good way to keep the same fragrance character and solve the issue of color change. Addition of a chelating agent and ensuring the product is slightly acidic, if possible, can help.

Very grapey, fruity, or thick jasmine or tuberose-type fragrances may turn a product pink. Often the only solution in this case is to reduce the amount of that note in the fragrance, which may change the character of the fragrance.

b. Physical Product Stability

If a finished product is not phase-stable when the fragrance is incorporated, it may be that a change in carrier will solve the issue. A change in carrier or solvent will not affect the character of the fragrance to any appreciable extent. For example, in a bath oil the carrier dipropylene glycol will form a second phase in the product, whereas isopropyl myristate will yield a homogeneous product, due to the hydrophobicity of the carriers versus the product solvent. A very good carrier for all product types is diethyl phthalate, but many consumer-product companies no longer allow the use of this carrier. At this time, benzyl benzoate, isopropyl myristate, dipropylene glycol, triethyl citrate, and Polysorbate 20 are the most common carriers.

c. Odor

For odor changes in a stability test, typically either the fragrance just fades away without having any negative odors, or the fragrance “turns” into something that does not have a pleasant odor. Again, especially in the latter case, it is imperative to determine that the fragrance is the cause of the negative odor, and that this odor is not present in an unfragranced base subjected to the same conditions. If the fragrance is the issue, it may be reacting with the product base or active materials in the base and probably will have to be reformulated or exchanged for a different fragrance without the same issues. Overall, one must consider that the fragrance and the product base are basically in a dance with each other. When negative issues result from their combination, one must not jump too fast to the conclusion that the fragrance is the cause.

1.2.10 FRAGRANCE TYPES DEFINED

There are many different categories of “fragrance,” and knowing which one to ask for is important to make a successful and cost-effective product.

a. Traditional or Standard Fragrances

Traditional or standard fragrances are mixtures of natural and synthetic materials in some concentration and a carrier. If desired, the natural materials can be “called out” for inclusion on the label, but certainly the rest of the fragrance is listed on the ingredient label as “fragrance.” Most traditional fragrances contain from 5 to 20 percent carrier, which may act as a fluidizer/solvent in the event that the raw materials have a low melting point. Use of a fluidizer/solvent will prevent undesirable crystallization. This approach is useful for ease in production of the fragrance, or may be included by the Perfumer to ensure that any crystalline materials stay in solution when shipping during the colder months.

Traditional fragrances are the most common in the marketplace and they can be made in nearly every odor character imaginable, and for nearly any price point. They are also very versatile, and can usually be modified to work well in almost any type of product. If at all possible, this is the best alternative for a fragrance and one that will most likely be optimal regarding its relative cost-stability, versatility, adaptability, and robustness.

If a fragrance house is just asked for a fragrance without any other information, the supplied fragrance will be a traditional fragrance.

b. EU Allergen Label Free or Allergen Free

Based on a consensus of the Scientific Committee on Cosmetic Products and Non-Food Products (SCCNFPP), it has been reported that certain fragrances contain up to 26 dermal allergens as defined in http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_0.

As a result, for products sold in Europe, the European Union (EU) has mandated that if any of the 26 purportedly regulated dermal allergens are present above certain levels in a finished product, they will need to be listed as an ingredient on the product label in the Ingredients section per the European equivalent of the PCPC.

If a product will be marketed in Europe, these materials *must* be listed on the ingredient label. The fragrance house should be notified up front that a fragrance needs to be “EU Allergen Label Free” if none of the 26 allergens appear on the product label.

The ramifications of having an allergen-label-free fragrance is that the Perfumer's palette becomes limited, as no natural essential oils can be used in the creation of a fragrance containing them. As one might imagine, this regulation has had significant effects on many previously formulated, well-known fragrances.

c. Water-Soluble Fragrances

As described above, the vast majority of fragrance materials have no appreciable intrinsic water solubility, which means that fragrance oils, overall, are not water soluble. However, some finished products, especially those without much surfactant or much of an oil phase, require the use of “water-soluble fragrances” in order to yield a transparent product or one that will not separate into distinct phases. An example of this type of product is a water-based hair gel. The designation “Water-Soluble Fragrance” (often abbreviated by the fragrance house as WS appended onto the fragrance name) is in fact a bit of a misnomer. If asked for this type, a fragrance house will supply what is in essence a “presolubilized” fragrance. This is actually a concentrated fragrance oil highly diluted in an affordable surfactant such as one of the Polysorbates. The purpose of the surfactant as a diluent is to provide enough surface-active compatibilizing agent to solubilize the fragrance concentrate—irrespective of the amount of that material in the final product. The amount of surfactant-compatibilizing agent in the fragrance supplied can be appreciable, and is typically 70–80% of the blend,

since the house will likely overdilute to ensure enough surfactant is present to solubilize the fragrance oil. This means that only 20–30% of a “WS” fragrance is actually providing an olfactory quality to the product, and the rest is odorless surfactant of the fragrance house's choice. If the means are available, it may be much more cost effective to buy a concentrated fragrance from a fragrance house and presolubilize it with an appropriate surfactant for the product at only the required level. This amount could be less than the typical ~70% level supplied by the fragrance houses.

A good starting point to accomplish making a presolubilized fragrance is to mix two to three parts surfactant of choice to one part fragrance oil and check it in the final product at the desired dosage (taking into account the dilution). If the final product is not transparent enough, or if the final product forms two phases, then more surfactant is needed. For some types of fragrances, up to four–five parts of surfactant to one part fragrance oil may be necessary to yield a clear product. It is also appropriate to ask the fragrance house to use dipropylene glycol as a carrier if possible since it has the most water solubility of all the commonly used carriers and will result in requiring less surfactant to solubilize the fragrance. It is also important to note that all-natural fragrances are usually the most hydrophobic types and these will need the highest levels of surfactant-compatibilizers to solubilize them if in fact it is possible.

d. Water-Dispersible Fragrances

A “water-dispersible” fragrance (often abbreviated as WD appended onto the fragrance name) is one in which only enough of the surfactant is added (as described above) to form a semi-stable dispersion of the fragrance oil in water. Typically the “WD” fragrance is 50% surfactant and 50% fragrance oil, but can be as low as 30% surfactant and 70% fragrance. This dispersion will likely be hazy, but will not separate into two phases for some period of time so that it can be used in a production or application process. This type of dispersion is normally used in applying fragrances to products that are fragranced at a low level and require some kind of diluent for the ease of application and for accuracy in measurement of the fragrance dosage. For example, if a facial wipe is to be fragranced at 0.010%, the fragrance may need to be diluted in water so that 0.100% of the resulting mixture is used, and the diluting water is added into the water already in product formulation.

e. INCI Blends

INCI Blends are fragrance mixtures in which each individual material has an INCI name as listed by the Personal Care Products Council (PCPC, formerly the CTFA, website listed above), so that the label of a product, as provided to the consumer, does not need to include the word “fragrance,” and instead need only list the blend ingredients separately. The fragrance house should be asked to provide a listing of the ingredients used in these blends once they are approved. Normally in order to do so there is a confidentiality agreement or equivalent in place. It is absolutely necessary to ask the fragrance house for an INCI blend up front, as they are not the norm, and generally require special creation. These INCI blends are typically extremely simple compared to the average fragrance and have very simplistic and singular odor characters since there are so few materials used in their construction.

1. Natural INCI Blends

Not all natural materials or essential oils have an INCI name, so it is necessary to determine the exact purpose of asking for a natural fragrance. If the product needs to be “All Natural” *and* will list the fragrance ingredients on the ingredient label *and* must exclude the word “fragrance,” then asking a fragrance house for an “All Natural INCI Blend” would be appropriate. If the only requirement is a “Natural Fragrance” and the word “Fragrance” or “Natural Fragrance” can appear on the product ingredient label, it would be more appropriate to ask just for a “Natural Fragrance.”

A Natural INCI Blend is an extremely restrictive requirement, and the resulting blends are fairly limited in scope. Natural materials are generally the most expensive. If pricing restrictions are imposed on the fragrance house, typical cost-reducing carriers would be the highly hydrophobic hydrogenated soybean oil or medium-chain vegetable triglycerides or other inexpensive stable oils. Again, it may be best to have as concentrated a blend as can be afforded. The advantage of this is that one can use less of it as opposed to getting a less expensive diluted natural INCI blend. Such blends can cause phase stability or viscosity issues in the product or necessitate the use of more surfactant to incorporate it into the product. It should also be noted that natural fragrances, whether or not they are INCI blends, are subject to wild fluctuations in cost due to seasonal variations, crop yields, and availability of materials, as described previously.

2. Allergen-Label-Free INCI Blend

An “Allergen-Label-Free” INCI Blend is the most restrictive requirement for a

fragrance oil. In this case, no natural essential oils, absolutes, or other complex natural (i.e., must be from an essential oil, or an “absolute”) products can be used, but only chemicals that have an INCI name *and* are extracted from essential oils or have another natural source.

Nearly all natural essential oils or absolutes contain one of the currently recognized 26 EU dermal allergens at levels that would preclude their use completely. In the case of asking for an “Allergen-Label-Free” INCI Blend, one would need to list on the ingredient label the actual chemical names of the blend materials. This approach may defeat the overall purpose of having an INCI blend, since the long, unfamiliar names of the “chemicals” may seem onerous to the final consumer and will not differ from the negative impact and consumer perception of their synthetic counterparts. Most of these blends by definition must be fruity in character. They will have little to no base notes, a limited number of middle notes, and therefore will mostly be composed of volatile top notes. Further, they will not be appropriate for the most part to mask any base odors from the product. Such materials would be most appropriate for very clean bases that need a fleeting hint of fragrance.

3. Traditional INCI Blend

A traditional INCI blend will contain only fragrance materials that have INCI names as per the PCPC (Personal Care Products Council). They will likely contain some natural essentials oils and some artificial chemicals. These blends can be quite beautiful fragrance-wise but are typically somewhat limited in fragrance character. They do tend to be more affordable than natural INCI blends (and in many cases will have similar INCI names). It should be communicated to the fragrance house how many materials (or letters) can be supported on the ingredient label. Generally, the more ingredients, the more acceptable the fragrance character or odor.

4. “Unscented” INCI Blend

An “unscented” INCI blend is a very simple blend of neutral-smelling materials with INCI names that help mitigate the base odor of a product but do not add any recognizable fragrance quality to it. These blends typically contain two to three materials. These types of blends are useful for products like eye cream or facial cream or a type of product that has some odors from the actives but does not need a characteristic fragrance odor. Note that the base odor from the product will not be totally covered but will likely be more palatable to the final consumer.

f. Natural Fragrances

If the only labeling requirement is a “Natural Fragrance” and the word “Fragrance” or “Natural Fragrance” can appear on the product ingredient label, it would be appropriate to ask the fragrance house for a “Natural Fragrance.” If the product needs to be “all natural” *and* will list the fragrance ingredients on the ingredient label *and* must exclude the word “fragrance,” then asking a fragrance house for an “All Natural INCI Blend” would be appropriate (see above).

Natural fragrance materials can include essential oils, absolutes, concretes, and natural fractions of essential oils (which may be a single material, normally called an “isolate”). Natural materials may also use fermentation reactions and other ways to produce materials that are not synthetic.

Natural fragrances can vary dramatically in price from month to month and year to year, and shortages or quality problems in the starting essential oils can occur.

1. Traditional Natural Blends

Traditional natural blends use a combination of essential oils, absolutes, concretes, isolates, and fermentation products. The result can be a fragrance (although fairly limited in odor character) that is pleasant and somewhat complex and can be referred to on the label as a “natural fragrance.” If asked for a “natural fragrance,” most fragrance houses will send this type of traditional natural blend.

Natural fragrances are generally three to ten times more expensive than traditional fragrances and the odor characters are somewhat limited. Knockoffs of fine fragrances are impossible, but quite pleasant floral mélanges with some woody base notes and fruity top notes would be possible.

2. Essential Oil Natural Blends

Essential Oil Blends are a very limited version of a natural fragrance. These blends incorporate *only* the entire essential oils obtained from a plant or citrus fruit peels. They do not contain absolutes, concretes, or single material isolates. Such materials are very limited in character because they contain only citrus, leafy herbal, lavender, some woody, and a very few floral characters and their resulting blends. It is necessary to specify to a fragrance house that an “essential oil blend” is needed, and the fragrance house may have questions about the allowed types of materials.

Essential oil blends are the most expensive as well as the most limited in

character, and their pricing over the season varies wildly as many essential oils increase dramatically in price when not in season.

g. GRAS Fragrances (Generally Recognized as Safe)

GRAS fragrances are a niche specialty fragrance type necessary for lip balms or other products that may enter the mouth or penetrate the lips, or are for children's toys. GRAS fragrances are those in which each fragrance material has a flavor FEMA-GRAS number and/or is recognized as a flavor material. These fragrances also must meet all of skin contact requirements for the product category in which they are used. GRAS fragrances, nearly by definition, tend to be fruity or food-related in character. They tend not to be long lasting and are usually quite simplistic in character.

CONCLUSION

Fragrance development is very important to the success of a consumer product. Not only will the character and intensity of the fragrance reinforce the product's benefits, but they will also add appeal to the consumer. Understanding the basic technical requirements of a fragrance and knowing how to interact with a Fragrance house will greatly enhance the success of the cosmetic chemist and ultimately the product.

REFERENCES

http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_0

PART 1.3

FRAGRANCE PACKAGING DESIGN:

A Multi-Sensory Experience from Concept to Consumer

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ABSTRACT

The benefits of cosmetics and personal care products are both tactile and functional, providing a temporary benefit by enhancing the appearance of the face, body, or hair.

By contrast, fragrance transforms the moment, awakens the senses, creates indelible memories, and captivates the people in a room. The consumer's experience of fragrance is subjective, abstract, conceptual, and emotional. The combined expertise of the marketer, perfumer, and designer is dedicated to focusing on the development of a product that communicates an emotional link and provides a cohesive, holistic sensorial experience to the consumer. Sensory cues are used throughout the packaging to communicate the olfactive character of the product as well as the emotional story; and these cues are the foundation of a successful fragrance. In this chapter, experts have been interviewed across all components of the development process, and a review of research has been compiled in order to expand the knowledge base of product developers and educate further about the stages of a cohesive fine-fragrance development process.

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With over 1,000 fine fragrances launched globally in 2011, what is the best way for fragrance brands to stand out in the eye of the consumer and generate revenue for the brand? Expectations are set for the consumer through sensory cues such as the bottle, packaging, color story, and scent. If these elements are not aligned, consumers can experience sensory dissonance. The five senses are core to human experience and are essential to our perception of the world. In today's beauty industry, marketers, perfumers, and designers need to work in tandem in order to meet tight timelines and anniversary sales and to be

competitive in the marketplace. But is the final product always optimized? Were there connectivity points between the team to maintain synergy for the final product? Does it communicate the concept to the consumer?

Primary Question for Product and Brand Development:

What can companies do to integrate their internal process in order to produce a unified product the consumer will positively respond to, accept, and ultimately purchase?

1.3.1 INTEGRATED PROCESS: THE BRIEF

Led by Brand Development or Marketing, a document called the “brief” outlines the objective and strategy for the finished product. The brief is shared with Product Development to initiate the project. After the initial review, additional briefs can be developed, narrowing the focus and outlining evolving details of the project. The initial brief puts the project in action, and detailed briefs such as the fragrance brief and creative brief are viewed on a micro level for the designated department. Product Development also creates a project timeline and a component pricing analysis, which outlines the budget for the product.

Initial Brief or Project Profile

- Brand identity
- Target consumer (demographic and psychographic)
- Quantitative and qualitative research results, if applicable
- Product positioning and white-space opportunity
- Competitive analysis
- Pricing and cost targets
- Quantity
- Distribution
- Target ship date

Fragrance Brief

- Brand overview
- Target consumer with focus on psychographic
- Concept: Olfactive story
 - Mood board
 - Color direction
 - Product personality
- Olfactive category direction: Primary and Secondary
 - Primary: citrus, floral, fougere, woody, chypre, oriental
 - Secondary: citrus, marine, aldehydic, aromatic, green, floral, spicy, fruity, musky, gourmand, leather/animalic
- Oil cost target / Fragrance blend percentage
- Olfactive market inspiration or benchmarks
- Fragrance color
- Distribution (in order to understand compliancy restrictions)
- Regulatory and stability requirements
- Timeline for deliverables

Creative Brief

- Brand overview
- Target consumer
- Product positioning
- Concept
- Component direction
- Color direction
- Olfactive direction
- Existing brand assets (logos, fonts, imagery, color)
- Budget
- Timeline for deliverables

While all the departments are working simultaneously, it is important to have connectivity points throughout the process in order to confirm alignment and clear direction towards achieving the ultimate goal.

Throughout the development process of a product, it is essential to think about the left-brain and right-brain functions you want the consumer to experience with the product. One must ask, “How will the consumer react to the product emotionally, creatively? How will the product function? What will the consumer experience through sensory cues? How will the concept or story be successfully translated to the consumer?” In developing sensory cues it is important to consider how each cue will translate the scent to deliver the concept to the consumer.

1.3.2 SMELL THROUGH HEARING: CONSUMER TESTING

Curate and guide the development process based on consumer findings.

Gail Vance Civille, President of Sensory Spectrum Inc., has pioneered advanced sensory evaluation approaches for industry, academia, and government. She shares that ideas are coming directly from the consumer. Preliminary concept work should involve consumers in order to understand what types of messages are being communicated through product design. A hybrid approach using qualitative and quantitative testing can provide insights as to what the consumer wants and needs, and how the concept and marketing messages are being perceived.

Qualitative Testing

- Field Testing
- Shop-alongs
- In-home visits
- Diaries
- Focus groups
- Interviews
- In-store intercepts

Quantitative Testing

- Surveys
 - Multiple choice
 - Numeric

Mark Knitowski, the “Nose” behind all the Victoria’s Secret scents, does not consider himself the “expert” in developing fragrances for his consumers. Rather, he considers himself the “curator of fragrance” and the “protector of the consumer information.”

Further, Knitowski believes the consumer should not be “sold” a fragrance, but they should embrace a journey with a scent and connect to a well-told story. “The right fragrance should be part of an emotional journey, reflecting how a woman or man feels about themselves and how they want to project the image of themselves to the world.”

By using over 1,000 of the Victoria’s Secret stores as direct access to the consumer, Knitowski involves them in the development process. He spends time in the stores engaging consumers with general day-to-day conversations through in-store intercepts. He may also test two fragrances that he’s been developing to gain feedback and perspective. He first has the consumer smell on skin and then on a blotter, the scent strip used to test fragrances. The consumer then smells the fragrance completely blind without any sensory cues. From their response, Knitowski is looking to understand whether they would purchase the scent, and what adjectives they use to describe it. Through the qualitative learning extracted from his consumer studies, Knitowski further evolves the scents based on the comments from the consumer. Each component, including the fragrance, carton, bottle, and name, are tested independently and then together. If one of these elements does not align to create a holistic finished product, then the consumer will not align with the intended concept.

As an example, Victoria’s Secret product “Bombshell” was the perfect blend of fragrance, bottle, and packaging—but it did not start out this way. The fragrance tested so positively that there were no dislikes out of 150 paneled, yet when the scent launched under the name “Signature” in a rounded bottle, the results were less than successful. After analyzing the less-than-favorable response, the team knew the fragrance was not the problem. This observation became a turning point for creating a product success and is an excellent example of the role of the development team realigning and modifying the product based on consumer results.

The next test that was initiated included the same fragrance with a more faceted bottle and a name that aligned with a recent bra launch, Bombshell. Led by Marketing and Design together, these adjustments built the foundation for a successful fragrance that ultimately won a Fifi award for Best Packaging in the Specialty Market and a Consumer Choice Award; it was also voted Fragrance of the Year in 2011. The positioning within the Bombshell lingerie master brand enhanced the fragrance story by giving it depth and an emotional connection. The adjustments to the bottle further enhanced the cohesiveness of the product because it aligned with the overall product positioning in a way that resonated with consumers.



Marketing and Product Development need to consider both qualitative and quantitative testing. Throughout the testing process it is important for the developers to listen closely to what the consumers are saying, while also maintaining the integrity of the concept. By closely “smelling” the direction through listening to the consumer and curating the learning, a successful fragrance, Bombshell, made history.

1.3.3 SMELL THROUGH SEEING: FRAGRANCE NEEDS COLOR

“Used wisely, color is vitally important and is an instant attention grabber that succinctly gets the message across.” (Pantone Color Institute)

Color is perhaps the strongest cue in aligning the consumer’s expectations with the desired fragrance experience. Through the fragrance, carton, bottle, and

color, olfactory direction can be communicated, as well as the brand's emotional positioning. The relationship between color, scent, and emotion can impact a consumer's emotions. What does yellow smell like? What branding cues does red communicate? It is imperative to have color direction in the initial product profile or brief because disconnect in the color with the other elements of the fragrance can confuse the consumer.

Here are some examples of the message a color in a fragrance can communicate:

Blue—calm, watery, ozonic

Green—revitalizing, fresh, nature, eco-conscious

Red—sexy, powerful, confident, energizing

Purple—luxury, mystery, exotic, royalty

THAT WAS THEN...

...THIS IS NOW



Ylesight 2012)

Marketers can leverage color to communicate newness within a portfolio and to distinguish flankers, or fragrance variations, from the original, while also aligning with trends. Fashion color trends relate closely to the beauty industry and change based on the season and geographic location. Forecasting services are a key reference in aligning with trendsetting color palettes, Marc Jacobs's Daisy, a fragrance product, is an example of how color has extended the brand lifecycle by communicating a prestige eau de parfum version through the use of richer colors such as black and gold, silver and gold, and red and black. Also, seasonal color stories stimulated demand and further differentiated a limited-edition bottle. Once consumers see the color and packaging of a fragrance, they already form an opinion and their expectations are set.



Coty's Senior Vice President, Catherine Walsh, is known as the "scent whisperer." She is the generating force behind brands and celebrities such as Jennifer Lopez, Calvin Klein, Marc Jacobs, and Sarah Jessica Parker. Walsh has stressed that it is critical for all teams involved in the development of a fragrance to be aligned all the way through the process. Marc Jacobs's fragrance Dot was launched in 2012, but the initial concept and color direction the Coty team was working towards was very different than the polka dot red-and-black fragrance that hit the market. Marc Jacobs changed the direction because he was inspired by a stronger story that was more meaningful to him and tied to his fashion line. Polka dots have been a recurring theme for the designer, and were a major focus for his Fall 2012 ready-to-wear line. "One design that I always go back to in some way is the dot. I felt like this was something I'd never get tired of, and I'll always find a new way to interpret," Marc Jacobs said. He liked the idea of a hybrid butterfly/lady bug, which communicated the Dot story of free spirit, femininity, playfulness, and luck (Naughton, 2012).

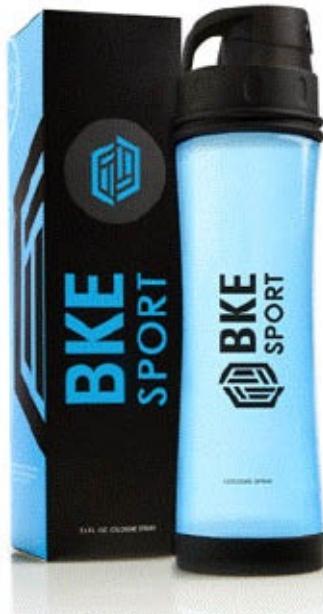
With this significant direction change, Coty communicated all the details of the new concept, the polka dot design and the red-and-black color story through all departments so that Marc Jacobs's strong vision and story could be communicated to the consumer.



As a trend leader in the fragrance industry, Coty utilizes consumer testing and research to better educate the marketers to learn how to lead consumers to the next trend. Trend-leading brands need to maintain the integrity of the brand identity and the character of the concept to offer consumers a product they did not know they wanted until they saw it—and of course smelled it.

1.3.4 SMELL THROUGH TOUCHING: HOW WILL THE CONSUMER FEEL?

The consumer will first see the packaging and the bottle. If the packaging and bottle appeal to the consumer, they will then pick it up: this is the first goal of the marketer. Bottle engineers need to think of the following when developing the bottle: how do you want the consumer to feel emotionally when they pick up the bottle? Does the bottle have soft edges and a lightweight feel or is it rigid and weighted? In one example, Tru Fragrance and Beauty worked with a bottle engineer to create a fragrance bottle for a men's apparel sport line. The Product Development and Creative teams needed to think about this product from an active man's perspective. The bottle was built so the male consumer could grab the bottle and go. Ergonomically tapering through the bottle gives it an aspect of comfort in the man's hand and the built-on spray-through cap allows for easy spraying any time without leaking. The weight was also considered, since the product was designed to be thrown in a gym bag and be a portable fragrance. While the glass is lightweight, the black base and overcap are weighted to give it a feeling of a dumbbell. The development team consciously thought about how the man would use the fragrance and how it would feel in his hand.



1.3.5 SMELL THROUGH SMELLING: TELL A STORY, PAINT A PICTURE

Fragrance is an art. Marketers provide a fragrance brief to the fragrance house. The brief includes as much information as possible about the brand DNA, packaging direction, color direction, target consumer, and store environment. Most importantly, the brief should contain a sensory story and paint a picture in the mind's eye of the consumer. The perfumer is no different than a poet or composer, so a marketer needs to convey the story of the product visually and verbally to stimulate the perfumer. To be successful, consideration should be given to an overall view of the use of the product anticipated. The marketer must set the stage in a variety of ways. Set an environment; describe the dream. What is the temperature? Is it hot like the sun or hot like a fire? How does it feel on skin? Is it humid or comforting? What is the mood of the environment? Is there music on the beach during the day in a tropical place with laughter and dancing, or are you warm and cozy next to a fire in a cabin in the mountains?

A fragrance evaluator, whose purpose is to work closely with the perfumers and optimize their ideas, reviews the brief. The evaluators represent the marketer, and the objective is to really put themselves not only in the shoes of the marketer but really to encompass their identity. They need to take on the role of the marketer, to learn the marketer's tastes and how they think, and to fully

understand them in order to achieve the best fragrance submissions.

CONCLUSION

Falling in love with fragrance. The perfumer begins to blend the “story” based on the brief and visual mood board and works closely with the evaluator throughout the process. Once the fragrance is chosen, it is the marketer’s responsibility to take the “story” that is bottled up and make sure that the story is released to the consumer through all the sensory cues so the consumer can experience the story and fall in love with the fragrance.

By means of the use of sensory cues and integrating the development process the concept is successfully shared with the consumer. The story becomes the consumer’s dream and each time the fragrance is used, the mood is impacted by how they desire to feel. Sophisticated, romantic, casual, masculine, sexual, playful...the consumer’s dream comes true—and this is why people love fragrance.

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UNDERSTANDING THE VALUE OF MOLECULAR CELL BIOLOGY AND GENE ANALYSIS FOR THE NEXT GENERATION OF COSMETIC PRODUCTS

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* *A note from Meyer R. Rosen*

Before we get started on perhaps one of the most complex chapters in this book, we call attention to the fact that many readers will not be familiar with numerous terms used in the text. To this end, a glossary has been created at the end of the chapter. To facilitate understanding, note that in the text all “big” words, unfamiliar to many, have been bolded, and a concise definition is presented in the glossary.

As Editor-in-Chief of this book—I take it as my duty to urge you, the reader (no disrespect intended) to grow in the 50-year-old tradition of Harry’s Cosmeticology. The contents of this chapter are deemed so important that,

taking editorial prerogative, it has been put in the first section of the book, “In the Beginning,” along with other key areas that provide the foundation for new ingredients and products that are essential for understanding and leading innovation in our industry.

This chapter introduces the basic fundamentals of the principles of molecular biology and why cosmetic industry professionals must understand them. It is our opinion, based on the developmental path being taken by ingredient suppliers and formulators, that, like learning to effectively use social media in the Internet age, if you do not at least have a basic understanding of these concepts, you will be left behind.

Consider this analogy, which is quite prevalent at the time of this writing. When young children age three or so and older, and teenagers, are walking around with their iPads and iPhones, etc., and you—the adult, the parent, the seeker of “how do I text, or use LinkedIn,” etc.—are your resource for how to do IT. If you do not use that brilliant mind of yours to lead the knowledge pack rather than be a follower, then, my friends, your foreseeable contribution to the development of new cosmetic and personal care products will wither into dust and be blown away by the wind.

And Now, to the Heart of the Matter...

1.4.1 INTRODUCTION

The Human Genome Project revolutionized scientific research in the field of molecular biology. Many of the techniques and gene interaction knowledge base currently in use are the result of the project. The cost and time to conduct such studies continues to decrease, resulting in the generation of information relevant to the formulations chemist. Molecular cell biology investigates how cells develop, operate, communicate, and control their activities. Cells communicate with body tissue, which is composed of cells. Tissues communicate with other tissues and organs throughout the body. Generally the transfer of information between cells, tissues, and organs is through proteins. We now know that genes consisting of deoxyribonucleic acid (DNA) are responsible for biological structure, maintenance, and integration of cellular function. DNA indirectly controls the synthesis of many small molecules including proteins. DNA may be considered a storage form of genetic information. Gene expression is how cells “know” to make the right proteins at the right time in the right quantities.

1.4.2 PRINCIPLES OF MOLECULAR BIOLOGY

Amino Acids and Proteins: Their Role in Cell and Tissue Communication

Proteins are the working molecules of the cell; they carry out the program of activities encoded by genes. To do this a coordinated effort of many different types of proteins is necessary (1). The three-dimensional structure of a protein enables it to regulate concentrations of metabolites, cause cell motion, and provide structural rigidity to the cell. The spatial organization of proteins is critical in understanding how they work (1).

Proteins consist of amino acids, and there are only twenty amino acids from which proteins derive their structure. The three-dimensional structure of the protein is determined by the amino acid sequence. Amino acids can be classified primarily on their solubility in water, which is influenced by the polarity of their side chains. Amino acids with polar side groups tend to be found on the cell surface and make proteins soluble in aqueous solutions. Amino acids with nonpolar side groups avoid water and aggregate to form the water-insoluble core of the protein. Water-soluble amino acids have ionized or polar side chains. At neutral pH, arginine and lysine are positively charged; aspartic acid and glutamic acid are negatively charged and exist as aspartate and glutamate. These four amino acids are the major contributors to the overall charge of a protein (1). Histidine has a pKa of 6.8, the pH of cytoplasm in the cell.

Small shifts in the pH of the cell will change the charge of histidine side chains. The activity of numerous proteins is modulated by pH through protonation of histidine side chains. Asparagine and glutamine are uncharged and have polar amide groups with extensive hydrogen-bonding capacities. Serine and threonine are uncharged and have polar hydroxyl groups facilitating hydrogen bonding with other polar molecules. Hydrophobic amino acids have aliphatic side chains that are insoluble or slightly soluble in water. These amino acids tend to pack in the interior of proteins away from the aqueous environment. Cysteine, glycine, and proline have side chains with unique properties. The cysteine side chain contains a reactive sulfhydryl that can oxidize to form a disulfide bond to a second cysteine. Disulfide bonds are commonly found in extracellular proteins where they facilitate maintaining a folded structure. Glycine, the smallest amino acid, has a single hydrogen atom as its R group. Its small size allows it to fit into tight spaces. Proline and glycine may be found at locations on a protein's surface where the chain loops back into the protein.

The Cell

All organisms are composed of cells. A cell is a membrane-bound unit that contains DNA and cytoplasm. A plasma membrane encloses each cell, separating its contents from its surroundings. The plasma membrane contains numerous proteins that are responsible for the ability of the cell to interact with its environment. For example, receptor proteins in the membrane interact with hormones and induce changes in the cell in response to the interaction. Transport proteins help molecules and ions move across the plasma membrane either from the membrane at the cell surface to inside the cell, or from inside the cell to the cell surface. Every cell contains DNA, which is the hereditary molecule containing the genes that code for the proteins being synthesized by the cell.

Cells found in animals referred to as eukaryotic cells contain numerous compartments termed organelles and smaller sac-like structures known as vesicles. The largest organelle in a eukaryotic cell is the nucleus, which is the repository of the genetic information that directs all the activities of a living eukaryotic cell. The nucleolus is an area found in the nucleus where intensive synthesis of ribosomal RNA is occurring. A nuclear envelope is located on the surface of the nucleus. The outer membrane of the nuclear envelope is continuous with the cytoplasm's interior membrane system called the endoplasmic reticulum. Scattered over the surface of the nuclear envelope are nuclear pores filled with proteins that act as molecular channels permitting certain molecules to pass into and out of the nucleus. Proteins moving into the nucleus may be incorporated into nuclear structures or catalyze nuclear activities; RNA and protein-RNA complexes are formed in the nucleus and exported to the cytoplasm. DNA of the eukaryotic cell is divided into linear chromosomes, which are fully extended into thread-like strands called chromatin. This arrangement allows proteins to attach to specific nucleotide sequences along the DNA. Without this protein interaction DNA could not regulate the day-to-day activities of the cell. Chromosomes are associated with packaging proteins called histones.

When a cell is ready to divide, the DNA coils up around the histones into a tight condensed structure; this initial aggregation is termed a nucleosome, and resembles a bead on a string (2). After cell division the chromosomes uncoil, permitting RNA polymerase—an enzyme that makes RNA copies of the DNA—to gain access to the DNA. RNA copies of DNA are the only way hereditary information in the DNA can be used to direct the synthesis of proteins. The eukaryotic cell is distinguished from plant cells with the presence of internal

membranes in the cell. The largest internal membrane is the endoplasmic reticulum (ER). The ER is composed of a lipid bi-layer embedded with proteins. The surface is embedded with ribosomes involved in protein synthesis. Proteins synthesize on the surface of the ER (termed rough ER because the surface appears pebbly, like the surface of sandpaper). These proteins contain special amino acid sequences called signal sequences. These protein sequences travel through the ER membrane to the interior of the ER and then move to a vesicle-forming system known as the Golgi apparatus, and is eventually released to the outside of the cell.

Eukaryotic cells contain a variety of vesicles. Lysosomes are membrane-bounded digestive vesicles that contain enzymes catalyzing the breakdown of proteins. Other vesicles, known as peroxisomes, contain the enzyme catalayse, which breaks down hydrogen peroxide into water and oxygen. The breakdown of hydrogen peroxide is important because hydrogen peroxide buildup in the cell is toxic. Mitochondria are organelles containing proteins that carry out oxidative metabolism of the cell. They have their own DNA-containing genes that produce proteins essential to the mitochondria's role as centers of oxidative metabolism. Most of the genes that produce the enzymes used in oxidative metabolism are located in the nucleus of the cell. The nucleus is the control center of the cell for direction of protein synthesis and cell reproduction. The nucleolus is the manufacturing site for ribosomal subunits, which are sites for rRNA synthesis, a process necessary for DNA replication. Ribosomes provide a framework for protein synthesis.

1.4.3 PROTEOMICS, GENOMICS AND EPIGENETICS

Protein Conformations and Cell Communication

Mammalian cells contain up to 10,000 different kinds of proteins (1). Proteins must be localized to the appropriate cellular membrane or aqueous compartment to function properly ([Figure 1](#)). Proteins are the working molecules of the cell; they carry out the program of activities encoded by genes. The structure of the protein gives rise to its function. The function of virtually all proteins depends on their ability to bind to other molecules, or ligands with specificity. There are numerous types of receptors on the cell surface, and each is essential for signaling between cells. Affinity refers to the strength of binding and specificity refers to the ability of a protein to bind one molecule in preference to another

molecule. In some cases a particular protein on one cell binds to a receptor protein on the surface of an adjacent cell, triggering differentiation.

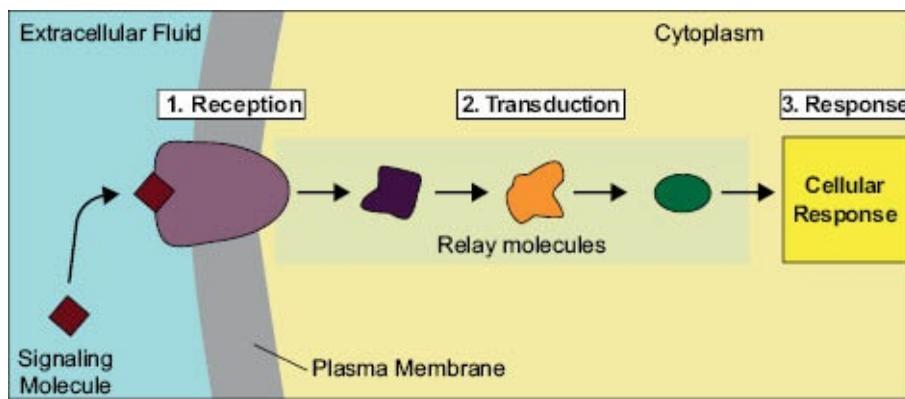


Figure 1: Overview of cell receptors and cell signaling

Cell signaling can be divided into three stages.

1. Reception: A cell detects a signaling molecule from the outside of the cell. A signal is detected when the chemical signal (also known as a ligand) binds to a receptor protein on the surface of the cell or inside the cell.

2. Transduction: When the signaling molecule binds the receptor, it changes the receptor protein in some way. This change initiates the process of transduction. Signal transduction is usually a pathway of several steps. Each relay molecule in the signal transduction pathway changes the next molecule in the pathway.

3. Response: Finally, the signal triggers a specific cellular response.

Source: Overview of Cell Signaling. <http://creativecommons.org/ns#>"

xmlns:dct="http://purl.org/dc/terms/"

about="http://www.hartnell.edu/tutorials/biology/signaltransduction.html

Available at: <http://www.hartnell.edu/tutorials/biology/signaltransduction.html>

Signal molecules are designated, endocrine, autocrine, or paracrine depending upon the distance over which the signal acts. Membrane-bound proteins on a neighboring cell can also signal to each other. In endocrine signaling the signaling molecules, or hormones, act on target cells distant from their site of synthesis. In paracrine signaling the molecules released are in close proximity to each other. In autocrine signaling cells respond to substances that they release themselves. There are compounds that may act by multiple types of signaling. Epidermal growth factor (EGF) is an example of a protein hormone synthesized in the plasma membrane. Membrane-bound EGF can bind to and signal an adjacent cell by direct contact. Cleavage by a protease releases secreted EGF,

which then acts as an endocrine signal on distant cells (1). There are four major classes of cell-surface receptors: 1) receptors with enzymatic regulatory function; 2) G-protein-coupled receptors, which activate or inhibit an enzyme that generates a specific second messenger or modulates an ion channel, causing a change in membrane potential; 3) ion channel receptors, which change the conformation of the receptor to enable specific ions to flow through the receptor resulting in a change of the electric potential across the cell membrane; and 4) tyrosine kinase-linked receptors, in which ligand binding stimulates formation of a dimeric receptor, which interacts with and activates one or more cytosolic protein tyrosine kinases. Receptors for many cytokines, interferons, and human growth factor are of this type. (See [Figure 1](#))

GTPase is another protein category involved in signal transduction. This group of proteins functions as molecular switches that turn cell signaling on or off. GTPase proteins are regulated in different ways. Protein kinases work through phosphorylation of proteins. Animal cells contain two kinds of protein kinases, those directed towards tyrosine and those directed towards either serine or threonine. Protein kinases become active in response to the stimulation of signaling pathways. The catalytic activity of kinases is modulated by phosphorylation, by direct binding to other proteins, and by changes in the levels of various second messengers. Adapter proteins function as docking sites for other proteins and mediate transport steps of structural proteins in the cell.

Epigenetics

Revising the Paradigm in the Age of Genomics

Epigenetics is defined as somatically heritable states of gene expression resulting from changes in chromatin structure without alterations in DNA sequence. Epigenetic changes are involved in inflammation, obesity, insulin resistance, aging, immune disorders, and cancer. The previous paragraphs summarized cell signaling through numerous protein messengers that activate a cascade of molecular events within the cell and between cells. Many cell-signaling mechanisms are not well understood. It is increasingly becoming evident that cell signaling modifies gene expression of critical genes associated with physiologic and pathologic processes. More recent observations in the field of epigenetics indicate that epigenetic modifications can be influenced by external or internal environmental factors.

It is anticipated that the emerging field of **epigenetics** will dominate the field

of genetics for the next decade and beyond. “Epi,” from the Greek language, means “above or over.” Epigenetics, above genetics, is an influence that affects appearance without changing the DNA code. In other words it is an influence that can affect gene expression without causing a mutation in the DNA code. The mechanisms of epigenetic gene expression can be profiled using a variety of techniques. Commonly used techniques measure the regulation of translation or stability of messenger RNA (mRNA), DNA methylation, and histone modification. Virtually all biological pathways and diseases may have an underlying epigenetic component. The epigenome is dynamic; it exhibits changes during the aging process. As an individual ages, gene promoters become hypermethylated, and many of the age-related DNA methylation markers are also associated with disease. Epigenetics is discussed in more depth elsewhere in this text.

A key element of epigenetics is chromatin and its structure. Chromatin is a DNA complex organized into repeating units called nucleosomes. Chromatin is wrapped around the DNA. The degree of winding plays a role in gene regulation. When the winding pattern is altered by a chemical or environmental stimulus, the change in winding pattern is termed histone modification. Modification of histone winding may result in an altered gene expression. Histone modifications regulate DNA transcription, repair, recombination, and replication. They can alter local chromatin architecture. Histones, proteins found in the cell nuclei of animals, package and order the DNA into structural units called nucleosomes. Nucleosomes are the chief protein components of chromatin.

The best-understood and most commonly studied histone modifications are acetylation, phosphorylation, methylation, and ubiquitination. Histone modification regulates DNA transcription, repair, recombination, and replication. It can alter chromatin structure. DNA methylation is a biochemical process involving the incorporation of a methyl group to the cytosine or adenine on the DNA nucleotide. DNA methylation alters the gene expression in cells and plays a role in many forms of cancer. DNA methylation is involved in the regulation of many cellular processes, including chromosome stability, chromatin structure, embryonic development, and transcription.

A study investigating aging and chronic sun exposure related to epigenetic changes in skin found that DNA methylation plays a role in aging skin exposed to the sun (4). DNA phosphorylation is the addition of a phosphate group to a protein, resulting in a conformational change that initiates protein activation or

deactivation of DNA regulatory mechanisms. DNA acetylation involves the removal of the positive charge on the histones, thereby decreasing the interaction of the N termini of histones with the negatively charged phosphate groups of DNA. As a consequence, the condensed chromatin is transformed into a more relaxed structure that is associated with greater levels of gene transcription. Translation is the process of DNA transferring genetic information via RNA that is processed by the ribosome into a chain of amino acids that become functioning proteins termed messenger RNA (mRNA). Transcription is the protein folding of the resulting mRNA proteins. The changes in gene expression and protein expression are the biological markers measured.

1.4.4 APPLICATION FOR SKIN CARE

The Broader Perspective of Molecular Biology

All life depends on three molecules: deoxyribonucleic acid (DNA), which holds information on how the cell functions; ribonucleic acid (RNA), which acts to transfer information to different parts of the cell and provides the template to synthesize proteins; and proteins, which form enzymes that send signals to other cells to regulate gene activity. Proteins also form the body's major components. A common feature of all organisms is the ability to store chemical energy as adenosine triphosphate (ATP). Genetic information is encoded by DNA, and the genetic information is transcribed by RNA. Translation involves ribosomes that use proteins via shared metabolic pathways. Gene expression is regulated through transcriptional controls that are proteins acting on the region of the gene termed a promoter site. These proteins are termed transcription factors, repressors/inhibitors, or enhancers, based on their influence of the gene. This activity results in epigenetic changes.

Skin is the primary interface between the body and the environment. It is the first line of defense against microbial pathogens, and against physical and chemical insults. There is a growing body of evidence suggesting that the immune responses in skin are important in the maintenance of homeostasis and occurrence of skin pathologies. Various cells present in the epidermis, including Langerhans, T-cells and macrophages, mast cells, and nerve-related cell types are also found in the dermis. The dermis is drained by lymphatic and vascular conduits through which migrating cells can traffic (3). Keratinocytes may be thought of as sensors of danger from pathogens, irritants, and other toxins. This

is carried out via numerous molecular pathways in skin. Activation of some of the receptors is through the inflammasome, which is a large multiprotein complex in the cell. Ultraviolet radiation activates inflammasomes in keratinocytes, leading to activation of various inflammatory pathways that in turn activate tissue-resident immune cells. Any type of barrier disruption, including UV light, trauma, irritants, or infection, triggers a coordinated immune response to maintain skin homeostasis. Skin-resident immune cells are important sentinels for homeostasis restoration and maintenance of normal cell activity.

Gene expression studies are currently providing insight into the biological mechanisms of skin aging and other aspects of skin health. The implication of these studies is that we have the capability to improve the appearance of aging skin through epigenetic therapy. Skin hydration, barrier repair, renewal of the extracellular matrix, pigmentation, and human antioxidant capacity are examples applicable to skin care. The cost and the time to conduct epigenetic studies continues to decrease, resulting in the publication of numerous papers focused on skin hydration, barrier repair, renewal of the extracellular matrix pigmentation, antioxidant capacity, and certain skin cancers. Common conclusions in the papers suggest that “epigenetic therapy” will revise skin care treatments, with additional strategies aimed at improving the appearance of skin. Nrf2 and AMPK signaling pathways are associated with epigenetic effects.

The Basics of Cell Signaling and Gene Expression Analysis

Transcription factors are a group of DNA-binding proteins that control which genes are turned on or off in the genome. They do so by binding to DNA and other proteins. Once bound to DNA, these proteins can promote or block the enzyme that controls the reading, or “transcription,” of genes, making genes more or less active. Frequently a specific cellular signal pathway can activate multiple transcription factors. The significance of this is that expression of a specific gene is under control of multiple transcription factors. Transcription factors are essential for the regulation of genes. For example, different genes turned on in liver cells than in skin cells. Different genes are turned on in cancer cells than in healthy cells. Through the action of transcription factors, the various cells of the body, which all have the same genome, can function differently. These proteins are so important to life that they are found in all living organisms. They play important roles in development, the sending of signals within the cell, and the events in a cell that lead to division and duplication, known as the cell cycle.

Several human diseases are linked to mutations in transcription factors, such as diabetes, autoimmune diseases, and cancer. Cytokines (cell motion) are signaling molecules that have key roles in several biological processes that include cellular growth, differentiation, gene expression, migration, immunity, and inflammation. Malfunction of cytokines can lead to a variety of diseases including arthritis, cardiac-related diseases, and cancer. Various cell types can secrete cytokines and other inflammatory mediators as part of an immune or inflammatory response. Gene expression analysis is the process by which information from a gene is used to direct the synthesis of a functional gene product, generally a protein. Gene expression analysis is the process of understanding the function of the gene, their protein products, and the complex regulatory network that affects biological processes of the cells.

Examples of Gene Expression Studies Relevant for the Cosmetic Chemist

Nrf2

Aging is a process that all living organisms experience. An aging human population is interested in products that promote health and maintain a younger-looking appearance. The molecular mechanism(s) of aging are not completely clear and are an area of intense molecular investigation. An area of current focus is on the Nrf2 signaling pathway. The Nrf2 signaling pathway is a mediator of multiple cell signaling cytoprotective pathways. It is expressed in numerous body tissues at different levels. It activates transcription of more than 200 genes crucial in the metabolism of drugs and toxins, signaling for protection against oxidative stress and inflammation. Nrf2 interacts with other key cell regulators including tumor suppressor protein 53 (p53) and nuclear factor kappa beta (NF-K β). These interactions are associated with age-related diseases (5). In 1956 Denham Harman postulated that reactive oxygen species (ROS) cause indiscriminate oxidative modification of cellular components leading to damage that cannot always be completely neutralized by antioxidants (6). Recent studies have resulted in producing data that question the validity of the Harman theory (7).

Many long-lived species of animals are found to have high levels of oxidative damage (8, 9). An emerging theory is that cellular stress resistance associated with extended aging tends to exist for numerous other nonoxidative stressors including heat, heavy metals, chemotherapeutic agents, dietary alterations, and xenobiotics (5). Another theory is that oxidative insult is just one factor of a multifactorial cytoprotective pathway that protects against cellular stressors

including synthesis of molecular chaperones, components of cell cycle surveillance, protein degradation, and oxidative stress. Nrf2 is a stress-sensing genetic transcription factor that appears to be a master regulator involved in many of these defense mechanisms (5). Gene expression studies have shown that phytochemicals work through the Nrf2 pathway. Sulforaphane, a component of broccoli, was found to be effective via this pathway (10). Nrf2 was shown to protect keratinocytes from ultraviolet A (UVA) irradiation by increasing the protein levels of Nrf2 in cell culture (11). Quercetin, resveratrol, caffeic acid, epicatechin, epigallocatechin, catechin chemical components found in polyphenolics are associated with Nrf2 activation (11). In summary it is hypothesized that long-lived species express higher levels of Nrf2. Nrf2 appears to be protective against numerous pathological conditions associated with reactive oxygen species (ROS) damage, inflammation immune responses, cell survival, and carcinogenesis (ROS).

Adenosine Monophosphate Activated Protein

Another signaling pathway potentially relevant to skin care is adenosine monophosphate activated protein (AMPK). AMPK is a master metabolic sensor and regulator of the cell, an enzyme that plays a role in cellular energy homeostasis. AMPK consists of three proteins that together make a functional enzyme. It is expressed in a number of tissues, including the liver, brain, skeletal muscle, and skin. AMPK has a key role in the regulation of cellular lipid and protein metabolism in response to stimuli, including physical exercise and changes in diet. The AMPK pathway interacts with other molecular pathways responsible for tumor formation and DNA damage repair (12). AMPK is switched on by metabolic stresses and various chemical compounds that cause a cellular energy imbalance. Energy status of the cell is critical for cell function, and AMPK has several downstream molecular targets that are modulated by changes in cell metabolism, cell growth, and other related functions that restore energy homeostasis to the cell. The AMPK pathway intersects with the Ras/PI3K/mTor and ERK pathways that are involved in tumor formation and cell growth. It also interacts with p53 and ATM pathways, which are tumor suppressor pathways and genomic gatekeepers (12). AMPK can be activated by resveratrol preventing hydrogen peroxide-induced premature senescence of keratinocyte cells when evaluated in human keratinocyte cell cultures (13). Another of its actions is to regulate the transcription of genes that function in the metabolism of glucose, fatty acids, and cholesterol. The AMPK signaling

pathway may also play a role in stimulating mitochondrial biosynthesis. In a keratinocyte cell culture study, resveratrol was found to activate AMPK and prevented the hydrogen peroxide–induced cellular senescence associated with cellular aging. The investigators note that cellular senescence is also linked with cancer in addition to aging (13). A variety of nutraceuticals and traditional medicines derived from plant products found in foods and beverages have been reported to activate AMPK in cells. In addition to resveratrol, epigallochatechin-3-gallate from green tea, curcumin, garlic oil, and berberines are reported in the literature (14).

1.4.5 (FUTURE PERSPECTIVES) CONCLUSION

Gene expression studies are providing a great amount of data that are not completely understood as applied to our health. While current knowledge of the use of gene expression is limited, the knowledge base is rapidly advancing. Published data indicate that bioactive ingredients have a biological effect that can be detected via various molecular biological assays using known markers. *In vitro* cell culture studies indicate that there may be a difference in biological activity depending upon the cell type studied. Further, it is hypothesized that there may be a difference in the efficacy of a biological active depending upon the existing state of the body when consumed or applied topically. An active may help to prevent a malignancy from developing; yet if applied or consumed after the malignancy has developed, it may in some cases have the opposite effect (12, 14).

In reviewing many of the published papers investigating gene expression, a common comment is “limitation of methodologies available to the investigator.” There are continuous variations in cells during the lifetime of an individual, and epigenetic changes may be a consequence of changes in the cellular composition as a person ages over time. There are also synergistic effects that may affect the outcome of a study leading to conflicting data, depending on the variation of study protocol, even if minor. As research techniques in gene expression improve, there is no doubt that data interpretation will improve—leading to enhanced skin care products.

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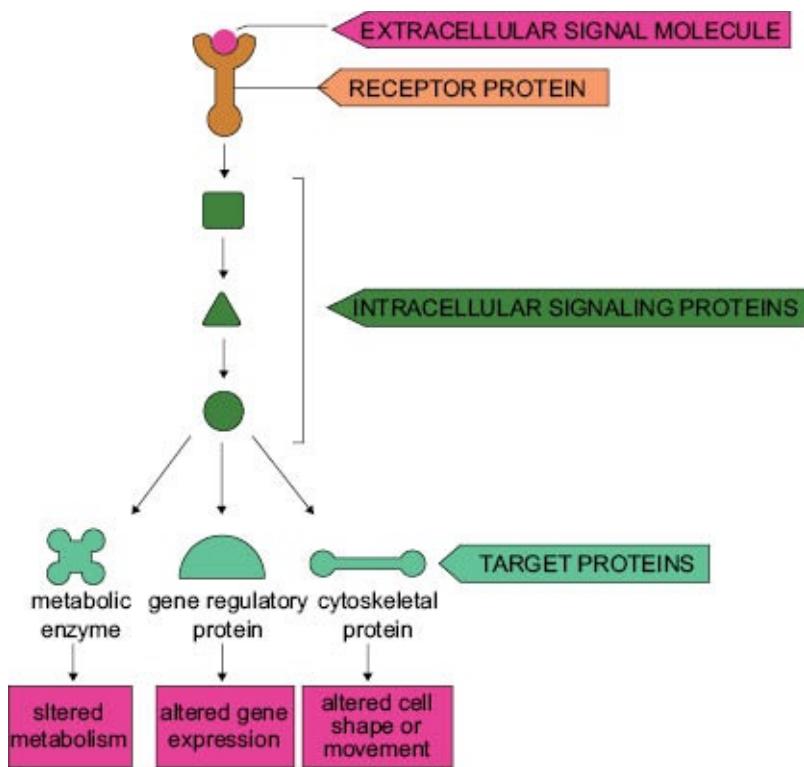


Figure 1 A schematic of an intracellular signaling pathway. Starting at the top, an extracellular signaling molecule binds with a receptor protein that then cascades a signal via intracellular signaling proteins that continue to cascade to target proteins, eventually resulting in modification of various cell activities as shown. Example modifications are altered metabolism of the cell, altered gene expression, and shape or movement of cells. From Molecular Biology of the Cell, 4th edition A.B. Johnson *et al.* (New York, Garland Science, 2002).

GLOSSARY

Biological Markers

A specific landmark that can be identified. It may be a substance, physiological characteristic, gene, *etc.* that indicates, or may indicate, the presence of disease or a physiological abnormality.

Chromatin

The material of which the chromosomes of organisms other than bacteria (i.e., eukaryotes) are composed.

DNA methylation

The modification of a strand of DNA after it is replicated, in which a methyl (CH_3) group is added to any cytosine molecule that stands directly before a guanine molecule in the same chain. Since methylation of cytosines in particular regions of a gene can cause that gene's suppression, DNA methylation is one of the methods used to regulate the expression of genes.

Endoplasmic reticulum

A membrane network within the cytoplasm of cells involved in the synthesis, modification, and transport of cellular materials.

Epigenetics

The study of inherited changes in appearance or gene expression that may be associated with diet and lifestyle.

ERK, MAPK, the MAPK/ERK pathway

A chain of proteins in the cell that communicates a signal from a receptor on the surface of the cell to the DNA in the nucleus of the cell. The signal starts when a signaling molecule binds to the receptor on the cell surface and ends when the DNA in the nucleus expresses a protein and produces some change in the cell, such as cell division. The pathway includes many proteins, including MAPK (Mitogen-activated protein kinases, originally called ERK, Extracellular signal-regulated kinases), which communicate by adding phosphate groups to a neighboring protein, which acts as an “on” or “off” switch.

Gene expression

Conversion of the information encoded in a gene first into messenger RNA and then to a protein.

Histones and histone modification

Low-molecular-weight proteins that form a complex with DNA in the chromatin and function in regulating gene activity. Histones are highly alkaline proteins found in eukaryotic cell nuclei that package and order the DNA into structural units called nucleosomes. Chromosomal processes such as transcription are

influenced by a variety of post-translational modifications to histones, including acetylation, phosphorylation, methylation, and ubiquitination. These modifications may act alone or in concert in a context-dependent manner to facilitate or repress chromatin-mediated processes.

Homeostasis

Homeostasis is the equilibrium of the internal environment within an organism. Disturbances in homeostasis cause decreased function of the cell and organism. It is the drive for efficiency of the cell, tissue, and body.

Inflammasomes

Large intracellular multiprotein complexes that play a central role in innate immunity.

Interferon (IFN)

Proteins made and released by host cells in response to the presence of pathogens such as viruses, bacteria, parasites, or tumor cells. They allow for communication between cells to trigger the protective defenses of the immune system that eradicate pathogens or tumors. IFNs belong to the large class of glycoproteins known as cytokines. Interferons are named after their ability to “interfere” with viral replication within host cells. IFNs have other functions: they activate immune cells, such as natural killer cells and macrophages; they increase recognition of infection or tumor cells by up-regulating antigen presentation to T-lymphocytes; and they increase the ability of uninfected host cells to resist new infection by virus. Certain symptoms, such as aching muscles and fever, are related to the production of IFNs during infection.

Ion channels

Ion channels are crucial components of living cells. Situated in the cell membrane, they allow ions to pass from one side of the membrane to the other.

Ligands (protein binding)

In molecular biology, a ligand (from the Latin *ligandum*, *binding*) is a substance (usually a small molecule) that forms a complex with a biomolecule to serve a biological purpose. In a narrower sense, it is a signal-triggering molecule, binding to a site on a target protein.

Lysosomes

Cellular organelles that contain acid hydrolase enzymes that break down waste materials and cellular debris. They can be described as the stomach of the cell. They are found in animal cells, while their existence in yeasts and plants is disputed.

mTOR

A protein that helps control several cell functions, including cell division and survival, and binds to rapamycin and other drugs. mTOR may be more active in some types of cancer cells than it is in normal cells. Blocking mTOR may cause the cancer cells to die. It is a type of serine/threonine protein kinase. Also called mammalian target of rapamycin.

Molecular chaperones

A large group of unrelated protein families whose role is to stabilize unfolded proteins, unfold them for translocation across membranes or for degradation, and/or to assist in their correct folding and assembly.

mRNA (messenger ribonucleic acid)

The type of RNA that codes for the chemical blueprint for a protein during protein synthesis.

Oxidative metabolism

Oxidative metabolism is often referred to as cellular respiration, the process during which chemical bonds of molecules such as glucose are converted into energy that life can use on a cellular level. It is referred to as “oxidative” because oxygen is a primary component of respiration. In the oxidative metabolism of glucose, for instance, glucose and oxygen combine to produce carbon dioxide, water, and energy. Oxidative metabolism occurs in gradual steps that convert the energy in bonds into chemical energy stored in adenosine triphosphate (ATP). ATP’s bonds carry energy throughout cells, releasing it when enzymes catalyze reactions.

Peroxisomes

A type of enzyme package similar to lysosomes. They are small vesicles found around the cell. They have a single membrane that contains digestive enzymes for breaking down toxic materials in the cell. They differ from lysosomes in the

type of enzyme they hold. Peroxisomes hold on to enzymes that require oxygen (**oxidative enzymes**). Lysosomes have enzymes that work in oxygen-poor areas and lower pH. Peroxisomes absorb nutrients that the cell has acquired. They are very well known for digesting **fatty acids**. They also play a part in the way organisms digest **alcohol** (ethanol). Because they do that job, you would expect liver cells to have more peroxisomes than most other cells in a human body. They also play a role in cholesterol synthesis and the digestion of amino acids.

P53 (also known as protein 53 or tumor protein 53)

A tumor suppressor protein that in humans is encoded by the TP53 gene. P53 is crucial in multicellular organisms, where it regulates the cell cycle and thus functions as a tumor suppressor that is involved in preventing cancer. As such, p53 has been described as “the guardian of the genome” because of its role in conserving stability by preventing genome mutation.

PI3K (PI3K/AKT/mTOR pathway)

An intracellular signaling pathway important in apoptosis and hence cancer.
http://en.wikipedia.org/wiki/PI3K/AKT/mTOR_pathway - cite_note-7. The PI3K/AKT/mTOR pathway is activated by IGF-1 and has a number of downstream effects that either promote protein synthesis or inhibit protein breakdown. PI3K activation activates AKT, which activates mTOR.

RAS

A protein belonging to a large super-family of proteins known as “low-molecular-weight G-proteins.” These proteins are called “G-proteins” because they bind guanine nucleotides (GTP and GDP). They are called “low-molecular-weight” to distinguish them from another, distinct, clan of guanine nucleotide-binding proteins, the heterotrimeric G-proteins.

Somatically heritable

A somatic epitype is a nonheritable epigenetic alteration in a gene. It is similar to conventional epigenetics in that it does not involve changes in the DNA primary sequence. Physically, the somatic epitype corresponds to changes in DNA methylation, oxidative damage (replacement of GTP with oxo-8-dGTP), or changes in DNA-chromatin structure that are not reversed by normal cellular or nuclear repair mechanisms. Somatic epitypes alter gene expression levels without altering the amino acid sequence of the expressed protein. Current

research suggests that somatic epitypes can be altered both before and after birth, and this alteration can be in response to exposure to heavy metals (such as lead), differences in maternal care, or nutritional or behavioral stress. There is no indication that somatic epitypes are heritable in a conventional epigenetic fashion. Some research suggests that methylation levels (and gene expression) can be reversed for some somatic epitypes by alterations in environmental factors such as diet.

Transcription

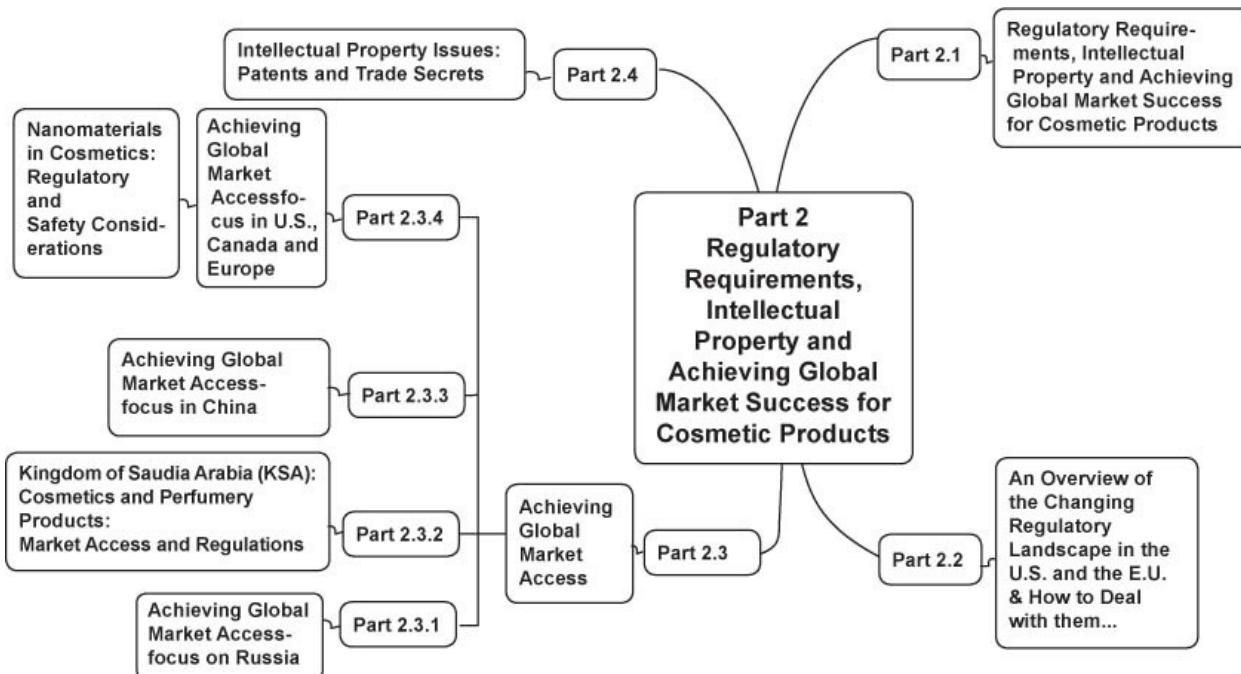
The first step of gene expression, in which a particular segment of DNA is copied into RNA by the enzyme, RNA polymerase. Both RNA and DNA are nucleic acids, which use base pairs of nucleotides as a complementary language that can be converted back and forth from DNA to RNA by the action of the correct enzymes.

Xenobiotics

A chemical that is found in an organism, but is not normally produced or expected to be present in it. It can also cover substances that are present in much higher concentrations than are usual. Specifically, drugs such as antibiotics are xenobiotics in humans because the human body does not produce them itself, nor are they part of a normal diet. Natural compounds can also become xenobiotics if they are taken up by another organism, such as the uptake of natural human hormones by fish found downstream of sewage treatment plant outfalls, or the chemical defenses produced by some organisms as protection against predators. However, the term xenobiotics is very often used in the context of pollutants such as dioxins and polychlorinated biphenols and their effect on biota, because xenobiotics are understood as substances foreign to an entire biological system, i.e., artificial substances, which did not exist in nature before their synthesis by humans.

PART 2

REGULATORY REQUIREMENTS, INTELLECTUAL PROPERTY AND ACHIEVING GLOBAL MARKET SUCCESS FOR COSMETIC PRODUCTS



REGULATORY REQUIREMENTS, INTELLECTUAL PROPERTY AND ACHIEVING GLOBAL MARKET SUCCESS FOR COSMETIC PRODUCTS

Co-Editors' Introduction
Co-Editors

Ruud Overbeek and Meyer R. Rosen

Part 2 of this book is unique since it covers the essential “nontechnical” information needed for achieving product success in a global environment. We have stepped out of the “normal” mode of presenting the technological aspects one might first expect, and thoughtfully placed this information here in recognition and emphasis that the technical aspects of ingredients are, of course, not the only areas of importance to achieving the goal of a successful product.

Once you have a stable product that works, looks good, and goes on well (all of which is covered later in this book), it is imperative that cosmetic and personal care professionals be aware of the rapidly changing world of regulations as well as making sure their technology is appropriately covered by patents.

We recognize that the regulatory environment is ever-changing and, after an overview of this area, we first cover thoughts on how to survive and thrive in this turmoil. We then turn to looking at what it currently takes to successfully market one’s products in the different geographical areas. Finally, we cover the essentials of global patenting in general and then by geographical area.

GLOBAL REGULATORY DEVELOPMENTS’THE NEED TO KNOW

For centuries, humankind has utilized cosmetics to enhance or alter physical

appearance, negotiate conceptions of femininity or masculinity, combat external manifestations of aging, and assert social status. This pursuit of beauty is a virtually timeless and universally enduring endeavor. The CTFA President's Message in the Cosmetic, Toiletry, and Fragrance Association (CTFA) 2005 Annual Report noted the wide reach of the industry and its potential for expansion. "In an era of globalization," the president stated, "we are truly one of the world's most global industries. Our products and our innovation know no boundaries. Whether it's Bangkok or Beijing, Baton Rouge or Baghdad, the products that we make are the products that women and families use every single day."

In addition to the global expansion regarding cosmetics for women, the men's cosmetic market is also growing exponentially. Beyond product efficacy, the highest priority for personal care products and cosmetics companies is the safety and health of consumers who use their products. To be able to support health, safety, and global expansion goals, the industry has a constant need and duty to inform itself of existing regulations, by geographical area. Further, it must cope with the seemingly endless evolution of newly developed and emerging changes in global regulatory developments.

Separately, in order to have a chance of success in each global market area, as well as each product category, the industry—ranging from ingredient developers, new product manufacturers, and claim writers—has an unavoidable need to understand the drivers for local and global regulation in order to be able not only to address, but also contribute to and impact the development of regulations. This challenge is a key step towards global alignment and regulatory harmonization.

Since regulatory hurdles affect cross-border trade of goods, reduce global competitiveness, and may harm manufacturers of cosmetics and personal care products, a reduction of international regulatory burdens will allow manufacturers improved access to foreign markets. Crossing these hurdles successfully is a key to global economic health. While regulatory harmonization has a positive impact for the protection of public health and safety, it also offers many benefits to both regulatory authorities and the cosmetics industry. The development of harmonized guidelines would streamline the regulatory assessment process and reduce product and ingredient development times and resources needed. Such harmonized guidelines prevent duplication and minimize use of *in vivo* testing without compromising safety and effectiveness.

The movement towards harmonization has recently been addressed among the

International Cooperation on Cosmetics Regulation (ICCR), an international group of regulatory authorities for cosmetics from Canada, the European Union, Japan, and the United States. The group has held multiple meetings to discuss issues related to cosmetics and cosmetic-like drug/quasi-drug products. This group seeks to promote regulatory convergence, while maintaining the highest level of global consumer protection and minimizing barriers to international trade. ICCR has chosen to include members of consumer organizations and industry associations, with an interest in regulatory issues involving cosmetics. Meetings have focused on key regulatory issues affecting the industry in general:

- Alternatives to Animal Testing
- *In silico* (computer) Prediction Models for Safety Assessment
- Nanomaterials
- Trace Impurities
- Endocrine Disruptors
- Allergens

At this time, the highest-priority topic for regulatory professionals in the cosmetics industry is the discussion around animal testing and alternatives to animal testing. This discussion includes the use of predictive models as well as *in vitro* testing methods. Historically, safety testing of chemicals has relied heavily on animal tests. However, in view of consumer pressure, and regulatory recognition of the lack of humaneness in testing on animals, this approach is in the process of being revolutionized. Today, and in the near future, safety assessment of chemicals will rely increasingly on pathway-based toxicity information largely informed by advanced, human-relevant, non-animal tests that are predictive of human health and safety.

The EU (European Union) has recently phased out the use of animals for cosmetics testing, culminating in a ban on the marketing of animal-tested products in 2013. China's Food & Drug Administration (CFDA) has announced that effective June 2014, China plans to remove its mandatory animal-test requirements for domestically manufactured cosmetic products. Initially the new rules will only apply to cosmetics manufactured in China. However, the CFDA has stated that, once the new system has been established, it may be expanded to include imported products and certain "special use" cosmetics as well. Chinese companies producing "non-special-use cosmetics," including shampoos and skin creams but not hair dyes, sunscreens, or other products with biological activity, will be exempt from carrying out animal tests. Manufacturers will now have the

option to substantiate product safety using existing safety data for raw ingredients, or European Union-validated non-animal tests. This would be a major step towards removing trade barriers between China and the EU. Clearly, legislative endeavors are underway to ensure more consistency between European and other markets, but this topic remains a major area of concern for companies wishing to sell products globally. To effectively address concerns about the safety of cosmetic ingredients, new regulations and harmonization should allow for the use of human-relevant, non-animal test methods. The development of such tests will not only provide international market security, but will help drive regulatory science forward.

The rapidly expanding field of Nanotechnology may be considered a second important area. Driven by health and environmental concerns, legislation is being introduced globally in an effort to “control” this new, exciting, efficacious, and emerging technology. As a result, the subject of nanomaterials has formed part of the recast EU cosmetic regulations and is being seriously discussed in various other countries. According to EU Regulation (EC) No. 1223/2009, nanomaterials are defined as insoluble or biopersistent and intentionally manufactured materials with one or more external dimensions, or an internal structure, on the scale from 1 nm to 100 nm. The European Union’s Commission sees in particular a need to better understand whether and how insoluble nanoparticles are used in cosmetic applications and has therefore agreed with cosmetic industry associations that it will set up an inventory of current applications of nanotechnology in cosmetic products together with the regulatory authorities from the U.S., Canada, and Japan. Results of this work will be assessed by the four authorities. Such measures are to help strengthen in-market control of products containing nanoparticles.

In the 2012 FDA Draft Guidance for Industry Safety of Nanomaterials in Cosmetic Products, the FDA summarized that inclusion of nanomaterials, or a change in the nanomaterials used, might affect the quality, safety, effectiveness, and/or public health impact of a nano-based product. Therefore, data needs and testing methods should be evaluated accordingly to address the unique properties and function of the nanomaterials used in cosmetic products. This need extends as well to the questions that continue to remain about the applicability of traditional safety testing methods to products that involve nanotechnology. Further, the FDA expects that the science surrounding nanomaterials will continue to evolve and will be used in the development of new testing methods. Although the FDA has not adopted a formal definition of “nanotechnology,”

“nanoscale,” or related terms, it has stated that the term is perhaps most commonly used to refer to the intentional manipulation, manufacture, or selection of materials that have at least one dimension in the size range of approximately 1 to 100 nanometers.

It is expected that the above topics, as well as other “hot” topics listed by the International Cooperation on Cosmetics Regulation (ICCR), will continue to play an important role in regulatory drivers and discussions. Discussion of such hot topics has especially reached a new level of impact with the emergence of social media. Also contributing to the acceleration of the discussion is an ever-growing, globally spread, public, social, regulatory, and political awareness that is expected to push faster regulatory change and possibly global harmonization. Any bad practice, emerging issue, or discussion about (the need for) new regulation will be very promptly published across social media, which can consequently oblige the regulator and industry to control, add, or remove ingredients from products and applications. Therefore, it is a prerequisite for any professional in the cosmetics industry to understand the regulatory framework and the potential social, political, and economic drivers for regulation in key economies around the globe.

This Section of the book has been carefully placed “up front” to insure that our industry *must* be aware of the significant impact of changing global regulations, as well as the need for an in-depth understanding of intellectual property matters in an environment that requires rapid evolution—nay, revolution—in products that really work and are safe and acceptable in the changing environmental context.

Thus, our belief is that this chapter is a must read for those industry professionals seeking globalization and desiring harmonization while pursuing the safety and health of consumers who use their products—be they “chemical-based,” “natural,” nano-type, or cosmeceutical—all of which may have biochemical impact on consumers.

ACHIEVING GLOBAL MARKET ACCESS-FOCUS ON RUSSIA AUTHOR

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Certification
INTERTEK FRANCE
Government & Trade Services

ABSTRACT

The expansion of the cosmetic and personal care market to all areas of the globe is indicative of the thirst of the peoples of the earth to look good, feel good, and have a healthy aging process. New markets beckon the world's ingredient developers, marketers, and product manufacturers for the need to expand into these "new" markets. Along with the development of new products for new needs, safety issues and regulations have proliferated. The bottom line here is that if a company wants to sell in a far-flung market, it must become knowledgeable about the changing requirements being promulgated by various countries. One such "new" market is Russia. The Russian and Customs Union markets are highly attractive for cosmetic manufacturers and exporters, as the demand for cosmetic goods increases by 5 or more percent each year. Actually, according to experts of the Russian press agency "Credinform," the annual growth rate could exceed 15% until 2015. As stated in the agency's study, import growth is associated not only with a steady growth in demand for foreign-made cosmetics, but also with a reduction of customs duty on imported products after Russia's WTO accession. The Russian market for cosmetics is one of the most dynamic in the world.

In this chapter we provide a detailed description of requirements for companies wishing to gain a part of this lucrative market, and have designed our description in the form of a series of questions and answers relevant to those seeking access to the expanded Russian market.

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References

2.3.1.1 INTRODUCTION

Much anticipated by the cosmetic world and generating many questions during the two last years, new Technical Regulation No. TR TC 009/2011 on the “Safety of perfumery and cosmetic products” (approved on 23.09.2011 by Customs Union Commission’s Decree no. 799) finally came into force on July 1, 2012. This document became applicable within the whole Customs Union of Russia, Belarus, and Kazakhstan.

We will discuss the general objectives of the Customs Union (hereafter CU) and the differences between the “old” procedure and the new one. We will address whether the new rules simplify access to Russian and CU markets; how

the new rules are different from European requirements; and how they will affect exports of cosmetics to Russia.

2.3.1.2 WHAT IS THE CUSTOMS UNION AND WHAT IS ITS AIM?

The new regulation does not concern only Russia, but the whole territory of the CU.

The Customs Union between Russia, Kazakhstan, and Belarus came into existence on January 1, 2010. These three states became one region by economic integration and removal of customs borders between Belarus, Kazakhstan, and Russia beginning on July 1, 2010. During the first stage of the integration process, the aim of the Customs Union was to create:

- Common customs tariffs without customs duties inside member-states
- Common customs code
- Other joint measures for foreign trade regulations

From January 1, 2012, the three states moved to the next step and established a single economic area through a number of inter-state agreements such as: “On single principles and rules of technical regulation”; “On single principles of regulating intellectual property rights protection”; “On coordinated macroeconomic policy”; “On single principles and rules of competition” and others. These principles were established in order to build a single market with a single macroeconomic policy that would simplify goods, services, capital, and manpower circulation.

The first joint document of the CU was issued in 2010, establishing unified hygienic requirements and unified assessment rules for various goods, including cosmetics: “Unified Sanitary-epidemiological requirements of the Customs Union” (also known as Decree no. 299 dated 28.05.2010). This document set up mandatory State Registration for the Customs Union instead of previous sanitary-epidemiological legislation in Russia.

Development and approval of the Technical Regulations of Customs Union for different categories of products, including cosmetics, have been the continuity of this reform.

2.3.1.3 WHAT WERE THE OLD REQUIREMENTS AND PROCEDURES OF PRODUCT CONFORMITY ASSESSMENT

AND HOW HAVE THEY PROGRESSED TO DATE?

1. Prior to July 1, 2012

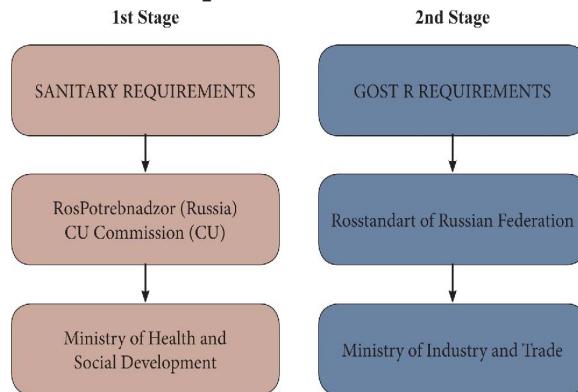
Before July 1, 2012, the majority of cosmetic products (with the exception of fragrances) were subject to a two-stage conformity assessment:

1. Customs Union State Registration
 2. GOST R Declaration of Conformity of Russia or GOST R Customs Union Certificate of Conformity
- with slight difference for fragrances, which used to be subject to:
1. Customs Union Sanitary Control (testing according single CU requirements);
 2. GOST R Declaration of Conformity of Russia or GOST R Customs Union Certificate of Conformity.

Both State Registration and Sanitary Control were done according to Unified Sanitary Requirements of the Customs Union (Decree no. 299).

Once the products passed the second step, i.e., GOST R certification, they used to be marked by the GOST R Mark of Conformity to demonstrate product compliance with the applicable Russian standards.

Relevant authorities for both steps of certification were:



The double conformity assessment takes its origin from the requirements for the Sanitary and GOST, provided by the Ministry of Health and Social Development and the Ministry of Industry and Trade respectively. The basic concern of both authorities and of the Russian certification system was to protect the safety, health, and environment of the Russian people and to prevent dangerous and noncompliant imported products from being placed on the Russian market. Consequently many products entering the territory of the

Russian Federation required double conformity assessment in order to:

1. Confirm that the products met Russian safety standards relative to the environment, life, and health of population

And in practice to:

1. Enable goods pass to through Russian customs
2. Enable goods to be used, sold, or marketed on Russian territory

Assessment documents issued according to the old legislation are still valid until July 1, 2014.

2. Since July 1, 2012

With introduction of the new Technical Regulation as of July 1, 2012, the function and aim of assessment documents remain the same. By contrast, the dual compliance system has been abolished. As of July 1, 2012, only one of following assessment documents is required:

- either CU State Registration
- or CU TR Declaration of Conformity

This topic is expounded in the next chapter.

2.3.1.4 OVERVIEW OF THE NEW CUSTOMS UNION TECHNICAL REGULATION “ABOUT SAFETY OF PERFUMERY AND COSMETIC PRODUCTS”

1. Definition of perfumes and cosmetics according to CU Technical Regulation

Perfumes and cosmetics are defined as “a substance or preparation intended to be applied directly on various external parts of the human body (epidermis, hair, nails, lips, and external genital organs), or on the teeth and the mucous membranes of the oral cavity with a single or primary purpose to cleaning them, changing their appearance, perfuming them, and/or correcting body odors and/or protecting them and/or keeping them in good condition and/or caring for them.”

2. Conformity assessment documents

As noted previously, the new Technical Regulation came into force on July 1,

2012. It is designed to unify certification requirements for the member states and to remove the double assessment system.

This Technical Regulation suggests two conformity assessment documents: **State Registration** or **TR Declaration of Conformity**.

a) State Registration

According to the text of the Technical Regulation, only a small list of products is subject to State Registration (higher-risk products). They include the following 13 categories:

1. artificial tanning products (autobronzants)
2. skin whitening (lightening) products
3. cosmetic products for tattoos
4. personal hygiene products
5. products for protection of skin from harmful industrial factors
6. cosmetic products for children
7. hair colorants, bleaches, and highlights
8. hair perms and hair straightening products
9. products made using nanomaterials
10. products for hair removal
11. peelings
12. products for dental and oral hygiene containing over 0.15% fluorine (or over 0.05% for liquid products)
13. Dental bleaching agents containing hydrogen peroxide and other ingredients producing hydrogen peroxide, including carbamide peroxide and zinc peroxide in the concentration of hydrogen peroxide (as an ingredient, or produced) 0.1–6.0%

State Registration certificate can be issued in the name of the manufacturer only, without mention of Russian or CU importer/distributor. By this means, the certificate can be used for the whole territory of the Customs Union and for different importers/distributors, regardless of their location. Its period of validity is not limited.

Information on registered products is entered in the unified CU register of State registration certificates before products are imported into the territory of the Customs Union.

b) TR Declaration of Conformity to Customs Union

All other cosmetics are subject to a CU TR Declaration of Conformity.

Declaration of Conformity is issued on the name of a legal entity registered in the Customs Union (buyer, importer, or distributor) and for one specific contract between the exporter and the importer, so that its validity is limited to one client and to the duration of the contract.

For both documents, testing of products at a CU-accredited laboratory is required. Conformity assessment shall be done before entering the Customs Union territory.

Both the Declaration of Conformity and State Registration to Customs Union are issued for a single product or a group of similar products and are valid until the product name, formulation, or manufacturer are changed. Products can be grouped in one conformity document upon condition that they are produced by the same manufacturer; according to the same technical requirements; and have similar ingredients composition, hygienic specification, and usage. However, they may differ by insignificant differences without impact on the hygienic value (for instance: different form or volume of goods, color or aroma resulting from the use of coloring and flavoring agents).

Requirements for obtaining both documents were brought into line with the most demanding conditions (i.e., those applied to previous State Registrations). All documents for conformity assessment must be presented with Russian legalized translations.

3. Requirements for perfumes and cosmetics

Safety of perfumes and cosmetics is ensured by a set of requirements for:

1. composition (*CU TR Appendices 1 to 5: lists of regulated ingredients*)
2. physical and chemical parameters (*Appendix 6*)
3. microbiological parameters (*Appendix 7, Article 5, item 5 of CU TR*)
4. the contents of toxic elements (*Article 5, item 5 of CU TR*)
5. toxicological parameters (*Appendix 7*)
6. clinical parameters (*Appendices 9, 10*)
7. production (*Article 5, item 7 of CU TR*)
8. consumer packaging (*Article 5, item 8 of CU TR*)
9. product marking (*Article 5, item 9 of CU TR*)

4. Labeling requirements

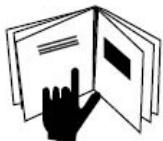
Perfumes and cosmetics labeling (marking) is "consumer information in the

form of inscriptions, digital, color, and pictograms to be applied to the consumer containers, label, sticker."

Marking of perfumes and cosmetics should contain the following information:

- Name, designation (if any) of perfumery and cosmetic products
- Intended use of perfumery and cosmetic products, unless it is clear from the product name
- Cosmetics for children should have the relevant information in the labeling
- Manufacturer's name and location (legal address, including country)
- Country of manufacture (if the country where the manufacturing facilities are located differs from the manufacturer's legal address)
- Name and location (legal address) of an enterprise authorized by the manufacturer to accept claims from the consumer (manufacturer's authorized representative, importer) if the manufacturer does not accept the claim itself in the territory of the member state of the CU
- Nominal amount (volume or mass) of product in a consumer pack (for solid toilet soap—the nominal mass of a piece, at the time of packaging), except for perfumery and cosmetic products with nominal mass less than 5 gr, or less than 5 ml nominal volume, or free samples of perfumery and cosmetic products
- Color and/or tone (for makeup products and coloring agents)
- Mass fraction of fluoride (% or mg/kg, or ppm) for oral hygiene products containing fluorine compounds
- Shelf life: manufacture date (month, year) and shelf life (months, years), or the notice "best before" (month, year) or "use before" (month, year)
- Description of storage conditions if different from the standard
- Special safety application measures (if necessary), including the warning information stated in Appendices 2–5 of the Technical Regulation
- Batch number or special code allowing identification of the perfumes and cosmetics
- Methods of application of the perfumes and cosmetics, which if not provided can lead to misuse by a consumer of the perfumes and cosmetics
- List of ingredients
- Ethyl alcohol by volume (%)

If the perfumes and cosmetics are accompanied with forwarding information, the products are marked with a graphic symbol of a hand on an open book:



All information must be written in the official language of the member state of the Customs Union in which the product will be marketed: Russian for Russia and Belarus, and Russian with Kazakh for Kazakhstan.

5. Mark of conformity

As per **Decision of the Customs Union Committee N 711 dated 15.07.2011 “On the uniform market circulation marking of products within the Customs Union,”** products that are certified according to the requirements of the Technical Regulations should bear the EAC conformity mark of the Customs Union (EAC stands for EurAsian Conformity), and no longer the Russian Gost-R mark.

Mark of conformity can be placed on each product, package, or accompanying documents in any reasonable way to make it indelible and clear.

Mark of conformity can be placed *only* after successful certification and *before* putting the product on the market. Minimum size allowed is 5 mm.



2.3.1.5 HOW DIFFERENT ARE THE NEW RULES FROM EUROPEAN REQUIREMENTS?

For cosmetic manufacturers and exporters, the new Technical Regulation provides a double advantage: bringing Russian regulation closer to regulation in the European Union, and ending “the dual compliance system.” Furthermore, this Technical Regulation also includes Belarus and Kazakhstan, two other members of the Customs Union, thus allowing access to more markets with one set of documents. Actually, this is true for State Registration but not for Declaration of Conformity, which is linked to specific importers or distributors.

In particular, one of the aims of cosmetic Technical Regulation was to harmonize requirements on cosmetic products for member countries of the Customs Union and make them more aligned with European rules, from the

manufacturing process to product labeling. The content and structure of these regulations have been inspired by both the European Directive 76/768/EEC and the European Regulations 1223/2009. The definition of cosmetics and the lists of regulated ingredients are almost identical (lists of substances prohibited in cosmetics, restrictive list of substances that cosmetic products may contain only under certain conditions, and lists of the only substances that can be used in cosmetics as coloring agents, preservatives, or UV filters).

However, the text still contains many requirements specific to Russia and to member countries of the Customs Union.

These include:

- Maintaining the obligation to confirm product compliance by a third party (Russian/CU-accredited laboratories and certification bodies)
- Maintaining animal testing
- Indicating expiry date and date of manufacture of the product
- Affixing Eurasian Conformity Mark (EAC)
- Indication of fluorine contents by weight (% or mg/kg or ppm) for oral hygiene products
- Setting limits for testing parameters in each category of products

In such manner, the Technical Regulation specifies the pH value of products, their microbiology, and various other aspects not detailed in European Directive 76/768 and its amendments, or in Regulation (EC) No. 1223/2009, which have replaced the Directive as of July 2013.

For instance, a **facial or body cream** or **liquid mascara and eyeliner** are regulated by the following:

	Facial or body cream	Liquid mascara and eyeliner
Physicochemical requirements		
pH value	5,0–9,0	5,5–8,5
Content of toxic elements:	perfumes and cosmetics containing natural herbal or natural mineral raw materials in amount exceeding 1% shall show the content of toxic elements not exceeding:	
arsenic	5.0 mg/kg	
mercury	1.0 mg/kg	
lead	5.0 mg/kg.	
Microbiology		
Total mesophilic and optionally	aerobic and anaerobic	

count: *CFU – colony-forming units per 1 g or 1 ml of a product	10^3 and less CFU* per 1 g (ml)	10^2 and less CFU* per 1 g (ml)
Candida albicans:	Not allowed in 0.1 g or 0.1 ml	Not allowed in 0.5 g or 0.5 ml
Escherichia coli:	Not allowed in 0.1 g or 0.1 ml	Not allowed in 0.5 g or 0.5 ml
Staphylococcus aureus:	Not allowed in 0.1 g or 0.1 ml	Not allowed in 0.5 g or 0.5 ml
Pseudomonas aeruginosa:	Not allowed in 0.1 g or 0.1 ml	Not allowed in 0.5 g or 0.5 ml
Toxicology:		
Skin irritant action:	0 points (none)	0 points (none)
Action on mucous membranes:	0 points (none)	0 points (none)
general toxic action measured by alternative methods <i>in vitro</i> :	none	none
Clinical testing:		
Irritant action:	0 points (none)	0 points (none)
Sensitizing action:	0 points (none)	0 points (none)

Similarly to these examples, each category of products has its own requirements. Categories vary depending on the type of testing. For example, cosmetics are divided into 33 categories for pH value, into three groups for microbiological requirements, into 21 categories for toxicological requirements, and into 23 categories (including oral hygiene products) for clinical requirements.

As can be seen from the above, the Technical Regulation requires several additional constraints compared to the rules of the European Union.

2.3.1.6 DO THE NEW RULES SIMPLIFY ACCESS TO THE COMBINED RUSSIAN AND CU MARKET? HOW DO NEW RULES AFFECT EXPORTS OF COSMETICS TO RUSSIA?

All cosmetic manufacturers exporting their products into Russia are expecting simplification of exportation rules and regulation requirements. In fact, the new Technical Regulation seems to simplify these procedures. However, experience

has shown that changes don't always mean simplification, as has been the case with the GOST R declaration of conformity in 2010. Presently, it is quite premature to confirm that simplification has resulted.

At this early stage of promulgation the introduction of new rules is accompanied by typical confusion among the main players involved. Thus, many issues continue to be discussed and amended. For instance, the following projects of amendments are currently under study:

- Rules for issuing of the Declaration of Conformity of Customs Union
- Rules for edition (filling out) of State Registration of Customs Union and its annexes
- Rectification of some terminology (e.g., definition of “peeling”)
- Elaboration of support standards for testing methods, labeling, etc.

Only recently the authority responsible for application and verification of right application of the Technical Regulation in Russia was named. This function was entrusted to RosPotrebnadzor, the Federal Service for supervision in the area of consumer rights and welfare protection (RF Government decree no. 989 dated September 27, 2012), acting under the Ministry of Health. The decision was made three months after the Technical Regulation came into force. In the same manner, controls under right application of Technical Regulations have been recently assigned to the newly created state authority “Rossakkreditatsia” (Federal Accreditation Service).

The end product of these changes says that the rules are not yet completely fixed.

2.3.1.7 BUSINESS CLIMATE IN RUSSIA AND REFORMS—RUSSIA JOINED WTO

On December 16, 2011, after 18 years of negotiations, Russia was accepted as a World Trade Organization (WTO) member after final ratification in August 2012. In acceding to the WTO, Russia embraces a series of rules and commitments and would ensure that all legislation related to sanitary and phytosanitary measures, technical regulations, standards, and conformity assessment procedures comply with the WTO SPS (Sanitary and Phytosanitary Measures) and TBT (Technical Barriers to Trade) agreements. This should be the next step for facilitating international trade and in Russian efforts to make a more favorable entrepreneurial climate. Today this is evidenced by reducing customs duties for imported goods.

REFERENCES:

- I. Decree No. 299 of 28.05.2010 of the Customs Union Commission (Single Sanitary Requirements of the Customs Union).
- II. Technical Regulation No. TR TC 009/2011, “Safety of cosmetic and perfume products,” adopted by the Decision of the Customs Union Commission No. 799 of September 23, 2011.
- III. Regulation (EC) No. 1223/2009 of the European Parliament and the Council of November 30, 2009 related to cosmetic products.
- IV. Ubifrance, the agency for international business development in France (December 2012).
- V. Russian press agency “Credinform” publication, July 2012.

KINGDOM OF SAUDI ARABIA (KSA): COSMETICS AND PERFUMERY PRODUCTS: MARKET ACCESS AND REGULATIONS BY

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ABSTRACT

Saudi Arabia is a Middle East country, part of the Gulf Cooperation Council (GCC), which includes five other countries (Kuwait, United Arab Emirates, Qatar, Bahrain, and Oman).

In the past few years, the Saudi market has become a key and strategic market for cosmetic and perfumery products, quickly growing and constantly increasing, with particular demand for international luxury products and attracting the world industry. It is however a specific market, where prevailing social customs play a big role, and in which particular precautions need to be observed.

This section proposes an overview of the cosmetics regulations and of the procedures implemented for the control of perfumery and cosmetic products and its enforcement. It also identifies some key issues that are useful in drawing a risk analysis.

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2.3.2.1 REGULATORY FRAMEWORK

[**a. Cosmetic ruling authority**](#)

In the Kingdom of Saudi Arabia (KSA), the responsibility for regulation of cosmetic products conformity falls under the Saudi Food and Drug Authority (SFDA), which was created in 2003. Their Cosmetic Department was established in 2008 and is today primarily responsible for issuing regulations and controlling cosmetic product safety (for imported and locally manufactured products), but also for the development and update of standards and for cosmetovigilance.

The national standardization body (Saudi Arabian Standard Organization—SASO) transferred all activities linked to food, drug, and cosmetics standards to the SFDA in 2011; but it should be noted that although the responsibility for creating new standards now lies with SFDA, the applicable standards in Saudi Arabia are still the ones issued or adopted by SASO.

SFDA however published a draft version of its first own Cosmetic Safety Standard in December 2012. This standard is not approved yet, but it is aimed to replace all current SASO standards for cosmetics, including the specific product standards.

[**b. Cosmetic regulations and sanctioned standards**](#)

[**1. Sanctioned Safety Standard**](#)

SFDA official sanctioned standard is the Gulf standard: “GSO 1943/2009 –

Cosmetic Products Safety Requirements.” This GSO Standard is equivalent to SASO 1953/2009; however, SFDA refers to the GSO reference in all their documents and communications.

This document is based on the EU Directive 76/768/EEC and follows the same structure:

- Table 1: Substances that must not form part of the composition of cosmetic products
- Table 2: Substances that must not be contained in cosmetic ingredients except subject to certain restriction and condition
- Table 3: Coloring agents allowed for use in cosmetic products
- Table 4: Preservatives that cosmetic products may contain
- Table 5: Permitted UV filter that cosmetic products may contain

In addition to this standard, SFDA has published other ingredient prohibition/restrictions through its Guidance for Products Classification. This document includes additional restrictions that were previously decided by SFDA through circulars.

2. Guidance for Products Classification

This guideline “SFDA Guidance for Products Classification,” published in 2012, determines rules for classifying products (from drugs to cosmetics) relative to definition of products, function, composition, and claims.

Among these rules, there are some significant ingredient restrictions to follow per product category. Cosmetic products are subject to the restrictions listed in the guideline, in addition to the tables under GSO 1943.

These additional restrictions for cosmetic products are:

1. Free of prohibited substances according to GSO 1943/2009 in addition to the following substances:
 - a. Tretinoin (Retinoic acid) and its salts not permitted at any concentration)
 - b. Hydroquinone (Dihydroxy Benzene)
 - c. Triclosan not permitted at any concentration)
2. Compatible with restricted substances according to GSO 1943/2009 in addition to the following restricted substances:
 - a. Vitamin A (Retinol) and its esters retinyl acetate, retinyl palmitate (permitted at concentrations equal to or less than 1%)
 - b. Sulfur (permitted at concentration equal to or less than 2%)
 - c. Urea (permitted at concentration equal to or less than 10%; cosmetics

intended to be diluted in bathwater may contain levels exceeding 10% urea)

- d. Salicylic acid (permitted at different concentrations according to its role as shown in the following table):

Role	Salicylic Acid permitted concentration
Active Ingredient	≤ 3% in rinse-off hair products ≤ 2% in other products
Preservative	< 0.5%

- e. Zinc oxide permitted at concentration less than 25% (unless it is used for a medical condition, in which case it will be classified as a drug)

- f. Alpha-hydroxy acids (AHAs):

a. Permitted at total concentrations equal to or less than 10%, with a pH equal to or greater than 3.5

b. The inner and outer labels of all leave-on skin products containing AHAs at concentrations equal to or greater than 3% shall carry cautionary statements in Arabic and English to the effect: “Use only as directed,” “Avoid contact with the eyes,” “If irritation persists, discontinue use and consult a physician,” “It is recommended that prior to exposure to the sun, users cover areas where AHAs have been applied with sunscreen,” “Contact of the . . . product with the skin must be of limited frequency and duration.”

c. Preparations intended to be diluted in bathwater may contain levels of citric acid exceeding 10%.

Products falling under the above restrictions will generally be classified as low-risk health products and will be subject to listing at SFDA.

3. Product-specific standards

In addition to the general safety standard, some specific product standards still exist in the KSA. Even if these are not mandatory and not sanctioned officially by SFDA, it should be noted that they may be used for local assessment (for samples drawn by customs and tested in local laboratories). Some of these parameters may not be aligned with the SFDA-sanctioned standards. An acquaintance with the parameters included in these standards is therefore highly desirable in order to achieve a reliable risk analysis.

Example of specific parameters requested by a SASO standard: SASO

1512:2010 (equivalence GSO 1152:2010)

- pH value shall be in the range of 5–9,5.
- The total content of fatty substance shall be not less than 15% by mass.
- The water content shall be not more than 85% by mass.
- The utility date shall not be more than three years.

Note: All the SASO standards have equivalence at a GCC level with the GSO standards, which may be applicable in other countries within the GCC.

2.3.2.2 DEFINITION AND SCOPE OF APPLICATION

a. Definition

As per GSO 1943/2009, “Cosmetic Products—Cosmetic Products Safety Requirement,” the written definition of a cosmetic product is identical to the European definition: *“Any substance or preparation intended to be placed in contact with the various external parts of human body (epidermis, hair system, nails, lips, and external genital organs) or with the teeth and the mucous membranes of the oral cavity with a view exclusively or mainly to cleaning them, perfuming them, changing their appearance and/or correcting body odors and/or protecting them or keeping them in good condition.”*

This standard provides an illustrative list of products considered as cosmetic products, which include but are not limited to the following:

- Creams, emulsions, lotions, gels, and oils for the skin (hands, face, feet, etc.)
- Face masks (with the exception of peeling products)
- Tinted bases (liquids, pastes, powders)

- Makeup powders, after-bath powders, hygienic powders, etc.
- Toilet soaps, deodorant soaps, etc.
- Perfumes, toilet waters, and eau de cologne
- Bath and shower preparations (salts, foams, oils, gels, etc.) – Depilatories
- Deodorants and antiperspirants
- Hair care products: hair tints and bleaches; products for waving, straightening, and fixing; setting products; cleansing products (lotions, powders, shampoos); conditioning products (lotions, creams, oils); hairdressing products (lotions, lacquers, brilliantines) – Shaving products (creams, foams, lotions, etc.)

- Products for making up and removing makeup from the face and eyes –
Products intended for application to lips
- Products for care of teeth and mouth (mouthwash products containing alcohol are considered banned products) – Products for nail care and makeup
- Products for external intimate hygiene that do not contain ingredients that have antimicrobial activity – Sunbathing products
- Products for tanning without sun
- Skin-whitening products (except Hydroquinone-containing products) –
Anti-wrinkle products

Note: There is a ban on alcohol in mouthwash; the decision to ban these types of products was taken by SFDA in 2011 due to the existence of permissible and available alcohol-free alternatives.

b. Classification

In 2012, the SFDA Drug sector issued another method to define the cosmetic by issuing the Guidance for Product Classification, which defines cosmetics but also the following product categories:

- Drug products
- Herbal products
- Health products
- Cosmetic products

The product category can be generally identified by referring to this Guideline; however, manufacturers can submit a classification request to SFDA to get an official Classification Letter to be sure of the product status.

The other categories are handled directly by SFDA locally and are subject to SFDA registration (for high-risk products: drug, herbal, and certain health products) or to SFDA listing (low-risk health products, cosmetics).

2.3.2.3 LABELING

a. General rules

Product labeling shall comply with the general safety standard GSO 1943/2009. This standard lists the following markings as mandatory:

- Name of the product, Trademark

- Function of the product (in Arabic if the function is not clear from the product's presentation)
- Name and address of manufacturer
- Date of production or batch number
- Durability information (PAO or Best before date)
- Weight/volume
- Country of origin
- Ingredients list (INCI)
- Instructions of use and storage (in Arabic if special instruction that may have an effect on the use of the product for consumer safety)
- Any warning (in Arabic)

Note: A minimum of Arabic translation is always required, such as for the product's function, and safety warnings or special instructions for use.

b. Specific rules

Particular attention needs to be paid to some labeling rules, in order to be in conformity with the Saudi requirements. Among those we can find the following: **Medical claims:**

Medical claims are not allowed, and terms like “treatment” are generally not accepted. Any other claims relative to “regeneration of cells,” or “stimulation of collagen production,” should be avoided.

Pictures and illustrations: Any pictures or illustrations that are inconsistent with the prevailing social customs and values should not be used.

Country of origin The country of origin marking is mandatory on all products and its immediate packaging; the importance of this marking was reinforced in 2010 by a MoCI decree (MoCI Royal Decree No. M/5) and is applicable to every product entering the KSA, not only cosmetic products. This mandatory requirement is strictly controlled in the country of origin/supply by the inspection agent and by customs in the KSA.

It should be noted that an indication such as “Made in EU” is not accepted; the exact country of origin must be clearly indicated. The rule is also very strict as regards to dual country of origin and labeling such as “Brand of Paris” + made in China; or “designed in France, manufactured in China,” which should be avoided.

Expiry date

The marking of the expiration date is not mandatory as per GSO 1943/2009,

which only requests a “best before date” or indication “Period After Opening” (PAO). However, the expiration date is required by some SASO-specific standards, and this contradictory instruction occasionally causes issues during clearance.

Perfumery products Perfumery products standard (SASO 585/2000 – perfumery products based on ethanol) is applied in addition to the general safety standard, due to the presence of alcohol in such products, because of the sensitive issue relative to alcoholic products. This standard imposes the denaturation of ethanol, and some restrictions for products containing a percentage of essential oil/fragrance compounds lower than 8%: volume shall not be more than 250ml, and must be fitted with nonremovable pumps. Furthermore, perfumery products having a percentage of essential oil/fragrance compounds lower than 8% must bear a specific warning in Arabic “for external use only.”

Products packed in ampoules or vials Cosmetic products can be packed in ampoules or vials, even if this may sometimes be considered a medical form, provided that such products bear the following instructions in Arabic and English:

- For external use only.
- Avoid contact with eyes.
- Open with caution.

2.3.2.4 MARKET ACCESS

Any cosmetic product imported into the Saudi market must present a Certificate of Conformity by the manufacturer (hereafter referred to as CoC) during customs clearance. This certificate is requested by law through the MoCI Decree 6386 as well as per the instructions published by SFDA.

a. Certification process

The SFDA regulations that outline the conformity assessment procedures demand that every shipment of cosmetic and perfumery products needs a CoC, issued by an approved inspection company, for each customs entry. The CoC needs to be issued prior to shipment, following a full evaluation of conformity against Saudi Standards and SFDA requirements. Each CoC is valid for one shipment only, and bears all reference specific to this particular shipment (such

as final invoice reference).

In order to perform certification of the country of origin, since 2011 the SFDA has officially subcontracted the evaluation of conformity and the related Certification to approved inspection bodies.

The SFDA requirements, and consequently the inspection bodies' scope of work, are that each shipment should be tested and inspected against the sanctioned standards. The Certificate issued by the inspection bodies should thereafter be presented by the importer to the local SFDA agent and Customs official at the port of entry, in order to ensure that the regulations are fully respected for any cosmetic or perfumery products entering the Kingdom.

It should be noted that the appointed inspection and certification bodies only certify cosmetic products, and not the other products' categories (herbal, health products, and drugs).

b. Conformity assessment

The first step of the certification process in the country of origin/supply, by the inspection agents, is the preclassification of the product. In certain borderline cases, manufacturers can be asked to refer directly to SFDA to obtain a written confirmation of their classification of the relevant product. Once the product is clearly identified as a cosmetic product, it will be assessed as per the sanctioned standards and SFDA requirements. This assessment is done through a documentary review, product testing, and inspection.

A nonmandatory registration system can be implemented by the inspection bodies in order to avoid testing of each shipment.

Once the document review is fulfilled and the products are found compliant, the products are physically inspected prior to shipment, in order to verify the physical integrity of the shipment against the shipping documents as well a verification of the marking of the individual products.

c. Key issues

At the points of entry, a full review of the conformity of the products can happen and samples can be randomly drawn for shipments holding a Certificate of Conformity, to ensure product and certification are correct (if a shipment of cosmetics arrives without a CoC, samples will automatically be drawn for testing).

When this situation occurs, it can happen that the local laboratories do not follow the SFDA-sanctioned safety standard and the local tests are done as per other SASO standards. The risks with such a local test is that a product may fail due to a nonsafety-related test requirement such as fatty matter for a skin cream. It is therefore recommended that a risk analysis be performed as per the specific SASO standards for specifications and labeling requirements, even if these are not officially sanctioned by the ruling authority.

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- Saudi Arabia Standard Organization. Cosmetic Products – Perfumery products based on ethanol. SASO 585/2000, 6 p.
- GCC Standardization Organization. Skin Cream. GSO 1152/2010, 4 p.
- Saudi Food & Drug Authority. Guidance for Products Classification–version 1. 23 May 2012, 21 p.

Abbreviations

CoC: Certificate of Conformity

GCC: Gulf Cooperation Council

GSO: Gulf Standard Organization

KSA: Kingdom of Saudi Arabia

SASO: Saudi Arabia Standard Organization

SFDA: Saudi Food and Drug Authority

PAO: Period After Opening

ACHIEVING GLOBAL MARKET ACCESS-FOCUS ON CHINA AUTHOR

**Mr. Zhongrui Li (Mr. Ray Li), Toxicological Risk Assessor,
Intertek ABSTRACT**

The purpose of this chapter is to give a brief overview of the cosmetic legislation and regulatory status for importers who are interested in supplying their cosmetic products to the Chinese market. The difference and similarity between EU and Chinese regulations are summarized. Cosmetic legislation, definitions, the registration process, and new ingredient requirements are also discussed to help the reader understand how to enter the marketplace in Mainland China.

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2.3.3.1 CATEGORY OF COSMETICS IN CHINA: SPECIAL USE AND NONSPECIAL USE COSMETIC

Cosmetic regulation in China is based on Regulations Concerning the Hygiene Supervision over Cosmetics (1990). “Cosmetic products” are defined as those daily-used chemical products (substance and mixture) applied on the surface of any part of the human body (such as skin, hair, nails, oral, and lips) by way of

smearing, spraying, or other similar methods to keep the body clean; to get rid of undesirable smell; to protect the skin; to make up the face, and to increase appearance and beauty. The definition is similar to that of the EU Cosmetic Directive 76/768/EEC and includes prohibited and restricted substances that are listed in the EU Hygienic Standard for Cosmetics (2007). The main difference between the EU and Chinese regulations is that cosmetic products are divided and managed in two categories: “special use” and “nonspecial use” products. Nonspecial-use cosmetics include hair care, nail care, skin care, perfumes, and makeup. Special-use products include hair growth, hair dye, hair perm, hair removal, breast shaping, fitness, deodorizing, spots removal, and sun block.

2.3.3.2 ORAL PRODUCTS REQUIREMENTS

Oral care products are not listed in either of the two categories, and instead are regulated according to other separate national standards (e.g., GB 8372-2008 gives specified requirements for toothpaste). Furthermore, there are guidelines, national standards, and industry standards on cosmetic products that present specific requirements. These include, but are not limited to, testing methods, labeling, and registration. Consequently, compliance with the legal requirements is made difficult to understand for importers or distributors foreign to China. The authorities have noticed that and now are discussing the matter with industry; it is very likely that oral products will be treated as “cosmetic” and regulated by China FDA.

2.3.3.3 DOCUMENT AND TESTING REQUIREMENTS DURING PRODUCT REGISTRATION

For imported cosmetic products, except oral care products, and unlike the trend in the EU and U.S., registration and animal testing are required by China’s State Food & Drug Administration (CFDA) before marketing. Such testing can take several months or more to perform before registration is possible. The nonspecial-use cosmetics do not require registration, but notice should be provided to provincial authorities if the products were manufactured by domestic companies, including internal brands that have manufactured products in mainland China. The Registration Documents are listed in the [Table 1](#). The application form and all other documents should be written in simplified Chinese and the original language of the technical document should also be submitted to

CFDA together with its translation.

Table 1: China FDA required registration documents for cosmetics

Nonspecial-use cosmetics	Special-use cosmetics
Application form for imported cosmetics (nonspecial use)	Application form of imported cosmetics (special use)
Product formula—INCI name in both English and Chinese	Product formula—INCI name in both English and Chinese
Quality standard of product (specification)	For products with the function of slimming, breast care, and hair growth, documents should provide the effective ingredient based on scientific data and a brief introduction and sketch of production process.
Testing report of inspection institute accredited by Ministry of Health (MOH) and relative documents	Brief description of manufacture technology and procedure chart
Original package (including label) or package (including label) designed for the Chinese Market	Quality standard of product (specification)
Certification documents of sale permission for the registered product in country of origin (region) or manufacturing country (region)-free sale certification	If products contain ingredients that endanger health, documents should provide the related safety assessment data (test report for concerned impurities).
If products contain ingredients that endanger health, documents should provide the related safety assessment data (test report for concerned impurities)	Testing report of inspection institute accredited by Ministry of Health (MOH) and relative documents
“BSE” certificate (prepared by responsible unit of China)	Original package (including labeling) or package (including labeling) designed for the Chinese market and instruction for use in Chinese
Business license copy of responsible unit in China	Certification documents of sale permission for the registered product in country of origin (region) or manufacturing country (region)-free sale certification
Authorization letter—it will be needed if the unit responsible is to apply for registration	“BSE” certificate (prepared by responsible unit of China)
Consigned manufacturing agreement, GMP, or ISO certificate	Business license copy of responsible unit in China

Certificate of an agency issued by the unit responsible if needed	Authorization letter—it will be needed if the unit responsible is to apply for registration
Other documents that may be helpful for the record	Consigned manufacturing agreement, GMP or ISO certificate
Unopened samples	Certificate of an agency issued by the unit responsible if needed
	Other documents that may be helpful for the record
	Unopened samples

2.3.3.4 ANIMAL TESTING REQUIREMENTS IN CHINA

Animal testing for finished cosmetic products is a mandatory requirement and should be conducted by Ministry of Health (MOH) accredited laboratories in China. Rats, rabbits, and guinea pigs are normally used under finished product registration. Alternative methods are normally not accepted by CFDA even if they have been validated by OECD or other international bodies; a good example is the Guinea Pig Maximum Test (GPMT), which has been prohibited in EU. Instead, the Local Lymph Node Assay (LLNA) is recommended in order to comply with the 3R principle (Replace, Refine, and Reduce) for investigating skin sensitization potential, but at the moment China FDA only accepts the GPMT method. As a consequence, all cosmetic products that have not been tested on animals are excluded from the Chinese cosmetic market. Under pressure of international animal rights organizations and the cosmetic industry, the CFDA finally changed its attitude and approved the first *in vitro* method of 3T3 Neutral Red Uptake Phototoxicity Test in February 2012. However, a CFDA prohibition of animal testing is currently thought to be a long way off.

In the European Union, all animal testing has been banned for finished products, as well as ingredients, as of March 2013, but it is clearly mentioned that the ban is only applicable to those animal tests designed for *this regulation* in article 18 of EU cosmetic regulation EC 1223/2009. Therefore, exemption conditions exist for those tests designed to fulfill other countries' legal requirements (such as China's); the relevant data should be documented in a Product Information File (PIF) and made as transparent as possible. The authorities may not consider these animal tests to be in violation of EU cosmetic regulation.

2.3.3.5 SAFETY ASSESSMENT FOR INGREDIENTS AND FINISH PRODUCTS

A toxicological risk assessment (cosmetic safety assessment) is required in China, but this report mainly focuses on identifying prohibited substances or toxic impurities in the ingredients. For example, phenol is a common impurity in phenoxyethanol, and a testing report of phenol would normally be provided either for phenoxyethanol itself or a finished cosmetic product. Other routine test substances such as Dioxane, Methanol, Acrylamide, and N-nitrosodiethanolamine (NDELA) and their safety levels (Margin of Safety) should be calculated to demonstrate their safety if trace levels are present in the finished formulation. This is different from the EU cosmetic Regulation EC 1223/2009, which emphasizes the toxicity of intentionally added ingredients rather than the impurities. Comparable EU and China regulation requirements are summarized in [Table 2](#).

2.3.3.6 COMPARISON OF EU AND CHINA REGULATION REQUIREMENTS

Table 2 comparison of EU and China regulation requirements

Requirement	EU cosmetic regulation/directive	China legislation
Category	Leave-on and rinse-off products	Special and nonspecial-use products
Oral products	Cosmetics	National standards
Animal testing	Banned	Mandatory
<i>In vitro</i> test methods	Accepted	3T3 Neutral Red Uptake Phototoxicity Test only
Notification	Yes	Yes, but for domestic manufacturers' nonspecial-use products
Registration	No	Yes, all imported cosmetics and domestic manufacturers' special-use products
Duration of notification or registration	Minimum	Notification is minimum but take several months, possibly years, for registration.
Claim of effects	Yes, document evidences in the Product Information File	Yes, reviewed by committee during registration

Testing report(s) for high-risk impurities	Not mandatory	Yes
Hygienic testing report(s)	Not mandatory	Yes
Testing samples	Not mandatory	Yes, for animal and clinical testing
Language	English and member-state official language	Simple Chinese
Organic cosmetic	Not regulated	Prohibited
Labeling	26 allergen	Ingredient INCI Chinese name in the label
Infant or child products	No specific requirements	Yes
Existing cosmetic ingredient	No	Yes, 3667 in three batches
New cosmetic ingredient registration	No	Must be approved before use
Nanomaterials	Notify authorities and provide safety data	Not specified, not recommended in infant or child products

Any claims of performance effect(s) are reviewed by the CFDA committee, and substantiation of these claims, such as clinical research reports for the ingredients or products themselves, shall accompany the submitted dossier. Cosmetic products intended for pregnant women and mothers during lactation are not accepted by CFDA as they cannot be justified or assessed for potential risk.

The principle of “organic cosmetics” is not recognized in China according to GB/T 19630 Organic Products, the authority AQSIQ (General Administration of Quality Supervision, Inspection and Quarantine of the People’s Republic of China). Products containing 95% or above “organic ingredient” may use the “organic” mark after the official certification process. Third party “organic” certifications are not accepted and cosmetic brands are basically prohibited from advertising their products as “organic” in mainland China.

This approach is similar to that used for “cosmeceuticals,” which is also not a recognized term. If a product has drug-like properties, it must meet the requirements for drugs as defined in the Drug Control Law of the Peoples Republic of China.

Cosmetic labeling should comply with GB5296.3-2008, and normally the approach that industry takes is to design a “patch” in Chinese and then paste it onto the original label and package. However, warning statements will not be covered by this patch. The CFDA committee obligation is to check the claim(s) on the patch but not to check the original language or the company’s official websites. Importers or suppliers to the Chinese market therefore simply need to design this “patch” rather than modify their products’ claim(s) in the original market.

To protect consumers against allergic contact dermatitis in the EU, the seventh amendment to the cosmetic directive required the listing of any one of 26 allergen names on the packaging should the concentration exceed 0.01% for rinse-off products and 0.001% for leave-on products. No such labeling is required in China. The unique requirement for labeling is that ingredients be listed by their Chinese INCI names, and if a Chinese INCI name does not exist for an ingredient, importers must apply for one. The Chinese INCI name can be identified in the Catalogue of International Cosmetic Ingredients published by the Ministry of Health (MOH).

“Nanomaterial” in cosmetics has been a hot topic in recent years, understandably so as they often provide unique and fascinating properties for products. Unique or not, the safety of these materials requires further investigation. Elsewhere in this text we cover the growing and complex field of the characterization of nanomaterials. While notification is required for products containing nanomaterials according to EU Cosmetic Regulation EC 1223/2009, the Chinese CFDA has not yet given any official opinion on use or labeling—although use of nanomaterials is not encouraged in products intended for infant or child cosmetics.

A new guide for products used on infants and children was published on October 12, 2012. One important requirement is that these types of cosmetic products should minimize the total number of ingredients relative to classes of colors, fragrances, and preservatives. It also requires proof of need for their inclusion.

2.3.3.7 EXISTING AND NEW COSMETIC INGREDIENTS IN CHINA

Existing and new cosmetic ingredients are defined in the Guidelines for the Registration and Evaluation of New Cosmetic Ingredients (2011). A new

ingredient is defined as any ingredient that is used in cosmetics for the first time in China. Basically, a “new ingredient” can be defined as any ingredient that is currently not included in the current CFDA cosmetic ingredient list—even if it is already being used in EU or U.S. markets. China FDA has published an Inventory of Existing Cosmetic Ingredients in China (IECIC 2003 & 2011), and three additional batches of existing ingredients were released in May, July, and September 2012 containing respectively 1674, 637, and 1356 substances. The lists are not exhaustive, and CFDA experts have suggested that more lists will be released over time.

Maximum approved concentration (MAC) and/or maximum used concentration in approved cosmetic products (MUCAP) are available for each ingredient. In these, if MUCAP limits are exceeded then evidence will be required to prove the safety of the finished cosmetic product during use. New ingredient registration requiring exhausted toxicity and safety data and process is extremely time consuming and expensive. CFDA took over the regulation of cosmetics from the Ministry of Health (MoH) in 2008, and since then only three new cosmetic ingredients have been approved for use. Therefore, industry has no choice but to design formulations based on the currently existing list; otherwise companies may have to wait for prolonged periods of time—sometimes years—to gain authorization to market cosmetic products containing “new” ingredient(s).

For all EU and U.S. cosmetic companies, tolerance and patience with the complex registration process are, regrettably, key factors needed to enter this huge and important market.

PART 2.3.4

NANOMATERIALS IN COSMETICS: REGULATORY AND SAFETY CONSIDERATIONS

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ABSTRACT

Nanomaterials are present as ingredients in numerous types of cosmetic products that are marketed worldwide. Regulatory and safety evaluations of nanomaterials and nanomaterial-containing cosmetic products continue to evolve with the accumulation of knowledge on the physicochemical and toxicological properties of particles at the nanoscale. This chapter provides an overview of the current state of regulation of nanomaterial-containing cosmetic products in Europe, the United States (U.S.), and Canada. It also outlines some considerations for the design and conduct of evaluations of nanomaterial toxicity that can support cosmetic product safety.

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2.3.4.1 REGULATION OF COSMETICS CONTAINING NANOMATERIALS

a. Definition of Nanomaterial

The regulation of cosmetics containing nanomaterial ingredients depends, in part, on the definition of a nanomaterial, which in and of itself varies among jurisdictions. For reference, [Table 1](#) presents a summary of the current definition of a nanomaterial for cosmetic regulation purposes in Europe, the U.S., and Canada.

Table 1 Nanomaterial Definition in Different Jurisdictions

Jurisdiction	Definition of Nanomaterial	Reference
Europe	<ul style="list-style-type: none">“Nanomaterial” means an insoluble or biopersistent and intentionally manufactured material with one or more external dimensions, or an internal structure, on the scale from 1 to 100 nm.	1
United States	<ul style="list-style-type: none">FDA has not adopted a formal definition of “nanotechnology,” “nanoscale,” or related terms.	2
	<ul style="list-style-type: none">Although there are numerous definitions of “nanotechnology,” the term is perhaps most commonly used to refer to the intentional manipulation, manufacture, or selection of materials that have at least one dimension in the size range of approximately 1 to 100 nanometers.	
	<ul style="list-style-type: none">While one definition for “nanotechnology,” “nanoscale material,” or a related term or concept may offer meaningful guidance in one context, that definition may be too narrow or broad to be of use in another.	
	Any manufactured substance is a nanomaterial if:	

<p>Canada</p> <p>For the purposes of this definition:</p> <ul style="list-style-type: none"> • It is at or within the nanoscale in at least one external dimension, or has internal or surface structure at the nanoscale; <p>or</p> <ul style="list-style-type: none"> • It is smaller or larger than the nanoscale in all dimensions and exhibits one or more nanoscale properties/phenomena. <p>3</p>

b. Regulation in Europe

The European Commission was the first regulatory body to formally regulate the use of nanomaterials in cosmetics. As of July 11, 2013, cosmetic products placed on the European market must conform to the procedures and data requirements contained in Regulation (EC) No. 1223/2009 of the European Parliament and of the Council 1. As of this date, a Product Information File (PIF) for each cosmetic product marketed in Europe must be maintained by a "Responsible Person" within the Community (defined as "a legal or natural person established within the Community"). Each PIF contains a cosmetic product safety report and other pertinent information for a given cosmetic product and must be made available to regulatory authorities if requested. Moreover, the Responsible Person must notify the Commission prior to the introduction of a cosmetic product to the European market. While notification for cosmetic products that do not contain nanomaterials can be made immediately prior to placement on the market, notification for cosmetic products containing nanomaterials must be made six months prior to placement on the market 1. In the latter case, the notified information must contain the following:

1. The identification of the nanomaterial including its chemical name (IUPAC) and other descriptors
2. The specification of the nanomaterial including size of particles and

physical and chemical properties

3. An estimate of the quantity of nanomaterial contained in cosmetic products intended to be placed on the market per year
4. The toxicological profile of the nanomaterial
5. The safety data of the nanomaterial relating to the category of cosmetic product, as used in such products
6. The reasonably foreseeable exposure conditions

The Regulation does not provide specific guidance on how to address some of the items listed above (e.g., the specific types of toxicological data required), but such guidance is available from the European Commission's Scientific Committee on Consumer Safety (SCCS). Specifically, guidance is available that assists with understanding what needs to be considered when evaluating the safety of cosmetic ingredients in general 4 and when conducting physicochemical characterization, exposure assessment, hazard identification and dose response, and overall risk assessment evaluations for nanomaterials in cosmetics 5. Based on SCCS guidance, the following parameters are considered important for identification and characterization of nanomaterials in cosmetic products 5:

1. Chemical identity
2. Chemical composition and purity
3. Size and size distribution
4. Morphology
5. Surface characteristics (charge, modifications, etc.)
6. Solubility
7. Surface area
8. Catalytic activity
9. Concentration
10. Dustiness (for dry powder products)
11. Density and pore density
12. Redox potential
13. pH
14. Viscosity
15. Stability
16. Other aspects (e.g., ultraviolet absorption, light reflection)

In the event that information supplied for a given nanomaterial raises concerns about its safety, the Commission may request the SCCS to issue a formal opinion. These opinions focus on the safety of the nanomaterial for use in the

relevant categories of cosmetic products and on the reasonably foreseeable exposure conditions. Timelines have been established for the SCCS to request additional information to assist with its evaluation of a given nanomaterial, for such information to be provided, and for a final opinion to be issued, all of which is made publicly available.

Unlike in other regions, special labeling requirements have been established for nanomaterial-containing cosmetics in Europe. Specifically, all nanomaterial ingredients present in a cosmetic product marketed in Europe must be clearly indicated in the list of ingredients and be followed by the word “nano” in brackets. Moreover, the Regulation calls for the creation and maintenance of a publically available catalogue of all nanomaterials that are used in cosmetic products placed on the market. This catalogue will be made available by January 11, 2014 and will indicate categories of cosmetic products that contain nanomaterials and the reasonably foreseeable exposure conditions. In recognizing that understanding nanomaterial safety is a pragmatic process, the Commission has set a deadline of July 11, 2018 for undertaking the first review of the provisions of the Regulation that concern nanomaterials, and will propose suitable amendments to the provisions where it is deemed necessary.

c. Regulation in the United States

As outlined in a recent draft guidance document on the safety of nanomaterials in cosmetics 2, cosmetic ingredients (with the exception of color additives) and cosmetic products are not required to be notified and do not require premarket approval from the U.S. Food and Drug Administration (FDA) in order to be placed on the U.S. market. With some exceptions (e.g., color additives and ingredients that are prohibited or restricted from use by regulation), a manufacturer may use any ingredient in a cosmetic product formulation as long as the use of the ingredient does not otherwise cause the product to be adulterated or misbranded. The manufacturer is responsible for ensuring that the cosmetic product is not misbranded or adulterated, and manufacturers or distributors should have obtained all data and information needed to substantiate the safety of the product before marketing.

The safety of any cosmetic product to be marketed in the U.S. should be evaluated by assessing the physicochemical properties and relevant toxicological endpoints for each ingredient in relation to the expected exposure levels that will result from the intended use of the finished product. For cosmetic products

containing nanomaterials, the FDA recommends that safety assessments address several important factors, including the following 2:

1. Physicochemical characteristics (including size and size distribution, aggregation/agglomeration, surface chemistry, morphology, solubility, density, stability, and porosity)
2. Agglomeration and size distribution of nanomaterials under the toxicity-testing conditions that should correspond to those of a final product
3. Impurities
4. Potential product exposure levels, and the potential for agglomeration of nanoparticles in the final product
5. Dosimetry for in vitro and in vivo toxicology studies
6. In vitro and in vivo toxicological data on ingredients and their impurities, dermal penetration, irritation (skin and eye) and sensitization studies, and mutagenicity/genotoxicity studies
7. Clinical studies to test the ingredient, or finished product, in human volunteers under controlled conditions

With regard to toxicity testing for nanomaterials, FDA has indicated that manufacturers should consider modifying traditional methods by giving consideration to appropriate solvents and dosing formulations, methods to prevent agglomeration of nanoparticles, purity and stability conditions, and other variables. FDA recommends that, at a minimum, evaluations be conducted for acute toxicity, skin irritation, dermal photo-irritation, skin sensitization, mutagenicity/genotoxicity, repeat-dose toxicity (21 to 28 days), and subchronic toxicity (90 days), and take into consideration toxicokinetics and toxicodynamics with regard to different exposure routes 2.

Manufacturers of cosmetic products are encouraged to contact the FDA if they are considering using a new nanomaterial or an altered (i.e., nanoscale) version of an ingredient already on the market so that discussions can be held regarding test methods and data needed to substantiate a given product's safety.

d. Regulation in Canada

Notification is a mandatory requirement for the sale of cosmetics in Canada. A Cosmetic Notification Form must be submitted to Health Canada within ten days of making a cosmetic product available for sale in Canada. This form provides information specific to each cosmetic product, including the address and contact information of associated companies, the purpose of the cosmetic (shampoo,

lipstick, etc.), the form of the cosmetic (gel, liquid, etc.), and a list of the ingredients and their concentrations.

Ultimately, manufacturers are responsible for ensuring that their cosmetic products meet the requirements for cosmetic products under the Canadian Food and Drugs Act and the Cosmetic Regulations. To assist manufacturers in determining whether ingredients are safe for use in cosmetics, Health Canada maintains a list of prohibited and restricted cosmetic ingredients (the “hotlist”). While there were no nanomaterials listed in the cosmetic ingredient hotlist as of late 2012, this list is not exhaustive, and Section 16 of Canada’s Food and Drugs Act permits Health Canada to restrict or prohibit any ingredient it deems unsafe.

Specific guidance is not readily available regarding the regulation of nanomaterials in cosmetics in Canada. In late 2011, Health Canada released a policy statement on its working definition of nanomaterial 3. At that time, Health Canada indicated that existing risk assessment methodologies are applicable to nanomaterials, and that guidance documents would be developed over time for nanomaterials specific to products, substances, or commodity groups. Given its authority within existing legislative and regulatory frameworks, Health Canada may request the submission of information on specific nanomaterials in cosmetics and other regulated products to assist with assessing potential risks due to exposure to nanomaterials via the use of such products. Such information may include the following (when relevant) 3:

1. Intended use, function, and purpose of the nanomaterial, and information regarding any end product in which it will be used
2. Manufacturing methods
3. Characteristics and physicochemical properties of the nanomaterial such as:
 - a. composition
 - b. identity
 - c. purity
 - d. morphology
 - e. structural integrity
 - f. catalytic or photo-catalytic activity
 - g. particle size/size distribution
 - h. electrical/mechanical/optical properties
 - i. surface-to-volume ratio
 - j. chemical reactivity
 - k. surface area/chemistry/charge/structure/shape
 - l. water solubility/dispersibility

- m. agglomeration/aggregation (or other properties)
 - n. descriptions of the methods used to assign these determinations
4. Toxicological, eco-toxicological, metabolism, and environmental fate data that may be both generic and specific to the nanomaterial if applicable
 5. Risk assessment and risk management strategies, if considered or implemented

Manufacturers and other stakeholders are encouraged to establish communication with Health Canada early in the development process for products (including cosmetics) that are, contain, or make use of nanomaterials.

On September 13, 2001, the Minister of Health confirmed the government's intention to proceed with the development of new environmental legislation for all products regulated under the Food and Drugs Act, including cosmetics, as their ingredients would no longer be exempt from the requirements of the Canadian Environmental Protection Act, 1999 (CEPA). Health Canada is in the process of developing Environmental Assessment Regulations to address the unique properties of ingredients in cosmetics and other products. However, until the new Regulations are developed, these substances are subject to notification and assessment requirements under the New Substances Notification Regulations (NSNR) of CEPA. The NSNR represent a volume-based, premanufacture/preimport notification program that requires the submission and government assessment of physical and toxicological information for nonexempt chemicals and polymers that are not listed on the Domestic Substances List (DSL). The amount of information and the studies that must be submitted to the government are described in a series of NSNR "Schedules." The specific Schedule that applies is dependent on a variety of factors including, but not limited to: the anticipated manufacture or import quantity of the notifiable substance (i.e., in kg/year); the inherent nature of the substance (e.g., chemical, polymer, or low-concern polymer); the intended use of the substance (e.g., research & development, site-limited, export-only); and whether the substance is listed on the Non-Domestic Substances List (NDSL).

Within the NSNR program, nanomaterials that are manufactured in or imported into Canada that are not listed on the DSL are considered new. Although there is no internationally recognized definition of this type of substance, for purposes of the NSNR, nanomaterials can be described generally as substances having one or more dimensions in a nanoscale range, typically between 1 and 100 nanometers. The nanoscale form of a substance on the DSL is considered a "new" substance if it has unique structures or molecular

arrangements. Substances listed on the DSL whose nanoscale forms do not have unique structures or molecular arrangements are considered existing and are not subject to the NSNR. In addition, incidentally produced or naturally occurring nanomaterials are not subject to notification.

2.3.4.2 SAFETY ASSESSMENT CONSIDERATIONS FOR NANOMATERIALS

a. Study Design Aspects

The safety of nanomaterials contained in cosmetics and other products must be supported by data generated in studies that are of high quality in terms of their design and reporting 6. While not always feasible, such studies ideally adhere to established testing guidelines, such as those published by the Organisation for Economic Cooperation and Development (OECD), and to the principles of Good Laboratory Practice. Tiered strategies have been proposed to evaluate the safety of nanomaterials wherein *in vitro* and *in vivo* screening studies are followed by pivotal *in vivo* studies and supportive mechanistic studies 7–9. While this may be an ideal scenario, the evaluation of nanomaterial toxicity is particularly challenging in Europe given the restrictions that are in place on animal testing in Europe and the fact that validated alternative methods currently available for conventional chemical substances (e.g., skin corrosion, skin irritation, mutagenicity, photomutagenicity, phototoxicity, and dermal absorption) have not been validated specifically for nanomaterials 5.

At the present time there are insufficient data on nanomaterial properties, behavior, and effects to allow for nanomaterial safety to be evaluated using a read-across approach (i.e., based on structural surrogates or categories of substances). Thus, substance-specific data (i.e., those generated with the substance of interest) are generally required to support the safety of a given nanomaterial. In cases where specific endpoints have not been evaluated with the substance of interest, information from the published literature may be useful if it is deemed to be relevant (i.e., based on a substance that is sufficiently similar in terms of physicochemical properties) and of suitable quality. In this respect, it is worth noting that published scientific articles do not always provide data that can be used for safety assessment purposes. Physicochemical characterization is a key element to nanomaterial study design and its importance is discussed further below. In addition, as for any study, those that are conducted with

nanomaterials need to consider the importance of general study design and adequate documentation of methods, materials, and results. Unfortunately, as summarized in a review of the published literature on the safety of food-related nanomaterials 10, there are many examples of peer-reviewed published articles in which it is difficult or impossible to discern simple yet critical experimental details, such as the number of animals per treatment group, the method/route of exposure, or the doses or concentrations of the substance that was tested. Thus, caution must be exercised when attempting to use publicly available information to support the safety of a particular nanomaterial.

b. Nanomaterial Characterization

Characterization of the physicochemical properties of nanomaterials is paramount to understanding their behavior in biological systems and conducting risk assessments related to potential exposure. Lack of adequate nanomaterial characterization limits the value and significance of a given study and renders it impossible to compare studies and recognize parameters that might influence toxicity. The physicochemical characteristics of a given nanomaterial may differ considerably in the “raw” versus the “tested” state and may be influenced by the solvent, test media, or biological environment that is utilized in a particular evaluation. As such, the SCCS has recommended that, unless otherwise justified, characterization of nanomaterials for use in cosmetics be undertaken under at least the following three conditions: i) in the raw form as manufactured; ii) as present in the final cosmetic formulation; and iii) as present during toxicological evaluations (in the dose formulation, cell culture, etc.) 5.

The importance of nanomaterial characterization was highlighted in a review of the published literature on the safety of food-related nanomaterials 10. Among other findings, this review identified that information on nanomaterial purity was lacking in more than half of the published articles that reported on studies of the oral toxicity of food-related nanomaterials. This seemingly simple oversight in many published articles renders the results of such nanomaterial toxicology studies difficult to interpret, and emphasizes the need for sufficient physicochemical characterization in nanomaterial toxicology studies to allow for their inclusion in risk assessments.

Toxicological properties of food-related nanomaterials is of particular importance as the cosmetic and personal care industries move increasingly towards the concept of “beauty from within” and the growing use of

nutraceuticals. Concerns of nanoparticle incorporation in organs of the body and elsewhere would appear to be very important since the size of a nanoparticle is at a level where cell penetration may be of significance. Thus, there remains much to do regarding the reliability of measuring and reporting toxicological impacts of ingested nutraceuticals.

c. Dose Metrics

Due to their large surface area per particle mass or particle volume, traditional means of expressing dose levels as mass or volume units (e.g., mg/kg body weight or mg/L) may not be appropriate for nanomaterials, particularly those that are insoluble in aqueous systems. Other metrics, such as particle number or relative surface area, may be more relevant to dose-response relationships and need to be considered when designing and evaluating the results of toxicology studies 2, 5, 7, 11. Thus, characterization of the physicochemical properties of nanomaterials should be conducted in a manner that allows for the derivation of such alternate dose metrics.

The results of a study reported by Oberdorster *et al.* 12 provide an example of the influence of dose metrics on data interpretation. When expressed in terms of equivalent mass, lung inflammation in rats was observed to be more severe (i.e., the dose-response curve was steeper) following installation of 25 nm TiO₂ nanoparticles than following installation of 250 nm TiO₂ nanoparticles. But when the dose was expressed in terms of relative surface area of the instilled nanoparticles, the dose-response curves for the two nanoparticle sizes overlapped. Thus, for particles with the same chemistry, but of different sizes, particle surface area may be a more relevant dose metric and should be considered when evaluating the results of toxicological studies. Such studies are of particular importance for nano-size TiO₂ particles in view of their wide use in sunscreen agents and other cosmetics.

d. Assay Interference

Various types of nanomaterials may interfere with assays commonly used to determine cellular or toxic effects. Numerous examples have been cited in the published literature and include interference with optical or other detector measurements, interference with colorimetric or fluorometric dyes used in cytotoxicity assays, interference with measurement of reactive oxygen species, and adsorbance to essential growth factors and nutrients in cell culture media

that leads to indirect growth inhibition and apparent cytotoxicity 13–15. Such effects may lead to false positive or false negative readouts, examples of which are presented in [Table 2](#).

Table 2 Examples of Assay Interference by Nanomaterials

Assay	Interference	Altered Readout	Nanomaterial
MTT (cell viability)	Adsorption of substrate	Decreased indication of cell viability (false positive)	Carbon nanoparticles
LDH (cell viability)	Inhibit LDH activity	Decreased indication of cytotoxicity (false negative)	Trace metal-containing nanoparticles
ELISAs (inflammatory response)	Adsorption of cytokines	Decreased indication of inflammation (false negative)	Metal oxide nanoparticles

Abbreviation: ELISA, enzyme-linked immunosorbent assay; LDH, lactate dehydrogenase, MTT, (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide).

It is of particular interest to note that the MTT cell viability assay has been reported to be affected by some nanomaterials. This is one of the recommended assays for assessing cell viability in OECD test guideline number 431 (*In Vitro Skin Corrosion: Human Skin Model Test*) 16. As such, the possibility of false positives from this assay, and others, needs to be carefully considered when evaluating skin corrosion and other toxicological endpoints for nanomaterials.

CONCLUSION

Regulatory and safety evaluations of nanomaterials and cosmetic products that contain nanomaterials are evolving processes. Considering that nanomaterials can, and have the potential, to possess properties quite unlike their macroscopic counterparts, the unique physicochemical properties of nanomaterials present many difficulties with respect to characterization and toxicity testing. These differences contribute to the challenges associated with conducting risk assessments for cosmetic products containing nanomaterial ingredients. The continued accumulation of knowledge on nanomaterial properties and safety and the development of additional validated methods for evaluating these aspects will assist regulatory communities around the world in ensuring that cosmetic

products containing nanomaterial ingredients are safe for use by consumers worldwide.

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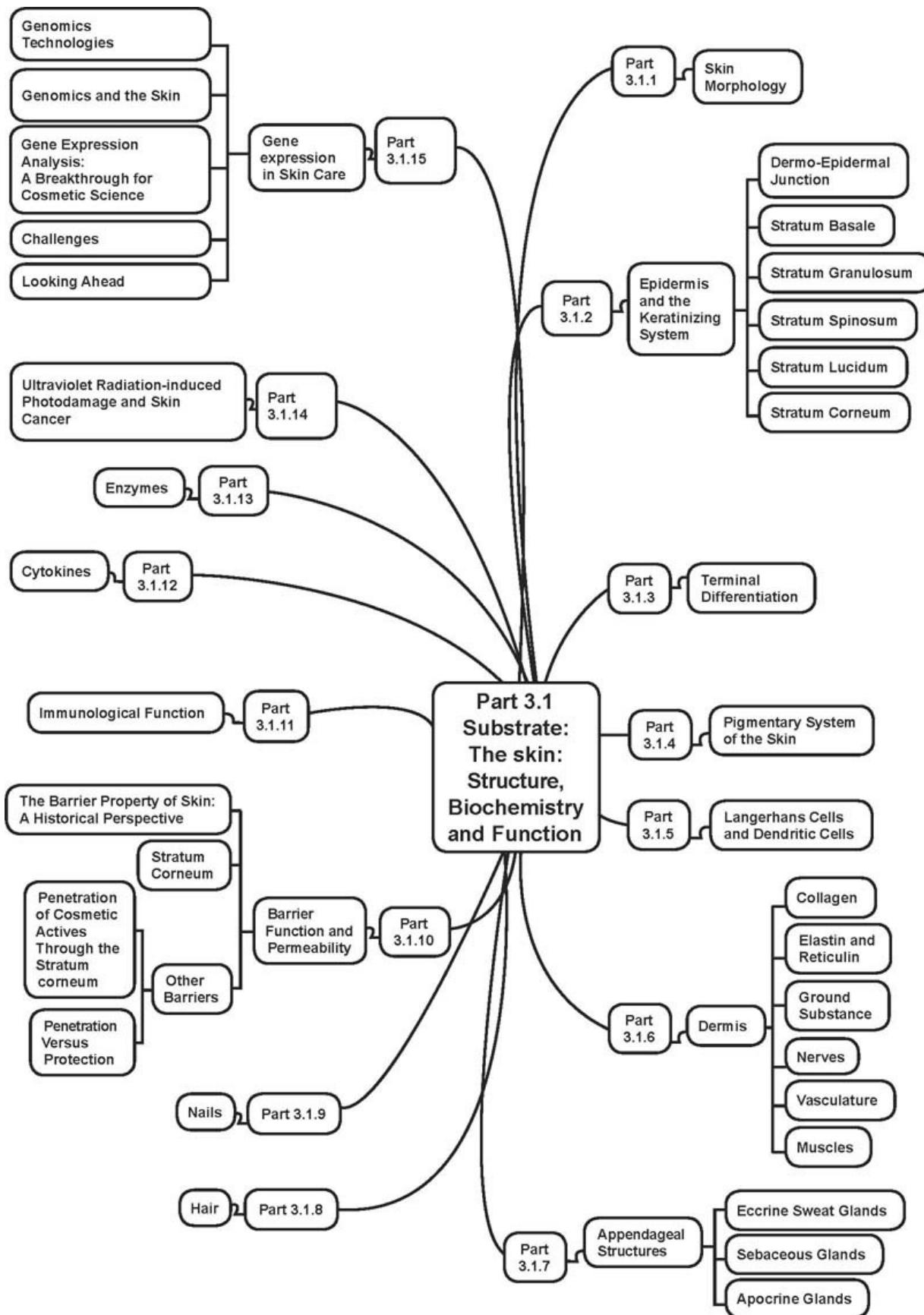
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PART 3

**SUBSTRATE:
THE SKIN:
STRUCTURE, BIOCHEMISTRY
AND FUNCTION**

PART 3.1

SKIN MORPHOLOGY



PART 3.1

THE SKIN: STRUCTURE, BIOCHEMISTRY, AND FUNCTION

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ABSTRACT

The skin is the organ that forms the border between the organism and the environment. Skin prevents dehydration, stops the penetration of noxious foreign materials and microorganisms, cushions against mechanical shock, helps to maintain a constant body temperature, and transduces incoming stimuli. In order to perform these functions, skin must be maintained in good condition, an important objective for cosmetic formulators. For cosmetic and personal care scientists, whether they are concerned with the improvement of the skin by pharmacology or cosmeceuticals, with the prevention or repair of damage, an understanding of skin structure and function is essential. As such, this section has a primary focus upon these issues.

Further, the impact of light on skin and on skin aging has become so important in cosmetics that it too requires a complete discussion. This chapter is carefully and thoroughly designed to provide a basic knowledge of skin structure and function, thereby forming a sound foundation for the reader to understand key issues and provide stimulation towards the generation of new ideas and novel approaches for new ingredients, addressing the global quest for advanced cosmetic and personal care products.

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PART 3.1.1 SKIN MORPHOLOGY

The skin is divided into three layers: the epidermis, the dermis, and the subcutaneous tissue. The epidermis is the outermost layer of the skin and consists of a stratified squamous epithelium. Its thickness varies, depending on location, and varies from 0.05 mm to 1.5 mm. The epidermis is made up primarily of keratinocytes, whose basic function is to produce a filamentous protein, keratin, to serve as a protective barrier in combination with various lipid components. These cells also produce several other proteins, for example, cytokines, which play a role in the cutaneous inflammatory response. Separated from the epidermis by the basement membrane, the dermis is composed primarily of the so-called ground substance, which includes glycosaminoglycans (GAGs) and the structural protein collagen. Its thickness also varies with location from 0.3 mm to 3.0 mm. The dermis is divided into two layers: the papillary layer, which interdigitates with the epidermal rete ridges, and the reticular layer, which extends to the subcutaneous tissue. This deepest layer of the skin, also known as the subcutis or hypodermis, is composed primarily of lipocytes with fibrous septae providing structure and support ([Fig. 1.1](#)).

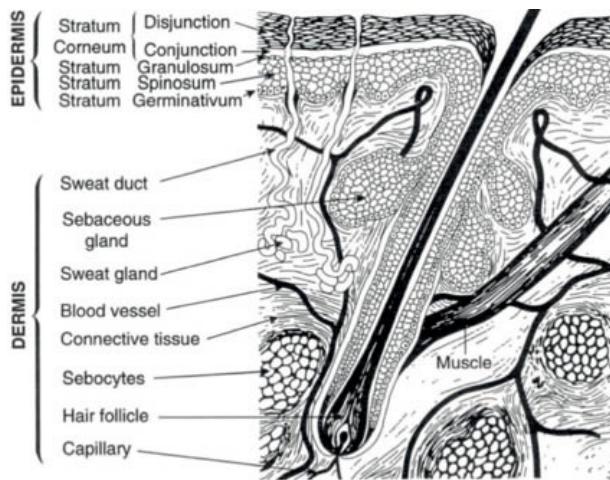


Figure 1.1 Diagram of normal human skin

(Reprinted from Harry's Cosmeticology 8th Ed.2000)

PART 3.1.2 EPIDERMIS AND THE KERATINIZING SYSTEM

The epidermis measures approximately 100 micrometers in thickness and consists of a number of layers: the innermost basal layer (*stratum basale* or *stratum germinativum*) which attaches to the basement membrane at the dermoepidermal junction, the prickle layer (*stratum spinosum*), the granular layer (*stratum granulosum*), the clear layer (*stratum lucidum*) and the horny layer (*stratum corneum*). Together the basal layer and the prickle layer comprise the Malpighian layer. The viable epidermis (the basal, prickle, and granular layers) consists of about 10 biologically active keratinocytes. The stratum corneum is located on top of the viable epidermis. The stratification into the layers is the result of changes in the keratinocytes as they mature and move outward from the basal layer, in which they are continuously formed by the mitosis of self-renewing progenitor cells, which lose their nuclei and are shed from the skin surface (Fig. 1.2).

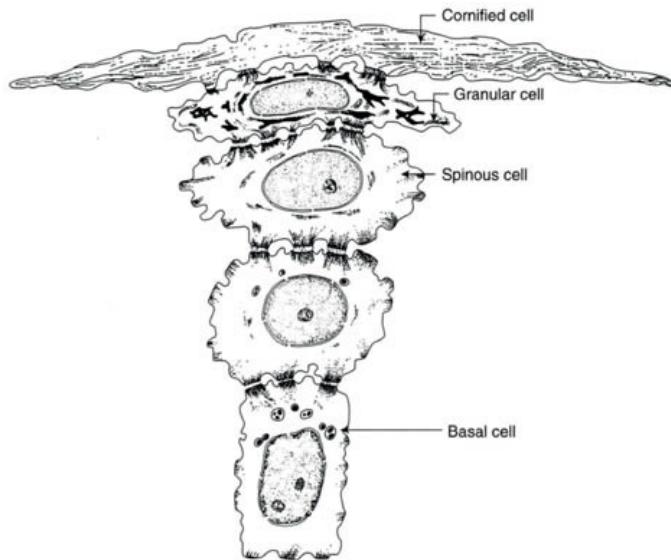


Figure 1.2. Conversion of individual basal keratinocytes into the flat cells of the stratum corneum (Reprinted from Harry's Cosmeticology 8th Ed.2000)

The replacement time for the whole epidermis is about 42 days and it is about 14 days for the *stratum corneum*. While the process of keratinization remains incompletely understood, in normal skin the desquamation of keratinocytes is in equilibrium with the generation of keratinocytes by mitosis of the proliferating cells. The importance of this equilibrium is best understood by studying examples of those skin diseases with abnormal keratinization. Abnormally rapid transformation of basal cells into horny cells of the *stratum corneum* occurs in psoriasis. *Ichthyosis vulgaris*, on the other hand, is a genetic disorder that results from abnormal retention of keratinocytes.

Three other cell types are present in the epidermis: melanocytes, the dendritic pigment-synthesizing cells; Langerhans cells, which are colorless and dendritic in form and perform functions related to immunology; and Merkel cells, which are concerned with sensation. The melanocytes and the Merkel cells are confined mainly to the basal layer, while the Langerhans cells are distributed in the basal, spinous, and granular layers.

a. DermoEpidermal Junction

The basement membrane zone forms the junction between the epidermis and the dermis. Under the electron microscope, it is seen to be composed of four components, listed from the outermost layer: the plasma membrane of the basal keratinocytes, the clear lamina lucida, the electron-dense basal lamina, and the dermal fibrils and bundles of fine filaments [1]. Some details of the components of this junction are provided in a later segment of this section.

b. Stratum Basale

The basal layer is a continuous layer of cells that gives rise to all the keratinocytes through mitotic activity. It is usually described as being one cell thick, but in thick normal or in pathological epidermis it appears that mitosis may not only be confined to cells in contact with the basement membrane. A portion of the basal cells is proliferative; these are the cells that differentiate and move up through the epidermis, eventually to become the components of the *stratum corneum* (essentially “dead” cells that are anuclear) and later desquamate at the top of the skin.

Cells of the *stratum basale* are cuboidal and have a basophilic cytoplasm and dark-staining, elongated nuclei; under the electron microscope their cytoplasm reveals many ribosomes, mitochondria, and in some cases smooth membranes. In addition, they contain numerous fine tonofilaments, about five nm in diameter, which form the developing cytoskeleton. The basal cells also often contain melanin, transferred from adjacent melanocytes. Intercellular bridges, or desmosomes, connect basal cells with one another and with the overlying squamous cells. Modified desmosomes, or hemidesmosomes, connect the basal cells to the underlying basement membrane zone.

c. Stratum Spinosum

The *stratum spinosum* is so called because the cells are given a spiny appearance by the numerous desmosomes. These desmosomes, or specialized attachment

plates for the cellular tonofilaments, correlate with the intercellular bridges between keratinocytes. The glycocalyx is the intercellular cement between keratinocytes and is composed of glycoproteins. In the upper region of the stratum spinosum, lamellar granules, also known as keratosomes or Ödland bodies, make their appearance. These are ovoid bodies about 100–500 nm long. In the *stratum granulosum* they ultimately migrate toward the periphery of the cell and are discharged into the intercellular spaces. Their appearance there correlates with the degradation of keratinocytes. Their lipid contents act to establish a barrier to water loss and may participate in *stratum corneum* cellular cohesion. Water loss, as is well known, is a critical phenomenon that is measured in the development of new skin care products and relates to some aspects of skin aging.

d. Stratum Granulosum

The thickness of the granular cell layer is usually proportional to the thickness of the *stratum corneum*. It may be only one cell layer thick in thin skin and up to ten layers in the thicker skin found on the palms and soles of the feet. The cells contain basophilic granules of a material called keratohyalin, a material thought to be responsible for keratin filament aggregation. The “hard” keratins of hair and nail lack these keratohyalin granules.

e. Stratum Lucidum

The *stratum lucidum*, not seen in most formalin-fixed sections, is located at the deepest portion of the *stratum corneum*. It can be recognized only in palmar and plantar skin.

f. Stratum Corneum

In the *stratum corneum* the keratinocytes have lost their nuclei and virtually all of their cytoplasmic organelles and contents, including the keratohyalin granules. This layer of cells is about 10–15 cells thick (approximately 10 micrometers) and is located on top of the viable epidermis. The corneal cell layer stains eosinophilic with the hematoxylin and eosin stain due to the absence of basophilic nuclei. The cells are flattened and completely filled with keratin. The keratin is in the form of bundles of filaments embedded in an opaque interfilamentous material. They align into disulfide crosslinked macrofibers under the influence of filaggrin, the protein component of the keratohyalin granule responsible for keratin filament aggregation [2].

The structure of the *stratum corneum* has been compared to that of a brick wall, with the corneocytes as bricks and the intercellular lipids as mortar [3]. Horny cells are continuously shed from the skin surface. *Stratum corneum* lipids have been characterized and consist of phospholipids, glucosylceramides, cholesterol, cholesterol sulfate, cholesterol esters, ceramides, and fatty acids [4]. The makeup of lipids suggests that hydrophilic lipids are excluded from the *stratum corneum* to provide a hydrophobic surface on the skin.

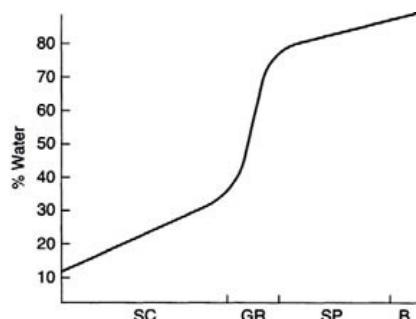
PART 3.1.3 TERMINAL DIFFERENTIATION

Terminal differentiation describes the change of the cuboidal keratinocytes (on the basement membrane) to the flat cellular remnants that are shed from the skin surface. The progressive changes of keratinocytes as illustrated in [Figure 1.2](#) are accompanied by biochemical changes, formation of keratins, formation and hydrolytic changes in lipids, loss of water, and crosslinking of cell envelopes. The formation of keratins proceeds from the intermediate filaments present in keratinocytes. Intermediate filaments of more than 50 types are synthesized in human tissues. In skin, two types (I and II) are specifically expressed in epithelial cells. In this classification, acidic keratins (cytokeratins K9–K20) are identified as type I, while the basic keratins (cytokeratins K1–K8) are classified as type II. In skin, the keratins are customarily dimers of one type I and one type II. The fundamental structure of the intermediate filaments includes coil-coil alpha-helical segments bonded to each other by nonhelical linker segments. Both ends of the rod-shaped filament are terminated by peptides. The exact modus of attachment of these filaments to each other to form the keratins within the keratinocytes is still under investigation. As noted, epithelial cytoskeletal filaments generally belong to one of two keratin (acidic or neutral-basic) groups ranging in molecular weight from about 40 to 70 kDa. Filaggrin has been identified as one of the keratohyalin proteins forms in differentiating keratinocytes. Filaggrin is involved in the aggregation of keratin filaments to form the keratins found in mature keratinocytes or corneal cells. After filaggrin has served its function as a matrix between intermediate filaments, it is hydrolyzed enzymatically to create various free amino acids that form part of the natural moisturizing factor (NMF).

The hydrolytic changes of the epidermal lipids are also controlled by keratinocytes, which discharge lipids into the intercellular space after forming the lamellar granules. These lipids are distinctly different from the sebaceous

lipids secreted by the sebaceous glands. In the process of terminal differentiation, which requires about three to four weeks, the basal keratinocyte generates a remarkable set of complex lipids (e.g., ceramides). During the cells' passage outward, these lipids are modified (become more hydrophobic) to contribute to the biphasic structure commonly called *stratum corneum*.

During their ascent to the skin surface, the keratinocytes shrink, primarily through loss of water. The fate of this water is not known, but one may safely assume that it becomes part of the evaporating water generally described as trans-epidermal water loss. The loss of water during the maturation of keratinocytes is an important phenomenon that must be considered in studies of skin moisture levels ([Fig. 1.3](#)) and the impact of ingredients used in cosmetic and personal care products to improve the skin's ability to retain its moisture. The level of water in the basal layer is about that found in internal tissues, that is, about 80–85%. The water level drops stepwise to about 35% at the border between the *stratum granulosum* and the *stratum corneum*. The water level in the topmost layers of the skin is variable and is under the control of the environment and the evaporative flux from lower skin layers.



[Figure 1.3](#). Water concentration profile in epidermal layers [SC—*stratum corneum*; GR—*stratum granulosum*; SP—*stratum spinosum*; B—*basal layer* (*stratum germinativum*). (Reprinted from Harry's Cosmeticology 8th Ed.2000)

Finally, the proteins in the cell membranes of the maturing keratinocytes undergo drastic changes due to crosslinking. This provides the terminally differentiated corneal cell with a rigid cell envelope that is chemically resistant and acts as a protective coating. The most important enzymes that play a role in this process are transglutaminases that catalyze epsilon- (gamma-glutamyl) lysine crosslinking. Involucrin is the primary cytoplasmic precursor to the protein making up the cell envelope. Other crosslinked proteins are present and have been identified—for example, loricrin.

Biologists have further identified some layers within the *stratum corneum*.

The desquamating layer at the surface is frequently called the *stratum corneum* disjunction, while the layer below it is known as *stratum corneum* conjunction. To complete this highly simplified discussion of the skin, it is important to note that the latter is frequently identified as viable epidermis. Cells from this layer can be cultured and are commonly used to study the release of cytokines and the like as well as the impact of drugs and/or cosmetic ingredients. In contrast, the nonviable epidermis includes only the dead cells of the *stratum corneum*.

Before leaving the life history of the epidermis, the process of differentiation should be considered as proceeding from the inside to the exterior. The outward movement of biological debris, water, and lipids directly opposes human efforts to drive ingredients/drugs into and through the skin unless special efforts are made to create molecules that are shaped and manipulated to permeate.

PART 3.1.4 PIGMENTARY SYSTEM OF THE SKIN

Melanocytes are dendritic cells that produce and secrete melanosomes, which contain melanin. Melanin is the major determinant of skin color. The number of melanocytes in the epidermis is the same—regardless of skin color; it is rather the number and size of melanosomes produced that determine the color of one's skin. Melanosomes in dark skin are nonaggregated, whereas they are smaller and form membrane-bound complexes in light skin. Melanocytes are derived from the neural crest in the embryo and are seen in the basal layer of the epidermis by the eighth week of gestation. They differ from the other cells of the *stratum basale* by the possession of dendritic processes, by which they transfer pigment to a group of keratinocytes; the whole forming the “epidermal melanin unit” [5]. Typically each melanocyte is associated with about 36–40 keratinocytes in the human epidermis. Melanocytes have no desmosomes and thus, when stained with hematoxylin and eosin, appear to have a halo due to the separation from adjacent keratinocytes. The concentration of melanocytes, though, does vary in different areas of the skin, with the highest concentration on the face and the male genitalia and the lowest concentration on the trunk.

The characteristic feature of melanocytes is a special cytoplasmic organelle known as a melanosome in which the melanin is formed by the action of the enzyme tyrosinase. The melanosomes arise as spherical, membrane-bound vesicles in the zone of the Golgi apparatus and eventually appear as densely pigmented granules [6].

Melanins consist of two kinds of quinoid polymers: pheomelanins and

eumelanins. Pheomelanins are yellow or red in color, and eumelanins produce the brown or black color. Both are formed by the same initial steps, which involve oxidation of tyrosine to 3,4-dihydroxyphenylalanine (DOPA) and its dehydrogenation to DOPAquinone. The formation of eumelanins then involves several further steps to produce indole-5,6-quinone, which polymerizes and becomes linked to protein. It is now believed that eumelanin is not a homopolymer composed solely of indole-5,6-quinone units but rather a poikilopolymer that includes several intermediates. Pheomelanins are formed by a different route. The DOPAquinone interacts with cysteine to form 5-S- and 2-S-cysteinyldopa, and these isomers are further oxidized to a series of intermediates that then polymerize [7].

The formation of melanin depends on the generation of free radical species. The biochemical pathways leading to the formation of melanin pigments *in vivo* were described by Raper [8] and more recently by Prota [9, 10]. This information can be found in most textbooks of biochemistry and is not repeated here. Once formed, melanin has been identified as a (stable?) free radical that can react with superoxide.

The significance of melanin as a purported photoprotectant and the response of skin to ultraviolet (UV) irradiation are critically important to skin appearance and health, especially with regard to sun exposure.

PART 3.1.5 LANGERHANS CELLS AND DENDRITIC CELLS

The skin provides the first line of immune surveillance against biologic pathogens and chemical irritants through a network of sophisticated and specialized antigen-presenting cells. These include Langerhans cells (LCs) in the epidermis and several distinct types of interstitial dendritic cells (DCs) in the dermis. LCs and DCs are the sentinels that recognize certain antigens as well as recognizing tumors. For example, they recognize foreign environmental antigens such as irritating chemicals (thereby inducing contact hypersensitivity), microorganisms (thus protecting through antimicrobial immunity). They also recognize tumors (providing antitumor immunity) and allogeneic markers (causing skin graft rejection).

The extended dendrites of these unique cells form a network within the epidermis and in the dermis they create an immunologic barrier primed to recognize, capture, and process pathogens and foreign antigens for presentation to naïve and memory T-lymphocytes, thereby initiating and then modulating the

subsequent immune response. Furthermore, the LCs and DCs play a significant role in inducing peripheral immunologic tolerance to innocuous environmental proteins and to self-antigens, thus helping to prevent autoimmune disease.

LCs are the epidermal resident dendritic cell population. They make up about 3–5% of the suprabasal, *stratum spinosum* layer of the epidermis [11] including the mucosal epithelia lining the ocular oral and vaginal surfaces [12]. LCs are scattered without desmosomal connections between keratinocytes. In light microscopic sections, LCs cannot be detected by routine histologic staining, but only by staining with gold chloride (for human LCs).

LCs are characteristically stellate with extensive dendritic fibers stretching in all directions, forming a network within the *stratum spinosum* layer. When viewed by phase contrast microscopy, the DCs extend delicate flower-like projections that bend, retract, and reextend. This shape and motility enable them to accomplish their function of capturing antigens and of selecting and processing the antigens for specific T-cells [13]. By means of high-powered electron microscopy, LCs have been shown to be a folded nucleus and are identified by their distinctive, intracytoplasmic *Birbeck granules* that (when mature) are rod shaped with a vacuole at one end, resembling a tennis racquet. Antigen capture seems to be a consequence of binding to langerin, a calcium-dependent lectin that specifically binds mannose as well as other sugars and is found only in LCs and some dermal DCs [14]. This binding actually induces formation of Birbeck granules and then routes the antigen into the Birbeck granules for further processing.

Langerin is a recognition receptor for carbohydrates on the surfaces of pathogens that routes antigens to different cellular compartments involved in antigen presentation in order to promote recognition by different specific T-cells [15].

In addition to LCs, the skin has at least four other subclasses of DCs in the dermis. The characterization of these DCs is quite complex because DCs can arise from several types of progenitor cells, and different functional phenotypes of DCs can be generated from the same precursor cell (as determined by function required, generating a sequential encounter with specific cytokines). Furthermore, as the LCs and DCs encounter a foreign antigen and migrate, they mature, and their surface markers as well as their functional interactions change. Thus, the steady-state population is different from the inflammatory response populations.

Langerhans cell function is impaired by UVB radiation, resulting in a decrease in the antigen-presenting capacity and in the production of cytokines. This UV suppression leads not only to adverse consequences by impairing endogenous antimicrobial activity and tumor recognition and suppression, but also to possible advantageous therapeutic treatments for skin allergy and inflammatory disease.

PART 3.1.6 DERMIS

The dermis is a tough and resilient tissue that cushions the body against mechanical injury and provides nutrients to the epidermis and cutaneous appendages [16]. It consists of an association of protein fibers within an amorphous ground substance containing glycosaminoglycans (GAGs), previously known as mucopolysaccharides. There are few cells in this matrix; most of them are fibroblasts, which secrete the dermal constituents. Fibroblasts are derived from the mesenchyme. The mast cell, also of mesenchymal origin, houses granules that contain heparin, histamine, and other active substances. The mast cell is an active participant in skin inflammation and irritation, as well as in several other skin disorders. The dermis also houses blood, contains lymphatic and nervous systems, and surrounds the invaginated epidermal appendages. The GAGs in the dermis can hold copious amounts of water and tend to surround the other constituents of the matrix. Together with the fibrous portion of the matrix, these substances account for the skin's flexibility and resistance to deformation.

a. Collagen

The major fibrous constituent of the dermis, accounting for 75% of the dry weight and 18–30% of the volume, is collagen [17]. Under the light microscope, collagen fibers appear as eosinophilic branching wavy bands. Collagen fibers are loosely arranged in the papillary dermis and are tightly bundled in the reticular dermis. Pilosebaceous units, eccrine glands, and apocrine and dermal blood vessels are surrounded by a thin meshwork of collagen. Collagen fibers display characteristic cross-striations with a periodicity of 60–70 nm. Collagen is rich in the amino acids hydroxyproline, hydroxylysine, and glycine. The fibroblasts produce a precursor known as procollagen, which includes 300–400 additional amino acids in each of its chains; these extensions are removed after secretion, which results in the conversion to the collagen molecule. Collagen fibrils form by the association of collagen molecules. Vitamin C and copper are two of

several cofactors required in the biosynthesis of collagen. Collagen production is a dynamic process that involves continual synthesis by fibroblasts and degradation by collagenases.

b. Elastin and Reticulin

Elastic fibers make up only 4% of the dry weight and 1% of the volume of the dermis. They are delicate, straight, freely branching fibers that prove very resilient. These fibers are thicker in the lower portion of the dermis and become thinner as they approach the epidermis. Elastin differs from collagen not only structurally but also chemically. Desmosine is an amino acid unique to elastin. About 0.4% of the dry weight of the dermis is made up of fine branching fibers that, unlike collagen, stain black with silver stains and are known as reticulin. Their axial periodicity is identical to that of collagen. Reticulin fibers in the papillary dermis serve to anchor the basal lamina [17].

c. Ground Substance

The amorphous ground substance in which the fibers and cells lie contains acidic GAGs. In dermis the major forms are hyaluronic acid, chondroitin sulfate, and dermatan sulfate.

d. Nerves

The skin is supplied with both sensory and autonomic nerves. It is innervated with about one million afferent nerve fibers; most terminate in the face and extremities, and relatively few supply the back. The sensory nerves, unlike autonomic nerves, possess a myelin sheath up to their terminal ramifications.

The papillary dermis is heavily innervated with unmyelinated nerve fibers that transmit the sensations of temperature, pain, and pruritus. Three types of special nerve end organs also exist in the dermis. Vater-Pacini corpuscles are large end-organs that are located in the deeper portions of the dermis and subcutis and mediate a sense of pressure. They measure up to 1 mm in diameter and have their greatest concentration at the tips of the fingers and toes. A few are present in the nipple and anogenital regions. Meissner corpuscles are located in the dermal papillae and mediate the sense of touch. They occur only on the ventral aspects of the hands and feet and are most concentrated in the fingertips. The mucocutaneous end-organs are found in the papillary dermis of the modified hairless skin of the glans, the prepuce, the clitoris, the labia minora, the perianal region, and the vermillion border. The autonomic nervous system supplies fibers

to the arrector pili muscles, the blood vessels, and the eccrine and apocrine glands. The sebaceous glands are not innervated, and their functioning depends on endocrine stimuli. The autonomic nervous system controls vasoconstriction, contraction of the arrector pili muscles, and glandular secretion.

e. Vasculature

The dermal vasculature consists of intercommunicating plexuses. The subpapillary plexus lies within the papillary dermis and runs parallel to the epidermis to furnish a supply of capillaries, arterioles, and venules to the dermal papillae. The deeper plexuses are composed of larger vessels and surround hair follicles and eccrine glands. The dermal lymphatics are associated with the vascular plexuses.

f. Muscles

Smooth muscle occurs in the skin as the arrector pili muscles of the hair to pull the follicle upward with contraction. There are also smooth muscles fibers in the scrotum and the areolas. Striated muscle occurs in the skin within the neck as the platysmas and in the muscles of expression of the face. Special aggregates of smooth muscles are found between the arterioles and the venules in the skin. These serve to shunt blood from the arterial to the venous system directly and thus bypass the capillary system.

PART 3.1.7 APPENDAGEAL STRUCTURES

a. Eccrine Sweat Glands

Humans have several million eccrine sweat glands distributed over most skin sites, but they are more concentrated in the axilla, forehead, palms, and soles. In some areas they number as many as 600 glands per square cm. Eccrine sweat glands are the most numerous skin appendages and are responsible for the production of sweat. They have a cylindrical spiral duct lined with epidermal cells extending from their visible opening in the epidermis down into the deep dermis, where the duct becomes coiled and convoluted into a ball [18]. This secretory coil manufactures the odorless sweat, which rises up the duct to be released on the skin surface. It is thought that the duct of the gland has the ability to modify the sweat as it flows upward by removing salts or water.

The sweat glands control both body temperature and excretion, and they are

under the control of the cholinergic nervous system. The evaporation of sweat has a cooling effect. The glands respond to environmental temperature but also to other stimuli, such as UV light, emotional stress, and increases in body temperature. On the palms and soles, the secretion from the glands serves to increase surface friction.

Sweating appears to involve activation of myoepithelial cells, which line the ducts of the glands. Although sweating is considered to be a continuous process, it seems that sweat is ejected in small bursts, suggesting a peristaltic action by the ducts. The composition of eccrine sweat is similar to that of plasma, although more dilute, and was documented about 40 years ago. It includes, in decreasing relative concentration (mg %): Cl⁻ (320), Na⁺ (200), lactic acid (35), K⁺ (20), urea (15), ammonia (5), Ca⁺⁺ (2), glucose (2), Mg⁺⁺ (1), amino acid (1), and creatinine (0.3).

b. Apocrine Glands

The apocrine glands are tubular glands attached to the hair follicle and, like the sebaceous glands, develop in association with it [19]. Although rudiments are found covering the entire surface of the fetus, the glands become canalized and functional almost exclusively in selective areas including: the axillae, the anogenital regions, the areola, the external auditory canal, and the eyelids. In humans, apocrine gland secretions are milky and viscous but without odor. Odor production is related to bacterial action on apocrine secretions at the skin surface.

After puberty, apocrine gland secretion is in response to emotive stimuli. Adrenergic nerves control secretory activity, in contrast to the cholinergic control of eccrine function. The function of the glands in the human species has been much debated; studies have demonstrated that the apocrine skin glands of mammals produce various glycoconjugates and antimicrobial substances, which may serve as a nonspecific defense on the skin surface [20]. In other mammals the glands serve a sexual function.

c. Sebaceous Glands

Sebaceous glands [18] secrete sebum, which forms the majority of the lipid that covers the skin and hair. They are found in all areas of human skin except the palms, soles, and dorsum of the feet. Sebaceous glands are usually associated with hair follicles, except for those on the nipples, areola, and labia minora. The greatest concentrations (reportedly as high as 400–900/cm) are found on the

scalp, face, upper chest, and shoulders.

The glands are holocrine and thus form their secretion by decomposition of their cells. New cells are formed continually from the lining of the gland by cell division to replace those lost. No motor innervation has been demonstrated in humans. During the generation of sebum, cells at the periphery of the lobule undergo division. As the daughter cell moves toward the center of the lobule, it synthesizes lipids. As the sebum accumulates, the cell increases in volume as much as 150-fold. When synthesis is complete cell rupture occurs. This process from cell division to rupture requires approximately 14 days. The relatively long delay must be taken into consideration when designing drugs, cosmeceuticals, and therapies aimed at altering sebum.

Sebaceous gland activity is under hormonal control. It is stimulated by androgens of both gonadal and adrenal origin. In human males the glands are minute during the prepubertal period but undergo vast enlargement at puberty, when their output increases more than fivefold. Eunuchs secrete about half as much sebum as normal males but substantially more than boys; it seems that the secretion is dependent on adrenal androgens. Adult women secrete only a little less than men; their sebaceous activity appears to be maintained by androgens from the ovary and the adrenal cortex. Estrogens and anti-androgens, such as cyproterone acetate, inhibit sebaceous secretion in humans. On the other hand, relatively small doses of potent androgens can cause enlargement of the glands and an increase in sebum production.

Human sebum is composed of triglycerides (57.5%), wax esters (26.0%), squalene (12.0%), free fatty acids (10%), and, to a minor extent, cholesterol and cholesterol esters. Epidermally derived lipids differ in lacking wax esters and squalene and having much higher proportions of cholesterol esters and cholesterol. There are marked differences in sebum composition among species. The origins of sebaceous lipids and their composition are different from those of the epidermal lipids [21]. The purpose of sebum is not entirely understood, but sebum has been shown to have thermoregulatory functions, and plays a role in the regulation of immunological functions and inflammatory processes. In addition, sebum contains lipids with antimicrobial activity [22]. While excessive sebum production has been associated with the development of acne vulgaris, lack of sebum production in prepubertal children is not associated with any skin abnormalities.

PART 3.1.8 HAIR

The hair follicle is quite variable, depending on its location. In adults, deep terminal hairs are found on the scalp and male beard area. Hair on the extremities and trunk is located more superficially in the skin. Vellus hairs are present on the female face and on the nonbearded areas of the male face. Lanugo is soft fine hair that covers the fetus and is shed prior to birth.

In general, the cross-sectional shape of terminal Caucasian scalp hair is round and somewhat curly; African American scalp hair is oval, sometimes flattened, and usually kinky; hair in Asians is round in cross-section and straight. These relationships do not apply to pubic hair, beard hair, and eyelashes, which have similar features in all races and are typically oval.

Hair color is due to the distribution of melanosomes within the hair shaft produced by melanocytes in the hair bulb. These are transferred to cells of the hair matrix similar to the transfer from melanocytes to keratinocyte in the epidermis. Three types of melanosomes are present in hair. Eumelanins are seen in dark hair, and pheomelanins predominate in blond hair. The intensity of color is related to the number of fully melanized melanosomes produced. Gray hair and white hair are due to a decreased number of melanocytes that produce fewer melanosomes.

Despite the vast body of knowledge regarding the anatomy, biology, and function of hair, humans are still unable to induce hair regrowth in the many disorders that result in hair loss, or to induce permanent hair removal in states of excessive hair. The cost of hair care in terms of time and money spent is huge in many cultures, and the psychological impact of hair disorders should not be underestimated.

PART 3.1.9 NAILS

The nail plate is composed of keratinized cells that originate in the nail matrix. As discussed previously, there are no keratohyalin granules. The proximal nail fold forms the cuticle. The nailbed does not contribute to the development of the nail plate but serves as a base for the adherent keratinous nail plate.

PART 3.1.10 BARRIER FUNCTION AND PERMEABILITY

For the cosmetic industry, by and large, the skin has been synonymous with the epidermal permeability barrier, located in the *stratum corneum*. This is a

somewhat restricted view, but nevertheless remains in place due to the fact that skin moisturization—a function that is of prime interest to the industry—is dependent on a functional permeability barrier. As the saying goes, a good moisturizer is not necessarily a barrier product, but all barrier products are good moisturizers. The two-compartment, “brick and mortar” organization of the healthy *stratum corneum*, with its organized lipid membranes, do “lock in” moisture and prevent dry, chapped skin. However, the significance of other types of barriers in the skin, i.e., UV barrier, antioxidant, and antimicrobial defenses have all begun to be appreciated and emphasized, as evidenced by the proliferation of active ingredients targeted to them. This growing recognition, in concert with an improved understanding of the other areas of the skin, is critically important to novel thinking leading to new functional ingredients for this important cosmetic and personal care category.

a. The Barrier Property of Skin: A Historical Perspective

The barrier property of skin against influx and efflux of water was acknowledged as early as the 1800s, but the conclusive evidence was provided by the work of Irvin Blank and Scheuplein, who convincingly demonstrated its location in the *stratum corneum* (horny layer) [23]. The barrier properties are of interest to different scientific disciplines and industry; especially the pharmaceutical and personal care sectors that are interested in delivering drugs transdermally or active ingredients for topical delivery to improve the skin conditions (anti-aging, moisturizers, skin lighteners, etc.) respectively. For the dermatologists, inherited and acquired skin diseases such as various types of ichthyosis, atopic dermatitis, and psoriasis all have a compelling barrier dysfunction associated with it [24]. Extensive damage to the barrier, as in third-degree burns, can be lethal; attesting to the significance of a functional permeability barrier to prevent life-threatening dehydration of the body.

Details of barrier (and *stratum corneum*) formation, its structural complexity, and chemical characterization

Early investigators interpreted what appeared as a “basket weave” structure of the SC in routine histological studies as an indication that it is a passively occluding structure on the skin surface—a more or less uniform membrane functioning like “Saran wrap.” Investigators who followed them have dispelled the notion of the SC as a “basket weave” structure passively occluding the surface. A battery of investigative techniques (electron microscopy, laser confocal microscopy, two-photon microscopy, as well as biochemical

characterization of lipids and proteins) have led to the understanding first of a “brick and mortar” organization of SC, and a later concept of the SC as being a composite material with several characteristics of a “smart” material [24]. In this latest model, the bulk of the SC is made up of corneocytes, which are the “bricks.”

Before discussing the secretion of the lamellar lipids (pro-barrier lipids) and the further processing of the lipids in the SC extracellular domains (the “mortar”), a brief description of a multiplicity of the phenomena that occur in the SC is warranted.

These phenomena include: a description of the keratinocyte differentiation, synthesis of proteins (keratins, filaggrin, involucrin, loricrin, desmosomal components, enzymes involved in cornification) and lipids (which are packaged with lipid metabolizing enzymes and antimicrobial peptides within secretory organelles) in the form of lamellar bodies (Odland bodies, keratinosomes).

Keratinocytes (keratinizing epithelia) account for about 95% of the stratified epidermis (other cell types are melanocytes, Langerhans cells, Merkel cells), and based on its position within the strata as well as its progressive differentiation, is designated as belonging to several layers including: the basal layer (*stratum basale*), the spinous layer (*stratum spinosum*), the granular layer (*stratum granulosum*), and the cornified layer (*stratum corneum*). Epidermal stem cells that usually contribute to the maintenance of the interfollicular epidermis are located within the *stratum basale*. Their division (either symmetric or asymmetric) replenishes the keratinocyte population as cells from the basal layer move to the suprabasal layers and progressively undergo differentiation.

Stratum basale is made up of one layer of columnar basal cells attached to the basement membrane via hemidesmosomes. These cells display a high nucleocytoplasmic ratio, cell organelles like mitochondria and RER, as well as keratin filaments inserting into the hemidesmosomes. The cells also connect to the adjacent cells as well as those of the suprabasal cells via desmosomes. Biomarker proteins for the basal cells are keratins K14 and K5.

The *spinous* or prickly layer (*stratum spinosum*) above the *stratum basale* is named for its characteristic histological appearance due to the large numbers of desmosomes. In addition to the typical cell organelles, these cells often display a few lipid-enriched epidermal lamellar bodies (Odland bodies, keratinosomes, membrane coating granules). These will be described later in this section.

The biomarkers that distinguish this layer from the basal layer are keratins K1

and K10. *S.spinosum* also shows an increase in keratin filament content compared to *S.basale*. In the upper layers of *S.spinosum*, the cells begin to elongate and become slightly flattened. Above this layer is the *stratum granulosum*, a few layers of cells that are characterized by the presence of distinct, darkly staining (histological preparations) keratohyalin granules (KHK), composed of profilaggrin, loricrin, and a cystine-rich protein as well as keratins 1 and 10. The filaggrin subunits of profilaggrin play the role of matrix molecules to aggregate and align the keratin filaments.

In the upper granular layers of *stratum spinosum*, the keratin filaments are highly phosphorylated and have an extensive population of disulfide bonds compared to the cell layers below. As the cells of *stratum granulosum* stratify and differentiate, both the protein and lipid synthesis is ramped up, as evidenced from the boost in involucrin, KHKs, and LBs. In fact, the uppermost SG cells show an abundance of LBs (about 20% of the cell volume), which are apically polarized and poised to be secreted out. These details are not seen in histological preparations, but electron microscopy reveals both the internal structure of LBs, as well as tortuous and complex invaginations of the apical cell membrane at the *stratum granulosum*-*stratum corneum* interface. Secreted contents of LBs fill the interstices between the SG, and the overlying layer of *stratum corneum*, which still remain connected by means of desmosomes. The transition between the granular cell and the corneocyte presumably occurs within the span of a few hours, in response to several signals that involve but are not limited to an influx of calcium ions. The calcium signaling activates Ca⁺⁺ dependant transglutaminase, which catalyzes the crosslinking of involucrin and loricrin. This process then forms a toughened “cornified envelope” immediately subjacent to the cell membrane. The phenomenon is also accompanied by dissolution of the nucleus, as well as cytosolic organelles, mediated by caspases, especially caspase 14. In fortuitous sections, electron microscopy captures the images of the “transitional cell,” while the images primarily display an abrupt transition between the nucleated SG and the non-nucleated, flattened, organelle-depleted and keratin-filled corneocytes ([Figure 1](#)).

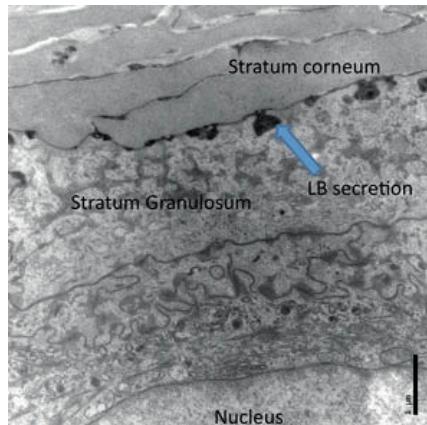


Figure 1 (Conventional TEM shows terminal differentiation of nucleated keratinocytes to keratin filled, non-nucleated corneocytes)

Conventional electron microscopy has illustrated the nature of the SC organization (Reference [Fig 1](#)), which consists of the secretion of epidermal lamellar bodies (enriched in cholesterol, sphingolipids, fatty acids, and a battery of enzymes and antimicrobial peptides) at the *stratum granulosum–stratum corneum* interface. Hydrophilic tracers (such as colloidal lanthanum) injected subcutaneously have been used to visualize its efflux (and hence of water) through the epidermis. This efflux is blocked at the SG–SC interface where the LB contents occlude the extracellular space [25].

The lamellar bodies are organelles, ranging from 0.2 to 0.5 microns in diameter, which originate from the Golgi apparatus, and begin to appear from the *stratum spinosum* and increase in numbers towards the upper *stratum granulosum*. In the uppermost SG, they occupy nearly 20% of the cell volume, and are polarized towards the apical surface. A recent proteomic investigation [26] identified up to 985 different proteins within the LBs. Freeze fracture studies [27] as well as ruthenium tetroxide post-fixation techniques [28, 29] have unraveled the sequence of events that follow the secretion of epidermal lamellar bodies ([Figure 2](#)) (i.e., the fusion of disk-like contents of the LBs into multiple lamellae that occlude the intercellular spaces of the SC). Cytochemical localization, as well as experimental studies with various enzyme inhibitors, delineated the role of enzymes (lipases, phospholipase, sphingomyelinase, B glucocerebrosidase, cholesterol sulfatase) in conversion of these disks into compact lamellar structures that surround the corneocytes akin to the mortar in a brick (corneocytes) wall [4, 30-35]. Investigations on animal models with specific gene defects or knockouts further extended the knowledge on enzymes such as B glucocerebrosidase [36], stearoyl CoA desaturase (SCD) [37], and

Elov 14, which are involved in elongation of long-chain fatty acids crucial for ceramides [38, 39] as critical to barrier competency. In addition, proteolytic enzymes like kallikreins that lead to progressive dissolution of the corneodesmosomes for orderly desquamation of corneocytes [40, 41] and antimicrobial peptides such as beta defensins and cathelicidins [42] are co-localized to the lamellar bodies and within the extracellular matrix of SC, linking the formation of permeability barrier with an antimicrobial barrier [43] and innate immunity.



Figure 2 (Arrow indicates transformation of secreted LB contents to tight lamellae in upper SC)

The epidermal lamellar bodies initially considered to be discrete organelles (0.2 to 0.5 micrometers in diameter) were later found to form an interconnected network within the cells of the *stratum granulosum* [44], enabling a well-coordinated secretion in response to acute barrier disruption, as in tape stripping. Conventional TEM of a lamellar body in perfect cross-section shows the disc structures well, but not all LBs show such crisp morphology, a fact thought to reflect the plane of sectioning (oblique sections or grazing sections along the outer surface). LBs deficient in internal contents (“motheaten appearance”) characterize a variety of skin dysfunctions [25], which correlate with permeability barrier defects and increased TEWL. Lamellar bodies are also crucial to the formation of “acid mantle” of the SC, which is of considerable interest to the cosmetic and personal care industry. These fascinating organelles consist of something resembling a cross between a lysosome and a secretory vesicle. They transport and secrete a diverse variety of molecules including: lipids, lipases, proteases, protease inhibitors, beta-glucocerebrosidase, antimicrobial peptides, etc.). An exhaustive proteomic and immunocytochemical analysis of lamellar-body enriched fractions and skin [26] identified about 985

proteins from the LB-enriched fraction: some secreted proteins, those with potential roles in desquamation, lysosomal proteins, related to transport and secretion of LBs, as well as proteins with putative roles in sorting of the LB cargo. It is possible that several subpopulations of LBs exist, and that the morphological diversity seen in electron micrographs may indeed reflect the pleomorphism of LBs, relating to either their contents or functional status. For example, hBD-2, which is virtually absent in normal skin keratinocytes, is induced when stimulated by cytokines or bacteria, and are localized to LB [45]. Most probably, some LBs synthesized in response to the bacterial challenge (LBS designed for microbial barrier?) must have a disproportionate content of the antimicrobial peptide (AMP), and it was speculated that pro-barrier lipids may not be the predominant components of this subpopulation [46]. They also may look different (deficient in lipids) or imperfect under the electron microscope. Could this be the basis of some of the abnormal, deficient LBs seen in various skin diseases such as psoriasis where keratinocytes show a high expression of AMPs? The study by Raymond *et al.* [26] showing 31 LB proteins corresponded to lysosomal components suggest that LBs are a new class of secretory lysosomes. Hence on a broad biological basis, they could be considered as “exolysosomes” involved in the matrix remodeling of the SC, or its exfoliation (the same way that lysosomes function in the molt of crustacean integument).

b. Stratum corneum

As the major barrier to flux of chemicals, water and other molecules into the skin, SC penetration pathways have attracted considerable attention. Since the SC is a two-compartment, composite system, three different routes of penetration have been proposed: transcellular, intercellular, and trans-appendageal (follicular) pathways (see for more details in the next section of this chapter). Much of the experimental results support the follicular and the intercellular pathway models. The intercellular pathway through the lipid domains is a tortuous path, and possibly both nonpolar and polar pathways are localized to this region. A true transcellular pathway (envisioned as a network of microor nano-channels) may only be opened up with the aid of microneedles or power-jet (gene gun) types of devices. The polar pathway may be located intercellularly and comprises aqueous regions surrounded by polar lipids, which create the walls of such microchannels as surmised by results of experiments designed to elucidate the contribution of extracellular lipids and intracellular

keratin to the structure of this pathway. This has been demonstrated by means of using baclofen, a model zwitterion, *in vitro* using human cadaver skin [47]. This contention is in conformity with the morphological characterization of a “pore pathway” through the mortar lipids, as proposed by Menon and Elias [48].

c. Other Barriers:

Other effective barriers in skin are antimicrobial barriers (AMP-mediated), immune barriers (innate and adaptive) mediated by Langerhans cells, oxidant barriers (keratinocyte and sebocyte derived antioxidants), and barrier to UV radiation (melanin-mediated). These diverse barriers are interdependent components of the skin barrier, as exemplified by facts that the permeability barrier recovery is faster/more efficient in darkly pigmented skin; the Langerhans cells are affected by a breach in the permeability barrier; and antimicrobial peptide synthesis is also up-regulated by challenges to permeability barrier.

As the tight junctions are also crucial for epidermal permeability barrier (the knockout models develop defective epidermal lipid synthesis and secretion), it is interesting to note that the Langerhans cells have been reported to extend their dendrites through the TJs, during the “search” mode when they scan the sub-SC domains for any sensitizers that have crossed the primary barrier. Such phenomena have only recently gained the attention of scientists and the appreciation of the cosmetic and personal care industry.

d. Penetration of Cosmetic Actives Through the Stratum Corneum

One of the most recurring debates in cosmetics has to do with the penetration of ingredients. How do manufacturers ensure optimal and targeted penetration of ingredients for efficacy at specific locations within the structure? What is the optimal penetration? How do water, lipophilic molecules, liposomes, or nanoparticles penetrate (if at all) through the *stratum corneum*, deep in the epidermis and below? From a scientific point of view, these questions are imperative towards the industry’s goals of achieving improvements in compromised skin or anti-aging effects. We point out that the (global) regulatory definitions and distinctions drawn between “drugs” and “cosmetics” are carefully excluded in this scientific discussion and merely note that if one is to actually impact the multiplicity of biological phenomena occurring in the skin, then, inevitably, the schism between the legal definitions of “drug” and “cosmetic” must merge into a single “zone,” and the difficulties in defining the

zone are indeed a challenge for all concerned.

In this section, we will take a somewhat different approach to exploring the question of penetration. We will focus on the *stratum corneum*, the outermost layer of human skin, which optimizes the delivery and efficacy of topical products in the skin. We will investigate now only how molecules are indeed able to penetrate this outermost layer, but also focus on the fact that even if they penetrate no deeper, they play an essential role in skin health and youthfulness.

As described above, the *stratum corneum* is the outermost layer of the skin. It consists of two distinct structural components: corneocytes and the intercellular lipid matrix. The corneocytes are differentiated keratinocytes (i.e., anucleated cells without intracellular organelles such as nuclei or mitochondria). They provide the structural support for the *stratum corneum* and act as a barrier against environmental aggression. Corneodesmosomes interconnect corneocytes as desmosomes interconnect keratinocytes in the epidermis. Essential corneodesmosomal proteins include desmocollin and desmoglein. The intercellular lipid matrix is located between corneocytes. The lipid species are composed of ceramides, fatty acids, cholesterol, and glucosylceramide derivatives. These lipids are synthesized by the keratinocytes in the upper layer of *stratum granulosum*. The covalent bonds between the ceramides of the intercellular lipid matrix and the structural proteins of corneocytes membrane create, through ester bonds, a dense and impermeable network [49].

Having identified what the *stratum corneum* is, let us now turn to the question at hand, namely: How do molecules of different sizes and forms, which have different physicochemical properties, penetrate into the *stratum corneum*?

Penetration

In general:

As discussed above, there are various ways for molecules (using passive diffusion) to penetrate the *stratum corneum*: intercellular, transcellular, intrafollicular, and polar. The intercellular pathway allows for a sinuous penetration of molecules between the corneocytes, through the intercellular lipids. The gaps between the intercellular lipids, which enable for penetration of molecules, measure 0.013 µm. This intercellular pathway is how most hydrophilic and lipophilic molecules penetrate through the *stratum corneum*.

The **transcellular pathway** involves corneodesmosomes creating bridges between cells and becoming sufficiently amphiphilic to enable transportation of molecules. The transcellular pathway is how amphiphilic molecules, as well as small other molecules, penetrate through the *stratum corneum*. These substances are able to integrate at the double layer of phospholipids constituting the cell membrane and can penetrate into the corneocytes. The speed of penetration by this pathway is very slow, but it is offset by the fact that the surface area is significant.

The **intrafollicular pathway** enables penetration through hair follicles (which account for 1 to 2% of the total skin surface). As the pilosebaceous follicles are located in deep invaginations of the epidermis, in the dermis, they lead molecules to the reticular dermis. Furthermore, hair follicles do not have a highly developed *stratum corneum*, which further facilitates penetration at this level. This intrafollicular pathway is how large molecules, which are typically highly insoluble, penetrate through the *stratum corneum*.

Finally, the **polar pathway** enables penetration of molecules through the polar pores. These pores are present between cells and are surrounded by polar lipids, which generate discontinuities in the organization of the lipids. This pathway is hydrophilic by nature. For example, sucrose penetrates into the epidermis this way.

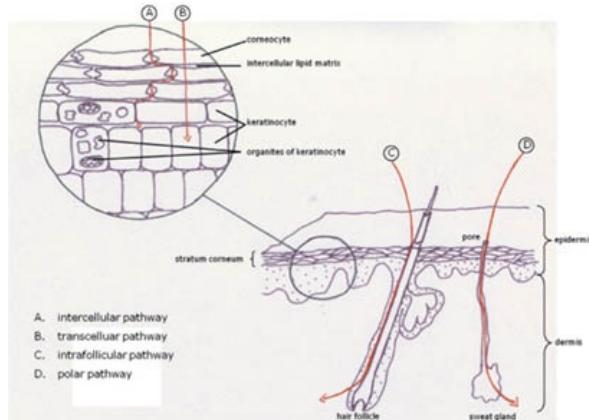


Figure 1: Various pathways of penetration through the *stratum corneum*. There are various pathways for molecules to penetrate the *stratum corneum*: intercellular (penetration between the corneocytes through the intercellular lipids), transcellular (penetration through the corneocytes), intrafollicular (penetration through hair follicles), and polar (penetration through the polar pores).

Penetration of a substance through the skin depends on many parameters that can interact with each other. These parameters are biological factors such as skin condition and age, the site of application, and skin hydration. Physicochemical factors, in particular those related to the substances themselves, also play a role, for example the size of the molecules or their attraction with the surface of the skin. These different pathways are passive diffusions through the *stratum corneum*. Only small molecules (molecular weight below 500 Da) penetrate through the pathways. It should be noted that the vehicle that carries the active ingredient can facilitate the penetration through the *stratum corneum*.

Penetration of water into the *stratum corneum*

The *stratum corneum* is virtually impermeable except for a small quantity of water that is delivered to it by the epidermis. This transferrable water hydrates the SC's external layers, helps maintain its flexibility, and enables adequate enzymatic reactions and corneodesmolysis. The passage of water in the other direction, from the body through the skin into the outside environment, is called trans-epidermal water loss or TEWL. Transepidermal water loss is influenced by the concentration of water in the epidermis, by cellular integrity, by relative humidity, by diffusivity of water in the *stratum corneum*, and by the thickness of the *stratum corneum*.

The ability to maintain water in the skin depends on the arrangement of corneocytes, the composition and structure of intercellular lipids, and the

presence of naturally moisturizing factors. Naturally moisturizing factors are made of water-soluble compounds and absorb water from atmosphere; they are typically amino acids or other chemical substances that hold water and rehydrate the *stratum corneum*. Naturally moisturizing factors are formed during the epidermis differentiation and represent 10% of corneocyte mass.

Penetration of lipophilic molecules into the *stratum corneum*

Lipophilic molecules of low molecular mass enter the *stratum corneum* essentially by the intercellular pathway (a sinuous penetration between the corneocytes through the intercellular lipids). These intercellular spaces are composed of lipids providing the last step of epidermic differentiation (such as free or esterified sterols, free fatty acids, triglycerides, sphingolipides), arranged in oriented bilayers bounding hydrophilic and lipophilic areas. The higher the concentration gradient of cutaneous lipids in lipid bilayers, the lower the penetration speed of hydrophilic molecules will be. Hence, by increasing the fluidity of cutaneous lipids, penetration can be facilitated.

Penetration of liposomes into the *stratum corneum*

Liposomes are lipid vesicles that can be used as carriers for hydrophilic substances solubilized into aqueous domains, or lipophilic substances solubilized into lipidic membranes. These colloidal carriers are formed as concentric bimolecular layers of phospholipids. The presence of cholesterol may improve the bilayer characteristics of liposomes by increasing the microviscosity/interfacial rheological behavior of the bilayer, thereby reducing the permeability of the membrane to water-soluble molecules, or stabilizing the membrane and increasing the rigidity of the vesicle.

Liposomes represent an alternative method to enhance the permeation of substances through the skin. There are five possible mechanisms for liposomes to penetrate into the *stratum corneum* [50]:

1. Liposomes release the active substances that then penetrate independently into the skin;
2. Liposomes are first absorbed and then fused with the skin's surface and constituents, then change the ultrastructure of the intercellular regions in the deeper layer of the *stratum corneum*. This decreases the impermeability of the *stratum corneum* and enhances the penetration of the encapsulated molecule [51];
3. Liposomes gravitate to the surface of the *stratum corneum*, resulting in a direct transfer of the molecule encapsulated from the liposome to the skin.

Vesicles could also fuse and mix with the lipids matrix of the *stratum corneum*, increasing the distribution of molecule into the skin [52];

4. Liposomes penetrate into the skin as an intact form. Although it is now generally accepted that conventional liposomes do not penetrate directly into the skin, this penetration mechanism has been described by Cevc *et al.* for deformable liposomes, also known as transfersomes [53].

In view of the presence of a surfactant in their bilayer, these vesicles exhibit viscoelastic properties that allow them to deform and pass between the cells of the *stratum corneum*, thus arriving intact into the epidermis. This mechanism is similar to the deformation of red blood cells as they change shape in order to pass through narrow blood vessels whose diameters are smaller than the undeformed blood cells. This penetration is made possible by the presence of a natural trans-epidermal water gradient, which is created by the hydrophilicity of phospholipids. Furthermore, these liposomes avoid dry environments and will follow the gradient of moisture, moving into the deeper layers and more hydrated skin.

5. Liposomes penetrate into the skin via cutaneous annexes such as sweat glands and hair follicles. An excellent review of these studies is provided in a review by Knorr *et al* [54].

The work with liposomes suggested that carrier/vesicle size may have an influence on skin penetration as well as deposition into hair follicles. The nanosizing approach has the potential to offer increased skin deposition of compounds and also the more efficient delivery of agents to hair follicles. Recent work by the group of Michniak-Kohn *et al.* suggests that some nanoparticulate carriers are able to deliver various lipophilic compounds to skin fairly efficiently while making sure that no compounds traverse the skin layers (no *transdermal* penetration occurs). Additional benefits of these carriers included high solubilization (in one case 5000-fold) and improved stability of the encapsulated compounds. There are many other such examples in the literature, and nanosized particles have been used for a long time in sunscreen formulations and facial foundations.

Penetration of nanoparticles into the *stratum corneum*

The penetration of nanoparticles occurs through two possible pathways: intercellular and intrafollicular pathways. While the transfollicular pathway plays a central role in the study of the penetration of nanoparticles [55], it is not yet clear if nanoparticles penetrate into the hair follicles, or if they are stored in the outer layer of these hair follicles.

Intelligent Targeting Carriers

Intelligent Targeting Carriers have the ability to be recognized by fibroblasts, melanocytes, or adipocytes, thus delivering anti-aging, whitening, or slimming active ingredients, directly to the cells where they have to act.

Capsules are formed by a matrix of poly (lactic-co-glycolic acid) PLGA with an outer polyvinyl alcohol (PVA) shell with the addition of N-pantethenic-peptide [56]. A proper ligand is covalently bonded to the surface of the capsule. These ligands (mostly peptides) are recognized by receptors in the surface of the target cells. The cells are thus able to uptake the particle by endocytosis, while the particles are able to escape from the endosomal compartment, then degraded and therefore able to deliver the active to the cytoplasm.

Cruz *et al.* suggested that the PLGA capsules used as intelligent targeting devices increase the activity of the encapsulated active ingredients by concentrating them solely in the place where they can be biologically active. This is thus of interest to personal care products (anti-aging, whitening, tanning, slimming) [57].

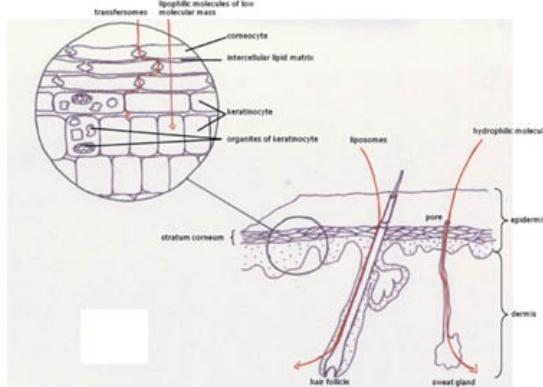


Figure 2: Examples of penetration pathways by various molecules. The lipophilic molecules of low molecular mass penetrate through the *stratum corneum* by the intracellular pathway while the hydrophilic molecules prefer the polar pathway. The liposomes may penetrate via the transfollicular pathway and the transfersomes (i.e., deformable liposomes), pass between cells, and prioritize the intercellular pathway.

e. Penetration Versus Protection

It is not clear yet whether there truly is real *in vivo* penetration of the ingredients incorporated in cosmetic and personal care formulations. While many will propose that this means cosmetic formulations lack clear efficacy, we propose rather that the lack of penetration of a product's active ingredients need not be a

negative characteristic. Indeed, these ingredients still possess the ability to protect the *stratum corneum* against environmental stressors and damage. This concept is significant since the *stratum corneum* constitutes only 10% of the entire skin, yet it contributes to over 80% of the cutaneous barrier function. For example, the use of antioxidant molecules in cosmetic products can help the *stratum corneum* regenerate and protect itself and thus also regenerating and protecting the underlying epidermis and dermis from the harmful effects of UV and other environmental toxins.

In summary, while the individual penetration of water, lipophilic molecules, liposomes, and nanoparticles through the *stratum corneum* is becoming better understood when viewed through their intercellular, transcellular, intrafollicular, and polar pathways, the penetration mechanisms of cosmetic formulations remain poorly understood and are an area in need of further exploration.

In this review, we presented not only the known and various pathways of penetration through the *stratum corneum*, but also the fact that even if cosmetic formulations penetration is no deeper than the SC, their efficacy is demonstrable. Indeed, the *stratum corneum* represents the barrier function of skin, and thus needs to be protected from environmental toxins. By regenerating and protecting the *stratum corneum*, active cosmetic ingredients protect the epidermis and dermis.

PART 3.1.11 IMMUNOLOGICAL FUNCTION

The skin is the largest organ of the human body and possesses the unique function of protecting the body from allergens, chemicals, and microbial infection [58]. It is an immunoprotective organ that comprises of specialized cells called Langerhans cells in the epidermis. The dermal collagen network comprises of dendritic cells, T-cells and T-cell subtypes, B cells and mast cells. Langerhans cells are activated in response to allergen-mediated contact sensitization that elicits an immune response [59, 60]. Resident dermal dendritic cells in addition are induced in this phenomenon. As a result, the Langerhans cells migrate into the lymph nodes in response to chemokines such as CCL19. The migrated cells activate T-cells that undergo differentiation and proliferation, which results in the selective expansion of clonal populations of allergen-specific T-cells. The activation of Th1 and/or Th2 clonal T-cell populations ultimately results in allergen-specific sensitivity [61].

After first phase of infection with a pathogen, dendritic cells resident in the

dermis present antigens to naïve T-cells in the skin-draining lymph node, initiating an adaptive immune response that generates central memory T-cells and effector memory T-cells. At second round of infection, dendritic cells present antigen to central memory T-cells in skin-draining lymph nodes, giving rise to another population of effector memory T-cells that migrate into skin and attempt to destroy the infection.

There is a marked decrease in number of Langerhans cells with age. This affects the ability of TNF-alpha to induce Langerhans cell migration in response to an infection. There is an overall reduced cutaneous immune function with aging, thus rendering aged skin more prone to infections. There is a reduction in dendrite formation, reduction in Birbeck granules, and reduced antigen trapping capacity. Thus, skin immune function is associated with and decreases with aging.

UV radiation results in changes in the immune response. It suppresses the immune response and results in skin cancer development [62]. In harsh circumstances, it also destroys the Langerhans cells on skin.

Psoriasis is a common skin condition characterized by immune and genetic factors. The symptoms of the disease include skin lesions, dryness, redness, and irritation associated with inflammation. The process is an inflammatory skin disorder that results in dermal infiltration of T-cells, dendritic cells, macrophages, and neutrophils [63]. Anti-TNF alpha as well as anti-IL-12 and anti-IL-23 therapy and methods to exfoliate the skin lesions including reduction of inflammation are suggested interventions to treat psoriasis. A systems biology approach to characterize the transcriptome of the diseased state versus normal skin can be indicative of suggested therapeutic options to combat the disease.

PART 3.1.12 CYTOKINES

Cytokines are small proteins or glycoproteins produced by epidermal keratinocytes, Langerhans cells, monocytes/macrophages, fibroblasts, and lymphocytes in the skin. The cytokines bind to receptors on target cells and initiate an active immune response. The cytokines produced by keratinocytes have been most extensively studied. Keratinocytes produce interleukins (IL-1, IL-3, IL-6, IL-7, IL-9, IL-10), interferons (IFN- α , IFN- β), cytotoxins (TNF- α , TNF- β), colony-stimulating factors (G-CSF, MCSF, MG-CSF), and growth factors (bFGF, EGF, KGF, MGSA, NGF, TGF- α , TGF- β , PDGF) [64]. There is a relatively low basal level of cytokine production in normal healthy skin;

however, in the presence of a challenge (e.g., viral, bacterial, fungal), cytokine production increases. This is regulated by membrane proteins, known as toll-like receptors, that bind certain molecular components of pathogens [65]. Different toll-like receptors recognize different molecular components of the pathogen. The ligands include bacterial DNA, lipopolysaccharide, flagellin, β -glucan, etc. Cytokine production is altered in a variety of skin diseases.

PART 3.1.13 ENZYMES

Enzymes within the skin layers play an important role in maintaining the biochemistry of the skin, supporting the synthesis of its components and hence cell replication. This results in skin lipid production and the formation of the *stratum corneum* barrier properties that play such a crucial role in the penetration of cosmetic actives into the skin layers. In addition, the presence of enzymes influences how cosmetic actives and formulation excipients are metabolized. Also, enzymes function as skin protectors by scavenging free radicals and maintaining a pH gradient that influences the number and type of bacterial flora on the skin surface. They are one of the most essential components of the skin.

Enzymes in the dermis are responsible for continuous remodeling of the extracellular matrix [66]. In the basal layer of the epidermis, the metabolic activity of most cells is low. The exceptions are the few basal cells undergoing replication, where enzyme activity is high. As cells move up from the basal layer and enter into differentiation, there is a general increase in the enzymatic activities necessary to synthesize the components of desmosomes, keratohyalin granules, keratin proteins, and lipids [67]. In the spinous layer most energy is produced by mitochondrial activity, but as cells reach the upper granular layer, the mitochondria are degraded and energy production is dependent on anaerobic glycolysis with lactic acid as a by-product. Most of the protein synthesized during differentiation is keratin, which makes up the keratinocyte cytoskeleton [68]. Most of the lipid that accumulates with differentiation is packaged into lamellar granules [69, 70]. In the late stages of differentiation, the lamellar granules migrate to the apical end of the cell, and the bounding membrane of the lamellar granule fuses into the cell plasma membrane [71, 72]. The lipids and a battery of hydrolytic enzymes are extruded into the intercellular space. At about this time, some proteins from the keratohyalin granules are deposited on the inner surface of the plasma membrane [73]. A high-molecular-weight, histidine-rich and highly phosphorylated protein, profilaggrin, is dephosphorylated and

proteolytically clipped to small filaggrin units [74]. On passage into the *stratum corneum*, the filaggrin units induce aggregation of the keratin filaments into bundles of keratin that lie parallel to the plane of the skin. The filaggrin proteins are subsequently acted upon by proteases until they are degraded to their component amino acids [75]. This results in a very high osmotic strength in the corneocytes. At about the time of the transition from the granular layer to the *stratum corneum*, a calcium-dependant transglutaminase crosslinks the proteins on the inner surface of the plasma membrane through formation of isopeptide linkages [73]. There is further crosslinking of these proteins through the spontaneous formation of disulfide linkages. ω -Hydroxyceramides derived from the bounding membrane of the lamellar granules becomes ester-linked to the outer surface of this crosslinked protein layer [76]. Thus the original plasma membrane of the keratinocyte is replaced by a compound envelope consisting of a thick crosslinked band of protein with a covalently bound lipid layer on the outer surface.

The main source of carbon for epidermal lipid biosynthesis appears to be acetate from the circulation [77]. The rate-limiting enzyme for synthesis of fatty acids is acetyl-CoA carboxylase [78]. This is a biotin-dependent enzyme that converts acetyl-CoA to malonyl-CoA at the expense of one ATP. The fatty acids are incorporated into phosphoglycerides, sphingomyelin, and glucosylceramides in the viable portion of the epidermis. The rate-limiting enzyme in synthesis of sphingolipids is serine palmitoyl transferase [79], and the rate-limiting enzyme in cholesterol biosynthesis is β -hydroxymethylglutaryl-CoA reductase [80]. HMG-CoA reductase uses two molecules of NADPH to reduce HMG to mevalonate-CoA.

The lipids found in isolated lamellar granules include phosphoglycerides, sphingomyelin, glucosylceramides, and cholesterol [69]. One unusual glucosylceramide contains 30-through 34-carbon long ω -hydroxyacids amide-linked to a mixture of sphingosines and dihydrosphingosines with linoleate ester-linked to the ω -hydroxyl group and glucose β -glycosidically linked to the primary hydroxyl group of the base [81]. This unusual lipid is thought to be a major component of the bounding membrane of the lamellar granule, and is the precursor of the aforementioned covalently bound lipid of the envelope. Enzymes associated with the lamellar granules include ceramide glucosyl transferase indicating that lamellar granules arise from the Golgi apparatus, since this enzyme is considered a biochemical marker for the Golgi apparatus [82]. Hydrolytic enzymes associated with the lamellar granules include β -glucosidase,

acid phosphatase, amino-glycosidases, galactosidase, aryl sulfatase, phospholipase A, sphingomyelinase, and several proteases [69, 70, 83]. The combined action of some of these enzymes converts the cholesterol-phospholipid-glucosylceramide mixture extruded into the intercellular space into the cholesterol-ceramide-fatty acid mixture of the *stratum corneum*.

A minor lipid in the *stratum corneum* is cholesterol sulfate. This lipid has been shown to be a serine protease inhibitor [84]. It is thought that this protease inhibitor protects the corneodesmosomes, until in the outer layers of the *stratum corneum*, sterol sulfatase degrades the cholesterol sulfate, and this permits the serine proteases to break down the corneodesmosomes leading to desquamation.

3.1.14 ULTRAVIOLET RADIATION-INDUCED PHOTODAMAGE AND SKIN CANCER

Photocarcinogenesis is a cosmetic concern because the UV light-absorbing agents used in cosmetic (over-the-counter) sunscreens help to lower the incidence of skin cancer.

Exposure to UV radiation causes not only photodamage with the appearance of premature aging, but also precancers and cancers of the skin. Actinic keratoses are premalignant lesions in which the epidermis proliferates, producing hyperkeratotic lesions characteristic of photodamaged skin, especially in individuals with skin types I, II, and III. These lesions have a high percentage of DNA mutations, particularly at sites p53 and p16 which are characteristic of nonmelanoma skin cancers (NMSC). Although actinic keratoses occasionally spontaneously disappear, they should be medically treated to prevent their becoming squamous cell carcinomas.

Skin cancer has been classified as nonmelanoma skin cancer, and as cutaneous malignant melanoma. NMSC has the highest incidence of any cancer, approximately one million new cases each year in the United States. One in five Americans will get a skin cancer in the course of a lifetime. Mortality from NMSC is low, but morbidity can be considerable with possible disfigurement, especially on the face and neck.

Basal cell carcinoma (BCC) is the most common NMSC (equal to about 75% of the incidence of all skin cancers). Although BCCs do not metastasize, they are locally invasive and disfiguring if not treated early. Squamous cell carcinomas (SCC) account for about 20% of all skin cancers; approximately 2% of SCCs (particularly those originating on the head and neck) metastasize with possible

mortality. Melanomas make up about 5% of all skin cancers. If excised before they become invasive, melanomas can be definitively treated; however, if allowed to invade the dermis, melanomas metastasize with high mortality.

Sun exposure (and the use of tanning beds) is linked to the skin cancer epidemic as the incidence of NMSC increases in proportion to the cumulative sunlight exposure and is highest in people with skin types I and II. In the case of melanoma, the effects of UV are not clear except in lentigo maligna melanoma (a subclass of cutaneous melanoma), for which sun exposure has been shown to increase the incidence.

UV radiation causes skin cancer by inducing mutations in DNA which lead to damaging the ability of the skin cells to control proliferation. UVB harms mainly the epidermis and UVA, the dermis. The primary damage by UVB is *via* direct absorption by DNA to generate mutations through the formation of dimers between adjacent pyrimidine residues, leading to specific UVB “signature” mutations that can accumulate over time. UVB can also indirectly induce oxidative damage by the formation of reactive oxygen species (ROS) that indirectly damage DNA. If not repaired, both the direct and indirect DNA mutations persist through subsequent subdivisions causing precancers (actinic keratoses) and skin cancers. Reactive protective mechanisms by the epidermis cause thickening of the outer layers of the skin to create a UV-protective “outer shell” that gives an unattractive leathery texture and increased pigmentation that results in mottled discoloration, both of which are characteristic of the appearance of photoaging. UVA penetrates deeper to harm the dermis. UVA damages DNA indirectly through absorption by chromophones that generate ROS which mutate DNA *via* mutagenic oxidative intermediates. UVA also activates metaloproteinases which directly break down collagen and elastic tissue and act on other transcription factors to inhibit repair, thereby causing the sagging and wrinkles of photoaged skin.

Furthermore, recent research shows that UVA in combination with common environmental pollutants is synergistic in generating oxidative damage, thereby significantly increasing visible photodamage and the risk of skin cancer [84.5]. Polycyclic aromatic hydrocarbons (PAHs) such as benzo[a]pyrene (BaP) are ubiquitous in the air, food, and water as a result of incomplete combustion of fossil fuels (from power plants, car exhaust, heaters) and from cigarette smoke (the most prevalent indoor pollutant). These chemical pollutants exist at levels below damaging or tumorigenic levels. However, when combined with UVA exposure, there is a synergistic increase in oxidative damage with the

consequence of even more severe photoaging and increased incidence of skin cancer.

Thus UV radiation is a complete carcinogen; it can act alone as an initiator and a promoter in formation of cancer. UV can also act as a promoter with initiating events inside the cell, such as DNA mutations arising from DNA polymerase incorporation errors, depurination, deamination of 5-methylcytosine, or oxidative damage from free radicals. The cell can respond to the damage by repairing the DNA to avoid the effects of the mutations or, if the damage is too great, by inducing cell death through apoptosis to remove potential cancer cells from the population. Immunosurveillance aids recognition of potentially damaging mutated cells so that apoptotic mechanisms can be initiated. UVA decreases this cellular immune recognition and response.

PART 3.1.15 GENE EXPRESSION IN SKIN CARE

The Human Genome Project (HGP) was a 13-year effort that was coordinated by the U.S. Department of Energy and the National Institutes of Health. The project, which was completed in 2003, produced sequence information for the entire 3 billion base pairs in the human genome. The work cost more than one U.S. dollar per base pair, but generated over 265 times that in economic impact and job creation [85]. More importantly, the HGP gave rise to new, high-throughput technologies used to analyze genes and ultimately, a new field of science—*genomics*. From genomics, other high-throughput technologies were developed, giving rise to a suite of “omics” disciplines (e.g., proteomics and metabolomics). In combination, “omics” data provide a comprehensive view of how cells and biological systems work together in health and in disease. This information has enabled advances in dermatology and in the cosmetic and personal care industry [86]. In this section, the application of genomics-based methods to cosmetics and personal care is reviewed.

a. Genomics Technologies

Researchers have been studying the function of genes and proteins in the skin for decades using technologies such as Northern and Western blotting, and traditional polymerase chain reaction (PCR). These methods are labor intensive and only allow for analysis of a few genes or proteins at a time [87-92].

More recent technologies, including microarrays and quantitative real time PCR (qPCR), have enabled simultaneous analysis of thousands of genes and

more accurate quantification. Microarrays are now the “go-to” method for screening cells and tissues to identify novel biomarkers and understanding mechanisms of action. Both microarrays and qPCR technologies are used to detect changes in the sequence of DNA (i.e., mutations), as well as gene expression (an indicator of which genes are “turned on or off”) in a given cell type. When a gene is “turned on,” or activated, it is transcribed from DNA into a complementary strand of RNA (ribonucleic acid)—the RNA strand serves as a template for protein synthesis, directing the ribosome as to which amino acids to put together to form specific proteins. On the other hand, when a gene is “turned off,” the cell stops transcribing the DNA strand and protein synthesis is terminated. Gene expression analysis is used to determine how cells and tissues are affected by specific conditions. The methods provide information at the molecular level as to how specific cellular functions are altered from normal homeostasis. Gene expression profiles can be used as a guide for creating products or targeted treatments that will convert an aberrant profile back to homeostasis.

b. Genomics and the Skin

To date, thousands of peer-reviewed research studies have been published using high-throughput genomics methods to elucidate molecular mechanisms associated with skin biology [93, 94]. Studies have been conducted across multiple disciplines, from embryonic development to aging, as well as all areas of skin ailments [95-97]. Many studies have identified unique gene expression profiles in skin disorders such as atopic dermatitis, acne, rosacea, and psoriasis [98-102]. A large number of whole-genome sequencing studies have been conducted to elucidate genetic mutations and predisposition related to rare skin conditions [103, 104].

Molecular studies of the epidermal skin barrier provide a good example of how genomics has improved our understanding of skin biology [105, 106]. The skin barrier is our body’s first line of defense against environmental insults such as chemicals, radiation, pathogens, and other stimuli. The formation and maintenance of a functionally intact epidermal barrier involves the coordination of complex regulatory networks that involve the expression and interaction of hundreds of molecules. Some of these major processes include keratinocyte differentiation, apoptosis and maturation into corneocytes, cell migration and desquamation [107]. Gene expression studies have been used to identify the expression of key molecules involved in each one of these processes [108, 109].

In other studies, microarrays have been used to identify large sets of genes [110]. For example, Taylor *et al.* [111] used microarrays to identify genes expressed by differentiating keratinocytes at different time intervals. The study identified 1282 genes differentially expressed; specific genes include those that code for structural molecules and innate immune molecules. Other studies have identified microRNAs that regulate genes, such as p63, in proliferating and differentiating keratinocytes [112, 113]. Again, a better understanding of how specific molecules and pathways interact will facilitate the development of high-performing topical products for cosmetics and for treating specific skin conditions.

Additional studies have identified genetic mutations associated with compromised barrier function [114, 115]. Identifying mutations in genes, such as filaggrin, may aid in creating future diagnostic tools and advanced therapies. Filaggrin is a filament-associated protein that binds keratin fibers in skin epithelial cells. In the *stratum corneum*, filaggrin monomers are incorporated into the lipid envelope that forms the skin barrier. Filaggrin undergoes further processing in the upper *stratum corneum* to release free [amino acids](#) that assist in barrier formation and water retention. These proteins can also interact with keratin intermediate filaments. Other research has shown that CYP26b1 is expressed in keratinocytes in the epidermis. CYP26b1 is a member of the cytochrome enzyme superfamily, a group of enzymes that are essential for performing metabolic functions in the cell. CYP26b1 is essential for metabolism of retinoic acid [116]. Okana *et al.* [115] has recently demonstrated that genetic ablation of CYP26b1 disrupts epidermal barrier formation by inhibiting retinoic acid metabolism.

c. Gene Expression Analysis: A Breakthrough for Cosmetic Science

Gene expression technologies in cosmetics and personal care are now routinely used in product development pipelines. Drawing on information produced in basic research studies that identify molecular biomarkers associated with phenotypic endpoints, cosmetics and personal care companies are performing studies to define the skin's response to topical materials and formulations [117-120]. Skin care scientists are currently utilizing gene expression methods to screen raw materials for biological effects, identify dose-response relationships, and understand how combinations of ingredients may work together—this may be additive, synergistic, or hormetic.

To date, gene expression studies have been used to:

- Demonstrate efficacy and understand mechanisms of action
- Streamline product formulations during development
- Create performance products that ultimately improve marketability, consumer confidence and brand loyalty

While most work has been conducted to improve product formulations and validate efficacy, genomics-based screening methods may also provide new and improved tools for assessing safety and toxicity [121]. This will require carefully planned studies to develop and validate improved tissue models that contain immune cells as well as sophisticated informatics and algorithms to easily analyze and interpret the datasets.

d. Challenges

Understanding how specific patterns of gene expression translate into a biological response is one of the greatest challenges in interpreting high throughput genomics data. For now, genomics data are best used as a roadmap to generate hypotheses and guide follow-on studies that utilize additional cell biology methods better suited for interrogating functional relationships.

Another challenge in conducting genomics-based studies for cosmetic science is the lack of fully representative experimental tissue models. Studies conducted for the cosmetics industry are typically carried out using *in vitro* cell cultures or artificial skin. While these models provide useful information regarding the keratinocyte and fibroblast response, they lack melanocytes, Langerhans cells, and an epidermal barrier consistent with *in vivo* human skin. *In vitro* models are an ideal tool for conducting early screening studies that should be followed up with *in vivo* clinical work.

e. Looking Ahead

Since the inception of genomics, newer, state-of-the-art technologies such as NextGen sequencing have been developed. These methods can rapidly sequence the entire genome in several days, perform RNA sequencing, and analyze epigenetic mechanisms (i.e., DNA methylation). While epigenetics is now a popular buzzword in cosmetic science, a greater understanding of these mechanisms in skin biology will help elucidate phenomena such as aging, photoaging, and skin disease. Groundbreaking epigenetic research in skin biology is beginning to emerge and will soon be translated into product development.

Another emerging concept is the promise of “personalized skin care” [122].

This approach uses an individual's genetic footprint to develop skin care products to address the specific needs of an individual. Companies are beginning to explore this opportunity, and there will most likely be an increased amount of data and ultimately, product development using the "personalized medicine" approach in the next few years.

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GLOSSARY

1. Epidermis - composed of the outermost layers of cells in the skin—"epi" in

Greek meaning “over” or “upon,” which together with the dermis forms the entire skin layer termed the cutis.

2. Dermis - a layer of skin between the epidermis and subcutaneous tissues that consists of connective tissue and cushions the body from mechanical stresses. It is divided into two layers, the area adjacent to the epidermis called the papillary region and a deeper and thicker area known as the reticular dermis.
3. *Stratum corneum* (Latin for “horned” or “horny” layer) - the outermost layer of the skin, consisting of dead cells (corneocytes) that lack nuclei and organelles. This layer is responsible for the barrier function of skin.
4. Glycoaminoglycan - any of a group of high-molecular-weight linear polysaccharides with various disaccharide repeating units and usually occurring in proteoglycans, including the chondroitin sulfates, dermatan sulfates, and hyaluronic acid. Abbreviated as GAG.
5. Collagen - protein made up of amino acids, which are in turn built of carbon, oxygen, and hydrogen. Collagen contains specific amino acids—glycine, proline, hydroxyproline, and arginine. There are many types of collagen that have been identified. Collagen is synthesized most commonly by fibroblasts.
6. Keratin - a family of fibrous structural proteins. Keratin is the key structural material making up the outer layer of human skin. It is also the key structural component of hair and nails.
7. Keratinocyte - the main cell type in the epidermis, the outermost layer of the skin. Those keratinocytes found in the basal layer (*stratum germinativum*) of the skin are sometimes referred to as “basal cells” or “basal keratinocytes.”
8. Corneocyte - a terminally differentiated keratinocyte that forms part of the stratum corneum, the outermost part of the skin. The cell is regularly replaced through desquamation and renewal from the lower epidermal layers, making it an essential part of the barrier property of the skin.
9. Fibroblast - a type of cell that synthesizes extracellular matrix and collagen, the structural framework for the skin, and plays a critical role in wound healing. Fibroblasts are the most common cells of connective tissue and in particular the dermal layer of the skin.
10. Cytokine - (Greek “cyto,” cell; and “kinos,” movement) are any of a variety of compounds secreted by specific cells of the immune system. They carry signals locally between cells and thus have an effect on other cells. They are a category of signaling molecules that are used extensively in cellular communication. They can be proteins, peptides, or glycoproteins.

11. Merkel cells - clear cells found in the *stratum basale* (at the bottom of sweat duct ridges) of the epidermis, approximately 10 µm in diameter. They also occur in epidermal invaginations of the plantar foot surface called rete ridges. Most often they are associated with sensory nerve endings, when they are known as Merkel nerve endings.
12. Langerhans cells - dendritic cells (antigen-presenting immune cells) of the skin, containing large granules called Birbeck granules. They are present in all layers of the epidermis, but are most prominent in the *stratum spinosum*. They also occur in the papillary dermis, particularly around blood vessels.
13. Dendritic cell - immune cell forming part of the immune system. Main function is to process antigen material and present it on the surface to other cells of the immune system. Dendritic cells are present in tissues in contact with the external environment, such as the skin (where there is a specialized dendritic cell type called Langerhans cells).
14. Birbeck granule (also known as Birbeck bodies) - rod-shaped cytoplasmic organelles with a central linear density and a striated appearance.
15. Filagrin - a filament-associated protein that binds to keratin fibers in epithelial cells.
16. Involucrin - a protein component of human skin. In humans it is encoded by the *IVL* gene. In binding the protein termed loricrin, involucrin is involved in the formation of the cell envelope that protects corneocytes in the skin.
17. Melanin (from the Greek “melas” meaning “black”) - a natural pigment in skin derived from the amino acid tyrosine.
18. Melosome - an organelle containing melanin, the most common light-absorbing pigment found in the mammalian kingdom.
19. Pheomelanin - yellow to red-brown pigment produced by melanocytes.
20. Eumelanin - black to brown pigment produced by melanin.
21. Golgi apparatus (also known as the Golgi complex, Golgi body, or simply the Golgi) - an organelle found in most eukaryotic cells. Part of the cellular endomembrane system, the Golgi apparatus packages proteins inside the cell before they are sent to their destination; it is particularly important in the processing of proteins for secretion.
22. Macrophage - any of the large, mononuclear, highly phagocytic cells derived from monocytes that occur in the walls of blood vessels and in loose connective tissue. Macrophages are usually immobile but become actively mobile when stimulated by inflammation; they also interact with lymphocytes

to facilitate antibody production.

23. Elastin - a key extracellular matrix protein that is critical to the elasticity and resilience of many tissues including skin.
24. Reticulin - name given to the chemical substance of reticular fibers, which were formerly thought to be distinct from collagen by reason of their distinctive structure and staining properties but are now regarded as type III collagen.
25. Apocrine gland - a sweat gland composed of a coiled secretory portion located at the junction of the dermis and subcutaneous fat, from which a portion inserts and secretes into the infundibular portion of the hair follicle.
26. Eccrine gland - a sweat gland that is not connected to hair follicles. It functions by responding to elevated body temperature due to heat or physical exercise.
27. Sebaceous gland - small oil-producing gland usually attached to hair follicles and releasing a fatty substance, sebum, into the follicular duct and thence to the surface of the skin.
28. Sebum - consists of a mixture of fats (triglycerides, wax esters, squalene, and cholesterol) and cellular debris, which is discharged through the sebaceous duct.
29. Kallikreins - these belong to a class of serine proteases with a high degree of substrate specificity. The enzymes are structurally related to trypsin. Kallikrein (kininogen, kininogenase = EC3.4.21.8) cleaves proteins between arginine and lysine residues.
30. Keratinosomes (otherwise known as membrane-coating granules [MCGs], lamellar bodies, lamellar granules or Odland bodies) - secretory organelles found in keratinocytes. Keratinosomes fuse with the cell membrane and release their contents into the extracellular space.
31. Androgen - a male sex hormone such as testosterone.
32. Ichthyosis - a family of genetic skin disorders characterized by dry, scaling skin that may be thickened or very thin.
33. Atopic dermatitis (eczema) - an itchy inflammation of the skin. It is usually a chronic condition that may be accompanied by other conditions such as asthma.
34. Psoriasis - a chronic skin disease that affects the life cycle of skin cells. It causes cells to build up rapidly on the surface of the skin, forming thick silvery scales and itchy, dry, red patches that are sometimes painful.

35. Transepidermal Water Loss = TEWL - This is defined as a measurement of the water amount that diffuses or evaporates from inside the body through the epidermis to the surrounding atmosphere. TEWL can increase due to disruption of the skin barrier through wounds, scratches, burns, exposure to solvents or surfactants, extreme dryness) and is affected by humidity, temperature, season, and moisture content of the skin.
36. Liposomes - nano/micro-size spherical drug carriers that can be produced from natural or synthetic phospholipids and cholesterol. They form spontaneously when phospholipids are combined with water and bilayered spheres form, since the phospholipid molecules orient themselves according to their polarity (one end of each molecule is water soluble, while the opposite end is water insoluble).
37. Rheology - a science dealing with the deformation and flow of matter and including the measurement of viscosity.
38. Colloids - a solution that has particles ranging between 1 and 1000 nanometers in diameter and evenly distributed throughout the solution.

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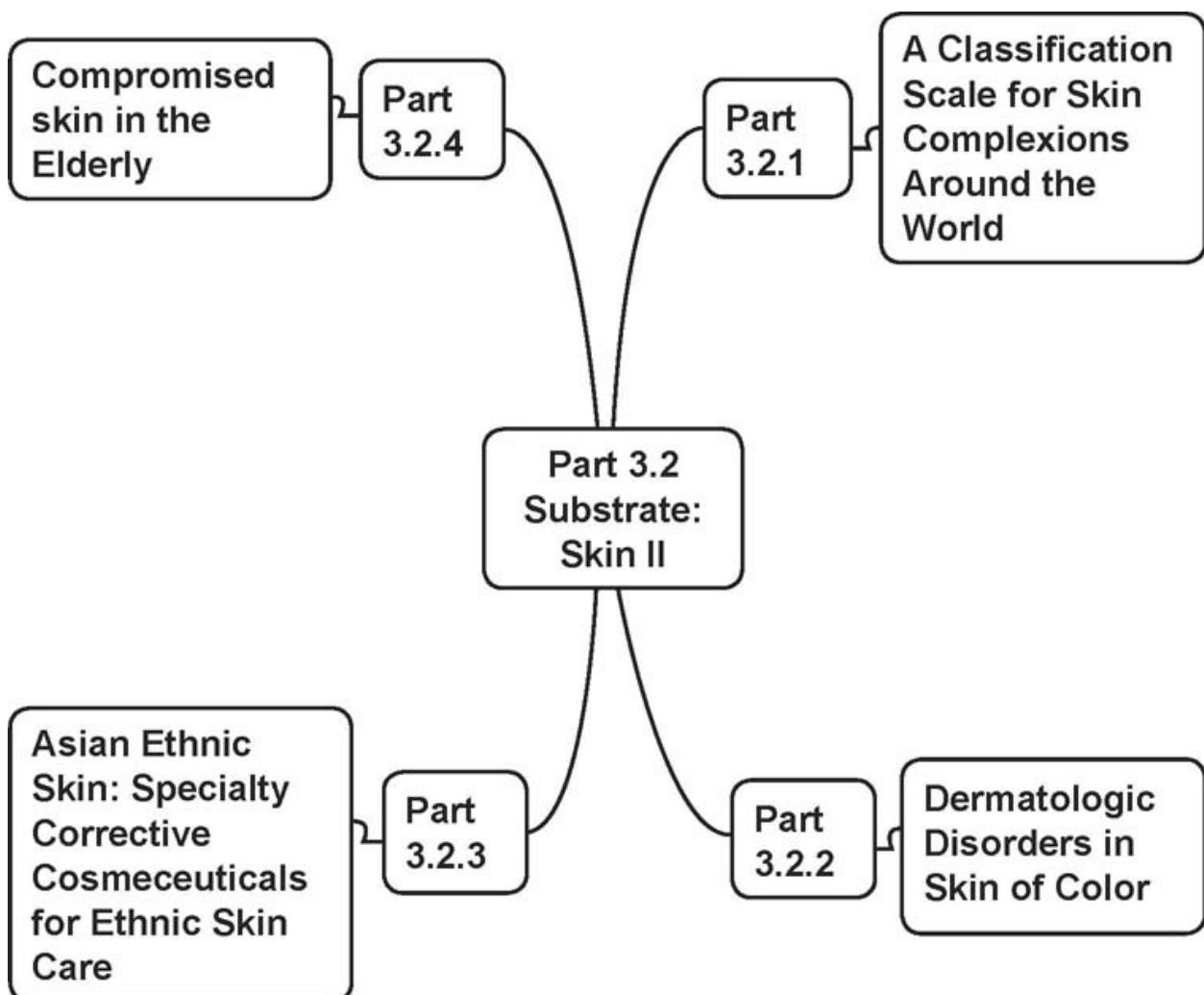
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Abbreviation: GAGs, glycosaminoglycans; NMF, natural moisturizing factor; DOPA, 3,4-dihydroxyphenylalanine; UV, ultraviolet; LCs, Langerhans cells; DCs, dendritic cells; SC, stratum corneum; RER, rough endoplasmic reticulum;

KHK, keratohyalin granules; LBs, lipid bodies; SG, stratum granulosum; SCD, stearoyl CoA desaturase; TEM, transmission electron microscopy; AMP, antimicrobial peptide; TJs, tight junctions; TEWL, TransEpidermal Water Loss; PLGA, poly(lactic-co-glycolic acid); PVA, polyvinyl alcohol; ATP, adenosine triphosphate; NADPH, nicotinamide adenine dinucleotide phosphate; HMG, Hydroxymethylglutaryl-CoA; HGP, Human Genome Project; PCR, polymerase chain reaction; DNA, deoxyribonucleic acid; RNA, ribonucleic acid.

PART 3.2

SUBSTRATE: SKIN II



CLASSIFICATION SCALE FOR SKIN COMPLEXIONS AROUND THE WORLD

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ABSTRACT

Cosmetologists and aestheticians are daily faced with a wide variety of clients. In order to provide their skin with optimal health and beauty services these practitioners must accurately look at skin of different races and determine appropriate treatment protocols. Early projections of the changing global skin color landscape have now been proven to be accurate, and the main categories of skin color are no longer just Caucasian-white, but have evolved towards a dramatic escalation of brown, black, yellow, and the many shades in between.

The current scale available to the industry is the Fitzpatrick scale (Figure 4), which was developed in 1975 by Harvard dermatologist Dr. Thomas B. Fitzpatrick. The Fitzpatrick scale is described in this chapter as a custom and practice foundation for current practitioners. It is simple and easy to use; however, the method leaves a gap in the ability to classify skin in light of the evolving epidemiological landscape, and the system fails to accurately predict skin type and precautions for race, complexions, and skin characteristics.

Extensive research over the last decade reveals that beyond color alone, other key factors such as race, country of origin, and ethnicity play an important role in how the skin will respond to cosmetic and personal care products and procedures. Further, when the world is viewed as a whole, and countries are thoughtfully divided into sections, there appears to be an order that helps define how skin from similar regions acts in similar ways. This observation provides the basis for a new, more detailed skin classification scale. A proposal for this

new scale is at the heart of the current chapter. The scale has been called the EP Global Skin Classification Scale (Figure 1) and is focused on an all-encompassing global approach that is comprehensive and easy to use for the skin care professional. ([Figures 2 & 3](#) show additions to EP Global Skin Classification Scale.) [**TABLE OF CONTENTS**](#)

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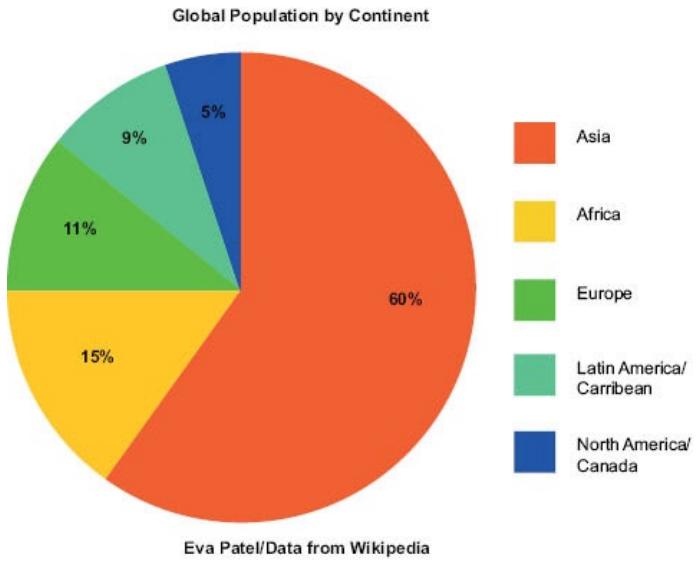
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3.2.1 WHY WE NEED AN UPDATED SKIN CLASSIFICATION SCALE

The changing of global skin color landscape is now a reality; the majority is no longer just Caucasian but Asian, with the many shades of yellow undertones and shades ranging from light to dark olive complexions. The common skin conditions among the different races also differ; therefore skin has to be diagnosed and treated according to color and race and a one-size-fits-all method of the past no longer works.

The world is divided geographically into continents:

- Asia is the most populous continent, with its 4.2 billion inhabitants accounting for over 60% of the world population. (The world's two most-populated countries alone, China and India, together constitute about 37% of the world's population).
- Africa is the second-most-populated continent, with around 1 billion people, or 15% of the world's population.
- Europe's 733 million people make up 11% of the world's population.
- Latin American and Caribbean regions are around 600 million (9%).
- Northern America, primarily consisting of the United States, and Canada, has a population of around 352 million (5%).
- Oceania, the least-populated region, has about 35 million inhabitants (0.5%).



Worlds Population by Race

East Asian 24% (Korea, Mongolia, China, Japan)

South Asian 21% (India, Sri Lanka, Pakistan, Bangladesh, Nepal)

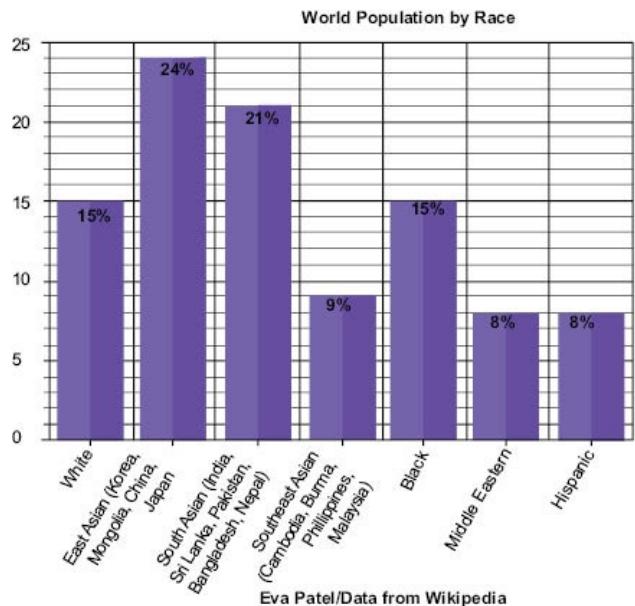
Southeast Asian 9% (Cambodia, Burma, Philippines, Malaysia)

Black 15%

White 15%

Hispanic 8%

Middle Eastern 8%



USA

The USA is a diverse country of many races; the chart below shows the growth and census population over the past decade.

Race	Census 2010, population	Percent of population	Census 2000, population	Percent of population
Total Population	308,745,538	100.0%	281,421,906	100.0%
Single race				
White	196,817,552	63.7	211,460,626	75.1
Black or African American	37,685,848	12.2	34,658,190	12.3
American Indian and Alaska Native	2,247,098	.7	2,475,956	0.9
Asian	14,465,124	4.7	10,242,998	3.6
Native Hawaiian and other Pacific Islander	481,576	0.15	398,835	0.1
Two or more races	5,966,481	1.9	6,826,228	2.4
Some other race	604,265	.2	15,359,073	5.5
Hispanic or Latino	50,477,594	16.3	35,305,818	12.5

NOTE: Percentages do not add up to 100% due to rounding and because Hispanics may be of any race and are therefore counted under more than one category.

Source: U.S. Census Bureau: National Population Estimates; Decennial Census.

The current scale that has been industry standard for the past 38 years is the Fitzpatrick scale.

The Fitzpatrick Classification Scale was developed in 1975 by Harvard Medical School dermatologist Thomas Fitzpatrick, MD, PhD. This scale classifies a person's complexion and their tolerance of sunlight. It is used by many practitioners to determine how someone will respond or react to facial treatments, and how likely they are to get skin cancer.

Skin Type	Skin Color	Characteristics
I	White; very fair; red or blond hair; blue eyes; freckles	Always burns, never tans
II	White; fair; red or blond hair; blue, hazel, or green eyes	Usually burns, tans with difficulty
III	Cream white; fair with any eye or hair color; very common	Sometimes mild burn, gradually tans
IV	Brown; typical Mediterranean caucasian skin	Rarely burns, tans with ease
V	Dark Brown; mid-eastern skin types	very rarely burns, tans very easily
VI	Black	Never burns, tans very easily

Fitzpatrick Classification Scale (Table 1)

The method leaves a gap in the ability to classify skin in light of the evolving epidemiological landscape, and the system fails to accurately predict skin type and precautions for race, complexions, and skin characteristics.

The EP Global Skin Classification tool was designed to fill the gaps by expanding the categories and taking into consideration global skin. The following categories were implemented:

- Race
- Country of Origin
- Complexion
- Skin Characteristics
- Skin Type
- Precautions

(See Figures 1-3)

EP Global Skin Classification Scale

EP #	Race	Country of Origin	Complexion	Skin Characteristics	Skin Type
I	Caucasian I	Belgium, Denmark, Estonia, Finland, Germany, Iceland, Ireland, Latvia, Liechtenstein,	Pale, Very Fair. Ruddy	Sensitive, thin fragile skin. Burns easily in sun, never tans, Age's rapidly. Fine wrinkling occurs early.	Hyper-Sensitivity Environmental Damage. Acne

		Lithuania, The Netherlands, Norway, Sweden, Switzerland, United Kingdom, Canada, Greenland	Freckled complexion.	Orange peel skin as they age. Scars usually thin. Skin tends to be dry.	Prone. Skin Cancer most common this type.
II	Caucasian II	Andorra, Austria, Belarus, Bosnia & Herzegovina, Croatia, Czech Rep. France, Greece, Hungary, Italy, Kosovo, Luxembourg, Moldova, Monaco, Montenegro, Poland, Portugal, Romania, Russia, San Marino, Serbia, Slovakia, Slovenia, Spain, Ukraine, Vatican City, USA.	Fair. Light Olive undertones.	Sensitive, fairly thin skin. When exposed to sun will first burn then tan. Signs of aging appear early. Nominal Scarring. Dry to normal skin.	Sensitive Photo Damage Premature Aging Acne Prone Greater chance of skin cancer.
III	Caucasian III	Australia, Bulgaria, Cyprus, Georgia, Greece, Macedonia, Malta, New Zealand, Turkey, South Africa, Zimbabwe	Fair Medium Olive undertones.	Seldom burns when exposed to sun, will tan, excessive exposure to sun will cause the skin to look leathery. Signs of aging appear later. Fine wrinkling less common. Scarring maybe thicker and darker. Normal to oily skin.	Environmental Damage Excessive Photo damage Prone Skin Cancer common
		Argentina, Belize,			

IV	Hispanic/Latino	Bolivia, Brazil, Chile, Colombia, Costa Rica, Ecuador, El Salvador, Falkland Islands (Malvinas), French Guiana, Guatemala, Guyana, Honduras, Mexico, Nicaragua, Paraguay, Panama, Peru, Suriname, Uruguay, Venezuela.	Olive undertones. Medium to Dark Olive; wide range of Olive tones.	Depending on skin tone will rarely burn; will always tan, aging process is moderate. Darker, thicker scarring. Normal to oily skin.
V	Mediterranean/Asian I	Afghanistan, Albania, Algeria, Armenia, Azerbaijan, Bahrain, Iraq, Iran, Israel, Jordon, Kazakhstan, Kuwait, Kyrgyzstan, Lebanon, Oman, Palestine, Qatar, Saudi Arabia, Syria, Tajikistan, Turkmenistan, UAE, Uzbekistan, Yemen	Medium-Dark Olive undertones	Seldom burns, will tan. Aging process is moderate. More advanced if over exposed to sun. Wrinkles appear later in more localized areas. Darker, thicker scarring. Normal to oily skin.
VI	Asian II	Brunei Darussalam, Cambodia, China, Hong Kong, Indonesia, Japan, Korea, Laos, Macao, Malaysia,	Medium – Dark Yellow-	Seldom burns, tans about average. Ages well, signs of aging appear late. Prone to

		Mongolia, Philippines, Singapore, Taiwan, Thailand, Timor Leste, Vietnam.	Olive undertones.	Pigmentation changes. Scarring can be thicker and darker. Normal to oily skin	Hyperpigment (PIH) Skin Ca very rare
VII	Asian III	Bangladesh, Bhutan, India, Maldives, Myanmar (Ex- Burma), Nepal, Pakistan, Sri- Lanka, Tibet.	Light to dark Olive tones. Yellow undertones	Rarely burns, always tans. Pigmentation changes may occur with over exposure to sun. Ages well, signs of aging appear late. Fine wrinkles do not occur. Darker thicker scars more common	Hyperpigment Melasma Activ Acne/ Acne Pr Post Inflamma Hyperpigment (PIH) Skin Ca very rare.
VIII	Native Hawaiian and other Pacific Islanders	Fiji, French Polynesia, Guam, Kiribati, Marshall Islands, Micronesia, New Caledonia, New Zealand, Papua New Guinea, Samoa, Solomon Islands, Tonga, Vanuatu.	Medium- dark Olive tones.	Seldom burns, tans well, thicker skin allows better sun tolerance. Ages moderately. Thicker and darker scars. Thick Oily skin.	Photo Damage Environmental Damage Hyperpigment Acne Prone Sk Cancer rare
IX	Native American Indian and Native Alaskan	There are over 70 Native American tribes and 8 Alaskan races *See attached list.	Medium Olive tones. Red undertones.	Rarely burns, tans well, high sun tolerance. Skin has a tendency to turn leathery with age and excessive sun exposure. Pigmentation	Environmental Damaged Excessive phot damage Hyperpigment / Melasma Skin

				changes may occur. Thick dark scarring. Normal oily skin.	Cancer rare
X	Black or African American	Eastern Africa, Middle Africa, Northern Africa, Southern Africa, Western Africa, The Caribbean. *See attached list.	Medium-Dark Brown Tones.	Never burns with sun exposure, tans well. Pigmentation issues common. Very little fine wrinkling. Formation of Keloids possible. Oily skin.	Hyperpigmentation Melasma Acne Prone Post inflammatory Hyperpigmentation Pseudo folliculitis Skin Cancer very rare
XI	Mix of two or more races	Any combination	Varies depending on race combination.	Take the predominant traits into consideration.	Treat with caution Use milder programs to start graduating to stronger products absence of irritation.

Figure 1 (EP Global Skin Classification Scale)

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EP Global Skin Classification Scale					
Race Section X (10) - Country of Origin					
Africa					
Eastern Africa	Middle Africa	Northern Africa	Southern Africa	Western Africa	The Caribbean
Burundi	Angola	Algeria	Botswana	Benin	Anguilla
Comoros	Cameroon	Egypt	Lesotho	Burkina Faso	Antigua and Barbuda
Djibouti	Central African Republic	Libyan Arab Jamahiriya	Namibia	Cape Verde	Aruba
Eritrea	Chad	Morocco	South Africa	Cote d'Ivoire (Ivory Coast)	Bahamas
Ethiopia	Congo (Brazzaville)	South Sudan	Swaziland	Gambia	Barbados
Kenya	Congo, Democratic Republic of the	Sudan		Ghana	Bonaire, Saint Eustatius and Saba

Madagascar	Equatorial Guinea	Tunisia		Guinea	British Virgin Islands
Malawi	Gabon	Western Sahara		Guinea-Bissau	Cayman Islands
Mauritius	Sao Tome and Principe			Liberia	Cuba
Mayotte				Mali	Curaçao
Mozambique				Mauritania	Dominica
Reunion				Niger	Dominican Republic
Rwanda				Nigeria	Grenada
Seychelles				Saint Helena	Guadeloupe
Somalia				Senegal	Haiti
Tanzania, United Republic of				Sierra Leone	Jamaica
Uganda				Togo	Martinique
Zambia					Monserrat
Zimbabwe					Puerto Rico
					Saint-Barthélemy
					St. Kitts and Nevis
					Saint Lucia
					Saint Martin
					Saint Vincent and the Grenadines
					Sint Maarten
					Trinidad and Tobago
					Turks and Caicos Islands
					Virgin Islands (US)

TABLE 2 (Race Section X (10) - Country of Origin)

EP Global Skin Classification Scale				
Race Section IX (9) - Country of Origin				
*American Indian and Alaska Native tribes				
Alaskan Athabascan	Crow	Menominee	Puget Sound Salish	Yaqui
Aleut	Delaware	Navajo	Seminole	Yuman
Apache	Eskimo	Osage	Shoshone	
Blackfeet	Houma	Ottawa	Sioux	
Cherokee	Iroquois	Paiute	Tlingit-Haida	
Chippewa	Kiowa	Pima	Tohono O'Odham	
Comanche	Latin American	Potawatomi	Ute	
Creek	Lumbee	Pueblo	Yakama	

TABLE 3 (Race Section IX (9) - Country of Origin) **3.2.2 THE**

METHODOLOGY IN WHICH THE STUDY WAS CONDUCTED:

The research was carried out at Skin Rejuvenation Clinic in San Francisco during 1997–2005. All subjects were residents of USA; a total of 588 subjects, both female and male (majority female) were chosen; they were of different races.

Charts and records were followed during this period to gather the information for the study. All data were manually collected through these charts and records collected from each individual client visit. The initial consultation is where the data were collected from the client. The minimum treatment process was eight visits over a four-month period, two visits a month. Thereafter monthly maintenance visits.

During the initial visit a client filled out a three-page Skin Evaluation questionnaire that asked several questions pertaining to several different factors, which included:

Skin Evaluation	
Skin Type	
<input type="checkbox"/> Very Dry	Dry all over—all the time, tight, easily irritated, sensitive
<input type="checkbox"/> Dry/Mature	Mostly dry, tight, maturing/aging, sun/photo-damaged, loss of tone
<input type="checkbox"/> Normal to Dry	Normal in the T-zone, dry on the sides of face
<input type="checkbox"/> Normal	Slightly oily on the T-zone, normal on the sides of face
<input type="checkbox"/> Normal to Oily	Oily in the T-Zone, slightly oily on sides of the face
<input type="checkbox"/> Oily	Very oily in the T-Zone, slightly oily on the sides of the face
<input type="checkbox"/> Very Oily	Very oily all over, all the time, prone to breakouts and acne
<input type="checkbox"/> Blemished	Oily with tendency to active acne or acne-prone skin

History/Pigmentation Country of origin and ancestral race

What is your ancestry? Parents: _____ Grandparents

Pigmentation (How do you tan?)

How do you tan? Burn Usually Burn Burn then Tan Usually Tan Always Tan

Pigmentation tendencies: Even Uneven Hyperpigmented

Melasma **Vascularity** Broken capillaries: Nose area Cheek area

Chin area Forehead

Entire face

Acne Do you have a history of any of the following?

Acne Whiteheads Blackheads Pimples Enlarges Pores
 Flakiness Scars Erythema Post-Inflammatory Hyperpigmentation
 Pits Cysts

Sun Exposure/Environment Do you spend time in the sun?

Seldom Occasionally Frequently Approximately how many hours do you spend in the sun a week?

Do you use a sunscreen on a daily basis? _____ What SPF? 15 30 30+

At what age did you start to use a sun block on a regular basis?

What percentage of time do you spend in the sun?

Summer _____ Winter _____

Where did you reside the first 20 years of your life?

Country _____ Region/City _____

Please note that most damage is accumulated during the first 18 years of your life.

Have you actively sunbathed in the past? Yes No Do you currently sunbathe? Yes No Do you use tanning

beds? Yes No Have you ever had skin cancer?

Yes No Do you have a family history of skin cancer?

Yes No Relationship _____

Free Radical Exposure Do you smoke?

Yes No How much? _____

Do you consume alcohol? Yes No How much? _____

Do you have a healthy diet? Yes No Do you exercise on a regular basis? Yes No Do you take vitamins? Yes No Which ones? _____

Lifestyle Sleeping habits Poor Good Minimal / 6 hours 7 hours 8 hours Stress level Minimal Moderate Excessive Do you meditate? Yes No How often? _____

Healing Ability Does your skin appear fragile and thin? Yes No

Do you bruise easily? Yes No Do you scar from

cuts or burns? Yes No Do you have a tendency to keloid? Yes No Have you had a deep

peel? [] Yes [] No Did you heal well? _____

Do you have facial laser treatments? [] Yes [] No When the skin evaluation was completed, the skin was further categorized and documented by complexion and skin characteristics of various skin types from different genetic backgrounds.

Complexion/ Skin Characteristics Skin Characteristics of people with Anglo-Saxon origins

Fair, dry thin-skinned

Scars heal well

Signs of aging appear earlier

Burn easily in the sun

Bruising more obvious

Greater chance of skin cancer

Skin Characteristics of people with Southern Mediterranean origins

Oily, olive-dark complexion

Signs of aging appear later

Cartilage tends to droop

Darker, thicker scars more common

Wrinkles appear later and in more localized areas Skin cancer rare

Skin Characteristics of people with Northern European origins / German and Scandinavian

Fair, blue-eyed, blonde

Thin skin

Scars heal well

Signs of aging appear early

Bruising more obvious

Greater chance of skin cancer

Skin Characteristics of people with Southern European origins

Dark, oily

brunette complexion

Signs of aging appear later

Fine wrinkling less common

Bruising lasts longer

Scars may be thicker and darker

Skin cancers less common

Skin Characteristics of people with Northern European/Irish and Northern England

Ruddy freckled complexion

Red hair
Scars usually thin
Signs of aging appear later
Bruises easily
Pigmentation problems
Skin cancers most common in this type

Skin Characteristics of people with African origins Signs of aging appear very late
Very little fine wrinkling
Formation of keloids is possible
Pigmentation changes may occur
Thicker cartilage hard to change
Skin cancers very rare

Genetically, this skin type is less susceptible to damage from UV radiation, although the skin can still get burned.

Skin Characteristics of people with Asian origins Signs of aging appear late

Fine wrinkling does not usually occur
Pigmentation changes may occur
Eyelid surgery more difficult
Skin cancers very rare

The completed questionnaire was then evaluated and categorized according to primary skin condition to be treated and the secondary skin condition to be treated. A woods lamp was used during the initial consultation to further check for photo-damaged, congested skin, hyperpigmentation/melasma. This data collected gave further clarity to the client's skin type.

A UV photo was taken through the canfield polarized camera; this visually showed the UV photo damage and was documented.

3.2.3 SUMMARY, ANALYSIS

During the years of 2005 and 2011 the collected data were reviewed and the conclusions were as follows:

- Subjects from a similar geographical region or ancestral region (country of origin) had similar skin types and characteristics.
- Common skin conditions were similar.
- Complexions had similar undertones according to region (lighter complexions in northern cooler climates and darker around the equator where it's the hottest). Undertones of Asian countries were yellow/olive and undertones of Europe more ruddy, red/pink.
- Migration to another country did not change the original skin type or characteristic.
- There were many mixed races that had combined skin types.

3.2.4 WHERE METHODOLOGY MEETS RESULTS

The method in which the data were collected and notes taken during office visits showed that skin from similar races or country of origin had similar skin types and skin characteristics.

Upon completion of the review of skin evaluation data, the skin type section of the EP Global Skin Classification was based on the collective findings of skin type, with sun exposure, environment, and lifestyle data combined.

The compiled data were then assembled into an organized chart, hence creating the **EP Global Skin Classification Scale**. (Refer to Figure 1.)

PART 3.2.2

DERMATOLOGIC DISORDERS IN SKIN OF COLOR

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3.2.2.1 INTRODUCTION

Skin of color is sometimes referred to as pigmented or ethnic skin. It simply means having enough pigment in the skin to impart a color other than white. In general, people of African, Asian, Latin, Native American, Pacific Islander, or mixed racial origins are considered to have skin of color. The demography of the United States is predicted to shift dramatically to the point where the majority of the population will fall under this category by 2050.¹

There are several dermatologic diseases that are more commonly encountered in this population. In particular, disorders of pigmentation such as melasma and

post-inflammatory hyperpigmentation are among the top skin concerns to prompt a visit to the dermatologist.²

3.2.2.2 SKIN PHOTOTYPES

The Fitzpatrick classification of skin types ([Table 1](#)) is based on an individual's ability to tan and propensity to sunburn. The skin phototype is generally correlated with degree of pigmentation. For example, Type I skin is typically seen in people of Celtic origin whereas Type VI skin is mainly seen in people of Sub-Saharan African ancestry.

Table 1: Fitzpatrick Skin Types

Skin type	Skin color	Sunburn and tanning history
I	White	Always burns, never tans
II	White	Always burns, tans minimally
III	White	Burns moderately, tans gradually
IV	Olive	Minimal burning, tans well
V	Brown	Rarely burns, tans darkly
VI	Dark brown	Never burns, tans darkly black

a. Melasma

Melasma is an acquired patchy hyperpigmentation predominantly occurring in individuals with Fitzpatrick skin phototypes III and IV. It is a common disorder affecting nearly 5 million people in the U.S. alone. Although most frequently seen in women, 10% of cases occur in men. The reported prevalence ranges from 8.8% of Hispanic women in the Southern U.S. to 40% of Southeast Asian females.³

Melasma occurs primarily in 3 clinical patterns: 1.) centrofacial, 2.) malar, and

3.) mandibular, although many patients have a mixture of these presentations ([Figure 1](#)). The most common is centrofacial and consists of light to dark brown patches occurring on the forehead, cheeks, nose, upper lip, or chin. The malar pattern affects the cheeks and nose, while the mandibular pattern occurs along the jawline.



[Figure 1](#) Melasma The clinical manifestations of melasma are thought to result from the presence of biologically hyperactive melanocytes in the affected skin. The exact etiology of melasma remains unknown, however, several risk factors have been identified. Affected individuals frequently have a family history suggesting the influence of genetics.

In addition, sun exposure is a common exacerbating factor most likely through upregulation of melanocyte-stimulating factors. The high incidence of melasma in women, especially those who are pregnant or taking oral contraceptives, suggests an important role for estrogen.

The location of excess pigment, which can be assessed using a Wood's lamp, tends to correlate with the response to therapy. In general, involvement of the more superficial epidermis is more amenable to treatment than that of the deeper dermis.

A critical component in the treatment of disorders of hyperpigmentation is sun protection. This is most commonly achieved by wearing sun-protective clothing and a broad-spectrum sunblock. Without these measures, any treatment regimen is bound to fail. Hydroquinone 4% cream is FDA-approved for the treatment of hyperpigmentation and considered by many to be the gold standard. This medication works by inhibiting the melanin-producing enzyme known as tyrosinase. Sometimes higher concentrations are needed which must be compounded by a pharmacy. The addition of tretinoin cream may enhance

efficacy. Non-FDA approved natural alternatives to hydroquinone include kojic acid, arbutin, soy, licorice extract, rucinol, mulberry, niacinamide, ellagic acid, resveratrol, and dioic acid. Other methods of treating hyperpigmentation include laser therapy and chemical peels.⁴

b. Post-inflammatory Hyperpigmentation

An increase in pigment occurring after an inflammatory event is known as post-inflammatory hyperpigmentation (PIH). This cosmetically distressful phenomenon is extremely common in people with skin of color. Frequent causes of PIH include acne, eczema, psoriasis, burns, and certain cosmetic procedures. It is of utmost importance that the underlying cause of PIH be addressed if still present. The treatment of PIH is otherwise similar to that of melasma.

c. Vitiligo

Vitiligo is an acquired disorder of depigmentation estimated to affect 0.5% to 1% of the population.⁵ Although vitiligo can occur at any age, almost half present before age 20. Both sexes are affected equally and there is no difference in rates of occurrence according to skin type or race. It can be a psychologically devastating, particularly in people with darker skin, because it is more noticeable.

Vitiligo is divided into three types: localized, generalized, and universal. The generalized type is most common, resulting in widespread symmetric white macules and patches. Areas of predilection include the fingers and wrists, axillae and groin, and body orifices, such as the mouth, eyes, and genitals. There is a propensity for lesions to occur in sites of trauma, which is a process known as koebnerization. The universal type is defined as having at least 80% skin involvement ([Figure 2](#)).⁵ Lesions begin insidiously and are generally slowly progressive either by centrifugal expansion of current lesions and/or the appearance of new lesions. Spontaneous repigmentation occurs in no more than 15% to 25% of cases.



Figure 2 Extensive facial depigmentation occurring in a patient with vitiligo. Under the microscope, vitiligo is characterized by the absence of melanocytes. The exact reason behind their disappearance is unclear but several theories regarding autoimmune, genetic, oxidative stress, and neural mechanisms have been proposed. Currently, the evidence is most strongly suggestive of an autoimmune process occurring in genetically predisposed persons. Indeed, nearly 20% of people with vitiligo have at least one first-degree relative also affected by the disease.⁵ Vitiligo may also be associated with other autoimmune diseases such as Addison's disease, pernicious anemia, diabetes mellitus, thyroid disease, and alopecia areata.

The treatment of vitiligo can be a frustrating process. Numerous treatment options exist but a cure remains elusive. As such, goals for treatment include halting disease progression, inducing repigmentation, and achieving an acceptable cosmetic result. It is also important to address the psychosocial impact of the disease. In general, the response to treatment is often slow and may be modest at best.

Current treatment options include topical and systemic steroids, topical calcineurin inhibitors, topical Vitamin D3 analogs, narrow-band UVB phototherapy, and excimer laser. Among these, the gold standard and most frequently used treatment is narrow-band UVB phototherapy.

Surgical therapy may be an option for people with focal non-progressive vitiligo who have failed phototherapy. Total depigmentation with monobenzene is a last resort reserved for individuals with extensive recalcitrant vitiligo who desire a uniform skin color. Cosmetic camouflage may be helpful in concealing areas of vitiligo occurring in particularly noticeable areas such as the face and neck.

d. Idiopathic Guttate Hypomelanosis

Idiopathic guttate hypomelanosis is a common disorder affecting nearly 80% of people over age 70. Although it occurs in all races and skin types, it is more noticeable in darker skinned individuals. It presents as several, well-defined, hypopigmented 1-6 mm flat spots with a smooth surface on the forearms and shins. The etiology is unknown but sun exposure is thought to play a role. Treatment with liquid nitrogen may be beneficial but must be weighed against the risk of causing post-inflammatory hyper-or hypopigmentation in darkly pigmented skin.

e. Pseudofolliculitis Barbae

Pseudofolliculitis barbae (PFB) is otherwise known as razor bumps or ingrown hairs. The disorder is associated with shaving and is particularly common in the beard area of black men or groin area in women. It is caused by newly shaved tightly curled hairs curving back into and piercing the skin, which results in an inflammatory reaction. PFB presents clinically as inflamed bumps and pustules, which may result in post-inflammatory hyperpigmentation and keloids.

The ideal treatment is to completely stop shaving and instead use a trimmer to leave 1-2mm of stubble. If this is not feasible for personal or professional reasons, a low potency topical steroid and topical antibiotic may be used to reduce inflammation. Other options for treatment include chemical depilatories, laser hair removal, and eflornithine cream.

CONCLUSION

The study of ethnic skin is absolutely necessary to meet the demands of our shifting demographics. Tremendous progress has been made in terms of understanding the biological mechanisms behind disorders of pigmentation. This, in turn, should spur the innovation of more effective medications and cosmeceuticals.

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ASIAN ETHNIC SKIN: SPECIALTY CORRECTIVE COSMECEUTICALS FOR ASIAN ETHNIC SKIN CARE

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ABSTRACT

The darkening of skin color in larger segments of the world's population is an emerging trend according to numerous projections. Concomitant with this change is that new, specific skin care needs are an emerging opportunity for product development in these growing markets. To serve these needs, professionals have to be abreast of the needs of this new and growing sector and they must understand this diverse global consumer. This chapter focuses on Asian ethnic skin, which is a new category as defined elsewhere in this book and known as EP V-VII in the EP Global Skin Classification Scale.

By 2014, skin care will increase its already-sizeable lead in the beauty market

to reach \$91 billion (*Euromonitor International*). This increase is driven primarily by expansion of opportunities in the Asian market. Even in USA the Asian population grew faster than any other major race group between 2000 and 2010, increasing by 43% (U.S. Census Bureau).

By 2050, the U.S. population projections are that 51% of Americans will have skin of olive tones and dark-skinned racial backgrounds (Hispanics, Black, Asian, others) according to the PEW Research Center.

The most apparent difference in the skin of those from different ethnicities is, of course, the color, although there are also differences in skin thickness, vascularity, and predispositions to certain skin conditions and diseases. Pigmentation disorders are a primary concern for Asian ethnic skin as well as a tendency for acne for those with normal to oily skin.

The ingredients and methodology applied in creating effective corrective cosmeceuticals for this segment of the population must take these ethnic skin differences into account. Multiple symptoms and conditions, such as hyperpigmentation with oily skin, can then be treated with today's sophisticated formulations.

Market research shows that consumers are streamlining the number of products they buy and focusing on products that are targeted to correct more than one condition. It is therefore important for industry professionals to focus on development of "necessity products" in these skin care ranges.

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INTRODUCTION:

In modern-day India, a woman's worth is all too often determined by the color of her skin. In India, beauty lies not only in the eyes of the beholder but in societal pressure to be fair-skinned. Arranged marriages are still popular and mothers seek fair-skinned brides for their sons; in fact everyone seeks such brides/grooms. Being dark is undesirable and affects the marriage process; a

darker-complexion girl, even with beautiful features, is not considered beautiful. Billboards all over India promote fairness, and ads on TV promote fairness with success and beauty.

Culturally this applies to Indians living in foreign countries as well: fair is considered beautiful. My personal experience in the west has been that Asians want lighter complexions and Caucasians want to be darker and tanned. It's like wanting what is not real. I feel we should all be happy with the complexion we are born with and not let societal or cultural pressure tell you otherwise. Taking care of your skin and bringing it back to optimum health by using good products is a necessity and no longer a luxury.

3.2.3.1 DEFINING THE ASIAN ETHNIC MARKET:

Pale skin has always been the ideal for women in Asian cultures for a long time. It has nothing at all to do with wanting to be more “white” in the racial or cultural sense. Up until the 1920s pale, unblemished skin for women was the ideal in western cultures as well. Browned skin was undesirable, since it was considered a trait of only the poor who endured hard labor outdoors. Well-off women used to apply all kinds of products to their skin to whiten them, and both Chinese and Japanese cultures have proverbs that imply “a white skin hides other flaws.” Those sayings have existed for ages.

All across Asia, fair skin is highly prized. In dark-skinned South Asia, a fair complexion is considered the epitome of beauty while in already pale-skinned North and East Asia, pearly translucent white skin is a sign of affluence and glamour. In Asia, many people associate dark and tanned skin with menial work in the fields under the hot sun, and a pale complexion with a higher social standing and cultural refinement. Since the 1970s, Asia has been the fastest-growing sector in the global skin-lightening market. Asia is a lucrative market with high-growth potential because of a rising middle-class with increasing disposable income and centuries-old entrenched cultural impressions of beauty.

India’s domestic cosmetics industry is expected to grow to US\$3.6 billion by 2014, according to the Associated Chambers of Commerce and Industry of India. The skin-lightening cream market alone was worth US\$432 million in 2010 and growing at 18% annually.

In Japan the cosmetics industry is growing at 17% a year and is expected to continue that rapid pace for a few more years at the very least, largely because of the hundreds of millions of emerging middle-class women who are spending

their money on cosmetics. (1)

In China, where the skin care market is worth more than 35 billion yuan (US\$5.5 billion), whitening products comprise a whopping 71% of the market. Elsewhere in Asia, a survey by the London-based market research firm Synovate found that four out of ten women in Hong Kong, Malaysia, the Philippines, South Korea, and Taiwan use a skin-whitening cream.

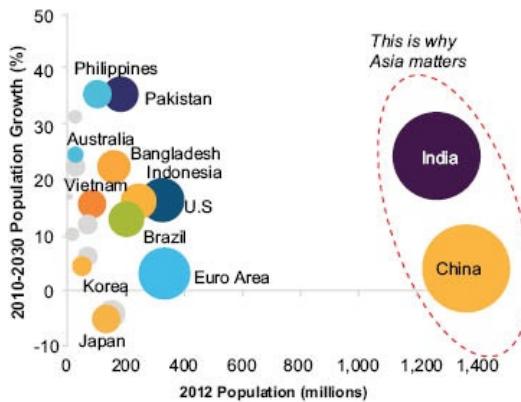
The cosmetics industry has also started to target men, selling them the same idea that a fairer complexion would make them more attractive. While skin-lightening products for women have been available for decades, products for men have only appeared in recent years, a trend that is rapidly growing. (2)

Japan dominates the global skin lighteners market with the lion's share, as stated in the new market research report from Global Industry Analysts, Inc. (GIA). The market for skin lighteners in Asia-Pacific is projected to cross the \$2 billion mark by 2012, driven by the fast-growing markets of China and India. Apart from Asia, Western countries such as the United States and the United Kingdom are emerging as potential markets for skin-whitening products. The growing proportion of ethnic groups—Asians, Hispanics, and African Americans—in these regions is a major contributor to the enhanced demand for skin lighteners.

In Asia, skin-whitening products are very popular. The West seems to believe that these products are made because Asians want to become more Western, which isn't true. Fair skin has been a staple in beauty for Asian cultures for centuries; even old paintings in Asian cultures portray women as fair with flawless complexions.

Western society's obsession with skin tanning is well recognized, but we're often less aware of the aggressive pursuit of fair skin by those with darker skin. In communities where light skin is associated with success, prestige, and envy, women commonly turn to skin-lightening products to achieve and maintain their desired complexion.

In India, the appeal of fair skin is deeply rooted in the nation's culture and the caste system. Higher caste members traditionally had lighter skin and were less likely to be involved in manual work. Many years later, the ruling colonials had fair skin. And in the last century, film, TV, and advertisements have been saturated with images of attractive western actresses with fair skin. Skin-lightening creams are reportedly the most popular product on the Indian skin care market, with around 60% of Indian women using the products daily.



Data as at May 11, 2011. Source: United Nations World Population Prospects, Haver. Size of bubble indicates 2012 population.

Global diversity continues to grow throughout the world as Asian ethnicity remains a constant that makes up the majority of the world's population. The need for an assortment of products that are relevant to the specific multicultural market continues to drive research and technology in the skin care industry. Although Asian ethnic skin care products exist, the market struggles to find skin care products that are optimal in addressing their particular needs. It's important for brands to connect with ethnic consumers and address specific concerns to a wide array of skin types. Women and men of color around the world experience very distinctive issues related to their skin types, and the amount of pigment varies significantly between many ethnic groups. As noted in the EP Global Skin Classification Scale, each region/race has its unique skin color, characteristics, and related skin conditions.

A. Mediterranean/Asian I (EP Global Skin Classification V)

Afghanistan, Albania, Algeria, Armenia, Azerbaijan, Bahrain, Iraq, Iran, Israel, Jordan, Kazakhstan, Kuwait, Kyrgyzstan, Lebanon, Oman, Palestine, Qatar, Saudi Arabia, Syria, Tajikistan, Turkmenistan, UAE, Uzbekistan, Yemen

B. Asian II (EP Global Skin Classification VI)

Brunei Darussalam, Cambodia, China, Hong Kong, Indonesia, Japan, Korea, Laos, Macao, Malaysia, Mongolia, Philippines, Singapore, Taiwan, Thailand, East Timor, Vietnam

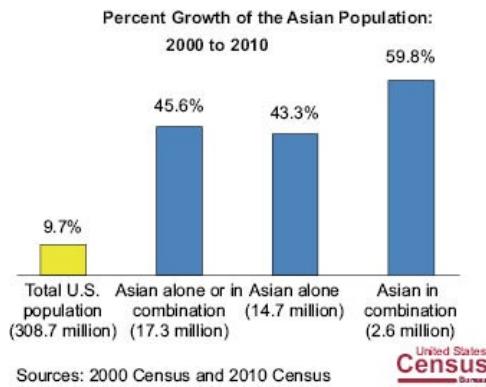
In Asia, a lighter skin color is associated with beauty and aristocracy. Asians have skin properties that set them apart from Caucasians. They have more yellow undertones, and skin color ranges from fair to dark. Sometimes Asian skin types can be deceiving and not appear to have a high content of melanin, which is why taking each person's ethnicity into account is crucial. Asian skin

does in fact contain more pheomelanin pigments, which determines the yellow undertone skin color. Despite how Asians of different regions seem to sport different skin colors (Japanese are the fairest, with little redness in their skin; Indians are the darkest, with more skin redness), all actually have yellow undertones. Some might be stronger than others, like Asians of Malay origins (e.g., many Malaysians and Filipinos) have the most yellow “tones.” Why yellow? It has to do with pheomelanin: high level of this type of melanin in the skin results in yellow undertones.

C. Asian III (EP Global Skin Classification VII)

Bangladesh, Bhutan, India, Maldives, Myanmar (Ex-Burma), Nepal, Pakistan, Sri-Lanka, Tibet

South Asians have a different skin type when compared to the Caucasians and require a different skin care schedule. Cosmetics used by Caucasians do not suit all Asian skin types. Asian skin is usually brownish, and skin tone varies according to the region of origin. Excessive melanin contributes to the brown color and also excessive melanin in the skin weaves a protection against skin cancer. In spite of excessive melanin content, South Asians are prone to pigmentation, age spots, freckles, etc. due to severe climatic conditions. Aging does not show up as wrinkles in South-Asians but rather as hyper pigmentation, dark spots, uneven skin tones, freckles, etc.



3.2.3.2 DIFFERENCES BETWEEN CONTENT LEVELS OF MELANIN IN THE SKIN

Differences between skin colors are determined by the amount of melanin produced by the melanocytes. Melanin comes in two different forms: pheomelanin, which gives the skin more red to yellow pigments, and eumelanin, which gives the skin dark brown to black pigment. The relative proportions of

these two types of melanin determine the skin color. For example, lighter skin types produce a lesser amount of melanin whereas darker skin types produce more melanin. Also, some Asian skin types might appear to be fairer with strong yellow undertones; therefore their skin contains higher levels of the pheomelanin pigment. No matter the amount of melanin produced, we all have the same number of melanocytes in our skin, which are the cells that produce melanin (pigment). In darker skin types the melanocytes are more sensitive to irritation and react more aggressively to injuries. An example of injury can be as simple as extracting a blackhead and applying too much pressure on the skin, which results in trauma to the skin where a dark spot is formed, also known as post-inflammatory hyperpigmentation. This is a common problem associated with those who contain more melanin in their skin. Hyperpigmentation occurs more often in those with darker skin, which can be a result from burns, inflammation, scraps, cuts, or even a pimple. Those who suffer with acne, eczema, and ingrown hairs will also result in uneven skin tone and hyperpigmented spots.

Another key point of difference in those with darker skin is the sebaceous glands that produce oil/sebum. These glands tend to produce higher levels of oil, which results in excessive oil production and ultimately results in overdrying the skin. A huge benefit for those with higher melanin content or more color in their skin is that they will tend to see signs of aging later on in life. Melanin acts as a protector for our skin and helps to ward off the UV rays of the sun by absorbing them, and keeping them from penetrating the skin. Whenever the skin detects excess solar radiation it produces more melanin and more melanin means darker skin, hence, the suntan. This not to say that people of color do not get wrinkles; it just means that aging takes place differently and the propensity for irritation and hyperpigmentation is magnified for their skin type. Therefore a greater knowledge is required for product choice and professional treatment options. Most commonly Caucasian women tend to seek out skin care products for anti-aging properties and moisturizing, whereas women with more color look for products to correct and prevent uneven skin tone and blemishes.

3.2.3.3 COMMON SKIN CONDITIONS FOR ETHNIC SKIN AND TREATMENT METHODS

With social media and companies paying attention to wanting to help the consumer solve a skin care problem, I feel that the time has come in the industry where companies can collaborate to help educate consumers on how important

prevention is and making lifestyle changes to support this. For example, if someone in their 30s wants to lighten their skin, it is mandatory that daily use of a SFP30 is also part of the regime. Direct sun avoidance during peak times is essential, and if not, a precaution such as a hat or additional protection measure should be taken.

With a collaborated campaign that is more educational, the message will reach out to consumers and change will happen. Social media plays a big role in that it can assist with getting the right message out to consumers, making it more fun and easier to grasp. In most Asian countries TV and media still play a big role in the lives of the masses, so some form of education via this method may also be very effective. Along with more education will come better preventative steps which will essentially be the most successful approach to reduce dark spots.

Prevention is a key to successfully reducing the risk of hyperpigmentation in the future. A good skin care regimen followed a.m. and p.m. will help provide preventative results. A preventative regimen must include some type of mechanical or chemical exfoliation three times a week, a melanin-suppression formula, and sunscreen. The first step is to exfoliate the skin so that the dead skin cells are removed and to reveal brighter skin tone. Exfoliation will help increase the cellular turnover, so results are seen more quickly from the skin-brightening product. The second step is to use a skin-brightening product twice a day after cleansing the skin. There are a variety of skin-lightening products on the market, so the best thing to look for is the product containing the most amount of skin-lightening active ingredients. Some formulas contain hydroquinone and others do not. There are many alternatives in the market to address different skin types and pigment disorders. Refer to the list of skin-lightening actives. The last step will be to protect the skin from the harmful UV rays. Sun is the number one precursor to pigment formation. Protecting the skin with a broad-spectrum SPF of 30 will provide the best protection from UV-A and UV-B rays. If exposed to the sun for a long period of time reapply sunscreen every two hours for adequate protection. Below are common skin conditions and different causes and treatments.

Melasma (also known as chloasma or “pregnancy mask”) is a common skin condition. It causes brown to gray patches of discoloration, very common in women during the reproductive years. Melasma is dark, irregular, well-demarcated hyperpigmented macules/patches commonly found on the upper cheek, nose, upper lip, and forehead. It can also appear on parts of the body that get excessive sun exposure such as forearms and neck and décolleté area.

Causes of Melasma: Hormonal changes in the body, genetics, and overexposure to sun. History of excessive or intermittent sun exposure. Pregnancy, birth control pills, HRT, other medications (diuretics, antibiotics, heart disease, diabetic, hypertension meds). Products or treatments that irritate the skin may cause an increase in melanin production and accelerate melasma symptoms.

People with olive or darker skin, like Hispanic, Asian, and Middle Eastern individuals, have higher incidences of melasma. People with a genetic predisposition or known family history of melasma are at an increased risk of developing melasma. Important prevention methods for these individuals include sun avoidance and application of extra sunblock to avoid stimulating pigment production.

An estimated 6 million women are living in the U.S. with melasma and 45–50 million women worldwide live with melasma; over 90% of all cases are women. Prevention is primarily aimed at facial sun protection and sun avoidance. Treatment requires regular UVA & UVB sunblock SPF 30 application and skin-lightening, -whitening and -brightening creams. (3)

Treatments for Melasma:

Microdermabrasion: utilizes vacuum suction and an abrasive material like fine diamond chips or aluminum oxide crystals to exfoliate the top layers of the skin. The vacuum pressure is adjusted depending on the sensitivity and tolerance of the skin. Typical microdermabrasion sessions can last anywhere from a few minutes to one hour. Minimal to no recovery time is needed after microdermabrasion. Microdermabrasion techniques can improve melasma, but dramatic results are not generally seen or expected after one or two treatments. Multiple treatments in combination with sunscreen and other creams yield best results.

Chemical peels: Many types and strengths of chemical peels are available for different skin types. The type of peel should be tailored for each individual and selected by a physician.

TCA: TCA peels are excellent and safe in the hands of an experienced dermatologist. This is a good option, particularly if you have severe melasma.

AHA +combinations: In treating melasma, 30–70% glycolic acid peels are very common. Various combination peels, including a mix of glycolic acid, kojic acid, lactic acid, etc., can be used to treat melasma. Usually six sittings at a gap of ten days produce good results.

Ultrasonic: Treatments with whitening jelly can be used in conjunction with chemical peels or MDA to further enhance results.

IPL treatments with pigmentation filter or currently Fractional Laser Treatments are being used to reduce pigmentation. Usually three to four treatments are needed at a gap of 15–20 days.

Although melasma tends to be a chronic disorder with periodic ups and downs, the prognosis for most cases is good. Just as melasma develops slowly, clearance also tends to be slow. The gradual disappearance of dark spots is based on establishing the right treatment combination for each individual skin type. Melasma that does not successfully respond to treatment is because sun exposure is not avoided.

Peri-Orbital Hypermelanosis (Undereye dark circles): This is a very common disorder among the Asian population, especially the Indian population. Undereye circles, which are dark pigments around the peri-orbital area, may be hereditary, or due to stress or eye strain. Also, those suffering from allergies seem to have dark circles. Smoking, drinking a lot of alcohol, coffee, soda, or caffeine beverages will usually make dark circles worse. Diet also plays a role and not having a healthy diet can affect it as well. A lack of sleep is a big culprit, so pulling all-nighters—whether for school or fun—can also contribute to dark circles around the eyes.

Treatments of Peri-Orbital Hypermelanosis: Once the cause has been identified there are several available solutions for treatment of this common disorder. Using a good eye cream that has ingredients specifically targeted to this delicate area, twice a day, will help prevent moisture loss, hydrate the area, and assist the delicate skin in absorbing the moisture and active ingredients, while stimulating the blood supply. Also wearing sunglasses can help prevent further pigmentation when exposed to the sun.

Photomelanosis: This is increased pigmentation due to sun exposure; it is also known as sun damage or photo-damage. The pigmentation occurs on exposed skin, commonly on the face, neck, and back. The pigmentation may be patchy or a diffused darkening of the exposed skin.

Hyperpigmentation: Hyperpigmentation is the production of excess melanin causing dark spots on the skin. Age spots, liver spots, freckles, sun spots, and pregnancy mask are all types of hyperpigmentation.

Causes of Hyperpigmentation: Common causes of skin hyperpigmentation are excessive sun exposure, hormonal changes, heredity, acne, and irritations

from skin treatments and products. Hyperpigmentation may also be caused by inflammation, or by other skin injuries, including those related to acne vulgaris. In situations where hyperpigmentation is caused by acne or some other cause of skin inflammation, it is known as PIH, or post-inflammatory hyperpigmentation. People with darker Asian, Mediterranean, or African skin tones are also more prone to hyperpigmentation, especially if they have excess sun exposure.

Many forms of hyperpigmentation are caused by an excess production of melanin. Hyperpigmentation can be diffuse or focal, affecting such areas as the face and the back of the hands. Melanin is produced by melanocytes at the lower layer of the epidermis. Melanin is a class of pigment responsible for producing color in the body in places such as the eyes, skin, and hair. As the body ages, melanocyte distribution becomes less diffuse and its regulation less controlled by the body. UV light stimulates melanocyte activity, and where concentrations of the cells are denser than surrounding areas, hyperpigmentation is affected. It can also be caused by using skin-lightening lotions. (4)

Post-Inflammatory Hyperpigmentation: a very common condition for those with more color in their skin. Many types of trauma, inflammation, medications, cosmetics containing alcohol or fragrance, and skin conditions including: acne, eczema, contact dermatitis. Systemic disease such as Addison disease, liver disease, pregnancy, and pituitary tumors can also cause hyperpigmentation.

Acne Vulgaris: one of the biggest challenges to women with brown skin who seek clear, glowing complexions. In addition to the acne, women with brown skin must also face hyperpigmentation—skin darkening in spots or patches—which occurs in response to the acne outbreak.

Acne Rosacea: not one of the skin problems typically associated with skin of color. A main feature of rosacea is redness or erythema of the face. But rosacea does occur in people of color, including African Americans, Latinos, and Asians, and is often undiagnosed or misdiagnosed.

Eczema: one of the more common skin problems for individuals with brown skin, including those of Asian, Latino, and African descent. It is felt to be the second-most-common skin disease in African Americans. Although it is unknown if the incidence of eczema is increased in Latinos, one study found a significantly higher percent of Mexican American adolescents with eczema than white and

African American adolescents.

Keloids: Abnormal healing of the skin occurs frequently in individuals with brown skin. When skin is injured, it may heal with one of several types of scars (keloids): normal (level with the surrounding skin), atrophic (depressed), hypertrophic (slightly raised), and keloidal (large and raised).

Seborrheic Dermatitis: often referred to as dandruff, is a common problem for many women with brown skin. Areas of involvement are the hairline and scalp, as well as the eyebrows, the area between the nose and corners of the mouth (nasolabial folds) and the ears.

Skin Cancer: People with brown skin often have a false sense of security when it comes to skin cancer. While individuals with increased skin pigmentation have added protection against the UV rays of the sun, it is dangerous to assume that darker skin exempts one from this serious skin problem.

Hypopigmentation: Areas of the skin that have lost their pigment completely when there are no longer functioning melanocytes in that area to create pigment, leaving white spots or patches on the skin. Hypopigmentation can result from overbleaching, or extensive trauma from UV light, lasers, or heat. Examples of hypopigmentation include:

Vitiligo: Vitiligo causes smooth, white patches on the skin. In some people, these patches can appear all over the body. It is an autoimmune disorder in which the pigment-producing cells are damaged. There is no cure for vitiligo, but there are several treatments, including cosmetic cover-ups, corticosteroid creams, or ultraviolet light treatments.

Albinism: Albinism is a rare inherited disorder caused by the absence of an enzyme that produces melanin. This results in a complete lack of pigmentation in skin, hair, or eyes. Albinos have an abnormal gene that restricts the body from producing melanin. There is no cure for albinism. People with albinism should use a sunscreen at all times because they are much more likely to get sun damage and skin cancer. This disorder can occur in any race, but is most common among whites.

Pigmentation loss as a result of skin damage: If you've had a skin infection, blisters, burns, or other trauma to your skin, you may have a loss of pigmentation in the affected area. The good news with this type of pigment loss is that it's frequently not permanent, but it may take a long time to repigment. Cosmetics can be used to cover the area, while the body regenerates the pigment.

(5)

3.2.3.4 TIPS TO ACHIEVE HEALTHY, BEAUTIFUL SKIN

- Mandatory daily use of broad-spectrum UVA & UVB Sunblock – SPF30+
- Wash skin daily with mild nonsurfactant cleanser, use a clean towel to pat dry, do *not* rub the face harshly, or share towels with others. The face should be washed twice, both morning and evening.
- Drink water—an average adult typically needs 10–12 eight-ounce glasses a day. I prefer to drink room-temperature or hot water with a slice of lemon.
- Use quality skin care products that hydrate and repair the skin, and avoid products that clog your pores.
- Use lightweight facial makeup foundation or powders; this will allow the skin to breathe and prevent further breakouts. Personally I feel the less used the better; if you take care of your skin you will have a natural radiance.
- The skin is a very sensitive organ and given the level of abuse it goes through, is remarkably resilient. However, this resilience decreases as we age, and opens the door for bacteria, viruses, microbes, parasites, *etc.* that invade the skin. In climates such as Asia where it is hot and polluted, especially in the cities, environmental damage also plays a role in the aging process.
- If you have a tendency to break out, seek professional help to treat it and go for the root cause of the blemish, rather than using makeup to cover skin blemishes and trauma. This can save you permanent damage with scars and or ice-pick marks.
- Avoid skin trauma like tattoos, body piercing, *etc.* These abuses can age the skin, and expose it to bacteria and other viruses.
- When you notice skin discomfort, dryness, itchiness, reddening, discoloration, blemishes, lesions, bruising, tags, moles, bumps, warts, corns, calluses, or other skin trauma, seek professional help, as some conditions may get worse over time if not treated.
- Diet plays a crucial part in skin health, so eat wisely. Your diet should contain plenty of green leafy vegetables. Eat seasonal fruits and add nuts to your diet; if you have health issues it is wise to seek a nutritionist, or dietician, to establish a healthy diet to suit your unique skin and bodily needs.
- Make regular monthly visits with a skin care professional to help maintain

healthy skin. Having topical treatments such as Lactic Peels, chemical peels, microdermabrasion, and laser therapy specific for Asian skin.

- Treat yourself to regular spa treatments; having a regular full-body massage is not only relaxing but good for you too. A deep massage is good to maintain healthy skin, since the kneading breaks up old fat deposits and releases toxins. The sauna and steam bath are recommended to help keep skin moist, supple, healthy, and toned. Drink water to hydrate before and after your sessions.
- Minimize exposure to tanning machines, lamps, beds, etc. The tanning process is actually a form of skin abuse, and can result in premature aging of the skin and promote hyperpigmentation.
- Always use high-quality and well-made skin care products. Using a daily program that consists of cleanser, exfoliator, moisturizer, and SPF 30 is highly recommended. The reward is to enjoy clear, healthy, blemish-free, radiant, glowing, beautiful skin for many years.
- Avoid cheaply made skin creams with a lot of chemicals. Products that are highly fragranced, with artificial colors, binders, emulsifiers, etc., can cause some people to develop allergies, leading to rashes and pigmentation issues.
- In order to maintain healthy skin and overall health, a total avoidance of alcohol, tobacco, and illegal drugs is advisable. These are all toxic to the body and after many years of such abuse, the skin can become wrinkled, saggy, dry, and itchy, and can lose its suppleness. Reducing or eliminating caffeine, sugar, and soft drinks will also benefit the skin and overall health.

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PART 3.2.4

COMPROMISED SKIN IN THE ELDERLY

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ABSTRACT

This chapter provides a thorough look at the structure and chemistry of the skin. It draws upon, and consolidates, the enormous body of literature on the subject and brings it together to form a solid foundation for the reader's understanding of skin aging as well as describing treatments of aging skin with selected effective "cosmeceuticals." We live in a culture that values a youthful appearance at a time when the population is aging rapidly in developed countries. According to the United States census there were ~57 million Americans age 60 and older in 2010, nearly a 25% increase in the ten years since the 2000 census. As people live longer, populations in Western Europe and Japan are also aging.

It is well known that the appearance of the skin can have dramatic effects on the overall impression of age. Both wrinkles (1, 2) and inhomogeneity of skin color (3–6) contribute significantly to the apparent age of individuals. The combination of the effect of skin appearance on apparent age and an aging population with the desire to look youthful has, not surprisingly, led to enormous interest in products that might make skin appear more youthful.

Skin aging is often broken into two categories referred to as intrinsic aging and extrinsic aging (7–9). *Intrinsic aging is the unavoidable effect of time.* It occurs on all body sites and is considered to be independent of lifestyle. Extrinsic aging results from lifestyle choices. The main factor is photoaging brought about by exposure to ultraviolet (UV) light from the sun (7, 10–12). While sun exposure is considered the main extrinsic factor leading to skin damage, it is becoming increasingly clear that exposure to cigarette smoke can lead to significant skin compromise both in smokers(13–21) and in nonsmoking spouses of smokers (22).

This chapter reviews the consequences of both intrinsic and extrinsic skin aging. Mechanisms underlying photoaging will be discussed, as will attempts to improve aging skin and at least partially reverse the effects of time and extrinsic insults.

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3.2.4.1 STRUCTURE OF THE SKIN

We begin with a brief review of skin structure to facilitate our discussion of skin aging. This subject is discussed in more detail in other chapters to this book. Discussion of the structure of skin will naturally refer to its various layers (23, 24) or strata. The skin’s multiple layers work together to perform its multiple functions. [Figure 1](#) is a hematoxylin and eosin (H&E) stained image of a cross-section of human skin.

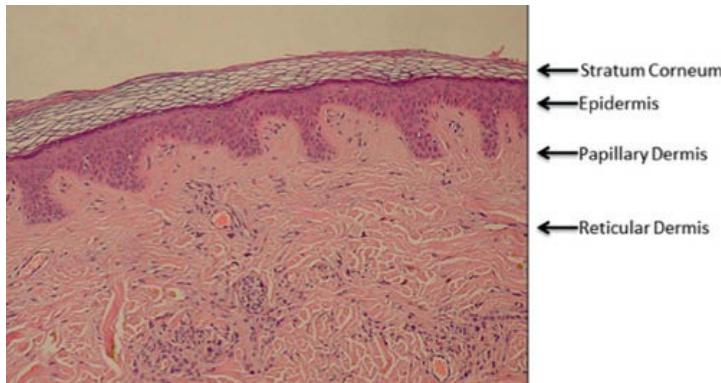


Figure 1 (H&E stained cross section of human skin labeled to show the various layers. The “basket weave” appearance of the *stratum corneum* is an artifact of processing. [http://en.wikipedia.org/wiki/Epidermis_\(skin\)](http://en.wikipedia.org/wiki/Epidermis_(skin)) public domain image)

The epidermis and dermis are fundamentally different types of tissue. The epidermis overlays the papillary dermis and is composed of cells. The main type of cell of the epidermis is the **keratinocyte**. Keratinocytes are found from the basal layer to the granular layer where they are transformed into the corneocytes of the *stratum corneum* (SC). Keratinocytes make many proteins including keratins, the main structural proteins of the SC (25). Two other important cells in the epidermis are melanocytes and Langerhans cells.

The **dermis** has two layers, papillary and reticular. The hypodermis or subcutaneous tissue is sometimes considered part of the dermis but will not be considered in this review. The dermis is a fibrous tissue composed primarily of collagen, elastin, and proteoglycans, which form the dermal matrix. The papillary dermis is named for the dermal papilla protruding into the epidermis (Figure 1). The fibroblasts, which make the proteins and proteoglycans of the dermis, are found in the dermal matrix (24).

The main structural protein of the dermis is collagen. Collagen has a unique amino acid sequence. Every third amino acid is glycine, which does not have a side chain. This allows collagen to form a compact triple-helix with three collagen chains winding around each other as if in a braid. Each triple helix is about 300 nm long and 1.5 nm in diameter (26). Collagen is synthesized in fibroblasts as procollagen and exported from the cell into the dermal matrix where it is processed by proteolytic enzymes, which remove sections from both ends of procollagen to form 300-nm filaments. These filaments then aggregate in the extracellular space to form collagen fibers.

The matrix in the papillary dermis is loosely packed with fibers compared to the tightly packed reticular dermis (24). The papillary dermis contains type III

collagen, which is found at relatively low levels in the reticular dermis where type I collagen predominates (27–29). Oxytalan fibers are thin (10–12nm) elastic fibers found perpendicular to the dermal-epidermal junction in the papillary dermis (30, 31). Elaunin fibers are also found in the papillary dermis parallel to the plane of the skin (30).

Elastin provides elasticity to skin (32). In elastin, four protein chains are bound together through the desmosine cross-link. Elastin has an amino acid residue called allysine, made from lysine by the enzyme lysyl oxidase. The desmosine cross-link is formed between three allylisine chains and one lysine chain. Amorphous elastin fibers surrounded by fibrillin microfibrils are found predominately in the reticular dermis (33, 34) ([Figure 2](#)).

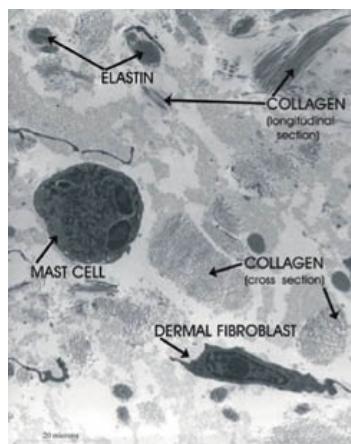


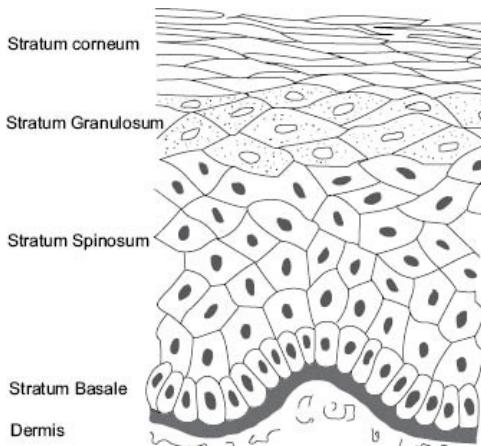
Figure 2: Electron micrograph of the reticular dermis showing collagen, elastin, a fibroblast, and a mast cell. (© Raymond Boissy, used with permission)

Glycosaminoglycans or GAGS bind water in the dermal matrix (35) keeping the dermis hydrated. Hyaluronic acid (HA), a major component of GAGS, is a very high molecular weight. GAGs such as chondroitin sulfate and keratan sulfate bind to core proteins to form proteoglycans. Proteins called link proteins bind proteoglycans to HA in a noncovalent proteoglycan complex that is very hygroscopic.

Figure 2 is an electron micrograph of the reticular dermis showing collagen and elastin fibers as well as a fibroblast and a mast cell. Mast cells contain histamine granules and release histamine in response to heat or irritants. The reticular dermis is much more densely packed with fibers than the papillary dermis.

The dermal matrix is significantly affected by both intrinsic and extrinsic aging as will be discussed below.

Skin barrier function resides primarily in the epidermis and barrier may be considered “*la raison d'être*” of the epidermis (36). Layers of the epidermis are shown schematically in [Figure 3](#).



[Figure 3](#): Diagram showing layers of the epidermis (illustration by Robin M. Wickett)

The epidermal barrier defends the body from water loss to the environment, absorption of noxious chemicals from the environment, and microbial infection. These defensive functions depend critically on the top layer, the *stratum corneum* (SC), and are thought to be integrated with SC formation and homeostasis (37, 38).

For 30 years the most common model used to organize thinking about the SC barrier has been the “bricks and mortar” model proposed by Elias (39). This model is based on the fact that corneocytes are surrounded by the extracellular lipids of the SC. Thus the corneocytes are modeled as bricks and the lipids as mortar. The model has proven useful for representing the phenomena associated with describing ingredient skin penetration. Moderately hydrophobic molecules are thought to penetrate the SC by diffusing through the lipid mortar, winding their way around the corneocytes bricks (40). Harding has described an updated version of the bricks-and-mortar model that includes the presence of the desmosomes that hold SC cells together prior to desquamation and the role of natural moisturizing factor in SC function (41). This model is illustrated in [Figure 4](#).

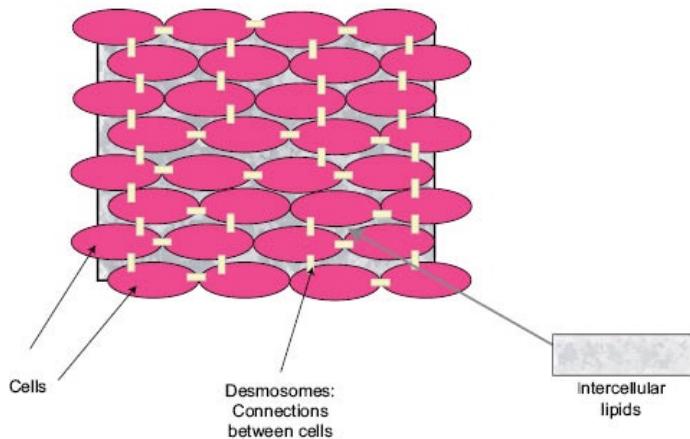


Figure 4: Schematic of the “bricks and mortar” model for human *stratum corneum* illustrating the corneocyte “bricks,” the intercellular lipid “mortar,” and the desmosomes connecting the corneocytes. (M.O. Visscher, used with permission)

The “bricks and mortar” of the SC barrier are formed in the *stratum granulosum* (SG) (Figure 4) where keratinocytes are transformed to form the corneocytes “bricks” and lipids are released into the intercellular space to form the “mortar.” The SG is named for the two types of granules that appear in the cells, keratohyalin granules (24) that are composed of protein and lamellar bodies that primarily contain lipids (42, 43).

A complex process occurs in the SG: the nucleus is digested, the cytoplasm disappears, and lipids are released into the intercellular space to form the mortar (39, 44, 45). The keratin intermediate filaments aggregate to form microfibrils (46, 47) inside the corneocytes (48, 49), aided by filaggrin from the keratohyalin granules.

Filaggrin is an acronym for filament-aggregating protein (50–53). Filaggrin contains a high level of positively charged amino acids, allowing it to interact with the negatively charged epidermal keratins (54). Filaggrin is then modified to come off the microfibrils, through conversion of the positively charged amino acid arginine to uncharged citrulline by the enzyme peptidyl arginine deminase (PAD) (55, 56). After release from the microfibrils filaggrin is digested by proteolytic enzymes to produce the amino acid components of the natural moisturizing factor (NMF) of the *stratum corneum* (57–62).

NMF consists of lactate, amino acids from filaggrin breakdown, and pyrrolidone carboxylic acid (PCA) formed from the amino acid glutamine (63–

65). These natural moisturizers are important to maintain proper hydration of the SC allowing it to be flexible and to desquamate properly (57, 66–69). Digestion of filaggrin to NMF is thought to happen in the mid-*stratum corneum* (70).

In the *stratum granulosum* (SG) the keratinocyte cell membrane is replaced by the corneocyte cell envelope, a very tough structure made of cross-linked protein with lipids covalently attached to its surface (71–75). The resulting corneocytes or squames (the bricks) are flat cells that tend to be in the shape of either a hexagon or pentagon. Corneocyte size and distribution have been investigated by several authors. A single corneocyte is about 25 μm on a side, with a surface area ranging from about 550 to 1300 μm^2 depending on age and body site (76–79), and a thickness of about 0.5–1.0 μm (80, 81). Rougier *et al.* (79) reported corneocyte surface area on most body sites investigated to range from 900 to 1100 μm^2 but to be as low as 500 μm^2 on the forehead. A negative correlation was found between corneocyte size and the penetration of benzoic acid through skin *in vivo*. The *stratum corneum* is 12–16 cell layers thick on most body sites, but it can vary from as little as nine cell layers on the forehead or eyelids to as much as 25 on the dorsum of the hand and 50 or more on the palms or the soles of the feet (82, 83).

The lipids that are released into the intercellular space as the SC forms are glucosyl ceramides, cholesterol, cholesterol esters, and long-chain fatty acids. In the intercellular space the glucosyl ceramides are converted to ceramides (84, 85). SC ceramides are polar lipids, but much less hydrophilic than the phospholipids of the original cell membrane. [Figure 5](#) shows the structure of some of the main SC ceramides with both the original and new nomenclature that is used for them. Phospholipids from the keratinocytes of the viable layers are broken down by phospholipases (86–88) in the lower *stratum corneum*. After this extracellular processing, SC lipids spontaneously organize into multiple layers between the SC cells (89). This layered structure is critical to the barrier function of the skin.

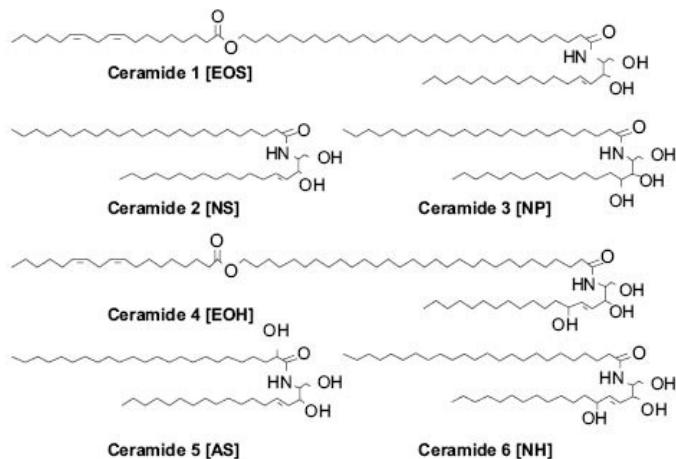


Figure 5: Key ceramides of the SC barrier.

3.2.4.2 SKIN AGING: CHANGES IN THE EPIDERMIS AND STRATUM CORNEUM

A general trend for corneocytes to increase in surface area with age has been reported by several groups (77, 79, 90-93). The reported increase in surface area between young adults and adults over 55 ranges from 8 to 14% depending on body site and method of measurement. [Figure 6](#) shows corneocyte size data from two different age groups reported by Grove *et al.* (91, 93).

Note the extreme variability in the data from the older age group compared to the narrower distribution in the younger group. The average size of corneocytes is increased in the older age group but there is considerable overlap between the two data sets.

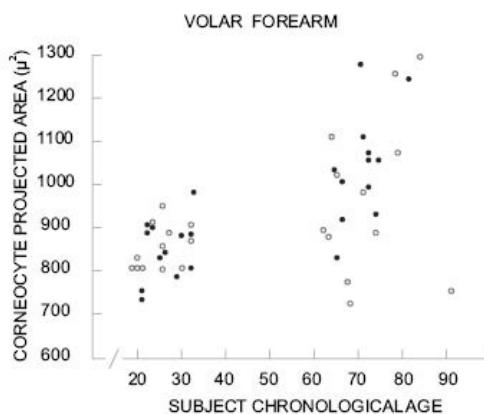


Figure 6: Projected corneocyte surface area for two age ranges for men (●) and women (○). Reprinted from Grove *et al.* (93) with permission of the Society of Cosmetic Chemists.

While the “bricks” of the SC may increase in size with age, the “mortar” content is apparently decreasing. Imokawa *et al.* reported a gradual decrease in total SC ceramides with age (94). Rogers *et al.* reported progressive reductions in fatty acids, ceramides and cholesterol with age in subjects ranging in age from 21–60 years on the hands, legs, and face (95). The reduction in ceramide I (EOS) linolate was especially noteworthy.

SC barrier function is affected by both intrinsic and extrinsic aging (96), but it is relatively robust to both and changes are not always in the direction that one might expect. The most common and best-validated method for determining SC barrier function *in vivo* is by measurement of the passive diffusion of water through the skin by measuring the rate water evaporates from the surface of dry nonsweating skin. This parameter is known as transepidermal water loss (TEWL) (97–99).

Several groups have studied the effects of age on SC barrier function by measuring TEWL. Results have varied. Roskos and Guy (100) reported no correlation between baseline TEWL on the ventral forearm and age for subjects ranging in age from 20 to 90 years, and Marks *et al.* (101) did not see a significant correlation to age on the forearm with a similar age range, but a trend to *lower* TEWL was observed for subjects above 55. Rougier *et al.* found no significant change in TEWL on the upper outer arm in subjects ranging in age from 20 to 80 years (79). Kligman (102) reported that TEWL on both the forearm and lower legs averaged slightly *lower* for a group of individuals aged 66–81 compared to a 19–26 year old cohort. Leveque *et al.* (77) reported TEWL from the arm to be approximately constant from 25 to 65 years of age but to decline slightly between ages 65 and 75.

Wilhelm *et al.* (103) compared TEWL data from a young group (26.7 ± 2.8 years) and an older group (70.5 ± 13.8 years) on several body sites. TEWL was significantly *lower* from the older age group on all sites except the palm. Saijo *et al.* (104) measured TEWL from the dorsum of the hand and the dorsum of foot on children from one to five years of age, young adults from 21 to 34 years of age, and older adults ranging from 63 to 78 years of age. Aged individuals had the lowest TEWL values on both body sites. There was no difference in TEWL from the dorsum of the hand between children and young adults. Ghadially *et al.* (105) reported average TEWL of 4.43 ± 0.16 g/(m²·hr) for a group of six individuals over 80 compared to 6.41 ± 0.93 g/(m²·hr) for a group of 21 subjects under 30.

Schwindt *et al.* (106) found significantly lower TEWL on the backs of

subjects averaging 69.8 years of age compared to subjects averaging 27.7 years old. In a recent study Luebberding *et al.* reported TEWL data from six different body sites on 150 women divided equally into five age groups, 18–29, 30–39, 40–49, 50–59, and 60–80 (107). No correlation was found between TEWL and age on the neck, forearm, or back of the hand. TEWL was negatively correlated to age on the forehead and cheek. Interestingly, TEWL on the décolleté area was positively correlated to age, ranging from 5.62 gm/m²-hr on the youngest group to 7.86 gm/m²-hr on the oldest, with a high level of statistical significance. In our review of the literature these data from the décolleté are the only ones to show a statistically significant increase in TEWL with age.

The SC barrier to penetration of compounds into the skin has also been evaluated as a function of age. Roskos *et al.* compared the penetration of six drugs of differing polarity into the skin of subjects 22–40 years of age and over 65 years old *in vivo* (108). Permeation rates for the two most hydrophobic drugs, testosterone and estradiol, were not significantly different between the two age groups but hydrocortisone, benzoic acid, acetylsalicylic acid, and caffeine all penetrated the skin of the older group at a significantly *lower* rate than into the younger. Rougier *et al.* also reported that benzoic acid penetrated older skin significantly more slowly than younger *in vivo* (79).

Analysis of all these results leads to the somewhat surprising conclusion that basal SC barrier function is relatively unaffected by age from childhood through late middle age and then may actually begin to increase slightly sometime after age 60. It is notable that this apparent improvement in baseline barrier function occurs in spite of the reduction in SC barrier lipids discussed above. Rogers *et al.* point out that while lipids decrease, the ratio of ceramides to other SC structural lipids remain approximately constant (95). It is also possible that skin permeation of some compounds through SC is slower because of the larger corneocytes. Rougier *et al.* found a negative correlation between corneocyte size and the skin permeation rate of benzoic acid *in vivo* (79).

Most studies indicate that the reactivity of skin to irritant may actually decrease with age, or at least is not more marked with age as one might expect. Bettley and Donoghue reported fewer reactions to patch testing with soap in subjects 50 and older compared to subjects 10–49 years of age (109). Grove *et al.* reported lower skin reactivity to a variety of irritants including dimethyl sulfoxide, ethyl nicotinate, and lactic acid (93, 110) in older subjects. Maibach and coworkers (106, 111) and Robinson (112) observed lower reactivity of older skin to sodium lauryl sulfate (SLS). Robinson also reported that reaction to

another strong irritant, octanoic acid, was significantly lower in subjects more than 55 years old while reactions to the weaker irritants acetic acid and decanol were only directionally reduced with age (112). Bowman *et al.* performed a ten-day cumulative irritation test comparing 26 subjects age 18–45 with 26 subjects age 65–80 using 11 products of widely varying irritancy (113). They found no statistically significant differences in response between the age groups, but the response was directionally less in the older age group for all the compounds that produced significant irritation.

While the static barrier function of the epidermis does not seem to diminish with age, recovery of barrier function after disruption has been found to be significantly slower in older skin. Ghadially *et al.* disrupted SC by either tape stripping or acetone treatment to the point that TEWL was increased to the range of 20–30 g/m²-hr (105). Recovery of the barrier was quantified by measuring TEWL over the next several days. After acetone treatment young skin (under age 30) recovered its barrier function by 50% at 24 hours, while older skin (over 80) had only recovered 15% at 24 hours. Younger subjects took about four days to recover to 90% of their original SC barrier to TEWL while older skin required seven days to reach this level of recovery regardless of the type of insult (tape or acetone). The slower recovery of barrier function may be related to slower rates of epidermal renewal in older skin.

It is well known that corneocytes are continually shed into the environment and are being continually replaced by cells generated in the SG (see above). The SC “turnover time” is most frequently measured by staining the SC with the fluorescent dye, dansyl chloride, and monitoring the rate of dye disappearance with a UV light. When all of the fluorescence has disappeared, the SC has turned over completely (114). Grove and coworkers reported that SC turnover time is increased in elderly skin (76, 93). The turnover time for SC on the volar forearm increased from 19.8 ± 1.4 days in cohort under 35 years old to 28.1 ± 2.7 days in an over 60 age group. Similar changes were seen on the inner arm. These results confirmed the positive correlation between age and the time to turn over the SC reported by Roberts and Marks (115).

Grove found the number of SC cell layers was approximately constant between age groups, indicating that the increased SC turnover time results from decreased epidermal cell proliferation rates with age. Marks reported a small but statistically significant decline with age in the number of epidermal cells in the DNA synthesis phase (116). Engelke *et al.* investigated differences between young normal skin, young dry skin, aged normal skin, and aged dry skin (117)

using an antibody that stains the nuclei of proliferating cells (Ki-S3) (118). Antibody staining indicated a significant reduction in epidermal proliferation rate in aged “normal skin” compared to young normal skin. A slower rate of epidermal cell proliferation is consistent with the increased time to repair a disrupted SC barrier as reported by Ghadially (105). Grove also reported that small chemically induced blisters healed more slowly in subjects between 65 and 70 years of age than in subjects 18–25 years of age (119).

The greater tendency of older subjects to develop dry skin is well known (120–122). Elderly dry skin seems to be especially prone to itching (120, 123–126), which can sometimes be severe. Dry skin in the elderly tends to develop some distinctive patterns, especially on the legs. [Figure 7](#) below shows dry skin that developed on the legs of three elderly subjects who lived in the desert Southwest. These pictures were taken after one week of washing twice daily with a standard soap and no use of moisturizers. Notice the distinct pattern of lines on some of the subjects.



Figure 7: Dry skin on the lower legs of elderly subjects (70–90 y.o.) after washing twice daily with a standard soap and avoidance of moisturizer use.

This pattern of lines frequently develops in elderly subjects but occurs rarely if at all in younger subjects (R.R. Wickett, personal observations). Treatment with an effective moisturizer reduces or eliminates the pattern in some subjects but not in others. In [Figure 8](#) below we see that moisturizer treatment of the right legs has nearly eliminated the pattern in the subject on the left but had little effect on the subject on the right.



Figure 8: Dry skin resulting from soap use on the legs two elderly subjects. After one week of washing with soap and no moisturizer use the right leg of each subject was treated with an effective moisturizing lotion for two weeks while soap treatment continued on both legs.

It is not entirely clear why the elderly are so much more prone to developing dry skin. One possibility is reduction of levels of NMF (see above) in elderly skin. It has been shown that NMF levels are lower in dry skin (62). Data on the effect of age on NMF levels do not present a consistent picture. One of the key moisturizing components of NMF is the very hydrophilic molecule pyrrolidone carboxylic acid (PCA) (56, 57) (see above). Harding *et al.* reported lower levels of PCA in elderly subjects and the difference was more marked deeper in the *stratum corneum* (70). Horii *et al.* reported a general decrease in free amino acids with increasing levels of dry skin in elderly subjects (127).

On the other hand, Jacobson *et al.* found that some SC amino acids decreased with age while others increased, but no difference was seen in total NMF free amino acids (FAA) normalized to protein content between young and old subjects (128). Takahashi and Tezuka (129) reported that NMF FAA actually increased in older subjects. However, both of these groups sampled the *stratum corneum* by scraping skin flakes from the surface rather than tape stripping to sample from lower levels of the SC as Harding *et al.* did. Further, as Tagami (96) has pointed out, Takahashi and Tezuka reported their results as FAA/corneocyte. Thus the higher levels of NMF FAA they report could be due to the presence of larger corneocytes in older skin (77, 78, 93) as discussed above.

Lactate is a component of the NMF that may be derived either from sweat (130, 131) or through anaerobic metabolism in the upper epidermis (132). Nakagawa *et al.* investigated the correlation between NMF components and SC stiffness, pH and hydration in summer and winter with healthy subjects (67). The only components that correlated significantly to SC stiffness and hydration were lactate and potassium. One might expect that the slower metabolism of aging skin reflected by the lower SC turnover rate might lead to lower levels of lactate in the SC. However, Prahl and coworkers reported that keratinocytes from aging skin produce higher levels of lactate *ex vivo* (133). It is well established that sweating rates decrease with age (134), which might lead to lower levels of lactate and thus stiffer, less well-hydrated SC.

Another factor in skin aging may be the reduction of sebum production that

occurs with age (135). Fluhr *et al.* have presented data from studies of asebic mice, indicating that glycerol derived from the hydrolysis of sebum contributes to skin hydration (136). Sebum production declines dramatically in women at menopause but does not really decline much in men before age 80 (135), so this seems unlikely to be the whole story either. It seems likely that the tendency for elderly subjects to develop dry skin has multiple contributing factors including lower PCA levels, the slower rate of SC renewal, lower levels of lactic acid due to reduced sweating, possibly reduced glycerol due to reduced sebum production, and other factors that have yet to be discovered.

There are been relatively few studies specifically comparing the effects of intrinsic and extrinsic aging on the SC. Tagami's group studied the dorsum of the hands of Japanese golfers who only wore a golfing glove on one hand (96, 137). Roughness of the skin surface was measured from silicon replicas. Interestingly, there was a significant negative correlation between the difference in roughness between exposed and covered hands and a golfer's handicap. Better golfers (lower handicap) had larger increases in roughness on their exposed hand. Hydration as measured by higher-frequency conductance was lower for the exposed site indicating a drier skin surface, but there was no difference in barrier function as measured by TEWL.

3.2.4.3 AGING AND THE DERMIS

Both intrinsic aging and photoaging affect the dermis far more dramatically than the epidermis. The papillary dermis is strongly affected by both intrinsic aging and chronic sun exposure. [Figure 9](#) shows the effect of aging on abdominal skin of 18-and 76-year-old females. At the time these micrographs were published (1955) the abdominal skin of women received very little sun exposure, so the changes represent the effect of intrinsic aging.

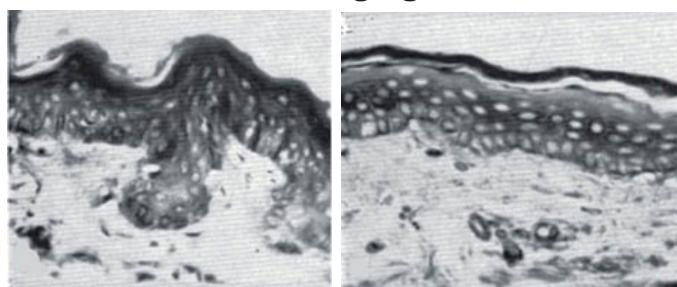


Figure 9: Abdominal skin from an 18-year-old female (left) and a 76-year-old female (right). Note the flattening of the dermal-epidermal junction as the papillary dermis diminishes with age.- (modified from Andrew [138] with

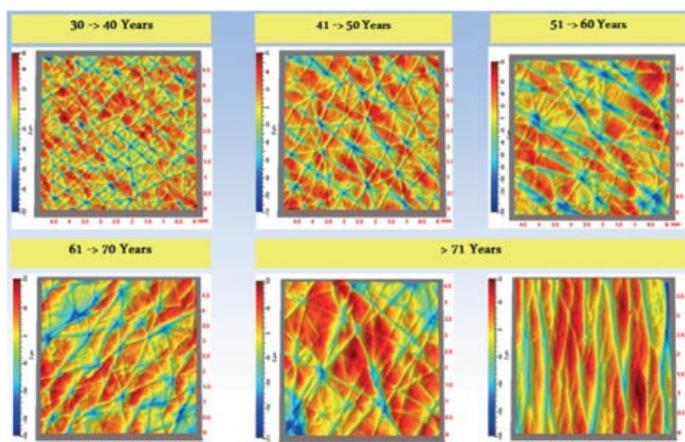
permission of the Society of Cosmetic Chemists).

This flattening of the papillary dermis makes the skin less resistant to shearing stress, and the skin of elderly people is more easily ulcerated than that of younger people.

The thickness of the skin varies with age and changes depend on body site. In general, skin thickness increases for the first two decades of life. After this initial period, skin thickness may be fairly constant until about age 60. De Rigal *et al.* reported ultrasound results showing that skin thickness on both the ventral and dorsal forearm decreased significantly with age, but only after age 60 (139). Gniadecka and Jemec, however, reported a gradual decline in skin thickness on the forearms and ankles starting at about age 20 (140). Skin on the forehead and buttocks was found to increase in thickness with age. Takema *et al.* also found skin thickness to decrease with age on the forearms and increase on the forehead, corners of the eyes, and the cheeks (141). They interpreted this increased thickness as the result of sun exposure on the face.

3.2.4.4 THE DERMATOGLYPHIC PATTERN OF THE SKIN

Young skin shows a complex pattern of shallow lines on the surface. This dermatoglyphic pattern is altered on aging and a pattern with fewer but deeper lines that become more oriented emerges (93). Natural lines of tension in the skin called Langer's lines may influence the directional orientation. Hassan Zahouani and coworkers have studied these line patterns in detail (142–144). [Figure 10](#) shows typical patterns from subject in various age ranges, pseudo-colored to indicate topography.



[Figure 10](#): Changes with age in the dermatoglyphic pattern of human skin.

Figure courtesy of Hassan Zahouani, used with permission.

A highly aligned dermatoglyphic pattern can also be seen on the legs of some of the subjects in Figure 8 above.

In addition to the flattened dermal-epidermal junction (Figure 9) and changes in the dermatoglyphic pattern, intrinsically aged skin has diminished collagen synthesis leading to reduced levels of collagen in the dermis (145–147). El-Domyati *et al.* reported that there is gradual loss of the transverse elaunin fiber network and shortening and eventual reduction of the oxytalin fibers, which were nearly gone by the ninth decade of life in sun-protected skin. Underlying elastic fibers in the mid to deep reticular dermis of sun-protected skin remained relatively unchanged in amount with age (146). Focal loss of elastin and clumping of elastin fiber bundles has been observed in sun-protected elderly skin in some individuals (148).

3.2.4.5 AGING AND MECHANICAL PROPERTIES OF SKIN

Skin is a viscoelastic material. Measurement of mechanical properties of skin *in vivo* is complicated by its complex, layered nature (Part 1). It is difficult to deform the SC without producing significant deformations in the underlying layers, and of course it is impossible to deform the dermis without deforming the SC. The general principle is that smaller deformations (minimum strain) will help to localize the response to the SC, and larger deformation will reflect more on the properties of the underlying layers (149).

The most commonly used instrument for measuring skin elasticity is the Cutometer® (150–152). The Cutometer uses negative pressure to pull the skin vertically into the opening of the probe (151, 152). Negative pressures can be controlled between 20 and 500 mbar. The height the skin raises into the probe is determined by an optical system. Probes with diameters of 2, 4, 6, and 8 mm are available. In the most widely used mode, deformation is measured while vacuum is applied quickly, held constant for a defined period of time, and then released ([Figure 11](#)).

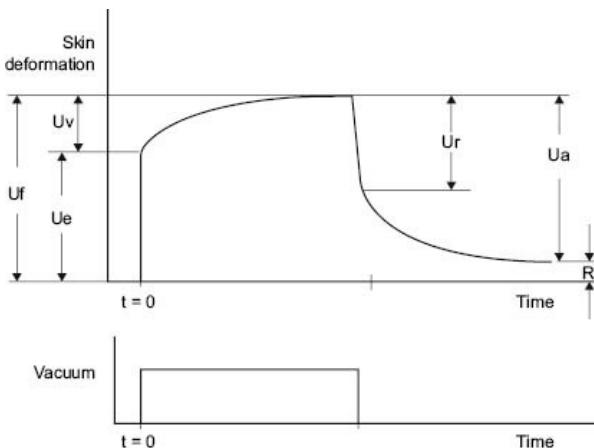


Figure 11: Schematic of Response of Skin to Cutometer

Table 1 defines parameters that can be obtained from the Cutometer curve.

TABLE 1 Cutometer Parameters from Figure 11

Ue	Elastic deformation of the skin due to the application of stress (vacuum)
Uv	Viscoelastic creep occurring after the elastic deformation
Uf	Total extensibility of the skin during stress application
Ur	Elastic recovery due to stress removal
Ua	Total recovery at the end of the stress-off period
R	Amount of deformation not recovered by the end of the stress-off period
Ua/Uf	Overall elasticity of the skin, including creep and creep recovery
Ur/Ue	Pure elasticity ignoring viscoelastic creep
Uv/Ue	Ratio of viscoelastic to elastic extension, called the viscoelastic ratio
Ur/Uf	Ratio of elastic recovery to total deformation

Cua, *et al.* (153) used the Cutometer® to evaluate age and body site differences in the viscoelastic properties of skin in a study with 33 subjects, 17 of them elderly averaging about 75 years, and 16 young subjects averaging about 28 years. Measurements were performed on 11 body sites. Generally, Uv/Ue increased while Ur/Uf decreased with age, but differences were not significant for all body sites.

Smalls *et al.* (154, 155) investigated the effects of age, body site and skin thickness on mechanical properties of skin with the Cutometer® using the 6-mm probe in a 30-subject study. Uf on the shoulder measured by the Cutometer® was negatively correlated to age with $r = -0.78$ and $p < 0.001$. Results are shown in [Figure 12](#).

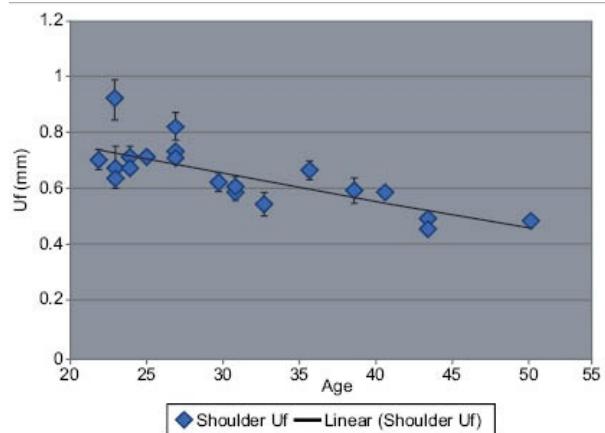


Figure 12: Uf on the shoulder versus age. Slope = -0.78 , Reprinted from (155) with permission.

Ur and Ur/Uf also showed significant negative correlations with age.

Krueger *et al.* (156) investigated the effect of age and body site on Cutometer parameters with a panel of 120 women divided evenly into four age groups as follows.

Group	Age Range
I	20–29
II	30–39
III	40–49
IV	50–65

All of the viscoelastic parameters in Table 1 were measured. The mean correlation of Uf with age was -0.56 . The parameter that had the strongest correlation with age was Ur/Uf, the ratio of immediate recovery to total deformation ($R^2 = 0.656$). [Figure 13](#) shows their Ur/Uf results for each age group and body site.

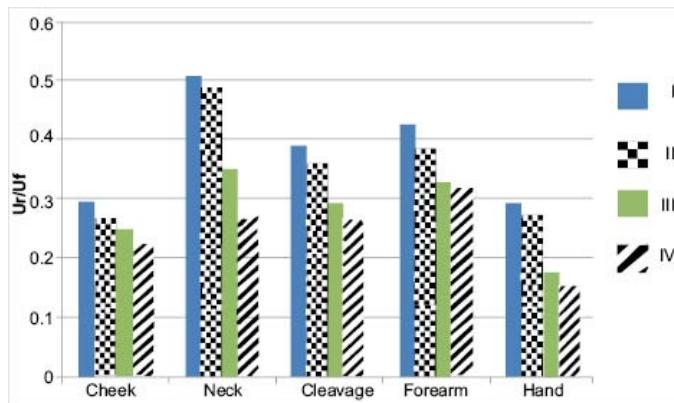


Figure 13: Ur/Uf as a function of age for five body sites. Data taken from

Krueger *et al.* (156).

Ryu *et al.* (157) also reported a strong negative correlation between Ur/Uf and age in a study of 96 Korean women ranging in age from 20 to 75 years.

The reduction in extensibility and even larger reduction in elastic recovery with age is consistent with studies showing changes in the elastic fiber network of skin with aging and photoaging (146, 158–161). Ezure *et al.* (162) reported that Ur/Uf decreased as “sagging” scores increased on the cheeks of Japanese women. Warrier *et al.* found significantly lower elastic recovery (Ur) on the cheeks of Caucasian women compared to age-matched African American women (163) and attributed the difference to less photodamage on the face of African American women because of sun protection by melanin. Other studies have also shown that Ur/Uf is negatively correlated with age (141, 164). Increases in Ur/Uf by product treatment could possibly be used to substantiate anti-aging effects on skin elasticity.

On the other hand, dynamic indentation experiments performed by Boyer *et al.* indicate that the skin becomes more extensible with age (165). The authors attribute this difference to the fact that the indentation experiments were made without a “guard ring.” The Cutometer deforms a small, well-defined area, determining the rheological properties of the skin, while dynamic indentation experiments deform a wider area and are proposed to be more reflective of the natural tension in the skin. Further discussion on the measurement and evaluation of skin elasticity and its relation to wrinkles and skin topology are extensively discussed in this book in the section on skin testing.

3.2.4.6 TELANGIECTASIA

Telangiectasia is the presence of visible blood vessels on the surface of the skin. It can occur both in sun-exposed and -protected areas of the skin with age. On sun-protected areas telangiectasia may develop because of thinning of the dermis.



Figure 14: Visible blood vessels (telangiectasia) on the outer thigh of a 64-year-

old male (nonsmoker).

Chronic sun exposure may lead to the development of telangiectasia (166, 167). Yano and coworkers have shown that acute sun exposure induces blood-vessel growth both by upregulation of vascular endothelial growth factor (VEGF) and downregulation of an important endogenous inhibitor of blood-vessel growth, thrombospondin (TSP-1) (168, 169). Upregulation of VEGF and downregulation of TSP-1 was seen in epidermal keratinocytes, endothelial cells, and dermal fibroblasts after UV-B exposure equivalent to two times the minimal erythema dose. Not surprisingly, new blood-vessel growth was also observed (169). The correlation between sun exposure and telangiectasia seems to be stronger in men than in women (21). Current smoking has also been reported to be strongly associated with the presence of telangiectasia (170).

3.2.4.7 PHOTOAGING MECHANISMS

The major factor in extrinsic aging of the skin is chronic sun exposure. It is well known that the majority of negative changes in skin's appearance, especially on the face, are primarily due to photoaging (171). Ultraviolet radiation affects skin cells through two mechanisms. The first is direct damage to DNA by UV-induced formation of DNA lesions, primarily pyrimidine dimers (7, 12, 172–174). DNA may also be damaged by reactive oxygen species (ROS) generated under the influence of UV exposure (175, 176). The ability to repair DNA damage through the Nucleotide Excision Repair system has been reported to decline with age (177), perhaps contributing to further compromise of sun-exposed elderly skin. Telomeres are especially sensitive to UV damage (178) and telomere shortening may significantly affect photoaging (172, 179, 180).

The second major factor in extrinsic aging is damage mediated by ROS, which in addition to damaging DNA, can cause skin changes either by direct action on cell surface receptors or by inducing the generation of cytokines (12, 173, 181–183). [Figure 15](#) illustrates the effect of ROS-induced cytokine release on keratinocytes and fibroblasts.

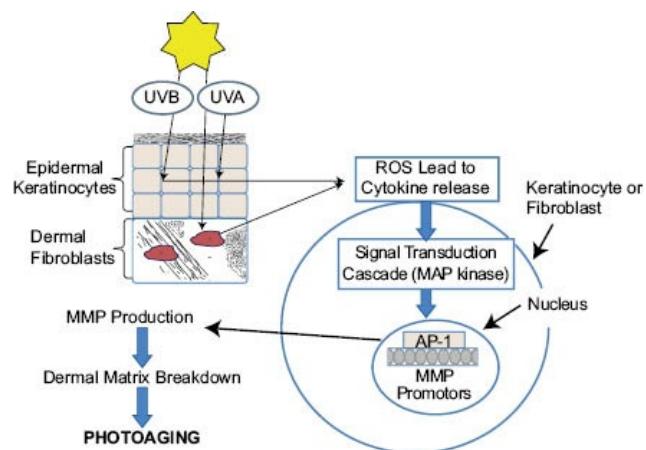


Figure 15: UV radiation affects keratinocytes and fibroblasts through similar mechanisms. Reactive Oxygen Species (ROS) generated by UV exposure lead to cytokine production that stimulates the Mitogen-Activated Protein kinase pathway. MAP kinases are generated that cause the production of the transcription factor AP-1 and promotion of the synthesis of Matrix Metallo Proteinases (MMP) that break down collagen in the dermal matrix.

In both cases the Mitogen-Activated Protein kinase pathway is activated (181). This pathway involves a complex cascade of protein kinases (enzymes that catalyze the phosphorylation of other enzymes) that leads to the formation of the transcription factor AP-1 (184, 185). AP-1 has two important effects that contribute to photoaging. It promotes the transcription of messenger RNA for the production of collagenase and other matrix metalloproteinases that break down the dermal matrix, and it reduces the sensitivity of fibroblasts to transforming growth factor beta (TGF- β), reducing collagen production (173).

The hallmarks of photoaging in histology are seen in [Figure 16](#). Both type I and type III collagen are reduced (146, 186, 187) except in the thin band right below the epidermis called the Grenz zone. The SC and epidermis may be slightly thickened, though not all authors agree on this point. The major characteristic difference between photoaging and intrinsic aging is the presence of elastosis (146–148, 188). There is actually a large accumulation of elastic fibers in the dermis of severely photoaged skin, but these fibers are dystrophic, with large accumulations of condensates including lysozyme and amyloid P protein (189), which protect the deranged elastin from digestion by elastase (190). The fibers that result from actinic elastosis provide little or no elasticity to the skin.

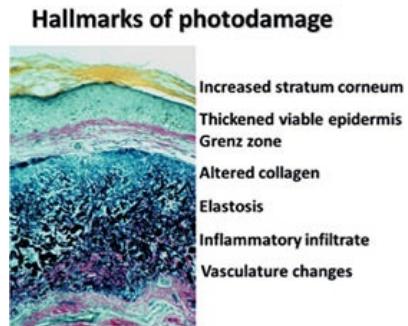


Figure 16: Hallmarks of photodamage as seen in histology. From Robert Lavker, MD. Skin aging and photoaging: Presented to Congress of the International Society for Bioengineering and the Skin, Orlando, FL. October 28, 2004, reprinted with permission.

As solar damage progresses, inflammatory cells migrate into the dermis. The vasculature changes that may result in telangiectasia have been discussed above.

3.2.4.8 PHOTOAGING AND APPEARANCE

The visual signs of photodamage are well known and include wrinkles around the eyes and mouth, hyperpigmentation (age spots), telangiectasia, and deep furrows in the skin in exposed body sites (158, 191–193). [Figure 17](#) shows classic signs of photoaging on some well-known faces.

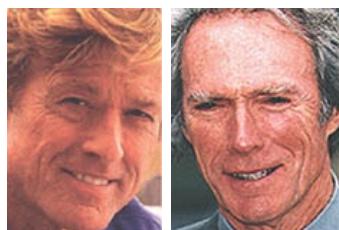


Figure 17: Classic signs of photoaging (public domain publicity stills taken from www.skinema.com)

Both subjects have extensive wrinkling in the peri-orbital or so-called crow's-foot area and around the mouth. The subject on the right spent much time filming in the sun while wearing a cowboy hat. Notice that the skin on the forehead looks better than the skin lower on the face.

Corcuff *et al.* analyzed wrinkling in the peri-orbital area on 81 subjects ranging in age from six to 76 years (194) using image analysis of silicone replicas. Results are reprinted in [Figure 18](#).

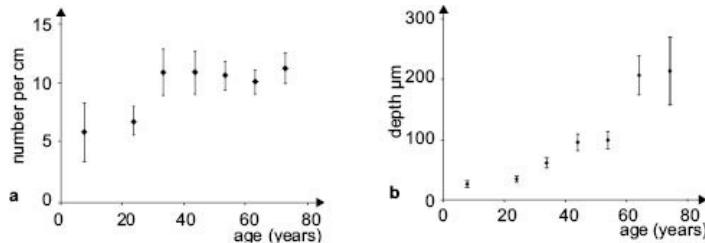


Figure 18: Evolution of “crow’s-foot” wrinkles with age. Number of wrinkles per $\text{cm}^2 \pm \text{S.E.}$ b. means depth of wrinkles ($\mu\text{m} \pm \text{S.E.}$). Reprinted from (194) with permission of the Society of Cosmetic Chemists.

Interestingly, the number of wrinkles peaked at about age 35, but mean wrinkle depth increased by eightfold between age 35 and 65.

Sun-induced changes can occur at an early age if sun exposure is sufficiently extreme. Corcuff, de Rigal, and Leveque did a very interesting study on riders in the 1985 Tour de France (195). Professional cyclists spend many hours in the sun and wear tight-fitting short-sleeved shirts while training, providing a sharp divide between exposed and sun-protected areas of skin. Even though the average age of the riders was 25 years, there were dramatic differences between protected and exposed skin. The line pattern on the exposed skin sites of these young cyclists resembled that seen in the >71-year age group in Figure 10. The average furrow depth in the exposed sites was $42 \pm 1.5 \text{ mm}$ on the exposed sites compared to $29.5 \pm 1.0 \text{ mm}$ on protected sites.

3.2.4.9 COMPROMISED ELDERLY SKIN TREATMENTS

a. Sunscreens—an ounce of prevention

Since sun exposure plays such an important role in compromising skin, it seems obvious that sunscreen use is a good strategy to prevent damage. However, this is difficult to prove in humans. Long-term studies are required, and it is not ethical to give people a “placebo” sunscreen.

The most convincing study on sunscreen use and photoaging that has been published to date was done as part of the Nambour Skin Cancer Prevention Trial, a randomized, community-based trial in Nambour, Australia (196, 197). In this study, conducted from 1992 to 1996, some subjects were required to apply an SPF 15+ sunscreen daily while others only used sunscreens at their discretion. The photoaging trial was conducted on a subset comprised of 903 subjects who were under 55 years of age to emphasize the effect of photoaging compared to intrinsic aging (198). Dietary supplementation with β -carotene was also

investigated. Photoaging was measured by using a validated method based on grading silicone replicas from the backs of the hands on a scale ranging from 1 (no damage) to 6 (deep horizontal lines and loss of vertical lines) (199, 200). Reported sun exposure was similar between the daily and discretionary sunscreen groups. Participants with initial grades of three or four showed reduced skin aging in the daily sunscreen group compared to discretionary use and significantly reduced skin aging after 4.5 years in the study, compared to those who only used sunscreens at their discretion. Results with the group given oral supplementation with β-carotene were not different from placebo.

The study described above showed that regular sunscreen use can have a significant effect on the rate of photoaging. When we consider that modern sunscreens tend to be higher SPF and broader spectrum compared to those used in the early 1990s, it seems logical that they could provide an even better protective effect, but the data indicate that very regular use is likely to be required, rather than just casual application when one goes out for prolonged exposure.

b. All-Trans Retinoic Acid (Tretinoin): The Gold Standard

Tretinoin is the only drug approved by the United States Food and Drug Administration for the treatment of wrinkles. It was first reported to have positive effects on photoaged skin by Albert Kligman and colleagues (201) in 1986. During the 1990s, tretinoin was investigated in double-blind, vehicle-controlled clinical studies (202–204). Olsen *et al.* performed a 320-subject multicenter study comparing 0.05%, 0.01%, and 0.001% tretinoin to vehicle on the face (205). After 24 weeks of treatment, 0.05% tretinoin produced statistically significant improvements in grades for overall severity of photodamage, mottled hyperpigmentation, fine wrinkling, and skin roughness compared to vehicle. Other studies showed that tretinoin was very effective for treating “liver spots” associated with photodamage (206) and hyperpigmented spots in Asian subjects (207). Grove and coworkers used image analysis of silicone replicas to demonstrate that 0.05% tretinoin consistently improved skin topography more than vehicle (208).

c. Cosmeceutical Treatments for Aging Skin

Tretinoin is a pharmaceutical product and an approved drug. Cosmetic treatments designed to improve aged skin are often referred to as “Cosmeceuticals.” While Albert Kligman, MD, is often said to have coined this term in the 1980s, it actually predates that time. The first published use of the

term we are aware of is by Raymond Reed, published in the *Journal of the Society of Cosmetic Chemists* in January 1962 (209). However, Reed's definition is so broad as to include simple moisturizers and other ingredients that probably wouldn't be considered "cosmeceuticals" today. Certainly Kligman popularized the term and played a significant role in the development of its current usage to refer to cosmetic ingredients that have activities that provide skin effects such as anti-aging (210). It is also important to keep in mind what the FDA says about "cosmeceuticals (211)"

The FD&C Act does not recognize any such category as "cosmeceuticals." A product can be a drug, a cosmetic, or a combination of both, but the term "cosmeceutical" has no meaning under the law.

Perhaps this is why, even today, the exact definition of cosmeceutical is not totally clear (212, 213). A significant literature exists on cosmeceuticals, even though the FDA says the term has no legal meaning and there are many reviews on the subject (212, 214–227). We will now briefly review a few of the many "cosmeceutical" actives.

d. Vitamin A and "Cosmeceutical" Derivatives

Retinol and retinol esters are popular ingredients in skin care products designed to treat the signs of skin aging. Retinol produces some changes in skin that are similar to tretinoin (228). It is much less effective but also less irritating (228, 229). Pierard-Franchimont *et al.* showed that retinol treatment can produce improvements in the mechanical properties and topography of skin (230). Retinol still has the potential for skin irritation and can be unstable. This has led to investigation of retinol derivatives such as acetate, propionate, and palmitate esters (216, 231). These esters are presumably metabolized to retinol and the free acid in the skin as has been demonstrated with retinyl palmitate (232). Bissett presented data showing that 0.15% retinol and 0.3% retinyl propionate can have positive effects on both wrinkling and hyperpigmentation after 12 weeks of treatment (216). Retinol has also been reported to improve intrinsically aged skin (233).

e. Niacinamide

Niacinamide, also known as nicotinamide, is the amide of nicotinic acid (vitamin B₃). In the cell it is incorporated in nicotinamide adenine dinucleotide and nicotinamide adenine dinucleotide phosphate, which are vital to energy metabolism. Topical application of niacinamide has been shown to have many positive effects on the skin, including improving barrier function (234–237),

reducing the appearance of fine lines and wrinkles (234, 238), and improving hyperpigmentation (239, 240). The improvement in skin pigmentation is apparently through a reversible effect on the transfer of melanosomes from melanocytes to keratinocytes (240, 241).

f. Alpha and Beta Hydroxy Acids

As pointed out above, the turnover rate of the SC decreases with age. Lactic acid had been well known as a moisturizer for many years (242–244), but the observation that lactic and glycolic acid increase the rate of SC turnover (245, 246) led to great interest in their application as potential anti-aging treatments. Stiller *et al.* published a double-blind placebo-controlled study comparing 8% lactic and 8% glycol acids to vehicle on signs of photoaging on both the face and forearm (247). Both lactic acid and glycol acid were significantly better than vehicle in improving global photoaging scores, and the two hydroxy acids were not different from each other. The so-called beta hydroxy acid products are based on salicylic acid, usually at about 2% concentration. This topic is described in great depth elsewhere in this book under the Ingredient section.

g. Antioxidants

Since reactive oxygen species play such an important role in photoaging, it is not surprising that antioxidants have been a major focus of cosmetic research. Vitamins E and C are probably the most studied for their potential to protect the skin from photoaging and treat its symptoms (216, 248–259). Raschke reported that 3% ascorbate had a significant effect on reduction of facial wrinkles compared to vehicle, but only when delivered from an “optimized formulation” (256). Antioxidants from plant sources are also under study. Green tea polyphenols have been the most studied ingredients in this regard. While studies in murine skin have shown promise (260, 261), human skin clinical trials of both topical (262) and oral (263) green tea treatment have failed to show visible benefits on skin aging. Histological evaluation after topical treatment for eight weeks did show significantly improved elastic tissue compared to placebo (262).

h. Other actives

A wide variety of other ingredients that can potentially improve the condition of compromised elderly skin are under investigation or have been investigated. They include peptides (221, 264, 265), topical application of estrogen cream (266) (a pharmaceutical not a cosmeceutical), anti-glycation compounds (267), and a variety of botanical ingredients (268).

CONCLUSIONS

As we age, our skin undergoes obvious changes. Some changes occur even on body sites not exposed to the sun or other insults. This intrinsic aging results from the unavoidable effects of the passage of time. Some of our daily activities not only accelerate the aging of skin but also alter its consequences, thereby leading to extrinsic aging. The most important contributor to extrinsic aging is exposure to the sun, though exposure to cigarette smoke may also contribute. Sun-exposed skin ages more rapidly than unexposed skin and differences in histology appear. For example, in chronically sun-exposed skin collagen decreases but elastin increases, though it is often highly dystrophic, while in intrinsic aging both collagen and elastin decrease.

This chapter has reviewed the effects of both intrinsic and extrinsic aging on the skin. Perhaps because of its vital function, the *stratum corneum* barrier is relatively unaffected by age, though barrier repair and wound healing may be slowed. The tendency of the Aged to develop dry skin is clear, and this may contribute to the appearance of fine lines and wrinkles. Changes in the dermis result in changes in the dermatoglyphic pattern of the skin and deeper wrinkles. Chronic sun exposure has profound effects on skin, leading to solar elastosis and the development of deep wrinkles and “age spots.”

We have also reviewed prevention and treatment of skin damage. Skin damage from the sun may be reduced or even prevented by frequent use of sunscreens. Tretinoin is the only FDA-approved drug for the treatment of wrinkles. Studies over the past three decades have shown the effect of tretinoin on photodamage, hyperpigmentation, wrinkles, and skin roughness.

Many cosmetic ingredients and products are alleged to provide effective treatment for the appearance of aged skin. The literature on some of the most common of these treatments was reviewed. Retinol derivatives, antioxidants, and niacinamide are among the treatments reviewed. While there is ample evidence that some “cosmeceutical” treatments may be effective for treatment of the signs and symptoms of skin aging, manufacturers must be careful that claims about product efficacy are restricted to those appropriate for cosmetics based on existing regulatory statutes in the U.S. and elsewhere.

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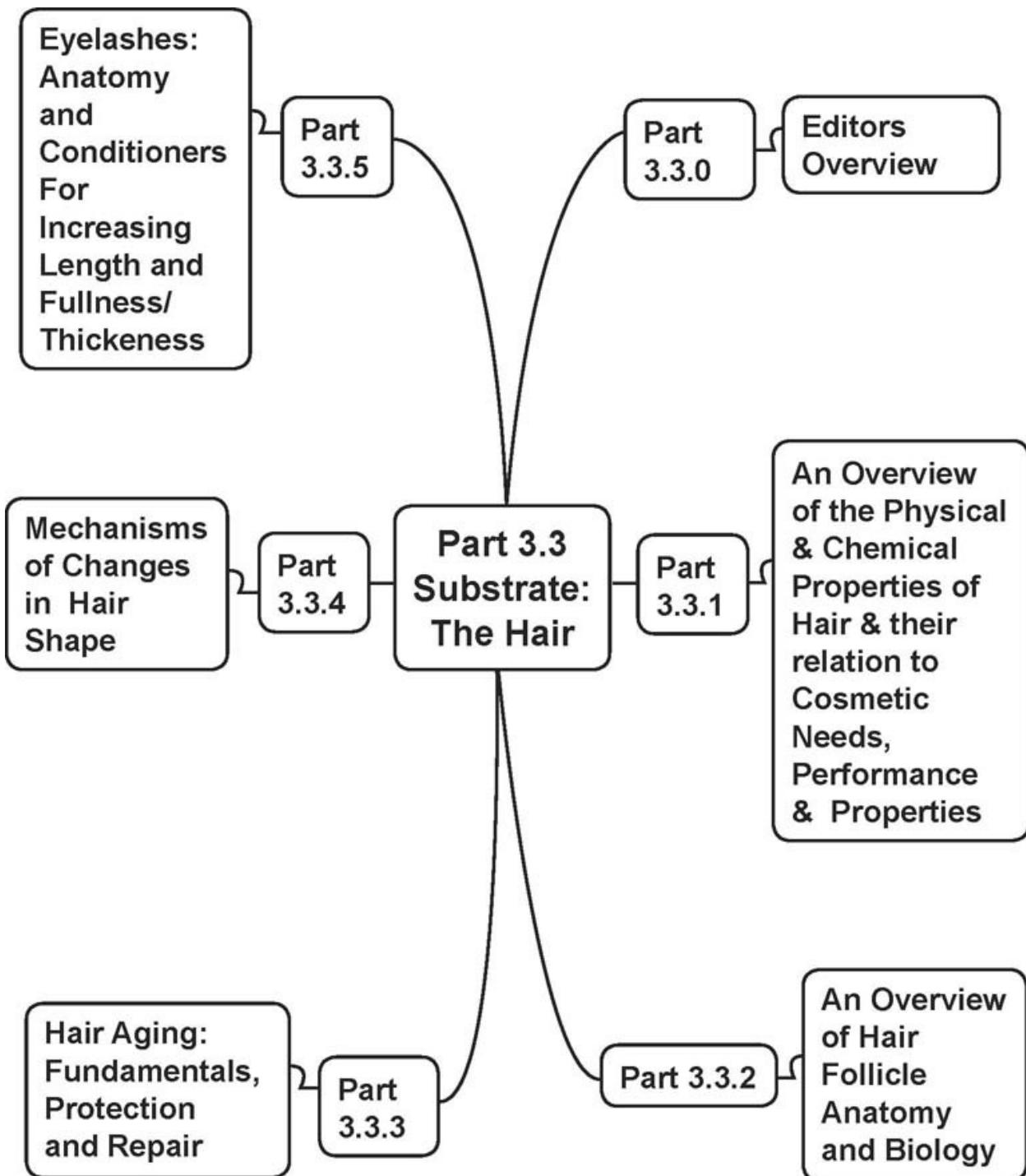
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PART 3.3

SUBSTRATE: THE HAIR



THE HAIR

Editors Overview

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Over the last ten years our understanding of hair and its care has developed significantly with the application of new scientific approaches and the resulting breakthroughs that have emerged.

In the area of cosmetic science, a large number of the aesthetic properties of hair have unequivocally been linked to the physical and viscoelastic properties of either the cuticle or the cortex. Various layers of the cuticle sheath have been shown to play an important role in hair lubricity, damage protection, and optical properties. The crystalline and amorphous phases contained in the ortho-and para-cortical cells of the hair shaft have been shown to be crucial for most hair-shape aesthetic modifications induced by consumers and cosmetologists' use of harsh cosmetic treatments such as the application of heat, exposure to alkaline formulations such as those used in hair relaxation, and other chemical-reducing aqueous solutions. Denaturation of crystalline regions, disulfide bond scission, and breakage of other protein bonds are all phenomena that have been carefully analyzed to assess their impact on the aesthetic aspects of hair and its damage.

In the area of hair biology, various important contributions have also been made. For instance, the role of stem cells, hormones, and other cytokines on hair growth; the discovery of additional stages in

the hair growth cycle; the biochemical basis for hair graying and aging, and the impact of new bioactives and other nutritional supplements in the health of hair are all relatively new discoveries just made within the last ten years.

With these rapid advances in the various hair-related areas, the science of hair care is unavoidably moving towards a more holistic approach. Such an approach considers the aesthetic properties of hair not only as a by-product of cosmetic treatments to the mature and keratinized form of the hair shaft, but also as a result of the action of bioactives that can enhance or improve the overall condition of hair while its cells are still alive in the hair follicle. This trend is quite significant when one considers that we used to think of hair as “dead,” and now our thinking and knowledge have evolved to focusing on the distinction that the cells in the mature hair shaft may be *s* “dead” but its proteins are still biologically active. Furthermore, by penetrating to the roots of the hair shaft, we have “discovered” that it is still *alive* while in the follicles, and this realization has created a paradigm shift in thinking of new approaches to bring beauty to what both men and women treasure as a major part of looking and feeling young.

It is within this context that the material in this section was chosen and organized. It starts with an overview of hair structure and function, followed by an overview of hair follicle biology. It then continues with a chapter on aging hair, hair loss, hair dyes, and bioactives, followed by a discussion on the mechanisms of changes in hair shape. The last chapter deals with eyelashes, including their anatomy and methods for increasing their growth.

The five chapters in this part of the book introduce fundamentals of hair science that range from its structure, mechanistic changes in shape and growth, to the action of bioactives to improve its health. This material provides a solid base in what we would now term “Hair Science” for beginners and fresh insights for seasoned researchers. The various contributors to this hair section were encouraged by the

book's editor-in-chief to not only give an in-depth overview of their various topics but also to present new insights and new ideas emerging in the area of hair care.

**AN OVERVIEW OF THE PHYSICAL AND
CHEMICAL
PROPERTIES OF HAIR AND THEIR RELATION
TO
COSMETIC NEEDS, PERFORMANCE AND
PROPERTIES**

Author

**Dr. Manuel Gamez-Garcia, Ph.D.,
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ABSTRACT

In this chapter we present an unusual approach to the subject wherein we consider the system of hair structure and its relation to hair function. The approach used for this purpose consists in reviewing the most recent and advanced hair structural concepts derived from the fields of chemistry and biology, while providing a strong emphasis on the physical, social, and cosmetic aspects of hair function.

The chapter is divided into six parts:

- The four first sections describe the chemical and biological composition of hair, which forms the foundation for its complex physical architecture. Through these sections a detailed analysis is made of the hair internal structure, its viscoelastic and optical properties, and its response to moisture. Wherever possible an effort is made to always connect physical properties and related processes with the functional aspects of hair.
- In the last two sections, a review is made of the various processes taking place in the follicle to create hair. We point out that the follicle is *alive*, and that the native proteins in mature hair still have biological activity and a

function to play. This provides access to cosmetic approaches to improve its beauty, and of course, it is a relatively new concept since the hair protein architecture has long been considered as a structure with no biological activity. There a description is made of the main biological activities occurring in the various zones and phases of the follicle to assemble cells and produce hair. As this description is made, special attention is given to the follicular activities that create, from its inception, the foundations that correlate hair architectural structure with hair function. We assert that this unique approach and a true understanding of the relationship between hair architecture and functional biology opens an entirely new field for the development of new concepts and products for the cosmetic and personal care industry catering to both men and women.

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3.3.1.1 INTRODUCTION

It is a general principle of nature that any living organism, or biological material created by nature, is made and structured in such a way that its properties are optimized to perform a specific function. In this sense, hair is not different from any other biomaterial found in the organs of our body. Contrary to the common belief that hair is a dead material and of little or no importance, hair is an optimized assembly of proteins, lipids, and polysaccharides organized into units or cells for a specific function. All cells in our body undergo a life cycle and are created to fulfill a function. However, in this respect there is a considerable difference between hair cells and other organ cells. Cells from other parts of the body undergo a programmed form of cell death, known also as “apoptosis,” once they have fulfilled their function. After this event, the cells are discarded and eliminated from the body. In contrast, when hair cells die by “apoptosis” in the follicle, their function is not finished; rather, it has just begun. Thus, when hair cells die, they are not discarded by the body because it is then that they are needed the most for their specialized biological function of scalp/skin protection and aesthetic purposes. The short-lived period of hair cells in the follicle is just a preparatory stage for their final function.

Another common belief is that since hair cells are inanimate or lifeless, their structure has no biological activity. This statement is somewhat incorrect because the protein structure of inanimate hair cells still possesses biological activity that is crucial for our living organism. Even when hair cells are dead and inanimate, if their protein structure is damaged, as in the case of enzyme structures, their biological function is compromised. Let us remember that enzymes are lifeless protein structures whose activity plays a critical role in many of our biological processes. Enzymes are not living organisms, and they lose their biological activity when their natural conformations are lost by denaturation due to harsh stresses, either thermal, chemical, or mechanical (1).

Likewise, the proteins composing our hair cells also lose their biological function/activity when they are subjected to harsh stresses, i.e., heat, chemical

treatments, mechanical fatigue, or excessive mechanical elongations. Hair treatments with hot irons and with other chemical processes such as perm waving, bleaching, and alkali straightening are known to cause denaturation and bond breakage in hair's native proteins (2). As a consequence, many hair properties are compromised, i.e., hair loses the ability to control moisture absorption. Its mechanical/thermal/optical response is compromised, and ultimately it loses its aesthetic purpose and ability to protect the scalp. Thus, the chemical composition and protein architecture of inanimate hair cells still possess biological activity enabling hair to fulfill the biological function for which it was created.

3.3.1.2 STRUCTURE AND CHEMICAL COMPOSITION OF HAIR

Hair is composed of proteins and a very small quantity of lipids and polysaccharides. These biomaterials form the hair cells, which are spatially separated and sequentially organized in a brick-and-mortar fashion in the cylindrical hair body. Consequently, they form membranes, amorphous and crystalline protein regions that extend all along the hair fiber, giving rise to the complex architecture of hair (see [Figure 1](#)). As will be seen later, these structures are the foundation of hair's remarkable physical properties. Table 1 displays the overall amino acid chemical composition of hair (3); the data in this table clearly show that hair is rich in half-cystine, serine, glutamic acid, and proline, i.e., the amino acids characteristic of a protein known as keratin. The next most abundant materials in hair, although at a smaller scale compared to the amount of protein, are lipids and polysaccharides, which account for 2–10% of the fiber weight. These materials, as will be discussed later, mainly appear in the cement layer gluing cortical and cuticle cells, as well as on the hair surface.

Another substance that is always present in hair is water. Incidentally, water is never considered part of the hair composition. Surprisingly, many scientists consider water to be an integral part of most biologically active proteins and, therefore, for all practical purposes water is also part of the hair composition. Native proteins contain a substantial amount of water in two forms, namely bound and free water. Free water, also referred to as “bulk water” or “loose water,” has only a minor influence in the native structure of proteins. In contrast, “bound water,” with its strong binding physical forces, attaches to specific protein groups stabilizing the protein secondary structure (4). Bound water plays

an important role in the conformation of exposed side chains, and because of its role on protein stabilization many scientists consider it to be part of the protein structure. Water is thus also part of the hair keratin composition (5) because it is needed for its stability. The role of water on hair behavior will be discussed in further detail later in this chapter.

Percentages amino acids in hair:

Alanine	4.6
Arginine	5.8
Aspartic acid	4.9
Half-cystine	17.8
Glutamic acid	11.4
Glycine	6.4
Histidine	0.9
Isoleucine	2.6
Leucine	5.8
Methionine	0.6
Phenylalanine	1.6
Proline	8.4
Serine	11.7
Threonine	6.8
Tyrosine	2.0
Valine	5.8

Table 1) Amino acid composition of unaltered human hair (3)

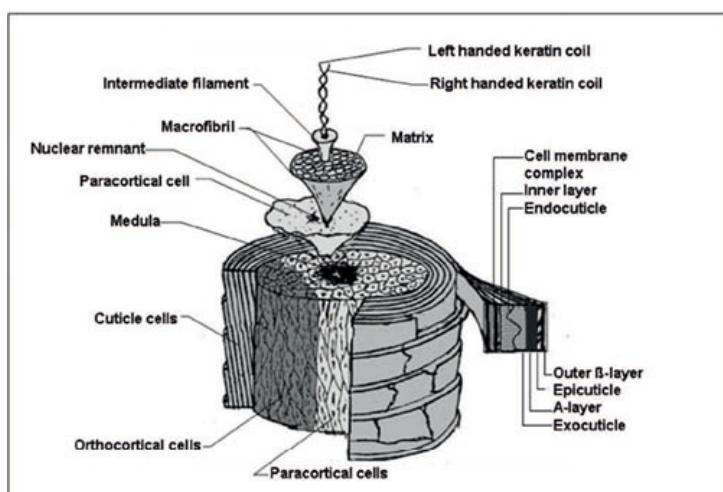


Figure 1: Diagram of a hair fiber showing its various components (60).

3.3.1.3 MAIN TYPES OF CELLS IN HAIR

A hair fiber is mainly composed of three types of cells, namely, cortical, cuticle, and medulla cells (see [Figure 2](#)). These three types of cells are made, organized, and assembled to form hair fibers by a tiny organ called the follicle (see [Figure 3](#)). This small organ sits on the scalp, and there are as many follicles as hair fibers. While hair cells are still alive in the follicle, they acquire a protein structure, composition, and architecture that prepare them for their final function. Before the hair cells undergo apoptosis the follicle carefully feeds and instructs them so they can arrange their internal protein structures into highly specialized conformations. For instance, a large percentage of the proteins found in the cortical cells of hair are arranged into a type of peptide sequence and conformation that is unique among all proteins. This unique protein is called keratin and its helical structure is such that, later, it allows hair to attain a high degree of mechanical strength (see [Figure 4](#)).

Keratin is a protein designed by nature to provide mechanical strength wherever needed in living organisms (6). Keratin proteins are usually coiled into filaments and constitute the micro-skeleton that gives mechanical stability to many cells. Without keratin filaments, many living cells would burst or collapse under the action of fluid pressure; i.e., in the liver they act as scaffolding structures that provide mechanical strength to the hepatocytes (7). Depending on their mechanical characteristics, keratins can be divided into alpha and beta keratins, or soft and hard keratins, respectively. Alpha or soft keratins are mostly found in hair, nails, skin, other body cells, wool, and the fur of other mammals, while the hard beta keratins are typical of reptile claws, the shell of turtles, etc. (8). Alpha keratins are also classified as type I (acidic) and type II (basic) keratins, and, in hair they are always coiled in pairs (9–10). Keratins can vary in structure and molecular weight, and therefore, there is a large variety of them; however, regardless of their type, nature always uses them when there is need for mechanical strength.

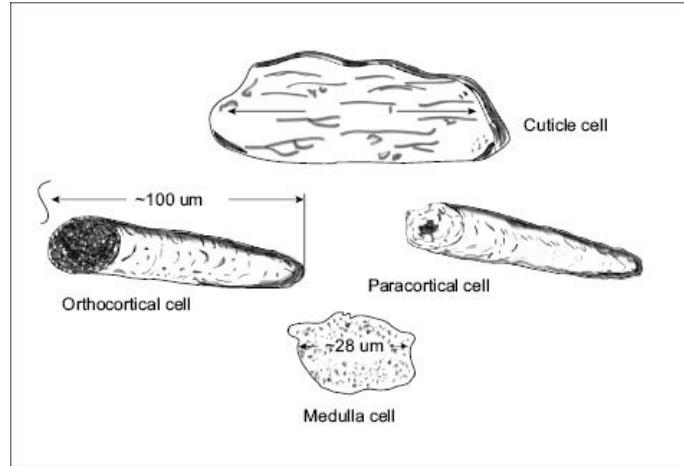


Figure 2: Pictographic representation of three types of cells in hair (60).

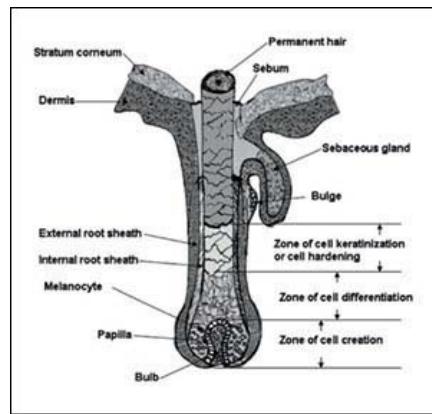


Figure 3: Diagrammatic representation of hair follicle with its main general components and the lipid gland (62).

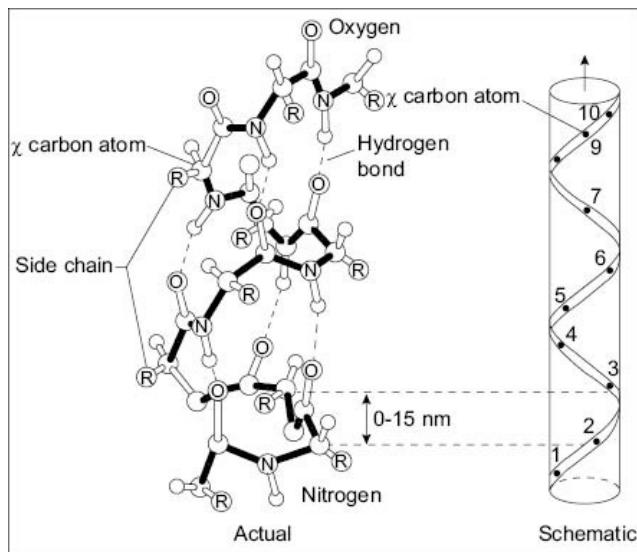


Figure 4: Helical structure of alpha helix in keratin.

3.3.1.4 CORTICAL CELLS AND THEIR ROLE IN HAIR PROPERTIES

a. Cortical cell structure and composition

The cortical cells are elongated and spindle-like in shape; they constitute the major cellular component of hair fibers and are responsible for most of the mechanical properties of hair (10–11). Their irregular circular shape in a grown hair fiber has an average diameter of approximately 20 μm ; their entire body length is approximately 100 μm long (see Figure 2). The cortical cells in grown hair fibers are not alive. However, before the hair fibers emerge from their follicles there is a point at which they are still alive. The main task of these cells during their life period is to synthesize keratin proteins in their bodies by assembling key amino acids into helical structures, strand them into filaments—also called intermediate filaments (IFs), and finally, bind them via disulfide bonds to an amorphous protein matrix to form macrofibrils (11–14). The amorphous protein matrix gluing the microfibrils will then be crosslinked with disulfide bonds and hardened as the cells die. The keratin filaments are crystalline in nature and are known to possess remarkable mechanical strength. To further maximize the mechanical resistance of the nascent hair fiber, the follicle facilitates the creation of hundreds of cortical cells and instructs them to interdigitate and produce an extracellular material, the cell membrane complex (CMC), which glues them all together in a cylindrical shape ([Figure 5](#)).

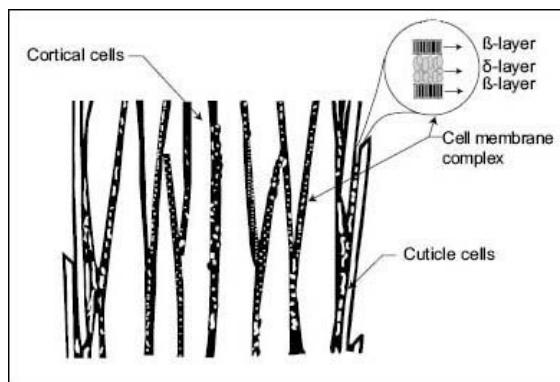


Figure 5: Schematic representation of cell membrane complex in hair fiber.

Because the cortical cells contain high levels of keratin, the resistance of a hair

fiber to mechanical breakage is comparable to that of a copper wire of the same diameter. Yet, in spite of this remarkable mechanical strength, the hair fibers do not behave like rigid metallic wires. We all know that hair fibers on the scalp are rather soft and easy to bend and move. This motion allows them to separate and create inter-fiber pockets of air needed for sweat transfer, thermal insulation, scalp protection, and aesthetic purposes (15, 16). Unlike a bundle of copper wires, hair is known for exchanging moisture with the environment by a physical process of absorption and desorption that involves heat transfer with the environment. This process, which occurs also in wool fibers, allows them not only to act as insulating materials, but also as heat buffers that effectively regulate skin/scalp temperature (17, 18). Furthermore, from a cosmetic point of view, hair fibers do not behave like copper fibers since an assembly of hair fibers can be deformed or styled into any desired shape with the aid of water, and it can later recover its original shape.

This particular behavior of hair, which combines mechanical strength with softness and moisture absorption/desorption with heat exchange, is at the base of its biological and cosmetic function. This combination of properties results from the biological activity of hair proteins with their unique architecture within both cortical and cuticle cells. The biggest contribution to this behavior comes, however, from hundreds of keratin microfibrils embedded in a protein-amorphous matrix in the cortical cells. The keratin microfibrils have a high degree of molecular order. Being crystalline, they do not swell in water, while the amorphous phase has no order, and a part of its structure is highly swellable by water. In terms of strength, the combination of keratin microfibrils embedded in an amorphous phase is akin to that of an epoxy composite material reinforced with glass fibers. In this sense a single hair fiber can be considered as an amorphous protein matrix reinforced with keratin microfibrils.

b. Viscoelasticity in hair and cortical cells

In 1959 a mechanical model was proposed by Max Feughelman (15, 20, 21) to explain the remarkable relationship existing between elasticity, softness, strength, and moisture displayed by wool and hair. This scientist considered that the outstanding mechanical behavior of hair and wool and, in general, of most keratin fibers, results mainly from the mechanical response of keratin microfibrils, or intermediate filaments (Ifs), embedded in an amorphous protein matrix forming macrofibrils in the cortical cells.

In order to explain the time-delayed elasticity associated with hair softness and

its dependence on moisture, Max Feughelman introduced the concept of viscoelasticity to keratin fibers. He proposed a two-phase mechanical model that didn't take into account the discrete nature of cortical cells, cell membrane complex (CMC), and cuticle cells. He thus generated a model of the hair fiber as if it were a continuum material—made only of keratin microfibrils embedded in an amorphous protein matrix (see [Figure 6](#)). In this model, now supported by a large amount of experimental evidence, the keratin microfibrils (C phase) are considered to act mechanically like rods in parallel with the amorphous phase (M phase M in which they are embedded) (15).

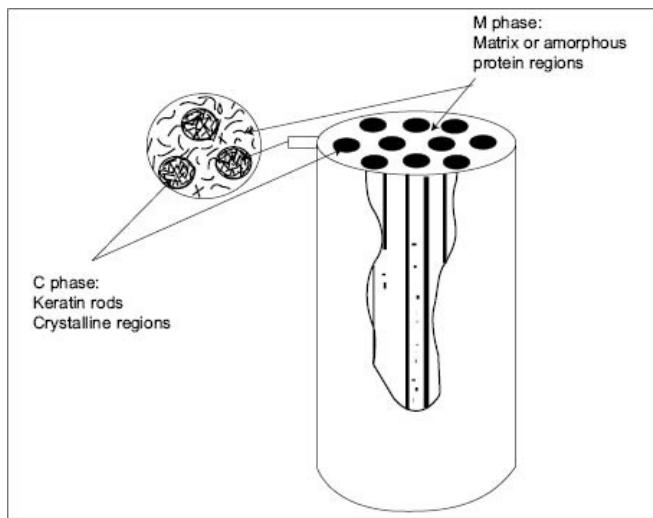


Figure 6: Representation of Max Feughelman mechanical model of keratin fibers showing keratin rods embedded in an amorphous matrix (20).

Since the introduction of the Max Feughelman model, three other models have been proposed to explain the mechanical behavior of keratin fibers. The main differences among all them seem to center on the way the amorphous phase interacts with the crystalline phase. A more detailed discussion of these differences can be found elsewhere (21). For the purpose of this chapter it suffices to emphasize, however, that all of these models recognize the existence of the amorphous and crystalline phases as two separate entities with different mechanical behavior. In all these models it is agreed that the amorphous protein phase corresponds to the zone of scrambled proteins surrounding the keratin microfibrils. The proteins in this zone constitute the “cement” that supports and interconnects, via disulfide bond crosslinking, hundreds of microfibrils to form the macrofibrils we know as “hair.”

Within the amorphous matrix, the two-phase mechanical model recognizes the

existence of two regions with different cystine content, namely, the high-and low-sulfur regions. The low-sulfur amorphous region is considered to be in close proximity to the microfibrils, while the high-sulfur region is further away from them. Furthermore, the amorphous phase contains hydrophilic and hydrophobic protein groups that form extended structures all along the cortical cells. Because of these two structures, the amorphous phase can display two distinct patterns of physical behavior. The first one consists of a softening effect usually observed when the amorphous phase interacts with water. The softening effect is due to the hydrophilic extended structure of the amorphous phase and is also referred to as the “gel structure” by Max Fueghelman (20). This structure absorbs large amounts of water, is susceptible to plasticization by moisture, and its viscoelastic response is moisture dependent. In contrast, the non-gel structure of the amorphous phase is hydrophobic, highly crosslinked, and softens at very high temperatures.

Phase C comprises the crystalline keratin rods forming the macrofibrils. As a result of their crystalline character, these rods do not absorb water, and therefore, cannot be plasticized by water. The macrofibrils thus act like strong micro-springs that respond only elastically to mechanical stresses. Thus, macrofibrils and amorphous phases combine their mechanical and water absorbing/desorbing properties to make hair a viscoelastic material. Because the content of crystalline keratin macrofibril protein in hair is approximately 95%, a hair fiber has a melting temperature (T_m) of $\sim 156^\circ\text{C}$. Hair has also a glass transition temperature (T_g) of $\sim 144^\circ\text{C}$, and it is primarily related to the noncrystalline amorphous matrix (23). Let us not forget that the T_g in polymers or biopolymers is nothing but the temperature at which a hard amorphous polymer becomes softer (24). Therefore polymers that are very hard and brittle at room temperatures can be softened, by raising their temperature to their respective T_g . They can also be softened by adding a plasticizer. In fact, hair fibers are biopolymers, which under dry and room-temperature conditions are mechanically hard but can become softer, either, by raising their temperature above 174°C , or by adding a plasticizer. We are all familiar with the fact that water is the natural plasticizer for proteins. Thus, hair without moisture is hard and brittle, but when it absorbs moisture it becomes softer because the gel structure of the amorphous phase becomes plasticized by water. This plasticization effect by water is clearly shown in [Figure 7](#), where it can be seen that the amount of force needed to deform a hair fiber is always lower when it has higher levels of moisture.

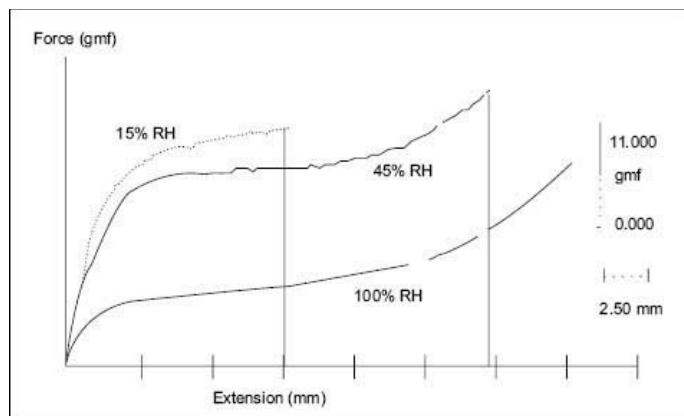


Figure 7: Typical stress-strain deformation curves of hair fibers obtained at three different moisture conditions (60).

The effects of water plasticization/softening in hair are more significant when hair fibers are deformed below 2% water level. The reason for this is that the main hair component that responds to low deformations is the gel structure of the amorphous phase with its large number of hydrogen bonds. At deformations (d), $2\% > d < 40\%$ the mechanical response of the fiber is not influenced by the presence of water. Rather, it is mainly controlled by the unfolding of the alpha-keratin into the beta-keratin configuration. Unfolding of the alpha helical keratin in the microfibrils thus allows for larger deformations in the hair fiber, thereby preventing it from immediately breaking in the yield region. The transformation of keratin from the alpha to beta configuration is a thermodynamic phase transition and can be induced in hair by many processes other than mechanical extension. When it is produced by heat or chemical damage, it is usually irreversible; however, when it is induced mechanically it is fully reversible. The beta configuration of keratin sometimes is also called the “ladder or platelet” keratin structure (22, 23). Deformations larger than 5%, giving rise to keratin unfolding, occur in hair in daily grooming practices. They occur mostly upon repetitive combing, in particular, when the hair is wet or when the hair is still wet and is being detangled.

The stress/strain curve of hair is shown in [Figure 8](#), where it can be seen that when we deform a single hair fiber from 0 to approximately 40% in length, the hair displays three different slope regions of force versus deformation. The first line is comprised between 0 and 2 or 5% deformation depending on the relative humidity of the environment (segment A). It is known as the Hookean region and has the steepest slope. This region represents the stress/strain mechanical response of the amorphous and crystalline (alpha keratin) phases. In particular, it

represents the mechanical response of protein region with hydrogen bonds. Since these levels of deformation are easily attained with everyday combing and grooming practices, we stress the amorphous and macrofibril phases of our hair almost everyday. If we continue to deform the hair fiber between 2 and 35% (segment B), the slope stress/strain changes because it is produced by a different phenomenon, namely, the opening of keratin coils in the macrofibrils. This section of the stress-strain curve of hair is known as the yield region. Hair deformations falling in this range are typical during excessive tangling when the hair is wet. Deformations higher than 35% cause the final change in slope before the fiber breaks; the physical phenomenon associated with these deformations seems to be a process of recrystallization of the amorphous phase (segment C) (11).

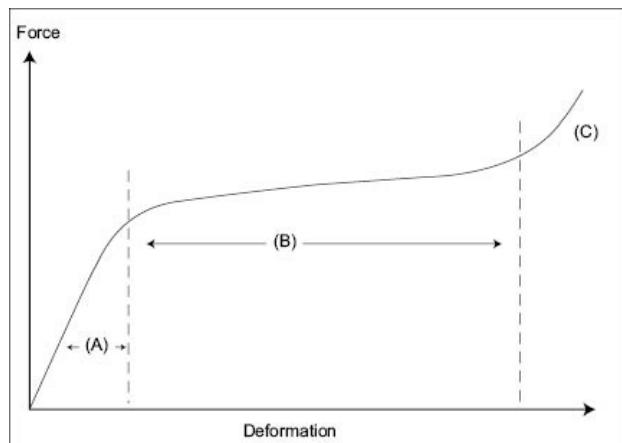


Figure 8: Stress strain curve for hair up to 30% deformation.

c. Shape-memory properties of hair

It is known from the field of polymer science that the mechanical behavior of polymers can be characterized as either being of the thermoset or thermoplastic type. Thermoset characteristics are those that do not respond to temperature changes, while thermoplastic characteristics refer to those materials that soften when heat is applied.

When polymers contain a high level of crosslinking bonds, they are usually of the thermoset type. In many cases these materials behave also as “smart” materials displaying shape-memory properties (24, 25). Since approximately 90% of the hair is composed of cortical cells filled with crystalline and amorphous proteins crosslinked by disulfide bonds, hair like any other polymeric crosslinked material behaves also as a thermoset, viscoelastic, shape-memory

biopolymer. Thus, hair has a permanent shape that may be temporarily changed, but can also be reversed later under the appropriate conditions. For instance, whether the hair is limp, wavy, curly, or extremely curly, it will always remember its native shape regardless of what type of styling shape has been imposed on it. Furthermore, the fact that the hair geometry can successfully be changed after styling, and held there for a certain period of time without the hair immediately recovering its shape, is by far one of the most important cosmetically useful examples of hair viscoelasticity and memory-shape properties. If the behavior of hair were purely elastic, hair would not be able to easily deform and accommodate for deformations needed for fiber separation, air pocket formation, thermal insulation, scalp protection, and aesthetic purposes. Also, hair would not be able to transiently change its shape during styling for cosmetic purposes. Thus, hair is a viscoelastic shape-memory material, *and failure to comprehend this will hamper our understanding of one of the most fundamental properties of hair's biological and cosmetic behavior.*

d. Viscoelasticity and the shape-memory properties of hair

Because of the great importance of viscoelasticity in the shape-memory properties of hair, a brief review of this concept will be helpful. We are all familiar with the fact that when a small force is applied to a pure elastic material, for instance, to a rubber band, it will deform a certain short distance. Then, when we release the force, the rubber band will recover almost instantaneously to its original shape. In this process it is obvious that the energy applied to deform the rubber band is stored as mechanical tension energy, and then returned when the force is released. In contrast, in a pure viscous material this does not happen. Rather, when we apply a similar force to a viscous material, for instance, to a string of chewing gum, a small deformation is also produced. However, contrary to the elastic material, the string of chewing gum will not recover its original shape once the force is released and it will stay deformed forever. Furthermore, the energy spent to deform a viscous material like the string of chewing gum is not returned but rather, it is dissipated as heat (26). In summary, an elastic material—when deformed—recovers its shape instantaneously when the force causing the deformation is released, while a viscous material does not.

A viscoelastic material, like hair, displays both behaviors simultaneously, namely, viscous and elastic. In fact, we all have daily encounters with materials that behave viscoelastically. For instance, when we apply a force for a short period of time, i.e., for one second, to a piece of plastic film from a sandwich

bag, it will deform slightly. The shape of the film will be recovered instantaneously again when we release the force. However, if we now apply the same force, but for a longer period of time, let's say five minutes, we will still produce the same deformation, but we will notice that when we release the force, the plastic film does not recover its original shape right away. If we wait for some time and pay careful attention to the plastic film, we will notice that it slowly crawls back and eventually recovers its original shape.

In the above example, with a one-second force application, the piece of film displayed typical elastic behavior; yet in the case of a five-minute force application the same plastic film showed initially a viscous behavior followed by a delayed, gradual, and slow elastic recovery. Thus, depending on the time period of force application, the plastic film displayed either pure elastic behavior or a combination of a viscous and elastic behavior. This is the universal characteristic of viscoelastic materials, namely, they either display elastic behavior in response to rapid deformations, or display a combination of viscous and elastic behavior in response to very slow deformations, or to deformations produced by forces applied for long time (27). Hair displays exactly the same behavior. When we deform it rapidly, as in a rapid head movement; or when we pass our fingers between the hair fibers, it behaves elastically, yet, the hair displays viscoelastic behavior when we deform it for long time. For instance, when we sleep with our hair bent or uncombed in an unwanted geometry, or in the morning when we wake up, our hair seems to stay bent and uncombed for long time, i.e., it does not recover immediately; but if we give it enough time it will remember its shape and recover gradually.

In summary, this remarkable mechanical behavior of hair is essentially due to the viscoelastic and shape-memory properties resulting from the disulfide crosslinked amorphous matrix and the keratin crystalline structure in the cortical cells. This behavior has been put in place by nature to provide an optimized assortment of mechanical properties for scalp protection and social communication—perhaps even as a part of the attractive mechanism between members of the opposite sex to facilitate propagation of the species.

The optimum mechanical and biological function of hair is thus achieved by a combination of an amorphous protein phase containing a gel and a non-gel structure, attached to the strong-coiled elastic keratin macrofibrils. For hair to maintain this optimum combination of softness, elasticity, strength, and shape recovery, it is necessary that we keep the biological activity of its proteins unchanged. We can achieve this, first, by helping hair to keep its amorphous

proteins moisturized so they are plasticized; and second, by keeping as long as possible the primary, secondary, and tertiary structure of the proteins in its native structure. We are all familiar with the fact that hair becomes hard and brittle when it lacks moisture, or with the fact that hair becomes weaker when its proteins oxidize or become brittle under the action of heat or prolonged UV radiation. We also know that hair becomes mechanically weaker after chemical treatments that we blithely apply in an attempt to look more beautiful, yet destroy strong hydrogen and covalent disulfide bonds that stabilize its tertiary structure, i.e., its crystalline structure (28, 29).

e. Water and moisture absorption/desorption by cortical cells

As in any other protein biomaterial, hair most of the time contains water, even at very low relative humidities. Water in general plays a crucial role on protein structure and function. It can be found in two states, namely, strongly bound and free or unbound water (30). In the case of hair, strongly bound water is critical for the conformation and mechanical stability of the helicoidal keratin fibrils that strand and form the macrofibrils in the cortical cells (31). This type of water is therefore critical in maintaining the mechanical properties of hair, and it can be found in hair even after it is subjected to zero relative humidity. This behavior is found even with a few water molecules that are strongly attached to protein functional groups and can only be removed from the hair by applying high temperatures close to its denaturation temperature (32). In fact, in the case of hair, denaturation of its proteins is caused by both losses of strongly bound water and breakage of disulfide bonds in the protein-amorphous regions.

In most cases, protein plasticization is ascribed to water molecules whose bond strength is weaker and can be broken by the movement of thermal motions of protein chains. This type of water molecule adsorbs onto the protein chains in multilayers and, at high moisture content, takes the form of bulk water. It is the water that causes swelling in hair and in other natural biopolymers (33–36). A great deal of information on the formation of water molecule multilayers and bulk water absorbed can be obtained by looking at the shape of the water absorption isotherm of a specific material. [Figure 9](#) shows the water absorption isotherm corresponding to hair. One can see it is sigmoidal in shape and presents a hysteresis effect (37, 38). Namely, the amount of water content in hair depends on whether we moisturize it from a dried state or whether we dry it from a wet state. In very dry hair there are a few water monolayer or nucleating sites for multilayers to form by a process of condensation, therefore, the amount of water

absorption is low (line A). However, when hair is fully wet, water multilayers are already formed *inside* the hair. Under these conditions the desorption of water occurs by an evaporative process (line B).

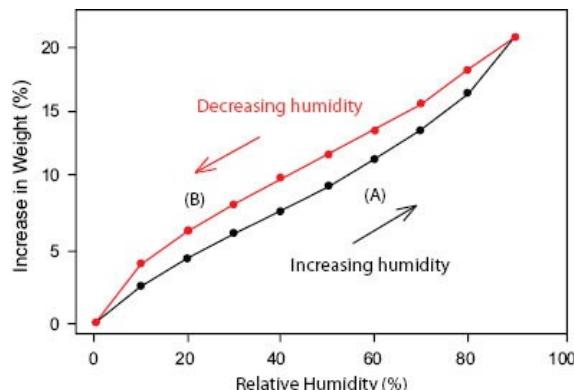


Figure 9: Water absorption isotherm of hair (37).

In total, at 100% relative humidity hair absorbs up to approximately 30% of water, and even when moisture is also absorbed by the cuticle cells and the cell membrane complex, most of the moisture is absorbed by the cortical cells (19). Many other synthetic fibers also have the ability to absorb and desorb moisture.

Surprisingly, the process of water absorption/desorption by human hair has an ulterior natural function and it is not like any other ordinary process of moisture interaction with solid matter. It is part of the physiological phenomena used by nature to provide for hair softness, thermal regulation, and sweat transport. Hair interacts with moisture in ways that no other fiber can emulate. Incidentally, wool is the only other fiber that performs like hair, but this is so because wool is also a keratin fiber with a cellular and protein architecture similar to that found in hair. Hair and wool both have water properties that, in principle, appear antagonistic, i.e., they have a hydrophobic exterior that repels water and a hydrophilic protein interior that allows it to absorb and retain water depending on the environmental moisture conditions. These unique and paradoxical properties are not shared by other synthetic fibers. This analogical behavior is at the root of why so much research in developing cosmetic hair products begins in literature reviews of wool.

Because of their crosslinked structure, hair and wool have the ability to absorb large amounts of moisture and sweat without changing their mechanical integrity. The absorption of large amounts of water by hair occurs without the hair feeling wet and soaked and without excessive weakening of its protein

structure. These properties of keratin in hair contrast with those found in keratin of the skin. If we simultaneously submerge a piece of *stratum corneum* and a hair fiber in water we will observe, after a few hours, that cornoecytes in the *stratum corneum* swell without limit and lose their mechanical integrity. In contrast, the cortical and cuticle cells in the hair do not swell without limit and keep their integrity. Furthermore, as the amorphous protein matrix in hair absorbs and desorbs moisture it is able to release and gain heat depending on temperature differences between scalp and external environment. This particular behavior allows it to act as a heat buffer against rapid environmental temperature changes.

The effects of heat release upon moisture absorption by hair and wool, and their ability to keep warm surfaces is well documented (39). For instance, it is considered that if 1 kg of dry wool absorbs moisture from 40 to 70% RH it will release approximately 164 kJ (18, 32). If the wool is dried and absorbs approximately 35% of water it will release ~960 kJ of heat; this is as much heat as an electric blanket will release during eight hours. For these heat effects to take place, the wool or hair has to be dried and absorb moisture from low to higher moisture conditions. Then, as the wool or hair absorbs moisture the temperature will increase due to heat release. The water absorption isotherm of hair associated to this remarkable behavior was already shown in Figure 9; the points in this curve represent equilibrium states. The proteins in the amorphous matrix of cortical cells have various active sites for water adsorption. They are amino, carboxyl, and other hydrogen-bonding groups (40, 41). These proteins also act as nucleation centers for water multilayer adsorption. It has been reported that most of the heat released by wool or hair occurs when their hydrophilic groups absorb moisture in multilayer form at low relative humidities. At high relative humidities, moisture condensation and bulk water formation take place and there is less heat released.

3.3.1.5 DIFFERENT TYPES OF CORTICAL CELLS AND HAIR SHAPE

There are two types of cortical cells in hair; these are the ortho-and para-cortical cells (see [Figure 10](#)). Hair fibers from other mammals contain also a third type of cortical cells, namely, the meso-cortical cell, but this type of cell does not seem to be part of the human hair. There must be a reason for nature to have created two different types of cortical cells and confining them in two different

sections of the cortex within the same hair fiber. Existing data, whose interpretation is still controversial, suggest that the presence of ortho-and para-cortical cells is associated to the creation and stabilization of hair shape (42–44). It appears that by creating these two types of cells, nature can control hair shape by making it straight, curly, or extremely curly as a response to environmental adaptation. For instance, the cortical cells of hair fibers that are straight and limp have been found to be mostly of the para type. In contrast, the cortical cells found in hair that is curly are of two types, namely para and ortho, and their location in the cortex is such that they fill two distinct separate regions within the cortex of the same fiber (see Figure 10). Furthermore, the ratio of para to ortho type cells varies with the degree of curliness, i.e., the higher the content of ortho-cortical cells the curlier the hair is.

In spite of their differences, the ortho-and para-cortical cells are still made mostly of keratin filaments embedded in an amorphous matrix. However, because of their different packing density, the viscoelastic response of the hair section filled with para-cortical cells should be different from the section filled with ortho-cortical cells. Furthermore, the ortho-cortical cells seem to absorb less moisture than the para-cortical cells and this should result in a lower degree of water plasticization for this type of cell. This difference in moisture plasticization and viscoelastic response between the two inner sections of a single fiber has been evoked to explain the curliness enhancement seen in wavy and curly hair when moisturized.

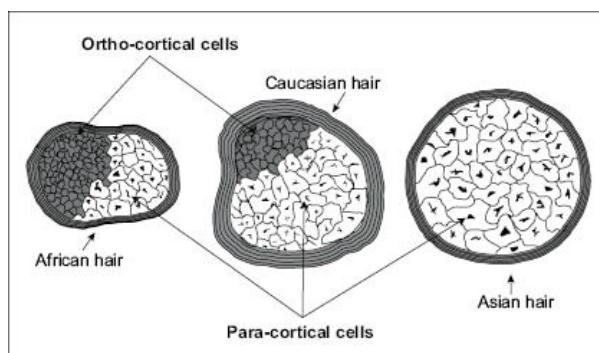


Figure 10: Schematic representation of different ratios of ortho- and para-cortical cells in African, Caucasian, and Asian hair (60).

3.3.1.6 CUTICLE CELLS AND THEIR ROLE IN HAIR PROPERTIES

a. Cuticle cell structure and composition

As the follicle produces the cortical cells from stem cells, it also creates the cuticle cells. Like the cortical cells, the activity of the living cuticle cells is also highly specialized. In the process of differentiation they are instructed to become layered and flat in shape, with a final thickness of approximately 0.5 µm (see Figure 1). Subsequently, the follicle arranges them in stacks of 5 to 11 cuticle cells like shingles on a roof all around the cortical cells to create a strong protective shield. Their flat and layered structure organized in ordered stacks allows them to form the cuticle sheath. This form of cellular envelope acts as an effective shield that protects the cortical cells against friction, mechanical abrasion, impact, and other environmental stresses. It has been put there by nature to extend the life of hair to fulfill its function. Without the cuticle sheath around the hair fibers, the cortical cells will fray out and become rapidly damaged by friction. The cuticle sheath acts also as a gate to control incoming and outgoing light from the cortex to provide protection against radiation and color for aesthetic and social communication.

All of these important property/functions of the cuticle cells would not be possible without the unique architecture of their various layers and their specific chemical composition. For instance, the very top layer of a hair fiber, called the F layer, is made up of a fatty-acid chain, the 18-methyl eicosanoic acid or 18-MEA (see [Figure 11](#)). This fatty acid chain is attached covalently to an ultra-high sulfur-rich protein on the epicuticle surface (45–47). The attachment is made in such a way that the waxy part of the 18 MEA protrudes away from the epicuticle surface to provide the highly needed lubricity and hydrophobicity of hair fibers. Beneath the monomolecular layer of 18-MEA is the epicuticle followed by the exocuticle. These two layers, which are 120 and 250 nm in thickness, respectively, have the highest degree of sulfur content in the whole hair fiber (48).

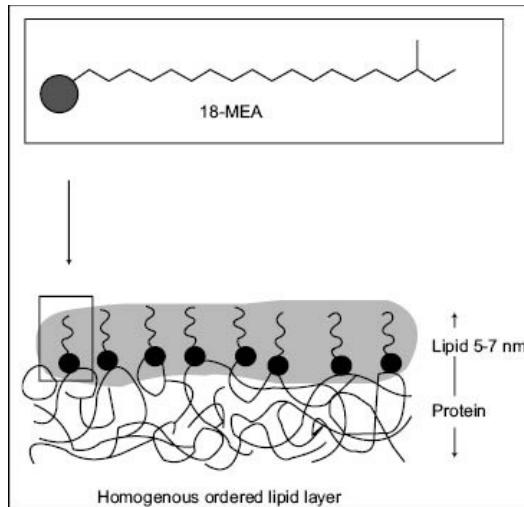


Figure 11: Representation of F layer on cuticle cell surface showing 18-MEA attached covalently to the epicuticle (47).

The high sulfur content in these layers is testimony to the high degree of crosslinking bonds needed by the proteins at the surface so that the cuticle sheath can become hard and strong in order to resist mechanical abrasion. Then, beneath the exocuticle we find the endocuticle, this layer is composed of a non-cross-linked negatively charged protein, which is very susceptible to water swelling (49). The absence of crosslinked and crystalline proteins in this layer makes it highly deformable, very soft, and somewhat weak to mechanical stresses. It has been speculated that the soft nature of this layer allows it to act as a soft viscoelastic cushion to protect the hair against mechanical impact.

Below the endocuticle one can find the cell membrane complex (CMC), which is also the extracellular material used by the cuticle cells to bind and cement together. The structure of this cementing substance is very important because it not only glues cuticle and cortical cells but also plays a crucial role in the diffusion of moisture and other chemicals into the hair cortex.

The cell membrane complex is essentially composed of three layers: the upper beta layer, the delta layer, and the lower beta layer (see [Figure 12](#)). The beta layers are made of lipid chains arranged in palisades and attached on one side covalently to their immediate near protein network on the epicuticle. The other side of the lipid palisade is not covalently bound to the proteins in the delta layer; it is rather attached by physical forces also called dispersion forces. For this reason, many researchers consider this junction to be the weakest mechanical link in the whole fiber assembly (49–52). Cuticle and cortical cells fail at this junction when hair fibers are elongated to their breaking point and

when the cuticle cells chip away during dry combing. When wet, the cuticle cell fails rather at the soft protein zones in the endocuticle and not at the cell membrane complex (CMC).

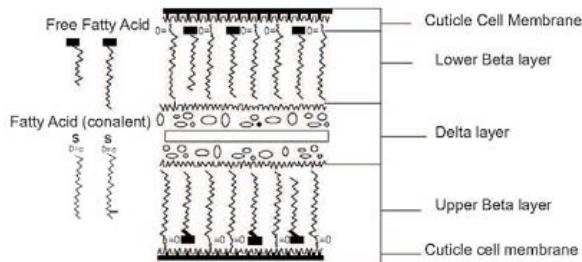


Figure 12: Representation of cell membrane complex between cuticle cells (53).

The delta layer in the CMC appears to be made of proteins that are partially hydrophilic and partially hydrophobic. This layer is considered to contribute, in great part, to the diffusion of many liquids into the cortex. In the past, it was thought that the overall composition of the CMC, with its delta layer forming a sandwich between the two beta layers, was uniform all across the hair fiber. However, recent analyses made by C. Robbins and others appear to suggest that its composition changes depending on whether the CMC is cementing the cuticle cells, the cortical cells, or the cuticle/cortical cells interface (53) (see [Figure 13](#)). It has been proposed that the beta layers in the CMC gluing the cuticle cells are made of a monolayer of 18-MEA. As it was previously explained, this fatty acid is attached covalently to the epicuticle and physically attached to the delta layer. In contrast, the beta layers gluing the cortical cells are made of bi-layers of fatty acids, cholesterol, and ceramides, all of which are only physically attached on both sides. This important proposition suggests that the lipid of choice by nature to provide for high levels of hydrophobicity and lubricity to the hair surface is 18-methyl eicosanoic acid (18-MEA).

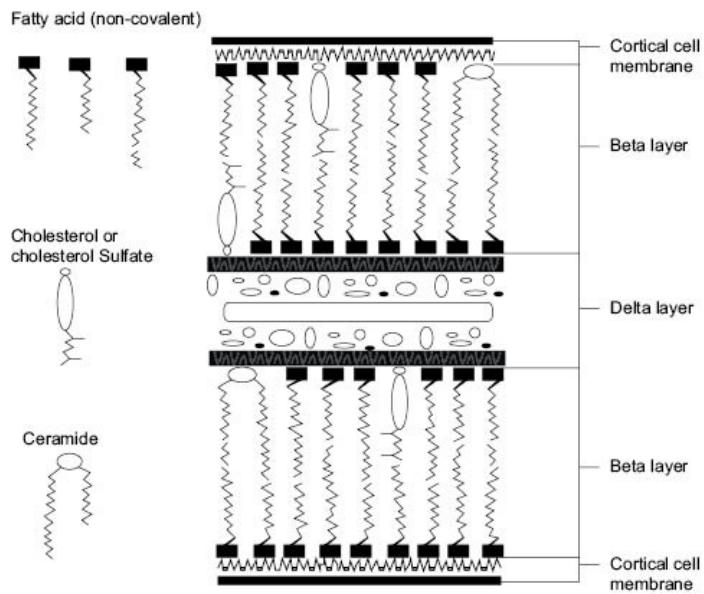


Figure 13: Representation of cell membrane complex between cortical cells of hair (53).

b. Viscoelasticity in hair and cuticle cells

The mechanical properties of the cuticle cells are just as important, if not more important, than those of the cortical cells for the function of hair. In fact, since hair is made of fibers whose length is comparably larger than their diameter, the fiber surface becomes predominant in controlling the mechanical properties of a hair assembly. Inter-fiber separation and air pocket formation for thermal regulation and aesthetic appearance are, therefore, mainly controlled by the mechanical behavior of the hair surface at the cuticle cells. Further, the integrity and mechanical stability of the cortical cells depends on the cuticle cells. Without the five to ten cuticle cells arranged in overlapping stacks to form the cuticle sheath, the cortical cells would rapidly collapse. Moreover, the surface of the cuticle cells not only has a high degree of hardness but also contains a lubricious fatty layer and is therefore very resistant to friction/abrasion. Hair fibers need this type of surface structure as they are constantly in motion, rubbing against each other.

Physical parameters relevant to these properties are the dynamic and static friction coefficients of the cuticle cells, the strength of the cuticle cell cement (CMC), and the Bending, Extension, and Compression Modulus of the cuticle cells. Unfortunately, data on the various mechanical modulus of the cuticle cells is scarce. Experimental studies carried out by various researchers have revealed, however, that the mechanical behavior of cuticle cells is not elastic but, as in the

case of the cortical cells, their behavior is also viscoelastic (54, 55). This is not surprising since the proteins making the various components in hair are biopolymers and, therefore, the overall behavior of each one of these components is also viscoelastic.

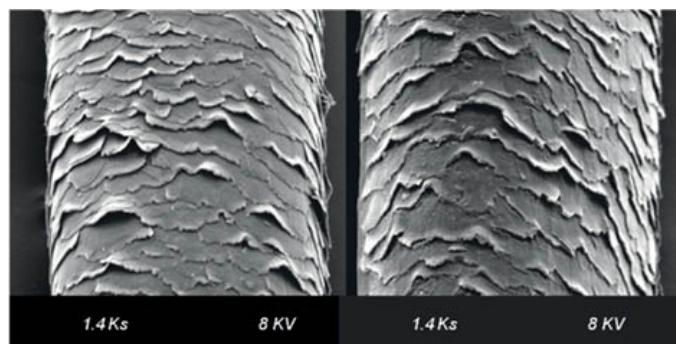
The phenomena of cuticle cell lifting and buckling produced by various environmental, mechanical, and thermal stresses, and the time it takes to recover from these deformations, are proof of cuticle cell viscoelasticity (56). Lifting and buckling of the cuticle cells occurs often in hair when subjected to continual elongation during combing, and also by thermal stresses after blow-drying or hot iron applications. Cuticle cell cement breakage and buckling are two of the most common manifestations of viscoelastic material mismatch between the various layers of the cuticle cells. When hair fibers are stressed, either thermally (by rapid compression due to water evaporation), or mechanically during excessive tensile elongation, the internal components of the single cuticle cells, i.e., the epicuticle, exocuticle, endocuticle, and cell membrane complex, do not respond in the same manner. This causes an excessive accumulation of shear stresses at their junctions and, consequently, the weakest link within the cuticle cell fails. Depending on moisture conditions, the endocuticle or the cell membrane complex fails, causing either cuticle cell buckling or breakage (52).

Cuticle cell damage in the form of cement breakage and buckling is commonplace in most people and it is significantly increased by various damaging hair treatments (54–60). This type of hair damage is almost absent in hair sections close to the fiber root and it is commonplace at its tips. Thus, a strong possibility exists that the “cement” becomes weaker by various environmental oxidative stresses during the life of a hair fiber. Gradual oxidation of the various cuticle cell layer components and their junctions will make them more prone to breakage by mechanical extensions during grooming.

[Figure 14](#)a shows a typical image of hair near its tip taken from a female subject with long hair (60). The same type of cuticle cell damage can be produced in virgin hair in the laboratory after subjecting it to large elongations and to thermal stresses (see Figure 14b). A very interesting aspect of cuticle cell buckling is that the deformations causing it can be either viscoelastically reversible or irreversible. For instance, it has been found that most of the buckling deformations produced during a hair-damaging process are reversible, i.e., the buckled cuticle cells can actually recover their original shape if enough time is allowed for recovery. There is, however, depending on the damaging process, a fraction of cuticle cells that cannot recover their shape and remain

permanently and irreversibly deformed (61).

It should be pointed out here that cuticle cell buckling always entails breakage of the “cement” or cell membrane complex (CMC). This is true regardless of whether the deformations in them are reversible or irreversible. In most cases, once the cuticle cells have undergone buckling their mechanical integrity is compromised. For instance, simple hair-strand swelling by water absorption and the resulting stresses are not capable of inducing cuticle cell lifting and buckling in virgin cuticle cells. Yet, after they undergo buckling, and after the cells have recovered from their deformations, they can be easily lifted and buckled again by simple water imbibition and resulting swelling. Studies have shown, however, that in certain cases the cell membrane complex by itself may have the ability to partially recement and re-glue the cuticle cells (61, 62). For instance, experiments have shown that, if deformed and lifted cuticle cells whose cement is broken are allowed to recover closing their gap, they will not lift again when immersed in water. In many cases the previously buckled and recovered cells will only buckle again when the levels of mechanical stresses are similar to the ones that initially produced cell lifting. This observation seems to indicate that, under certain conditions, the “cement” is able to partially recement the cuticle cells.

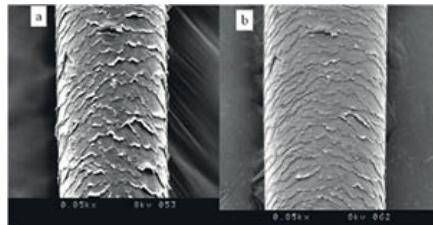


Figures 14a and 14b: SEM pictographs of cuticle cell buckling in hair as follows: (14a) as it appeared in a long hair of a female subject, and (14b) produced in the laboratory by mechanical stresses in an undamaged hair fiber (60).

c. Water and moisture absorption/desorption by cuticle cells

In the previous paragraphs we have pointed out that moisture absorption by the cortical cells has beneficial effects for the function of hair; among these benefits are scalp/skin temperature buffering, moisture transport, and a large contribution

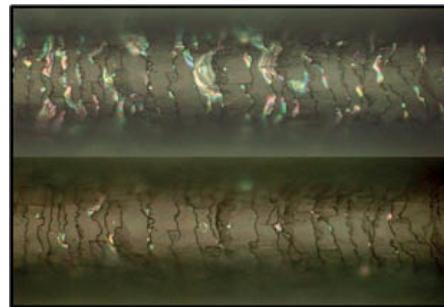
to softness/plasticization of hair fibers. We also pointed out that water is the main protein plasticizer, and we came to the conclusion that moisture makes hair softer because it plasticizes the amorphous protein in the cortex. An observation that is often ignored by many researchers studying keratin fibers is that the cuticle cells also absorb moisture and, most importantly, that their mechanical behavior is very sensitive to moisture. The strongest evidence of this sensitivity is the effect that moisture has on cuticle cell recovery from deformations that were caused by stress buckling. [Figure 15a](#) shows an SEM picture of a hair fiber whose cuticle cells underwent buckling deformations after the fiber was subjected to a mechanical elongation of about 15%. The same hair fiber with its cuticle cells recovered is shown in Figure 15b, where recovery of the buckled cuticle cells took place after the fiber was immersed in water absorption (61, 64). Likewise, [Figure 16a](#) shows a micrograph of deformed and buckled cuticle cells produced in hair by blow-drying in the presence of water and low-molecular-weight oils (58); while in Figure 16b the same hair fiber can be viewed with its cuticle cells recovered after they were allowed to absorb moisture. The pictures were obtained by optical microscopy using the technique of patterns of light interference analysis described in more detail in the following paragraphs.



[Figures 15a and 15b](#): SEM micrographs of cuticle cell buckling before (15a) and after water immersion (15b). Both micrographs correspond to the same spot in the hair fiber. As a point of reference, observe the elongated deep crack in the top section of the hair fiber (60).

Experimental observations made on those cuticle cells as depicted in Figures 16a show that their deformations do not recover if they are left alone for a good period of time at normal temperature (25°C) and under moisture conditions (53% RH). The experiments showed, rather, that the deformed and buckled cuticle cells recover only when moisture condenses on the cuticle cells, i.e., when the hair surface is above the dew point (65). These simple observations indicate the following: a) that cuticle cells need to absorb liquid water and not gaseous water molecules to recover by water plasticization and, b) that protein

moisturization in the cuticle cells only occurs when multilayer adsorption occurs at protein chains by moisture condensation.



Figures 16a and 16b: Optical micrographs of buckled cuticle cells observed in a hair fiber blow-dried in the presence of low-molecular-weight oils by using the LIP technique (54). Figure 26a represents the state of the cuticles after the hair fiber was blow-dried, and Figure 16b shows the cuticle cells after the hair was immersed in water for a period of one minute (60).

Moisture absorption by the cuticle cells is thus crucial for the cells to recover from deformations and to comply mechanically with the deformation of the cortex and the whole hair fiber. Just as in the case of cortical cells, cuticle cells are also able to perform their beneficial function in hair if they are plasticized by water, i.e., “moisturized.” It is important to mention here that the subjective perception of “hair dryness” is *de facto* associated to a lack of moisture in the cortical cells. However, studies have shown that this is an incorrect conception since hair swatches that were equilibrated at high relative humidities seemed to obtain higher “dryness perception” scores than those equilibrated at low relative humidities (66). An explanation of these contradictory facts has already been offered by some scientists. They have proposed that roughness on the hair surface, and not lack of moisture in the cortex, is what creates the perception of dryness in hair (66). Moisture content in the cuticle cells should, therefore, have a stronger correlation with the sensation of hair dryness.

d. Optical properties of the cuticle cells

Another important property of the cuticle cells is their optical set of properties. All the various layers composing the cuticle cells are transparent. When white light strikes a hair assembly the first component it encounters is the cuticle sheath. The main physical property that determines how a transparent material interacts with light is its index of refraction (IR). The IR of the cuticle sheath has

been reported to be ~ 1.53 (67); the IR of each cuticle layer is not known, but because each one has a different composition and architecture, it is expected that its behavior will be somewhat different. The behavior of white light striking the cuticle cell—just as in the case of other transparent materials—thus, will be dependent on the IR of each layer. Its value will thus determine how much light is reflected, refracted, and absorbed by the cuticle cells. Reflected light can be of two types, specular or diffuse. If the reflected light is specular it will give rise to shine; if it is diffuse it will give a matt and dull appearance to hair. Light that is refracted passes through the cuticle cells and reaches the cortex, where it interacts with the pigments of melanin. There, a certain wavelength component of the light will be absorbed and the remainder of the light, which will not be white any more but colored, will be reflected back into the cuticle cells. The light will pass, then, throughout the cuticle cells and will appear to the eye as hair color (see [Figure 17](#)). If part of the light diffracts, penetrates into the cortex, and is absorbed by the melanin granules, the hair color will appear shiny and black, brown, or blond depending on the type of melanin. As it will be seen later, brown and blond colors are produced by different amounts of a brown melanin granule called eumelanin.

The condition of the cuticle cells thus determines the optical appearance of the hair assembly. If the cuticle cell cement is broken and the cuticle cells are lifted, light will not be properly transmitted to the cortex, and neither will it be specularly reflected. Rather, the light will be scattered and diffused and the hair will partially lose its shine and color, thereby, appearing dull and lifeless. Recently, it has been found that, either, when the cuticle cells are lifted, or whenever there are micro-pockets of air within the cuticle cell layers, the cuticle cells produce colorful microscopic light-interference patterns (LIPs) (see [Figures 18a and 18b](#)) (56). It is important to mention here that the vivid colors found in insects, butterflies, birds, and also in some mammals str created by such light-interference phenomena. These colors are usually very strong and they are not created by subtraction of colors from white light due to absorption by pigments. Rather they are created by the same mechanism used by soap bubbles to create color—a phenomenon based on the constructive and destructive interference of light (see [Figure 19](#)). This phenomenon is also known as iridescence, and the colors are called structural colors (68, 69). In the case of hair, if the interference colors produced by lifted cuticle cells could be made coherent at the macroscopic level, they would create strong and vivid colors.

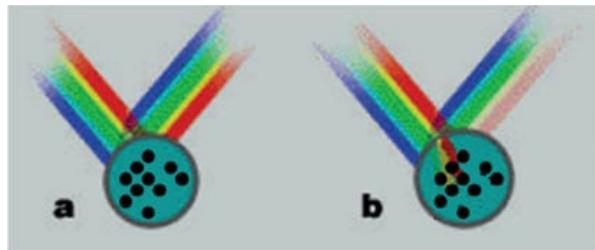


Figure 17: Diagrammatic representation of light interacting with hair showing (a) total reflection of colors, and (b) light reflection plus diffraction and absorption by melanin granules.

Unfortunately, the lifted cuticle cell areas containing air in their layers creating these strong LIPs are very small, and light-scattering edge effects dominate. In addition, because of the small size and discontinuity in the lifted cuticle cells, the strong interference colors are not coherent at the macroscopic level. Consequently, their final effect to hair appearance is rather deleterious. However, the weak patterns of iridescence produced by the layered structure of undamaged cuticle cells certainly will contribute to the final optical appearance of hair. Colored light coming from the melanin in the cortex will thus combine with colored light produced by the weak iridescent patterns of virgin cuticle cells, and both, just as in the case of cuticle cells of some mammals, should produce the final hair optical properties. Thus, the weak iridescence patterns of virgin cuticle cells contribute to the unique color properties of undamaged hair.

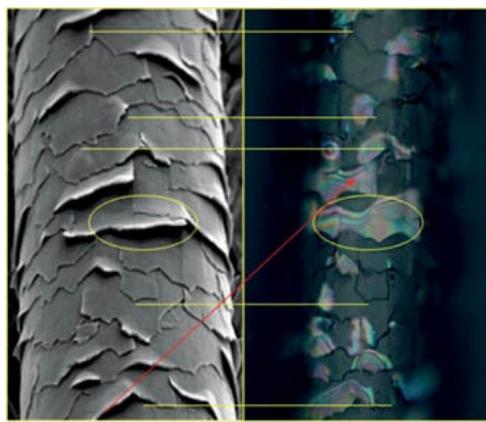


Figure 18: Micrographs showing lifted and buckled cuticle cells on the same fiber by SEM (left), and by optical microscopy (right) using the LIP technique. Lines and circles in the pictures identify same features (56).

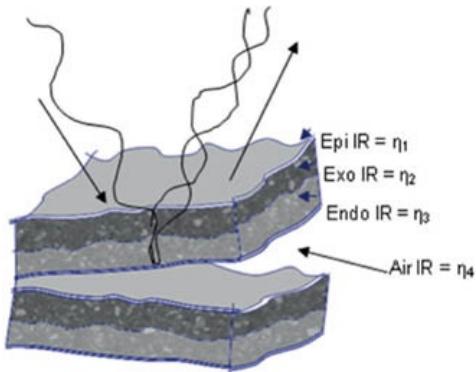


Figure 19: Diagram of lifted cuticle cells describing formation of light interference patterns by the phenomenon of thin-film light interference (56)

The color and shine enhancement often seen in hair when wet is a typical example of improved hair color perception due to changes in the IR of the cuticle sheath. Color enhancement in wet objects that absorb water can thus be explained by two mechanisms. The first is by the decrease in light scattering due to water filling of the otherwise empty pores containing air. The second is color and shine enhancement in hair by moisture absorption, also due to changes in the IR of the endocuticle, which enhances the phenomenon of iridescence. Polydimethylsiloxane polymers, oils, and some other high-molecular-weight polymers can also produce similar effects—as long as they are able either to penetrate into air gaps at the cuticle sheath or to smooth the cuticle cell surface to eliminate diffuse scattering.

e. The medulla cells

The medulla in hair refers to a region of very low density located at the center of a hair fiber (11). It is considered to be porous, irregular in shape; and, in human hair, in most cases, it is not continuous all along a single hair fiber. The regions of low density can be detected either by scanning electron microscopy or by optical microscopy (see [Figure 20](#)). In [Figure 21](#), we can see a photograph showing the discontinuous presence of medulla along the hair fiber by which the hair was analyzed by transmission using optical microscopy (70). Not all hair fibers have medulla, but it is frequently found in thicker hair. Analyses have shown that, even in the case of thick hair, hair fibers from the same head may or may not have medulla cells. The medulla region is made of cells that are very different in structure to the cortical and cuticular cells; they are large and cuboidal in shape, and often, but not always, they are very porous. The structure, composition, and role of the medulla cells are not very well known. Because of

their porous structure, the medulla cells are filled with air and it has been speculated that this cell-air system contributes to increase the hair's ability to provide thermal insulation. The medulla cells have also been reported to have a strong impact on hair optical properties (71).

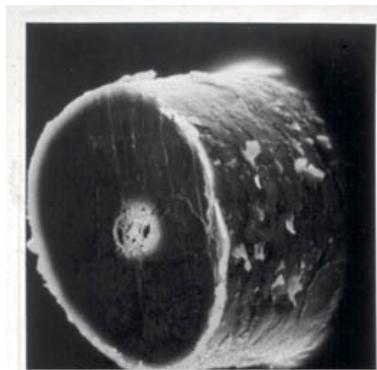


Figure 20: Micrograph of hair showing medulla region in hair (60).

3.3.1.7 MELANIN PIGMENTS IN HAIR

The melanin pigments in hair are made of very interesting molecules that play a crucial role in the function of hair. They act as free radical scavengers, antioxidants, UV light absorbers, heat radiation absorbers, and also as gamma rays absorbers (72–74). Melanin pigments not only protect the proteins in hair against oxidation and UV damage, but also help to protect the scalp from excessive harmful UV and heat radiation. They also provide color to hair for aesthetic and social communication. The protective action of melanin pigments is so important that nature does not use melanin only in hair, but also in the neurons of the brain as well as in the cells of eyes. Melanin has been found to provide important protection effects to the eyes; among these are the following: photoprotection, vision enhancement, decreases in the risk of cataract formation, and a lower risk of macular degeneration in the retina (73, 74).

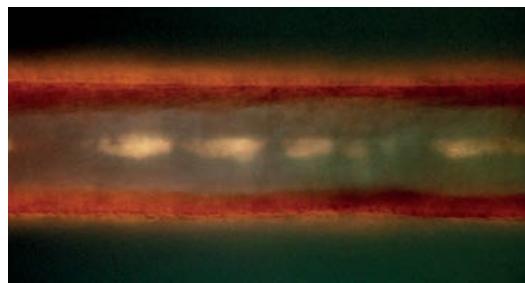


Figure 21: Micrograph showing discontinuity of medulla region along a hair fiber (60).

Melanin granules in hair are approximately 0.8 µm in diameter (see [Figure 22](#)). They are polymeric in nature and depending on their type they differ chemically. The various chemical moieties forming the melanin polymer are 5,6-dihydroxyindole (DHI) and 5,6-dihydroxyindole-2-carboxylic acid molecules (75, 76). A description of their composition is given in [Figure 23](#). Melanin granules isolated from hair or skin display brown, black, green, and yellow colors.

There are many types of melanin and they can be grouped into two main types, namely, eumelanins and pheomelanins. Eumelanins can be further divided into brown and dark eumelanins. Brown eumelanin is common in people with lighter or brown color while dark melanin is typical of people with dark hair. Gray hair is caused by the absence of melanin in general while blond hair is produced by low amounts of brown eumelanin. Red hair contains mainly pheomelanin as pigment, which is also found in small amounts in skin together with eumelanin. The melanin granules are made in the follicle not by the cortical or cuticular cells, but rather, by specialized cells called melanocytes. Once the pigment granules are made, the melanocytes get instructions to excrete them and pass them into the cortical cells, where they are incorporated by phagocytosis (76, 77).

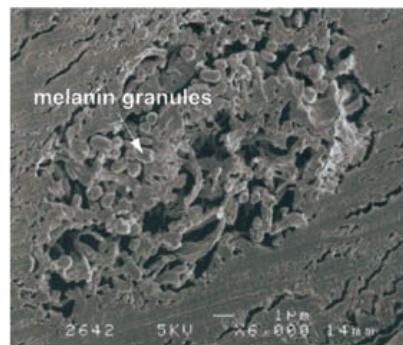
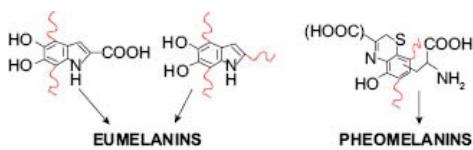


Figure 22: SEM micrograph showing physical aspect of melanin pigments in hair SEM (72–75).



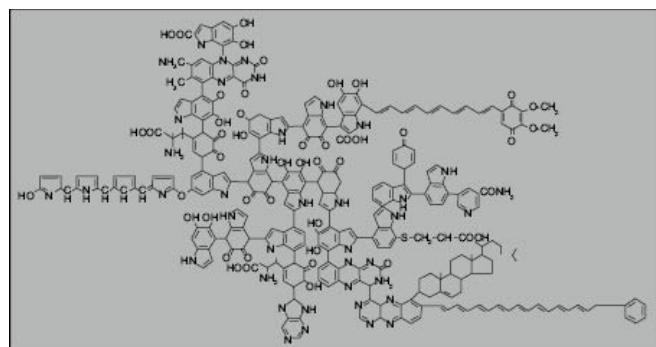


Figure 23: Schematic representation of melanin composition (75).

3.3.1.8 HAIR FUNCTION

As discussed in the previous paragraphs, the sequences of amino acids constituting the primary, secondary, tertiary, and quaternary structures in the proteins of hair are optimized so the fibers are capable of fulfilling their biological functions. Other biological substances in hair—such as lipids, polysaccharides, and complex polymeric molecules—are found in the cuticular cement and in the melanin pigments. These are also carefully structured in the hair cells to fulfill a specific function. This optimization of composition and structure in the cells occurs so that hair can, finally, serve its biological functions of temperature buffer by absorption/desorption of water, protection against harmful environmental radiation, thermal and mechanical insulation, and aesthetic agent in social communication. Hair with an undamaged structure is apt to perform its biological function, and it is not coincidental that undamaged hair is also aesthetically and cosmetically appealing.

3.3.1.9 THE FOLLICLE

As we have stated earlier, the follicle is the smallest organ in the body, and it is there where hair fibers are produced (see Figure 3). Also acknowledged previously, there is one single follicle for each hair fiber. This minute organ is essentially composed of various biological units, namely: bulb, dermal papilla, bulge, and external and internal root sheath (78). With these key basic units, follicles are able to produce and assemble hair cells to form hair fibers. There are between 100,000 and 150,000 follicles in a normal scalp, distributed on its surface in the form of clusters of three or four hair follicles (see [Figure 24](#)). Evidence suggests that the number of hair follicles is constant throughout one's life, so the number of hair fibers on a scalp will, at least, while one is alive,

remain constant. The hair follicle, with all its basic internal units, regenerates itself approximately every four to six years. At each regeneration cycle, the hair fiber that has been produced falls out, and a new one starts to grow.



Figure 24: Clusters of various follicles in scalp (60).

a. Different zones in the follicle

All cells forming a hair fiber are made in the follicle from stem cells (79–81). The manufacture of hair cells involves a very well-organized process of stem cell preparation, shaping, and assembly in a way that is akin to a car assembly line. These various processes occur from bottom to top of the follicle in three main zones; namely: a zone of cell proliferation; a zone of gene expression and differentiation, and, finally, a zone of cell hardening or keratinization (see Figure 3) (11).

Each zone within the follicle has specific tasks crucial for hair production. At the bottom of the follicle is the first zone, and it is constituted by the dermal papilla and basal membrane. It is in this zone where epithelial stem cells that descend from the bulge are gathered to receive instructions from the dermal papilla to proliferate by mitosis. Moving towards the tip, in the next contiguous zone, the cells obtain instructions also from the dermal papilla to become differentiated into cuticle, cortical, and medulla cells. During this process they are packed and glued together, forming a cylindrical shape. Moving further towards the tip in the following third zone, located in the middle portion of the follicle, the hair cells are instructed to undergo apoptosis and become hard through a process of keratinization. In this zone, proteins in the cortical and cuticle cells become crosslinked to attain their final optimized, mechanical, thermal, and physical properties. In the zone of keratinization the hair reaches its final form and emerges from the scalp.

Just as in a car assembly line, where total production is controlled by external demands and internal constraints, there has to be a very well-coordinated process of communication within the follicle itself and with other body glands to

successfully produce hair. For a period of approximately four to six years each hair follicle is instructed to keep its key components active making hair. The new portions of hair created in the follicle push out upper sections of the fibers and the hair grows. At the end of the sixth year of activity, the follicle gets instructions from the body's glands and genetic system to cease and stop the production of hair. The ancillary machinery in the various zones of the follicle is then shut down, and the three zones of the follicle become completely inactive. Subsequently, the key components of the follicle are dismantled and discarded as they are pushed out by new ones being formed underneath. In this way, the old grown hair fibers, together with their inactive follicle units, are pushed out from the scalp and the hair fibers fall out. The new components form a new follicle with new zones of cell proliferation, gene expression/differentiation, and cell keratinization, and a new hair fiber grows.

b. Life cycle of the follicle

From the time of its inception until it recedes, the regenerated follicle has a life cycle characterized by three different phases, namely: anagen, catagen, and telogen (see [Figure 25](#)) (82). In the anagen phase, the assembly line activity of the hair follicle is invigorated and has its maximum efficiency preparing and assembling cells into fibers. It is at this stage that the hair fibers in the three zones of cell creation, differentiation, and keratinization are continuously assembling and growing. An intensive production of cuticle and cortical cells via stem cell mitosis characterizes this stage. The produced cells are continuously shaped and assembled into hair fibers by instructions from the dermal papilla in coordination with other body glands. For instance, it is well known that the growth rate of hair is strongly dependent on certain hormones and that it is used by nature as a tool of social communication to assert gender differentiation. During the anagen phase most of the hair fibers thus acquire their main physical, mechanical, thermal, and optical properties. The overall characteristics of hair fibers are therefore dependent on nutritional and hormonal variations affecting this stage of follicle development. Then, in the catagen phase, which lasts only two to four weeks, the follicle lowers its cell production and a large part of its cells undergo apoptosis. In the telogen phase, which lasts only from two to three months, the inactive parts of the follicle are pushed out and the old hair falls out.

New research has actually shown that the activity of the follicle is even more complicated than described in the three stages mentioned above. Some researchers have already proposed the existence of various substages for each

one of the three main phases (83). There are two main reasons behind these proposals:

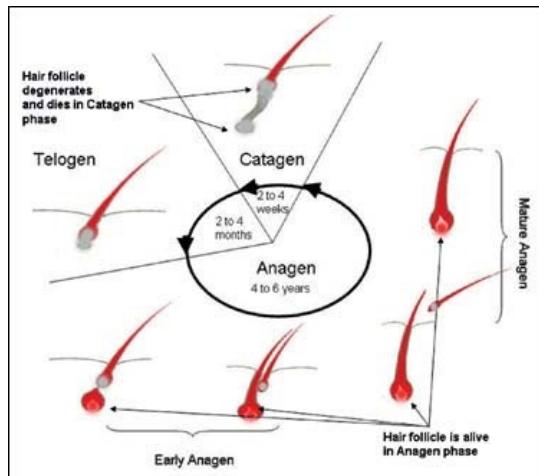


Figure 25: Life cycle of the follicle: anagen, catagen, and telogen (60).

namely, a) each stage has very well-differentiated activities that can be further divided, and b) it has been found that at the end of each stage there are already preparatory signals taking place for the next stage. In fact, the appearance of new follicle components producing a new hair fiber starts in the follicle even before the older fiber is pushed out; so the anagen phase of a new growing hair fiber in some cases overlaps with the telogen phase of a hair that is going to fall. Thus, some of the processes controlling the formation of new hair fibers and the shedding of old ones seem to overlap between phases. This process can easily be perceived in the picture of human scalp shown in Figure 24, where it can be seen that in certain areas there are two hair fibers emerging from the same follicle.

All processes taking place in the follicle, whether they occur in the anagen, catagen, or telogen phase, or in any of their substages, need to occur in synchrony. Their occurrence involves the timely interaction and participation of a large number of biopeptides, enzymes, and other biological factors that act as regulators, communicators, and controlling agents for the production of stem cells, cells division, cell apoptosis, keratinization, etc. Furthermore, the maintenance process of the hair shaft, after it has emerged from the scalp in all its length, is partially executed by the biological activity of the follicle. Sebum and enzymes are released from the sebaceous glands in concert with the follicle to maintain the integrity of the hair shaft.

Hair diseases, hair defects, or mechanical weaknesses in hair fibers are caused by internal or external factors that interfere, block, change, or damage the

various biological processes involved in normal hair growth and hair maintenance. Traction alopecia, androgenetic alopecia, chemical alopecia, trichorrhexis nodosa, etc. are all hair ailments caused by some sort of internal or external interference. It has recently been discovered that the follicle is the target of major hormonal pathways, and that the follicle and sebum glands, both together, referred to as the pilosebaceous unit, can also act as a peripheral hormone-producing unit for a wide range of steroid hormones, neuropeptides, and neutrophins (84). Thus, proper growth of new hair fibers and shedding of old ones require no obstruction or interference with the biochemical substances involved within the follicle. Any internal or external factor interfering with the function of these biochemical substances will cause some sort of malfunctioning or disease in hair growth.

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AN OVERVIEW OF HAIR FOLLICLE ANATOMY AND BIOLOGY

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ABSTRACT

Since ancient times, the human hair has been an attribute for health, youth, and attractiveness as it plays a vital role in people's self-perception ⁽¹⁾. As a result, changes in the appearance of hair such as hair loss, hair thinning, and premature graying often cause psychological distress to the affected individuals.

Hair is produced by a multicellular entity; the **hair follicle**. The hair follicle is one of the most fascinating and dynamic organs of the human body and, unlike the hair itself, which is composed of dead keratinocytes, hair follicles undergo a process of regeneration that controls the production of the hair fiber. One can distinguish between three main types of hair follicles: the **lanugo**, the **vellus**, and the **terminal hair follicle**:

- **Lanugo hair** is very fine and normally shed *in utero*, or during the first weeks of life.
- **Vellus hairs** are very short, nonpigmented, nonmedullated hairs found all over the body surface.

Terminal hair is the large, and usually pigmented and medullated hair that is predominantly found on the scalp. It is estimated that humans have around 5 million hair follicles, of which 80,000 to 150,000 are located on the scalp. Scalp hairs grow at a rate of 0.3 to 0.4 mm per day ⁽²⁾. **No additional follicles are produced after birth**, although the size of the follicles and hairs can alter with time, primarily under the influence of androgens. In pubic, axillary, and beard regions, androgens stimulate the conversion of vellus to terminal hairs, whereas

in frontal scalp regions of genetically predisposed individuals, androgens trigger miniaturization of hair follicles via conversion of terminal to vellus hair follicles⁽³⁾.

The most unique feature of hair growth is its cycle. Hair follicles undergo repeated cycles of anagen (growth), catagen (degeneration), and telogen (quiescence). During anagen, hair follicle stem cells sustain the steady production of matrix cells in the hair bulb. These proliferate several times and terminally differentiate into the companion layer, the inner root sheath, and the hair shaft.

Unwanted hair loss is a common problem and is suffered by much of the world's population in both women and men. The condition is characterized by premature hair loss and excessive hair shedding. The process is caused by an interruption in the normal hair growth cycle, which can lead to the development of baldness with insufficient replacement of hairs in human scalp. This can have negative effects on an individual's self-esteem and thus products that claim to be useful for treating hair loss target a steadily growing, multibillion-dollar market worldwide.

The field of human follicle biology is progressing rapidly largely because of technical advances in molecular and cellular biology as well as sophisticated cell culture techniques. This chapter reviews the structure of hair, the biological processes occurring during the hair growth cycle, and the changes that occur with hair aging.

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3.3.2.1 HAIR FOLLICLE STRUCTURE

The hair follicle is a three-dimensional tube, composed mainly of epithelial cells,

which protrude down through the epidermis and dermis of the skin ([Figure 1](#)).

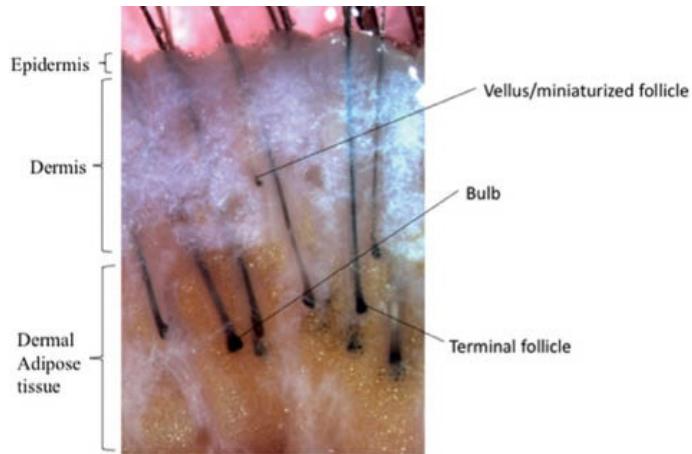


Figure 1. Human scalp tissue isolated from the scalp. Hair follicles protrude down through the epidermis and dermis of the skin (photo kindly provided by Dr Claire Higgins)

Hair follicles vary considerably in shape and size, depending on their location, but they all have common architecture. Anatomically, the hair follicle is most commonly described when it is in the mature anagen stage ([Figure 2A](#)). The mature anagen follicle can be divided into an upper permanent portion (infundibulum and isthmus), which does not cycle, and a lower part (bulb and suprabulbar), which is remodeled in each hair cycle. The infundibulum is the opening of the hair canal to the skin surface, and the isthmus is the permanent middle section extending from the sebaceous duct opening to the point of arrector pili insertion. The lower isthmus also contains the bulge region ([Figure 2B](#)), which is a region of functional importance as it is in this location that a reservoir of stem cells reside. Stem cells are unique, as they have the capacity to generate all the differentiated cell types within tissue and to self-renew in order to replenish their pool. Earlier studies have demonstrated that all epithelial layers within the hair follicle and hair originated from bulge cells ^(4, 5). Hair follicle stem cells therefore appear to be responsible for regenerating the hair follicle in each cycle. Up until recently the location of the bulge in human follicles was unclear. By contrast with the bulge in rodent follicles, human follicles are not morphologically distinct. However, identification of unique bulge markers in the human follicle has enabled the human anagen bulge to be defined as a distinct region ^(6, 7).

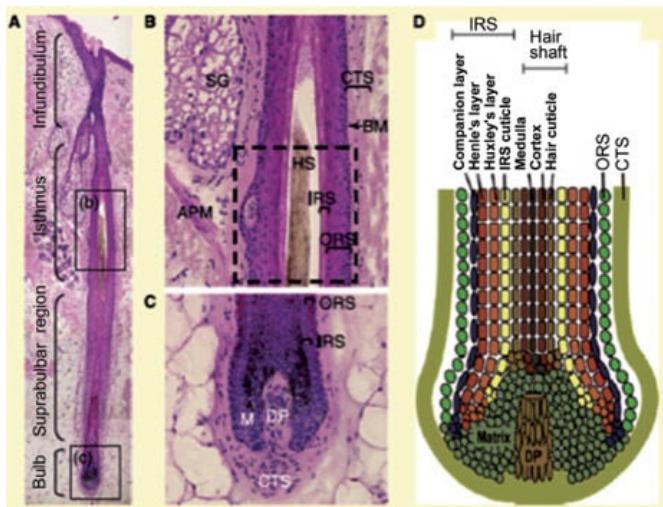


Figure 2. Structure of human anagen hair follicle. (A) Longitudinal section of a human scalp hair follicle showing the permanent (infundibulum and isthmus) and cycling (suprabulbar region and bulb) components of the hair follicle. (B) High magnification image of the isthmus. The dashed square indicates the approximate location of the bulge. (C) High magnification image of the bulb showing the outer root sheath (ORS), inner root sheath (IRS), matrix zone (M), dermal papilla (DP) and connective tissue sheath (CTS). (D) Schematic drawing illustrating the concentric layers of the ORS, IRS and shaft in the bulb. Current Biology 19, R132-R142 2009 ©2009 Elsevier

The anagen bulb (Figure 2C) is one of the most rapidly dividing tissues in the body. The bulb represents the hair shaft factory; it contains the proliferating matrix keratinocytes and pigment-producing melanocytes (matrix zone). The hair matrix keratinocytes move upwards and differentiate into the hair shaft, as well as into the inner root sheath; the melanocytes transfer pigment into the developing hair keratinocytes to give hair its color⁽⁸⁾. These cells are supplied by the pluripotent stem cells existing in the bulge. The hair bulb surrounds the dermal papilla, which comprises a group of specialized cells (dermal papilla cells) with important inductive properties. The dermal papilla is believed to be one of the most important drivers that instruct the hair follicle to grow, and dictates hair bulb size, hair shaft diameter, length, and anagen duration.⁽⁹⁾ The dermal papilla is known as the “conductor of the orchestra” or “command center,” and is an essential source of growth factors (keratinocyte growth factor, bone morphogenetic protein, hepatocyte growth factor, insulin-like growth factor, stem cell factor), all of which are critical for hair growth and melanogenesis⁽⁸⁾. Surgical removal of the dermal papilla and the lower dermal

sheath prevents hair growth, which indicates the importance of these specialized cells as the key signaling center in hair follicles⁽¹⁰⁾. Interestingly, dermal papilla cells from balding scalp have both higher levels of androgen receptor (AR) and type II 5-alpha reductase, which converts testosterone to dihydrotestosterone (DHT)⁽¹¹⁾. DHT can induce dermal papilla cells to secrete factors including transforming growth factor beta 1 (TGFb1) and DKK-1 that inhibit keratinocyte growth^(12, 13).

The hair shaft (Figure 2D) is divided into the hair cuticle on the outside and both the cortex and medulla in the center. The cells that make up the hair shaft are known as trichocytes, which are terminally differentiated hair follicle keratinocytes. The hair shaft cuticle consists of overlapping cells that are arranged like shingles pointing outward and upward. The hair shaft cortex, the bulk of the hair shaft, is composed of hair-specific keratin filaments and keratin-associated proteins with melanin produced by melanocytes in the matrix region⁽²⁾. The hair shaft medulla, the central part of the hair shaft, is composed of large, loosely connected keratinized cells with large intercellular air spaces. The hair shaft is surrounded and supported by the inner root sheath (IRS), the companion layer, and the outer root sheath (ORS).

The IRS consists of three distinct layers: the cuticle, Huxley's layer, and the Henle's layer (Figure 2D). The IRS cuticle layer adjoins the cuticle of the hair shaft and anchors the hair shaft to the follicle. IRS keratinocytes produce keratins and trichohyalin that serve as an intracellular "cement" giving strength to the IRS to support and mold the growing hair shaft, as well as guide its upward movement⁽⁸⁾.

The ORS surrounds the IRS and is continuous with the basal layer of the epidermis. Distinct from the other epithelial components, the ORS does not generate by upwards growth from the matrix zone, but rather directly from the stem cell bulge. The bulge region of the ORS is located just below the sebaceous gland and at the insertion site of the arrector pili muscle. Stem cells leave the bulge in the ORS and migrate toward the matrix zone, where they begin to proliferate and subsequently differentiate into new hair shafts⁽¹⁴⁾.

Hair follicles grow in an oblique angle towards the epidermal surface. This angle can be varied by the contraction of a bundle of muscle, collectively called the arrector pili muscle (APM). The APM is under adrenergic control and thus, in situations of cold temperature and emotional stressors, it gives rise to the interesting phenomenon of involuntary contraction resulting in the hair standing up!

3.3.2.2 HAIR FOLLICLE CYCLING

Hair growth and loss in humans is controlled by a specific follicular cell cycle ([Figure 3](#)) that includes three major stages ⁽⁹⁾:

- growth and differentiation (**anagen**)
- regression and apoptosis (**catagen**) and
- rest (**telogen**)

Additional phases recently discovered include exogen, in which the fiber is actively shed ⁽¹⁵⁾, and kenogen, when the follicle is empty ⁽¹⁶⁾.

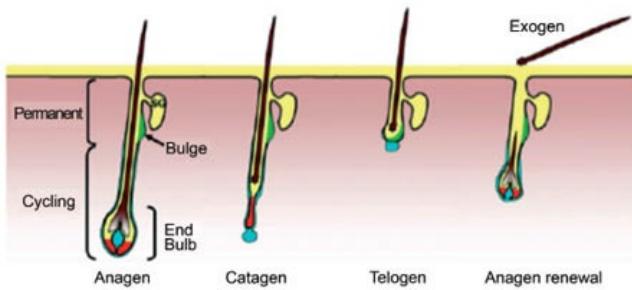


Figure 3. Hair Follicle Cycle. The different stages of the adult hair follicle are divided into three main categories known as anagen, catagen, telogen, which continually cycle throughout the life of the follicle. During anagen the matrix cells of the bulb (red) rapidly proliferate and differentiate to produce the hair fibre and layers of the inner root sheath. The hair follicle enters catagen which is characterized by apoptosis of the epithelial cells, and the formation of the club fiber. The dermal papilla (blue) moves up to the base of the permanent section of the follicle, in close proximity to the bulge (green). A resting phase known as telogen then occurs before the renewal of a subsequent anagen phase. Exogen describes the removal of the remaining club fiber which typically occurs close to the time of renewed anagen onset. Seminars in Cell & Developmental Biology 18 245-254 2007 ©2007 Elsevier

Hair follicle cycling is driven in response to continuing molecular interactions between the dermal papilla and the epithelial components of the follicle ^(17, 18).

The duration of the different growth phases depends on the type and localization of the hair follicle. Scalp follicles stay in the anagen stage for two to six years and produce long hairs, whereas eyebrow or eyelash hair follicles do so

for only one to three months and produce short hairs. An in-depth description of eyelash hair follicles may be found elsewhere in this book. What determines the clock mechanism and the duration of anagen in individual hair follicles is not known, although one candidate for consideration is the circadian clock, a molecular oscillatory system^(19, 20).

Following morphogenesis, the hair follicle enters the regression phase (catagen). The catagen stage lasts approximately two weeks, during which time the hair fiber ceases to grow. During the catagen stage, cells located in the lower portion of the hair follicle undergo programmed cell death (apoptosis). The hair follicle shortens and the hair bulb loses its internal and external sheaths. During this process the hair follicle acquires a club-shaped end and is subsequently referred to as the “club fiber.” Additionally the dermal papilla condenses, moves upward beneath the hair follicle bulge, and halts its activity. Follicular melanogenesis also ceases during this stage. Catagen has been suggested to occur as a consequence of decreased expression of anagen maintaining factors, such as insulin-like growth factor 1 (IGF-1), basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF), and increased expression of cytokines promoting apoptosis, such as TGFb 1, interleukin-1alpha (IL-1a), and tumor necrosis factor alpha (TNFa)⁽²¹⁾.

Upon catagen completion, hair follicles enter a resting stage (telogen) for an average of three months. In the telogen stage, the hair follicle has decreased to about half of its previous size and the stem cells are thought to be quiescent. However, by the end of the telogen stage, hair follicle stem cells become activated by Wnt and TGFb signals from the dermal papilla and form a new hair follicle^(22–24). Anagen involves the complete regrowth or regeneration of the lower, cycling portion of the follicle, i.e., the hair shaft factory. The cross-talk of dermal papilla with neighboring matrix cells results in the maintenance of hair fiber production (i.e., the hair continues to grow). Two secreted molecules that have important roles in hair follicle cycling are IGF-1 and bFGF. Both are produced by the dermal papilla, and their receptors are found predominantly in the overlying matrix cells⁽²⁵⁾.

Sometime after new shaft production begins, the club fiber is shed⁽²⁶⁾. The term ‘exogen’ has been introduced to identify the hair fiber shedding event as a separate process during hair follicle cycling⁽¹⁵⁾. Shedding of 100–150 hairs per day is normal⁽²⁾. Previously, hair fiber shedding was believed to be passive, but recent research suggests that shedding is an active and highly controlled process^(15, 27, 28). Empty hair follicles, after shedding of the club fiber, but before the onset

of renewed anagen, are in a stage called “kenogen.” Kenogen can be observed in healthy skin, but the frequency and duration are significantly greater in individuals with androgenetic alopecia⁽¹⁶⁾.

In addition to hair follicle tissue modeling, skin innervations and vascular networks also undergo substantial changes with the progression of the anagen stage^(17, 29). Perifollicular vascularization is significantly increased during anagen. It correlates with the up-regulation of the expression of vascular endothelial growth factor (VEGF) mRNA, a potent angiogenic growth factor, produced by keratinocytes of the outer root sheath⁽⁸⁾.

Each follicle can undergo repeated cycles until it eventually dies or miniaturizes to give rise to a vellus hair^(30, 31). **Disturbances of hair follicle cycling lie at the heart of most hair growth disorders, and have dramatic effects on visible hair growth and shedding.** For instance the most common alopecia, *androgenetic alopecia*, produces a patterned baldness involving a reduction in the percentage of scalp hair follicles in anagen, a reduction in the anagen growth phase duration, and a corresponding increase in the percentage of hair follicles in telogen and an increase in the duration of telogen. These alterations (in combination with a shift in the exogen phase from primarily occurring in early anagen to initiation primarily during telogen) result in thinning hair coverage^(32, 33). The most effective therapeutic strategies for hair loss therapy inhibit catagen development so as to prolong anagen, to induce anagen in telogen follicles, and/or to inhibit exogen⁽³⁴⁾.

3.3.2.3 AGING OF THE HAIR FOLLICLE

While consumers are increasingly concerned with diminishing the signs of aging, especially the aesthetic of hair, addressing the cellular mechanisms behind them represents a decisively new direction in hair care strategies. Like all biological systems, the hair follicle also undergoes an aging process. This process is not only characterized by loss of pigmentation, but changes in growth characteristics that include decreased hair follicle density, increased number of telogen hairs, a decrease in the diameter of hair shafts, and slower hair growth rates^(35–38).

The color of hair mainly relies on the presence or absence of melanin pigment. Two types of pigment can be produced by the hair follicle. The first type is eumelanin, which is the pigment in brown-black hairs, while the second type is pheomelanin, the pigment in red-blond hairs. Melanin is produced by the

melanocytes, and is the result of a complex biochemical pathway (melanogenesis) with tyrosinase being the rate-limiting enzyme. Hair melanins are formed in cytoplasmic organelles called melanosomes. The melanosomes travel out to the tips of the melanocyte's dendrites where they are transferred to the hair follicle keratinocytes of the hair cortex and medulla. Each hair cycle can produce a long pigmented terminal hair because the follicle is able to replace these melanocytes from a reserve found in the bulge region of the ORS⁽³⁹⁾. As individuals age, the melanin content of the hair follicle decreases, causing graying and eventual whitening of the hair. Although poorly understood, numerous mechanisms have been suggested for this loss of hair follicle pigment, including pigmentary machinery malfunction or loss, melanocyte apoptosis, anagen defects, the loss of melanocyte stem cells or their failure to differentiate, and melanocyte migration defects⁽⁴⁰⁾.

Hair graying, sometimes referred to as canities, is one of the most prominent signs of human aging. A recent worldwide survey showed that 74% of people between the ages of 45 and 65 have gray hair, and that it occurs earliest in people of Caucasian descent, followed by lesser occurrence in Asians and Africans⁽⁴¹⁾. The age of graying onset is genetically controlled and inheritable. Hair is considered to gray prematurely only if it occurs before the age of 20 years in Caucasians, before 25 years in Asians, and before 30 years in Africans⁽⁴²⁾. As hair color is socially important, premature graying imposes a psychosocial distress and is often perceived as a sign of stress, old age, ill health, and bodily decline^(42, 43).

Both the hair and scalp are constantly exposed to various environmental stressors. These include UV irradiation as well as oxidative and chemical stressors. The effect of external stressors on the hair fiber is quite well documented. In particular, repeated sun exposure impairs the structure of the hair shaft keratin fiber, while UV radiations induce hair protein loss and color changes⁽⁴⁴⁾. Recent *in vitro* and *ex vivo* studies have investigated the damage caused by UV radiation and oxidative stress on the hair follicle^(45, 46). Upon exposure to repetitive UVB irradiation isolated hair follicles in culture demonstrated reduced hair shaft elongation and premature entry into the catagen phase. Moreover, oxidative DNA damage was observed in hair follicle submitted to low UVB doses⁽⁴⁵⁾. Gondran *et al.* examined the effects of UV radiation and oxidative stress generated by H₂O₂ on human scalp skin grafts containing hair follicles^(46, 47). *Oxidative stress impaired hair follicle structure as indicated by increased number of vacuoles and edema mainly observed in the outer root*

sheath. Emerging evidence shows that reactive oxygen species (ROS) accumulate in human gray/white scalp follicles up to millimolar concentrations. The presence of ROS is believed to cause oxidative damage (free radical-induced aging) to the hair follicle melanocytes ^(48, 49). In addition catalase (the enzyme involved in cell response to oxidative stress), expression is reduced in unpigmented and older follicles ^(48, 50, 51). Altogether, the data strongly suggest that the hair follicle itself is a direct target of oxidative stress and underline the interest of applying a specifically developed cosmetic follicle ingredient to protect the hair follicle from endogenous or environmental stressful processes ^(46, 47).

CONCLUSION

Hair care formulations have traditionally relied on ingredients that treat the hair fiber to improve appearance. However, vibrant and healthy-looking hair starts at the roots and, therefore, cosmetic biofunctionals that target the hair follicle provide product manufacturers with new innovative ways to bring back beauty to the hair.

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GLOSSARY

Alopecia: Abnormal hair loss.

Androgenetic alopecia: Loss of hair caused by miniaturization of genetically predisposed follicles

Anagen: Growing stage of the hair follicle cycle.

Bulb: Lowermost portion of the anagen follicle containing rapidly proliferating cells that generate the hair fiber

Bulge: Site of stem cells within the follicle outer root sheath, located at the insertion site of the arrector pili muscle just below the sebaceous gland.

Catagen: Regression stage of the hair follicle cycle.

Club hair: Fully keratinized, dead hair formed during catagen.

Dermal papilla: Signaling control center of the hair follicle consisting of dermal papilla cells.

Exogen: Stage of the hair follicle cycle when hair is shed from the follicle.

Hair shaft: The hair *per se*, composed of trichocytes, which are terminally differentiated hair follicle keratinocytes. It is composed of the medulla and the cortex.

Infundibulum: Most proximal part of the hair follicle relative to the epidermis, extending from the sebaceous duct to the epidermal surface.

Inner root sheath: Surrounds and protects the growing hair.

Isthmus: Permanent middle section of hair follicle extending from the sebaceous duct to the point of arrector pili insertion

Kenogen: Stage of the hair follicle characterized by empty hair follicles after the hair fiber is shed before a new anagen hair emerges.

Outer root sheath: The outermost layer of the hair follicle. Merges

proximally with the basal layer of the epidermis and distally with the hair bulb.

Telogen: Resting stage of the hair follicle cycle.

Terminal hair: Large, usually pigmented hairs (e.g., those found on the scalp).

Vellus hair: Very short, nonpigmented hairs (e.g., those found diffusely over nonbeard area of the face and bald scalp as a result of miniaturization of terminal hairs).

HAIR AGING: FUNDAMENTALS, PROTECTION AND REPAIR

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ABSTRACT

Aging skin is a concept easily grasped; aging hair is not. However, changes in hair with aging are consumer perceivable and measurable. This chapter describes the structure of hair, fundamentals of aging hair, signs of aging hair, and causes of the visible signs of aging hair. Further, it also covers remedies for protection of hair fibers, the scalp from which it grows, as well as hair products and ingredients formulated to maintain and restore youthful hair. Beyond these approaches, the author has also provided a unique cosmeticological look at selected products and ingredients on the market with commentary on their performance.

While considerable attention has been paid to the many factors affecting aging skin, it is only recently that a shift in the perception that hair is “dead” has begun to accelerate. Actually, while this is true, in part, it is also true that the core of the hair fibers is alive as well as the skin from which it grows, and much has been done and needs doing to bring it to life again.

The understanding of the hair-scalp system described in this chapter provides important leads to ingredient-seekers and product formulators and presents an opportunity for a myriad of novel products that consumers long for. The chapter is also unique in that it provides thoughtful, practical recommendations for hair care and protection. This wisdom goes beyond the traditional approach of finding new ingredients or formulations to achieve the goals of beautiful hair. It is recommended that these approaches be incorporated in recommendations by

product manufacturers as important information to consumers.

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3.3.3.1 INTRODUCTION

Similar to skin, with aging, the scalp and hair are subject to intrinsic or chronological aging, and extrinsic aging due to environmental factors. The changes associated with aging skin and the mechanism of skin aging has been well researched and published extensively over the past decades. According to Mintel's global product database, nearly half of the skin care products in the market are anti-aging products.

While the aging of hair has not been studied extensively like skin aging, there are numerous studies published on hair aging¹⁻³, and the number of anti-aging hair care products continues to increase year by year⁴. The most obvious sign of hair aging is the appearance of gray hair that correlates closely with intrinsic aging. Melanin pigment is responsible for the natural color of hair and it is produced by melanocytes in the hair follicles. With aging, melanocytes stop producing melanin pigment, resulting in gray hair. The loss of melanin has been linked to a decrease in the number of melanocytes in the hair follicle as well as a possible decrease in the activity of the enzymes involved in melanogenesis⁵. A recent publication by Wood *et al.*⁶ showed for the first time high accumulation of hydrogen peroxide in human gray scalp hair shaft, thus supporting the theory

of oxidative stress in gray hair formation. These researchers also demonstrated that H₂O₂-mediated oxidation of methionine in tyrosinase enzyme limits its functionality, which leads to gradual loss of hair color.

In addition to graying of hair, with aging, your hair will become deficient in essential nutrients and the hair follicles shrink, it loses its elasticity, lipid and keratin production diminish, and many hair follicles stop producing new hairs altogether. These internal changes cause hair to become thin, dry, less manageable, brittle and less shiny, and ultimately lead to hair fall.

Externally, the hair shaft is exposed to sunlight, environmental pollutants, chemical and heat treatments, and subjected to daily grooming and mechanical aggression-in-search-of- “beauty.” These environmental insults and daily aggressors alter the chemical and physical properties of the hair fiber. When hair is exposed to sunlight, severe damage occurs due to lipid, protein, and melanin oxidation. This results in loss of mechanical strength, loss of color, an increase in porosity, as well as an increase in surface roughness. Other environmental pollutants such as cigarette smoke, car exhaust fumes, and smog also impart hair damage, resulting in dull and brittle hair. These physicochemical changes in hair structure and damage caused by aging are measurable and also perceivable to the consumer.

Several methods involving microscopic techniques, differential scanning calorimetric, wet and dry combing measurements, protein loss, and assessment of tensile properties of the hair are generally used to evaluate the various physicochemical changes to hair⁷. These approaches are useful in characterizing aging changes as well as the effect of “treatments” that may improve the detrimental effects of the various stressors.

Hair loss is another significant aspect of aging that occurs gradually with a progressive thinning of hair. With aging, less protein is produced in the hair follicle that can cause hair to fall out and not to be replaced as quickly as desirable. This causes baldness, as well as thinning of hair. Unlike the long term, initial focus on the skin of women, the baldness phenomenon is also a men’s “condition” and any impact of novel approaches to reduce hair loss and hair thinning represents an area of potential product growth for both men and women.

Experimental evidence supports the hypothesis that oxidative stress also plays a role in hair aging. The damaging effects of these reactive oxygen species are induced internally during normal metabolism and externally through exposure to

various oxidative stresses from the environment. The body possesses endogenous defense mechanisms, such as antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase) and non-enzymatic antioxidative molecules (e.g., vitamin E, vitamin C, glutathione, ubiquinone), protecting it from free radicals by reducing and neutralizing them.⁹ With age, the production of free radicals increases, while the endogenous defense mechanisms decrease. This imbalance leads to the progressive damage of cellular structures, presumably resulting in the aging phenotype.

Nourishment and proper grooming are very important to maintain the beauty of hair as perceived by: good hair texture, healthy growth, shine, and luster. Eating foods rich in vitamin A, B, minerals, and proteins is necessary for promoting a healthy scalp and hence healthy hair growth. Eating a diet rich in dark green vegetables, orange and yellow fruits, sprouted whole grains, cereals, meat, and soy helps maintain a healthy follicle. It is equally important to use good scalp care and hair care products to protect the hair shaft from damage due to sunlight, pollution, and other daily aggressors. For daily washes, use a shampoo that is mild, gentle, and moisturizing. Anti-aging hair care topical and ingestible products enriched with vitamins, lipids, proteins, vegetable oils, and antioxidant rejuvenate the scalp and follicle, and encourage new, more resilient growth from the root.

This chapter describes the fundamentals of aging hair, signs of aging hair, and the causes of visible signs of aging. Further, it also surveys current remedies for protection of the hair fiber, along with hair products formulated to maintain and restore youthful hair.

3.3.3.2 STRUCTURE, COMPOSITION, AND NATURAL COLOR OF HAIR

Hair is composed of two parts, the hair follicle and the hair shaft. The hair follicle is a tiny cup-shaped point located in the dermis from which the hair grows. The hair follicle consists of the hair bulb, the inner root sheath, and the hair shaft (see [Figure 1](#)).

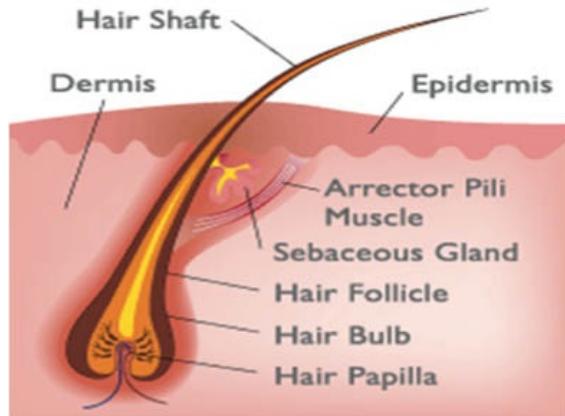


Figure 1. Cross-Sectional Image of Hair Bulb Structure

New hair is made inside the onion-shaped hair bulb that lies within the hair follicle. The hair bulb also contains some special cells called melanocytes that produce melanin, the pigment responsible for the natural color of hair. The part of the hair seen above the scalp is called the hair shaft. It consists of three layers; cuticle, cortex, and medulla (see [Figure 2](#)).

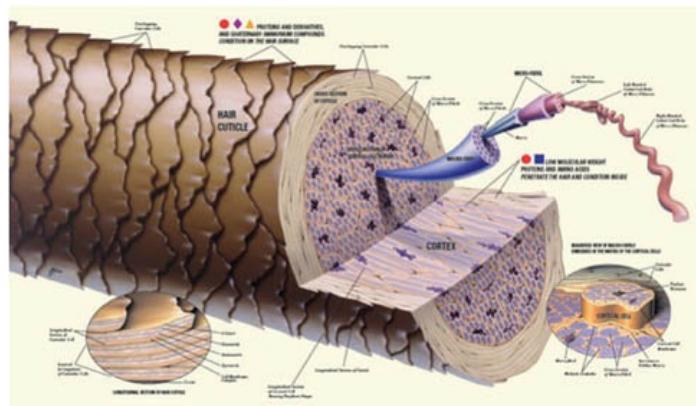


Figure 2. Structure of Human Hair Fiber

The outermost layer called cuticle is a thin and colorless layer made of several overlapping scale-type structures. It is responsible for the visual attributes of hair such as shine and smoothness. It is also responsible for the friction effect of hair, which determines manageability (i.e., how easy it is to comb or brush hair). The cuticle layer can be damaged by daily grooming such as combing and brushing as well as by chemical treatments such as bleaching or relaxing. The middle layer of the hair shaft is called the cortex, made of keratin fibers. The strength, color, and texture of a hair fiber are provided by the cortex. The innermost layer of the hair shaft called medulla is only present in large and thick hairs.

Melanocytes are the cells responsible for the natural color of hair, which are

large star-shaped cells with branches called dendrites. They are mainly found at the bottom of the hair follicle, where they produce melanin in the form of small grains of colored pigment. Melanocytes inject these pigments into the keratinocytes of the hair shaft by lengthening their dendrites. Melanocyte produces two types of melanin: eumelanin and phaeomelanin. Eumelanin occurs in the form of a small rice-like granule having a color varying between brown and black. Melanin is formed by the oxidation of an amino acid, tyrosine by the enzyme tyrosinase. The higher the concentration of eumelanin, the darker the color of hair.

The actual appearance of hair, and its overall reflective quality, is determined primarily by the pigment type, but also by the density and distribution of the pigment granules. Phaeomelanin has a less precise shape and can be seen in the form of diffuse spots. Its color varies from yellow to red. Phaeomelanin differs from eumelanin because, in addition to tyrosine, another amino acid, rich in sulfur (cysteine), is involved in its production. There are two different types of eumelanin (brown eumelanin and black eumelanin). A small amount of brown eumelanin in the absence of other pigments apparently causes blond hair.

Although people with dark hair may still produce the yellow-orange pheomelanin, it is largely masked by the dark eumelanin pigment and cannot easily be seen. However, the red-yellow pheomelanin is believed to cause the warm, golden, or auburn tones found in some types of brown hair. The range of colors produced by melanins is limited to shades of yellow, brown, red, and black. Gray hairs contain only a few melanin granules, spread throughout the hair. White hairs contain no melanin at all: their whiteness is an optical effect, due to the way they reflect the light.

3.3.3.3 FUNDAMENTALS AND SIGNS OF AGING HAIR

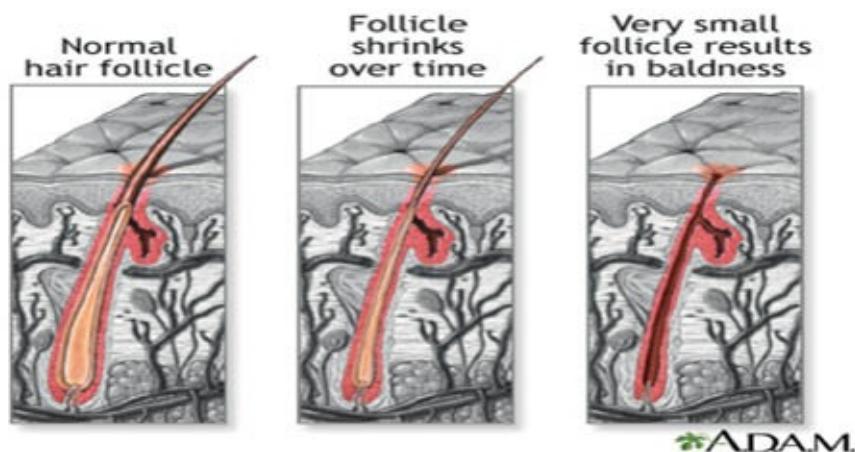
When considering the phenomenon of hair aging, genetic, hormonal, and environmental factors need to be taken into account. Similar to skin, the scalp and hair are subject to intrinsic aging depending on individual genetics and hormonal factors and extrinsic aging due to environmental factors. Intrinsic aging or chronological aging results in graying of hair due to a decrease in melanin production and loss of hair due to androgenic alopecia. Extrinsic factors include ultraviolet radiation, pollution, smoking, diet, and lifestyle. All these extrinsic factors progressively degrade the hair fiber through oxidation of lipids, cleavage of disulfide bond, degradation of amino acids, and oxidation/bleaching

of melanin pigment.

The effects of age-related changes in hair density, hair diameter, hair lipids, and hair color (graying) produce changes in hair fiber properties. These changes in properties impact important consumer hair assembly properties such as ease of combing, hair body, hair coverage, frizziness, shine, manageability, and style retention. There are many visible signs of aging hair that are measurable and consumer perceivable. *The most critical aging attributes are thinning of hair, loss of color, moisture, shine, and manageability.*

Thinning of Hair

Both men and women tend to lose hair thickness and amount as they age. Baldness is not usually caused by a disease. It has been found that poor diet, stress, aging, and pollution are the most common factors that damage hairs and cause hair loss. Scientifically, health experts have found that the growth of dihydrotestosterone (DHT) hormone on the scalp is responsible for hair loss. With aging, hair loss is experienced more rapidly because the amount of DHT increases with increase in age. This hormone makes hair follicles weaker and weaker by blocking the nutrients and water to the hairs. Over time, the follicle can shrink, causing the hair to become shorter and finer and finally the hairs fall from the scalp. [Figure 3](#) shows the picture of a normal hair follicle compared to a finer follicle and a very small hair follicle, which results in baldness.



[Figure 3](#). Picture of a hair follicle showing shrinkage over time

According to the American Hair Loss Association, around two-thirds of U.S. men will have experienced some degree of hair loss by the age of 35, and about 85% of men will have significantly thinning hair by the age of 50. Although hair loss is typically associated with men, figures state that women make up 40% of

all hair loss sufferers in the U.S., showing that hair loss is a condition that can affect us all. Recent studies claim that 16% of women under age 50 are affected, which increases to 30–40% of women aged 70 years and over¹⁰. The thinning of hair in women may become rather more pronounced after menopause when there are fewer estrogen hormones to counteract the androgens. Men tend to have baldness while women have thinning of hair and preservation of the frontal area.

Hair loss treatment products are mostly designed to act as DHT blockers. In recent years, two pharmacological agents have become available to treat male pattern baldness. Neither is fully effective in all cases. Both need long-term administration or there will be recurrence. Both must be prescribed on private prescription. Minoxidil is provided in 2% and 5% solutions that are applied to the scalp twice daily. The 5% solution is for men only. One study found that the 5% solution was 45% more effective than the weaker strength after a 48-week trial and was the preferred treatment for men¹¹. It may well be months before any improvement is seen and it should be discontinued if there is none after a year. A solution of 5% minoxidil in cutaneous foam is now available, which is developed for ease of use. The product instruction requires application of half a capful to the scalp twice a day but the treatment should be stopped if there is no improvement after four months.

Recently, the natural hair loss treatment, **Provillus™**, has gained significant popularity for its effective dual-step formula to treat the hairs of men and women accordingly as it offers different formulations for both. The treatment, clinically proven to regrow hair, consists of a powerful blend of natural herbs and has been approved as safe and beneficial formula by health experts and FDA. The two-step formula consists of a hair regrowth supplement and a hair regrowth spray with 5% minoxidil for men and 2% minoxidil for women, which performs dual action of preventing hair loss first and then promoting hair regrowth. The consumption of this supplement twice a day makes the hairs strong enough to fight against DHT hormone and to survive. The hair regrowth spray is a liquid to be applied over the scalp. The use of that spray twice a day promotes a thicker, stronger, and healthier hair regrowth. The product nourishes the overall health of hairs and makes them healthy, shiny, and beautiful, without any negative side effects.

Change in hair fiber diameter (fine-coarseness) is another parameter that is influenced by age. Large increases in hair fiber diameter occur during the first year in life and during the teenage years. Diameter tends to peak at about age 20 for men and in the mid-40s for women (see [Figure 4](#)). Figure 4 shows a steeper

drop for the scalp hair fiber diameter of Japanese males versus Caucasian males. A decrease in hair fiber diameter results in thin hair.

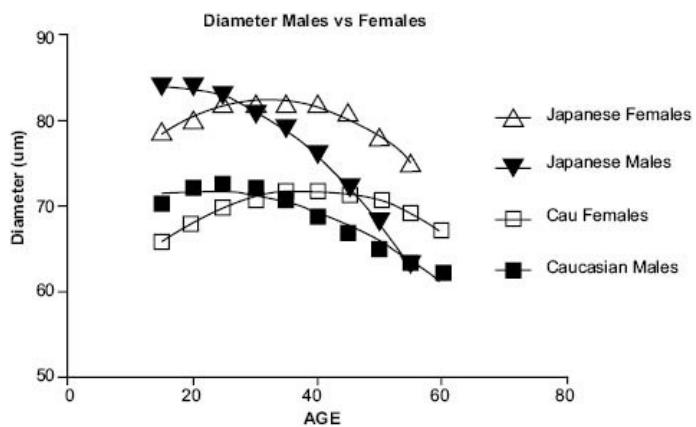


Figure 4. Hair fiber diameter of Japanese males vs. Caucasian males as a function of age (C.R Robbins pp 120)

Loss of Hair Color (Graying of Hair)

Graying of hair is a natural age-related phenomenon that varies between individuals and between different ethnicities. The biological process of hair aging has been attributed to the loss of pigment-forming melanocytes from the aging hair follicle¹². There are many theories that explain the gradual loss of pigmentation with aging, including inactivation of tyrosinase enzyme⁵ and decrease in stem cell factor¹³. Recently, a free radical theory of graying has been proposed by Arck *et al.*¹⁴. These authors found evidence of melanocyte apoptosis and increased oxidative stress in the pigmentary unit of graying hair follicles. Recently Wood *et al.*⁶ have shown for the first time accumulation of high levels, millimolar concentration, of H₂O₂ *in vivo* in human gray/white scalp hair shaft by FT-Raman Spectroscopy. These authors further demonstrated absence of catalase and methionine sulfoxide reductase A and B enzymes. In younger people, catalase enzyme breaks down hydrogen peroxide into water and oxygen, thereby protecting melanocytes from oxidative damage by hydrogen peroxide. As we age, the decrease in the levels of catalase, combined with lower levels of methionine sulfoxide reductase enzymes, MSR A and B, which repair hydrogen peroxide damage, cause hair to turn gray. A recent study by researchers at University of Bradford demonstrated repigmentation of eyelashes and skin after reduction of epidermal H₂O₂ with topically applied narrow-band UV activated pseudo-catalase PC-KUS.¹⁵ This further supports that accumulation of hydrogen peroxide due to decrease in catalase enzyme causes hair graying and reversal of

pigment formation is possible through introduction of pseudo-catalase type molecules through topically applied products.

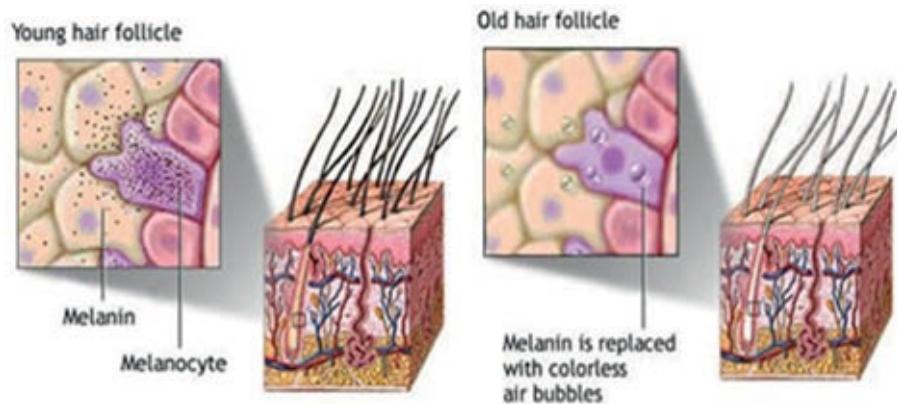


Figure 5. Picture of a young hair follicle vs. gray hair follicle showing melanin particles replaced with colorless air bubbles (www.nlm.nih.gov)

Hair Dryness, Shine, and Manageability

Hair dryness also results from both intrinsic and extrinsic aging. The sebaceous gland in the hair follicle produces sebum that normally hydrates the scalp and moisturizes the hair by surrounding it with a protecting microfilm. A deficiency or lack of sebum makes the scalp more fragile, resulting in irritation and itchiness. The loss of sebum also results in loss of hair elasticity and the hair becomes dry, dull, and prone to breakage. The intrinsic production of sebum from sebaceous glands in the follicle decreases with age, particularly in females as shown in [Figure 6](#) below.

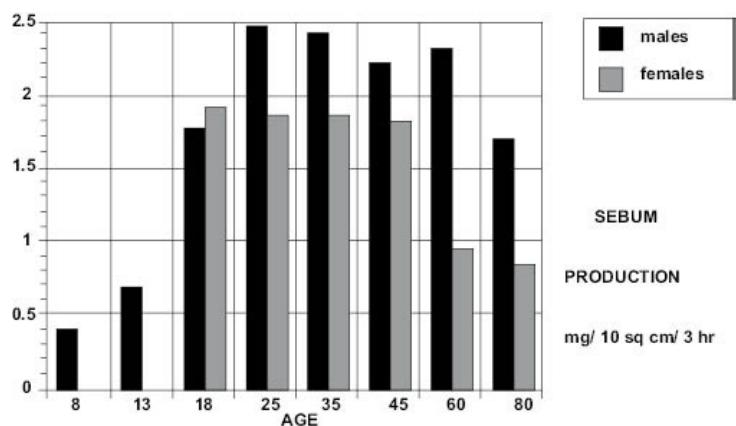


Figure 6. Variation of sebum production on foreheads with age. Data are from C.R. Robbins, *Chemical and Physical Behavior of Human Hair*, 4th edition, p. 79

Additionally, extrinsic parameters such as frequent washing of hair, use of alcohol-containing hair products, chemical treatments, exposure to sunlight, and frequent blow-drying also cause hair dryness. Each healthy hair strand has an outer protective layer (cuticle), which is composed of fatty acids, ceramides, and cholesterol. Together, these account for 0.7–1.3% of the total chemical content of hair. Integral hair lipids are located in the cell membrane complex (CMC) of hair cuticles, and are important in the maintenance of hair integrity due to hydrophobicity, moisturization, and stiffness.

Exposure to sunlight results in degradation of cuticle lipids by oxidative damage, and exposure to alcoholic cosmetic products solubilizes lipids and removes them from hair surface. Blow-drying and frequent washing with harsh surfactants also removes cuticle lipids. The appearance of your hair, and its reflective quality, depends heavily on the condition of the cuticle. Light reflection from a smooth and intact cuticle gives shine and glossiness, resulting in youthful appearance. Loss of cuticle also makes combing and brushing difficult. A flat and smooth cuticle structure reflects light, and it is important to keep healthy and shiny hair. It is reported that replacing the essential hair lipid, **18 MEA**, results in healthier-looking and -feeling hair through restoration of the hydrophobicity of hair fiber. Further, lipid replenishment protects the hair from further damage¹⁶.

Chemical treatments such as hair relaxing and permanent hair coloring chemically modify the hair keratin structure by disrupting the disulfide bond, which is responsible for the strength of hair. Curly hair has a higher degree of disulfide bond in the cortex compared to straight hair. The process of straightening or relaxing changes curly hair into straight hair using alkaline or other chemical relaxers. The chemical modification of the disulfide bond in the cortex of the hair shaft affects the physicochemical property of hair fiber. Frizzy hair, hair breakage, hair thinning, scalp irritation, scalp damage, and hair loss are some of the common consumer complaints due to the misuse or chronic use of chemical hair relaxers¹⁷. Permanent hair coloring also changes hair fiber properties due to oxidative damage of hair proteins and lipids due to the presence of ammonia and hydrogen peroxide. The oxidation of lipids makes the hair dry and dull and the oxidation of the disulfide linkage of the keratin protein makes the hair fiber weaker. However, recent hair color products are formulated with great care to minimize damage as much as possible. Additionally, the hair color kits provide after-color conditioners to be used right after coloring to provide conditioning, shine, and color protection. Regular use of products

formulated for colored hair provides conditioning, shine, and overall youthful appearance.

3.3.3.4 PHOTO-AGING OF HAIR

Photo-damaged hair shows dryness, an increase in fiber porosity, reduced strength and an increase in surface roughness. Long-term exposure to UV radiation causes severe chemical degradation to hair proteins and lipids. Photoaging of hair results in lipid oxidation, disulfide bond cleavage, tryptophan degradation, and cysteic acid formation. Hair exposed to sunlight is claimed to be more brittle, stiffer, and drier than before irradiation and exhibits a reduced water-absorption capacity. Hair pigments provide photochemical protection to hair proteins by absorbing and filtering the impinging radiation and subsequently dissipating this energy as heat. However, in the process of protecting the hair proteins from light, the pigments are degraded or bleached. Dark hair is more resistant to photo-degradation than light hair. This is due to the higher photostability of eumelanin compared to pheomelanin².

The most obvious effect of the photo-aging of human hair is hair lightening, an effect that is accelerated by moisture. The extent of this photochemical-induced color change is dependent on the nature of the hair pigment and is understood to involve an oxidative attack on the eumelanin or pheomelanin melanosomes. Black hair is more photostable than blond hair. This color of hair seems well protected against UV light, and light brown hair is obviously damaged by a wide range of natural sunlight. The protective action of melanin granules is limited to the melanin-rich cortex of black hair, which shows only a slight modification of fiber proteins under irradiation¹⁸.

3.3.3.5 PROTECTION OF HAIR FROM AGING

a. Scalp Care

Adequate care of scalp and shaft hair is critical to protect the hair from aging and to have healthy beautiful hair. Beautiful hair comes from a healthy scalp and hence taking care of the scalp is just as important as taking care of hair shaft. Hair problems such as thinning or falling of hair, premature graying or breakage, dandruff, and flaking could be symptoms that indicate an unhealthy scalp. Scalp care should be chosen based on the nature of your scalp, whether it is normal, oily, or dry. Normal scalp has the right balance of sebaceous glands, so it is

generally not prone to problems like dandruff or excessive oil deposition. Shampooing three to four times a week with a normal shampoo and a light conditioner will be sufficient for this type of scalp. A warm oil treatment and massaging the scalp one to two times a month stimulates the oil gland and the massaging action improves blood circulationⁱ. Coconut oil is great for all hair types. Avocado oil is one of the most emollient and moisturizing oils and is particularly good for really dry and frizzy hair.

If your scalp is oily, it is the most prone to dandruff and flaking. The excess oil that the sebaceous glands in the scalp produce tends to accumulate around the roots, clogging the pores. Such hair is usually limp and flat, making it almost impossible for volumizing or styling products to hold. Shampooing your hair every day with a deep cleansing shampoo is suitable for oily hair. If you suffer from dandruff, choose an anti-dandruff variety. Avoid using leave-in conditioners. A dry scalp causes constant itching and flaking, leaving the hair rough, dull and frizzy. As the oil secreted by the sebaceous glands is insufficient, apply oil externally and massage the scalp regularly to nourish the scalp. For dry hair, shampoo once or twice a week with a product that contains natural oils and moisturizing agents is sufficient. A thick cream-based conditioner and leave it on for about ten minutes before rinsing off makes hair smooth and soft. Get a warm oil treatment every week to stimulate the sebaceous glands. Massage your scalp thoroughly to stimulate oil secretion.

Unilever has recently launched Scalp and hair beauty therapy line of products formulated with Nutrium 10, an advanced moisturizing complex. A well-nourished scalp is the right foundation for strong, beautiful hair. For years women have been treating the wrong end of their hair. Clear Scalp & Hair Beauty Therapy made with Nutrium 10 nourishes the scalp, creating the right foundation for strong, beautiful hair. The regimen of shampoo and conditioner together is clinically proven to help restore scalp's natural moisture balance to create the right foundation for strong, beautiful hair from root to tip. There are various regimens of shampoo and conditioner to address the needs of different hair types.

b. Hair Care

Most women will see their hair become frizzier and less manageable due to chemical treatments such as coloring, straightening, perms, and frequent washing with harsh shampoos that strip the hair of natural oils. Everyday caring of hair starting with the right shampoo, conditioner, and styling product could

cosmetically improve the appearance of shaft hair. Nourishment and proper grooming is very important to keep the hair texture, growth, shine, and luster in a good condition. For daily care, choose cleansing products that are gentle and moisturizing, preferably without harsh surfactants like sodium lauryl sulphate. Daily massaging of your hair with your fingertips helps proper blood circulation in the scalp. Apply heat-styling products to your hair before blow-drying and try to keep the setting on medium, not high. Use UV-protection hair products or wear a hat if your hair is brittle or color-treated. Choose a demi-permanent hair color product for covering gray; it doesn't contain ammonia and has less hydrogen peroxide. This type of product may not be an option if you have a lot of gray hair (more than 50%) and want to cover it, in which case you'll need permanent color.

3.3.3.6 ANTI-AGING HAIR CARE PRODUCTS

One of the latest trends in the global beauty industry is the proliferation of anti-aging hair products in the marketplace. Consequently, beauty product developers, ingredient suppliers, and brands large and small have been busy addressing the graying population in hopes of attracting the baby boomer marketplace⁴.

Ingredients for hair care include the well-established ones such as: silicones for shine, quaternary ammonium compounds for ease of combing, thickening approaches such as proteins and high-molecular-weight polyquaternium polymers for conditioning¹⁹ and polyvinylpyrrolidone for shaping and styling²⁰.

The hair shaft lacks biochemical processes, which gave the traditional concept that hair is dead and cannot be repaired and restored. As the root of the hair shaft is alive, hair treatments using anti-aging ingredients such as small-molecular-weight peptides, alpha hydroxyl acids, certain vegetable oils, amino acids, peptides, and ceramides can provide benefits to both the shaft and the hair root. In recent times, the hair care sector is being enlivened by entirely new ingredients as well as proprietary packages that combine the old standbys in novel ways. Anti-aging hair care specifically focuses on ingredients and products (applied both topically and in ingestible form) that can repair hair, providing more manageable and healthy looking hair.

Many ingredients are recently launched to address hair and scalp aging at the cellular level, improve follicle health, reduce hair loss, and offer antioxidant properties. These low-molecular-weight compounds penetrate the cuticle layers

of the hair shaft, enhancing its softness, protection, and manageability. They can also penetrate the scalp, enhance scalp microcirculation, and improve follicle health, thereby promoting healthier new hair growth. Recently, researchers at DuPont have shown that hair care products with the well-known anti-aging ingredient, glycolic acid (marketed as DuPont™ Glypure), penetrates through the hair shaft and delivers enhanced protection and manageability to hair by conditioning, moisturizing, strengthening, and preventing breakage⁸. It also moisturizes and exfoliates the scalp, resulting in less flaking to give the scalp a healthy look and feel.

Natural vegetable oils provide nourishing conditioning and shine to hair. Coconut oil, being a triglyceride of lauric acid (principal fatty acid), has a high affinity for hair proteins and, because of its low molecular weight and straight linear chain, is able to penetrate inside the hair shaft²¹. It is an excellent conditioner and helps in the regrowth of damaged hair. It also provides the essential proteins required for nourishing damaged hair and it softens the hair and conditions the scalp. Avocado and Jojoba oil are other oils for nourishing, conditioning, and shine benefits.

Amino acid-like L-Arginine is a nitric oxide precursor found to stimulate hair regrowth by stimulating the production of hair follicles and also to strengthen hair by nourishing the hair roots. Sunscreens are another category of anti-aging ingredients now found in a variety of hair care products, including shampoos, conditioners, and hairsprays. They prevent breakdown of keratin due to sun exposure and also keep hair dye from fading in ultraviolet light. Ingredients like Crodasorb™ UV-HPP from Croda and PARSOL® SLX provide multiple benefits like prevention of color fading, strengthening hair body, and enhancing gloss when delivered through hair color, or hair care products, especially leave-on sprays. The table below lists some advanced functional hair care ingredients popular in today's hair care market.

Table 1. Advanced hair care ingredients popular in current hair care productsⁱⁱ

Hair Care Ingredients	Benefits	Supplier
Crodafos™ HCE	Increases color intensity & color uptake. Provides better color wash fastness. Reduces hair damage as shown by hydrophobicity and combing studies.	Croda Inc
Incroquat™ Behenyl TMS-50	Hair conditioning agent, suitable for cationic emulsions, provides soft touch.	Croda Inc

ChromAveil ®	Protects the hair from the damaging effects of sunlight. Gives color protection benefits to dyed hair from UVA and protects the mechanical properties of the hair from UVB.	Croda Inc
Crodasorb™ UV-HPP	Polyester polyquaternized ingredient that is substantive to hair and protects against the damaging effects of UV-B radiation.	Croda Inc
Lustreplex®	Creates lustrous, healthy-looking hair from anionic systems. Provides frizz control, shine, detangling, and conditioning benefits.	Croda Inc
Dow Corning® CE-8411 Smooth Plus Emulsion	Repairs colored, gray, and heat-damaged hair by restoring moisturized feel, shine, & alignment. Protects hair from further damage and color loss.	Dow Corning
Dow Corning® 5-7113 Silicone Quat Microemulsion	Restores smoothness, shine, and Alignment. Prolongs hair color. Protects hair from breakage.	Dow Corning
HYDROVANCE®	Improves water retention and helps to strengthen and even repair damaged hair, for benefits the consumer can clearly feel and see.	Akzo Nobel
STRUCTURE® PQ-37P	Cationic acrylic homopolymer multifunctional rheology modifier that provides thickening and conditioning.	Akzo Nobel
Procapil™	Reinforces hair anchoring. Reduces hair loss.	Sederma
Glypure® glycolic acid	Glypure® penetrates the hair shaft to enhance the softness and manageability of hair.	DuPont
L-Arginine	Helps hair regrowth by stimulating the production of new hair follicles. It nourishes the hair root and strengthens hair.	Ajinomoto
PARSOL® SLX	PARSOL® SLX is a silicone-based UV-B absorber. Delivers multiple benefits including prevention of color fading, gloss enhancement, and conditioning.	DSM

Table 1. Shows ingredient information obtained from supplier websites

Anti-aging hair care products are designed to address the consumer needs such

as thinning, coloring, breakage, and drying, with emphasis on particular ingredients that target specific hair issues. Such hair care formulations are designed to deliver conditioning, thickening, and color protection, strengthening, and nourishing properties through deposition of essential oils, cationic polymers, proteins, and other nutrients. For gray hair, so far, there are no solutions for reversing the color. Hair colorants are used for covering gray, to impart color and shine and hence youthful appearance. Therefore, hair colorants can also be classified as anti-aging hair color products.

Pantene has launched PRO-V Expert Age Defy shampoo, conditioner, and advanced thickening treatment designed to work together for thicker hair strands. These products are claimed to be gentle enough for daily use on color-treated hair. Formulated with a triple blend complex (mixture of amino acids), the shampoo and conditioner help fight seven signs of aging hair: reduces breakage, prevents split ends, reduces frizz, controls unruly grays, minimizes lackluster color, improves a thin look, and minimizes dryness.

There are several professional hair care products formulated with advanced technologies that restore oils vitamins and other essential nutrients. Nexxus has launched its first anti-aging range of products that combats eight signs of aging hair: volume loss, breakage, roughness, less shine, dryness, brittleness, unruliness, and loss of color vibrancy. It replenishes and revitalizes for more vibrant, youthful-looking hair.

Nexxus Youth Renewal™ Rejuvenating Shampoo gently cleanses hair to help rebuild volume, vibrancy, and vitality. Nexxus Youth Renewal™ Rejuvenating Conditioner provides lightweight nourishment to help preserve volume, vibrancy, and vitality. Youth Renewal™ Rejuvenating Elixir lightweight leave-in treatment helps rebuild hair's strength by reducing breakage while making hair look more youthful and vibrant in just seven days.

Alterna has launched CAVIAR Anti-Aging® Replenishing line of products comprising moisturizing shampoo and conditioner that transforms dry, brittle hair by sealing in a rich blend of lipids and essential ingredients to continually nourish and hydrate the hair. The shampoo is a luxurious, sulfate-free cleanser that restores moisture while protecting hair from color fade, daily stresses, and future damage. Both shampoo and conditioner are infused with Seasilk®, Age-Control Complex®, Enzymetherapy®, and Color Hold®. It not only helps maintain moisture, but also protects from daily stresses and delivers what your hair needs to become strong, healthy, and younger-looking. The anti-aging rapid repair spray helps add moisture and vibrancy to hair while combating natural,

chemical, and environmental stresses. It may be used as a thermal protectant when applied pre-styling, or as a finishing shine spray.

Keranique® has launched a hair revitalization system developed for women to strengthen, fortify, and thicken each and every hair shaft. There are three key components to the Keranique® System. Step 1 includes a revitalizing shampoo and volumizing conditioner. Step 2 has the hair regrowth treatment with clinically proven FDA approved ingredient, Minoxidil, and Step 3 is an amplifying lift spray to provide fullness, volume, and body.

CONCLUSION

Hair, like skin, undergoes aging both intrinsically due to chronological aging and extrinsically as a result of exposure to environmental factors such as sun, pollution, and daily grooming habits. The aging of hair results in a series of visual and measurable changes both in the scalp and hair shaft. The most obvious sign of hair aging is graying of hair while the consumer also perceives a series of changes such as thinning of hair, dryness, brittleness, loss of shine, and manageability. Prevention of scalp and hair aging is not completely possible, but there are ways to protect scalp and hair fiber from damage due to external aggressors and to minimize damage from daily grooming by choosing the right products. The chapter provides an overview of the cause of hair aging, the factors that contribute to hair aging, the visual and perceptual signs of aging, and finally a compilation of some hair ingredients and anti-aging products currently available in the market.

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GLOSSARY

1. **Gray Hair :** Hair without melanin pigment
2. **Hair Follicle:** An organ and part of the skin below the scalp that produces hair.
3. **Hair Shaft:** The part of the hair that is seen above the skin
4. **Oxidative Stress:** An imbalance between oxidants and antioxidants in favor of the oxidants, potentially leading to damage, is termed ‘oxidative stress’
5. **Chronological Aging:** Age of a person measured in years, months, and days from the date the person was born
6. **Androgenic Alopecia:** A common form of hair loss in both men and women
7. **DHT:** Dihydrotestosterone (a derivative of the male hormone testosterone)
8. **18-MEA:** 18-methyl eicosanoic acid (essential hair lipid responsible for hair shine and hair integrity)

PART 3.3.4

MECHANISMS OF CHANGES IN HAIR SHAPE

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A note from Meyer R. Rosen, Editor-in-Chief

* This chapter brings to mind a phrase that I hope will stick in your memory for a long, long time. At its heart it is Manuel Gamez-Garcia's unique ability to describe:

The Science of Hair Beauty and the Beauty of Hair Science

ABSTRACT

Changes in hair shape can be categorized as permanent and temporary. They can also be classified as cosmetically desirable and undesirable or by a combination of any of these two categories. For instance, examples of hair shape changes desired permanently are those produced by perm solutions and alkaline relaxers. Examples of desirable temporary changes are those resulting from water-setting, hot irons, and styling polymers. Undesirable permanent shape changes are, on the other hand, those leading to frizzy hair, excessive tangling, and lack of manageability.

This chapter provides an exceptionally detailed look at the development of scientific distinctions based on the integration of polymer physics and chemistry with that of hair behavior and what impacts it in our endless search for beauty.

Whether the changes in hair shape are desirable or undesirable, all of them result from a process consisting of three main steps:

- 1)the softening of hair, which may involve the breakage of chemical or physical bonds, and/or protein rearrangements in the amorphous and crystalline phases,
- 2)the mechanical imposition of a new shape, and
- 3)the fixing or locking of the new shape by the re-formation of broken chemical or physical bonds. In shape changes that are desirable the occurrence of these three steps is induced purposely to create a new shape, whereas in those that are undesirable the three steps occur mostly during hair damage. This chapter will focus primarily on those aspects that are desirable from a cosmetic point of view. Those aspects produced by damage have been reviewed elsewhere (1).

We would point out that technology transfer from the world of polymer physics and chemistry has been of great value in the development and application of “novel” concepts and their use as seminal thinking points for cosmetic hair scientists. We encourage the reader to continue this approach, as much of this type of “new” thinking will open fascinating and productive pathways to novel hair care products.

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References

3.3.4.1 INTRODUCTION

The key physical properties and mechanistic events related to changes in hair shape can be better understood when we recall that hair is a biopolymer that shares many of the physical and chemical features possessed by synthetic polymers. In fact, we can learn a great deal about hair's mechanical behavior by comparing its properties with those of synthetic polymers having a similar structure. For instance, synthetic semi-crystalline polymers, like hair, possess crystalline melting and amorphous phase glass transition temperatures (2–4).

Furthermore, the composition of hair's architecture is such that it can exhibit two types of physical behavior.

- One type of behavior is related to the hydrophilic structure of the globular proteins in the amorphous phase. The protein hydrophilic groups in this structure are capable of forming hydrogen bonds with water. This aspect of the structure makes it highly water swellable and susceptible to plasticization by moisture. It is responsible for the viscoelastic and softening effects produced in hair by water; Max Feughelman refers to this aspect as the “gel structure of the amorphous phase” (6).
- The other type of physical behavior stems from the non-gel structure of the intermediate filaments (IFPs). This structural aspect is comprised of an amorphous network of hydrophobic, and stiffer protein segments. These are covalently cross-linked by disulfide bonds, as well as an ordered arrangement of crystalline proteins regions, which are responsible for both shape stabilization and hair strength.

The entire amorphous phase acts mechanically in parallel with the crystalline phase and gives rise to the unique combination of viscoelasticity and strength typical of hair. This behavior is also found in semi-crystalline polymers and, incidentally, there is a remarkable similarity in the architecture and properties of hair, and a particular type of cross-linked semi-crystalline polymers, namely; those that have been synthesized to behave as shape memory polymers (SMP) (7). These polymers, like hair, also exhibit two and even more types of physical behaviors. These multiple types of behavior result in most cases from the various chemical groups or structures grafted onto main polymer chains introduced with the purpose of storing various temporary shapes (8–11).

As will be discussed later in this chapter, hair, with both its amorphous and crystalline structures, can also act as a shape memory polymer (SMP) and the mechanistic aspects related to changes in its shape can be better understood by using concepts from this field of polymer science. We would point out here that this cross-fertilization of ideas, or technology transfer, is an excellent approach for hair product developers to employ when seeking new ideas.

3.3.4.2 THE SHAPE MEMORY PROPERTIES OF HAIR

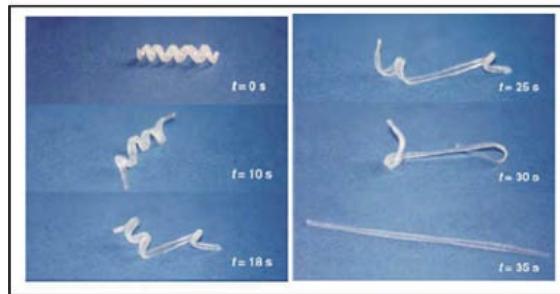
a. Definition of shape memory materials

In the scientific literature, C. Liu and C.B. Chun (8) define a shape memory material as follows:

“Shape-memory materials are those materials that have the ability to

‘memorize’ a macroscopic (permanent) shape, be manipulated and ‘fixed’ to a temporary and dormant shape under specific conditions of temperature and stress, and then, later, relax to the original, stress-free condition under thermal, electrical, or environmental command. This relaxation is associated with elastic deformation stored during prior manipulation” (2).

Currently, the most rapidly growing area in the field of shape memory materials are shape memory polymers (SMPs), and they can be either purely amorphous or semi-crystalline (12–14). [Figure 1](#) shows a sequence of captions displaying a shape memory polymer changing its shape from a coiled temporary to a straight permanent shape after this event is triggered by elevating its temperature (15). Shape memory polymers can store temporary shapes and can recover their permanent shape by a suitable stimulus, either electrical, chemical, or temperature.



[Figure 1](#)) Transition of shape from temporary (spiral) to permanent (rod) of a polymer synthesized from poly-(ϵ -caprolactone) dimethylacrylate and butyl acrylate (15).

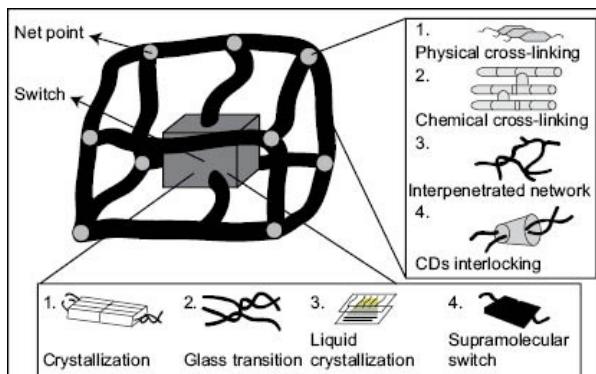
Various scientists working in this field have already listed the main physical requirements for a semi-crystalline polymer to display shape memory properties. According to C. Liu et al., these are as follows (8):

- 1)a relatively sharp glass transition and/or a high crystalline phase melting temperature that can be used to promptly fix the secondary shape at low temperatures and trigger shape recovery at high temperatures,
- 2)superelasticity (low loss modulus, high deformability) above the transition temperature that leads the shape recovery and avoids residual strain (permanent deformation) and,
- 3)complete and rapid fixing of the temporary shape by immobilizing polymeric chains without creep thereafter.

Synthetic polymers with shape memory properties consist primarily of two main features in their chain architecture. These are:

- A primary polymer network with network points, or cross-links that serve as a memory mechanism for the generation of a permanent shape, and
- A structure of secondary groups attached to the main network that serves as memory mechanism for the formation of temporary shapes (8).

These groups can be pendant functional moieties, grafted chains, polymer blocks, interpenetrating networks, or crystalline domains; they act as switches to release the temporary shape when activated by suitable external stimulus (see Figure 1) (7). Moisture, heat, light, and electric fields are examples of external stimulus that can release temporary shapes stored in SMPs with pendant groups or structures sensitive to them (see [Figure 2](#)) (8, 9).



[Figure 2](#)) Molecular mechanisms of shape memory polymers according to C. Liu *et al.* (8)

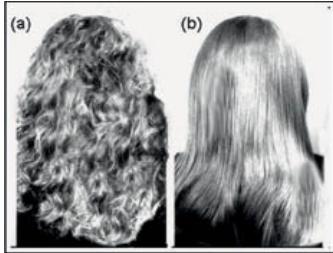
[b. Hair is a biopolymer with shape memory properties.](#)

In the scientific literature, biopolymers such as silk and collagen have also been reported to have shape memory properties, and thus, they have been classified as shape memory materials (16, 17). **Hair is a biopolymer that also behaves as a shape memory polymer.** Clear manifestations of this behavior and properties are the following:

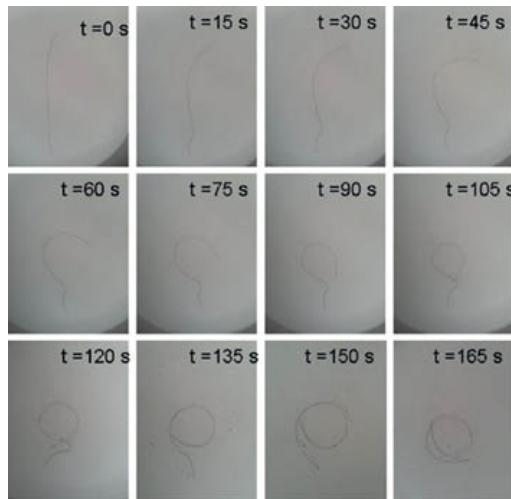
- 1) hair has a permanent shape,
- 2) its shape can be changed either temporarily or semi-permanently, and
- 3) it can “remember” or reverse to its permanent shape when treated appropriately.

Beauticians and cosmetic scientists, aware of these properties, have been creating fashionable hairstyles for years that can be reversed when desired. An example of hair’s ability to recover its permanent shape can be seen in [Figure 3](#), where it is shown that a curly temporary water-setting in a woman’s hair (Figure 3a) can return to its original straight shape after washing the hair (Figure 3b).

Also, [Figure 4](#) displays a sequence of micrographs of a hair fiber recovering its permanent shape from temporary straight to its curly permanent shape.



[Figure 3](#)) Captions of hair in a woman before (a) and after (b) it recovers its straight shape from a water temporary setting (18).



[Figure 4](#)) Captions showing sequence of a single hair fiber recovering its permanent shape from a straight temporary shape to a curly shape after water immersion (18).

As in the case of synthetic polymers, the permanent and temporary shape memory sites in hair reside in its crystalline and amorphous phases. In the case of hair, these polymeric entities are of natural origin and located in the macrofibrils of the cortical cells. These can be listed as follows:

- a) a protein crystalline phase forming intermediate filaments which serves as permanent shape memory and is also capable of storing a temporary shape when its crystalline structure unfolds or when it denatures above its melting temperature T_m .
- b) a disulfide bond cross-linked protein amorphous phase capable of storing temporary shapes when it becomes soft at the hair's glass transition temperature T_g , or when it undergoes disulfide bond interchange. This

structure also contributes to the stability of the crystalline phase, and c) an extended structure of hydrophilic protein groups located at the surface of globular proteins in the amorphous phase. Its structure forms a gel, is sensitive to moisture, and is also capable of storing a temporary shape.

c. Temporary shape memory in hair

The most common temporary or secondary shapes in hair are stored by physical means. The position of storage is in the protein regions with high levels of hydrogen bonding, i.e., in the soft gel structure of the amorphous phase (6). A typical example of this mechanism is water-setting. The gel structure in hair is ubiquitous in all types of hair and plays a crucial role in most temporary shapes imposed by hair movement and daily grooming practices. It is part of the amorphous phase, and behaves like a material that exhibits variable viscosity depending on the shear rate it is exposed to (i.e., pseudoplastic with a yield stress). This type of behavior can turn hair from very soft and flexible to very hard and brittle and, in doing so, it can virtually lock-in almost any temporary shape in hair. The viscosity of the gel structure depends on the hair's moisture content and, when hard and brittle, it presents a high opposition to the imposition or recovery of any type of shape in the hair. Temporary shapes in hair can also be stored by processes involving vitrification of the disulfide cross-linked amorphous phase and rearrangements of the crystalline phase. A typical example of this is the case of setting hair with hot irons.

The ideal creation of temporary shapes in hair shouldn't involve permanent and irreversible modifications to the protein architecture of hair. This means that after creating a temporary shape, the hair's permanent shape memory should remain intact, i.e., irreversible protein denaturation in crystalline filaments or irreversible breakage of covalent bonds in main protein chains and pendant groups should not be allowed to occur. If these irreversible phenomena occur, the hair may lose part or all of its permanent shape, becoming shapeless, and the imposition of a temporary shape will thus be irrelevant. As will be seen later, when hair is heated above its melting and glass transition temperatures with hot irons, temporary shapes can be created but at the expense of producing some of these unwanted irreversible phenomena.

d. The apparent permanent shape in hair

The hair shape that we normally perceive in our natural hair in everyday life is an apparent shape that results from a number of unavoidable mechanical effects.

These unavoidable effects are primarily caused by hair's own weight and by inter-fiber interactions in the hair assembly. Both effects produce a temporary shape that is stored in the gel structure of hair and is always present in our everyday life. It is a form of quasi-permanent water-setting. Therefore, the hair shape that we have in our natural hair is not the "real" hair's native shape, but rather it is a very close representation of it.

We call this close representation an "apparent permanent shape" or temporary shape in equilibrium with the permanent shape. Other factors that contribute to the formation of the "apparent permanent shape" are hair length, relative humidity, and type of hair; i.e., it also depends on whether the hair is naturally straight, wavy, or very curly.

The "real" permanent shape of hair is only manifested when we immerse a single hair fiber in water so that the temporary shape imposed by its own weight and other interactions is released from the gel structure. For instance, experiments have shown that a high percentage of hair fibers from Caucasian origin that appear wavy in shape, regardless of their degree of waviness or curliness, turn into almost perfect circular coils when immersed in water (18)!

We probably have all observed these effects at one time or another upon seeing our own hair fibers floating in a pool of water. These effects are shown in [Figure 5](#) where six single hair fibers from different origins are displayed as follows: (a) Oriental, (b), (c), (d) Caucasian, (e) Mulatto, and (f) of African origin. The shape of the Caucasian and Mulatto fibers was wavy/curly before equilibration in water. Observe in this Figure that the hair fibers of Caucasian, Mulatto, and African origin formed almost perfect circles after ten minutes of water immersion, except for the one of Asian origin. This means that the real permanent shape of most hair fibers that appear wavy in a hair assembly, is actually coiled and circular, and that the wavy pattern results from deformations mostly imposed by their own weight as the fibers hang from the scalp. Another important implication from these observations is that when hair emerges from the follicle, the shape of a high percentage of hair fibers of Caucasian origin is never wavy but rather circular with a predetermined radius of curvature. Thereafter, as the hair fiber grows longer it actually becomes wavy because it is deformed by both its own weight and the interaction with other hair fibers in the scalp.

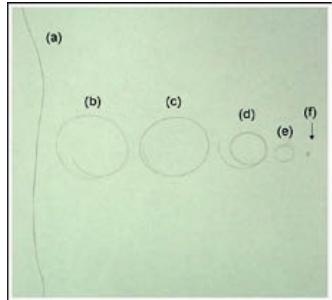


Figure 5) Picture showing six single hair fibers after their shape was equilibrated in water as follows: (a) Oriental, (b), (c), and (d) Caucasian, (e) Mulatto, and (f) of African origin (18).

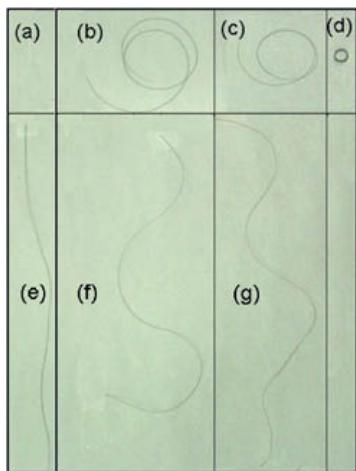


Figure 6) Picture showing four single hair fibers before and after they were hung vertically as follows: (a) Oriental, (b) and (c) Caucasian, and (d) of African origin (12). The shape of hair fibers depicted in (a), (b), (c), and (d) captions had been equilibrated by water immersion for ten minutes (18).

The above-mentioned effects are better visualized in [Figure 6](#) where it can be seen that the circular shapes of two equilibrated Caucasian hair fibers (b) and (c) turn into wavy patterns (f) and (g) as the hair fibers hang. In Figure 4 it can also be observed that the circular coil of an African hair fiber (d) presents a very small tight curly pattern, and does not open its coil upon hanging. The hair fiber of Oriental origin (e) did not change its straight shape very much after being hung.

The observations made on the behavior of African hair are not new and rather confirm what is already known by cosmetologists and beauticians; namely, that hair with a very tight coil is not readily opened by its own weight when hanging from the scalp regardless of its length. The tight curly hair will be opened,

however, when we impose a force, either with the fingers or with other devices. As will be seen later in this chapter, hair with very tight curls, such as that depicted in Figure 6d, has a very small radius of curvature, and its modification requires processes that modify a larger number of memory sites in the hair.

The modifications introduced to real permanent shapes in hair by its own weight also explain well-recognized hair shape variations often observed in people's hair when the environmental humidity changes. Higher levels of moisture result in hydrogen bond breakage in the gel structure and, therefore, moisture affects the "apparent permanent shape" of hair.

If the permanent shape is very strong, the hair will recover its real permanent shape, either curly or limp. For instance, people with wavy hair know very well that when the relative humidity is high their hair may become curlier. In contrast, people that have straight or fine straight hair as the real permanent shape may experience a higher level of hair limpness or straightening at high relative humidities. Furthermore, the formation of this "apparent permanent shape" also gives rise to differences in hair shape depending on its position on the head. For the sake of simplicity in the following paragraphs the term "real permanent shape" will be used when referring to hair shapes without any imposed deformation; and the term "permanent shape" will be used to refer to hair shapes in equilibrium.

e. Permanent shape memory in hair

The real permanent shape of hair is cast in the cortical cells during its life cycle through the anagen, catagen, and telogen phases (19–20). It is predetermined genetically and permanently imprinted in the hair follicle by the process of keratinization when the proteins in the amorphous phase are cross-linked by disulfide bonds. [Figure 7](#) shows typical examples of hair with different types of permanent shape. Whether the hair is straight, wavy, or extremely curly, its permanent memory shape essentially resides in the composition ratio of ortho-to para-cortical cells. At the microscopic level, however, the permanent shape of hair is imprinted in the two-phase architectural structure of hair which results from the crystalline keratin micro-fibrils embedded in a highly cross-linked amorphous network of proteins (see [Figure 8](#)). More specifically, it is imprinted in the primary, secondary, and tertiary protein structures of crystalline and amorphous phases, chemically and physically stabilized by disulfide and isopeptide covalent bonds and by hydrogen bonds, salt bridges, and dispersion forces. Both protein phases stabilize hair shape and also strengthen the fibers

mechanically.



Figure 7) Captions showing three types of natural permanent hair shape as follows: Oriental (left), Caucasian (center), and African (left).

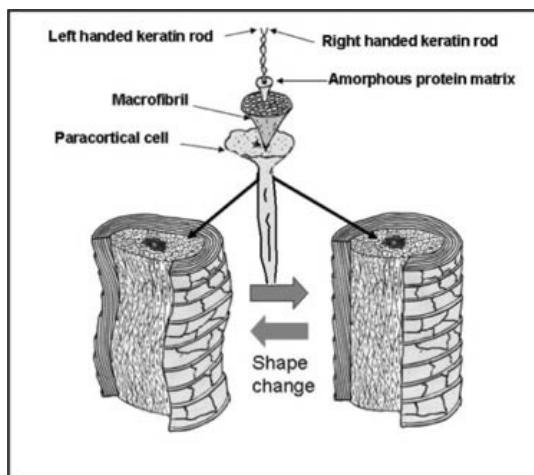


Figure 8) Schematic representation showing two hair fibers with different shape. The permanent shape of hair, either curly or straight, is imprinted in the hundreds of micro-fibrils and amorphous protein networks forming the two-phase protein arrangement in the cortical cells (18).

f. Changes to the permanent shape in hair

Permanent changes in hair shape have very different requirements than those already outlined for temporary shape changes. While temporary changes do not require irreversible modifications to the hair structure, permanent changes do require this process. Irreversible modifications are needed, first, because it is necessary to erase or delete the permanent shape from the memory of hair, and second, because it is necessary to build a new permanent shape in the hair structure when faced with consumer demands and needs. The latter step usually requires irreversible modifications to the crystalline phase, the introduction of new chemical bonds, and the rearrangement of proteins in the amorphous phase. **Thus, permanent changes in hair shape require irreversible modifications to**

one or two properties of sites where the permanent memory of hair shape resides.

The irreversible changes may be in the form of chain scission, chemical modification of protein-pendant groups, and breakage of disulfide bonds. They can also involve irreversible protein denaturation, irreversible melting of crystalline regions, or a combination of all these factors (21). Thioglycolate salt solutions and very high pH alkaline creams used in permanent waving and hair relaxers, respectively, involve exactly this type of irreversible protein denaturation. The former is selective to the breakage of disulfide bonds in the protein chains and does not have a strong impact on other chemical bonds. The latter also breaks disulfide bonds but is less specific to them and, it breaks other bonds in the protein chains as well. In addition, the phenomenon also produces irreversible modifications to the crystalline regions and to the whole hair architecture itself (22).

g. Shape reversion

One factor often encountered in treatments geared to produce a permanent change in the shape of hair is that in many instances, they do not produce a real durable permanent change. As a result, there is a partial and gradual reversion to the original fiber shape. Lack of performance of active ingredients, poor formulation design, or poor hair treatment conditions are often the cause of this reversal. Consequently, in the case of permanent waving, various treatments have to be applied periodically to maintain the wavy shape (18). In the case of hair straightening with alkaline relaxers, shape reversion is difficult to correct because after the straightening process, the hair fibers are left weakened due to the high degree of broken covalent and other protein bonds produced by these treatments.

Shape reversion phenomena occurring after permanent waving or alkaline relaxer treatments are usually difficult to explain when using conventional mechanisms of disulfide bond breakage and re-formation. However, the shape reversion processes can be easily explained when it is considered that hair is a shape memory biopolymer with a permanent shape stored in its “memory.” As will be discussed later in this chapter, in order to successfully induce permanent changes in hair shape, not only do disulfide bonds have to be broken, but also other chemical and key structural components of the hair memory need to be modified. Otherwise, the induced shape will be semi-permanent and eventually the fibers will undergo shape reversion.

Understanding the shape memory properties of hair can pave the way to optimize processes and produce successful permanent changes in hair shape. Therefore, in the following paragraphs shape memory polymer concepts will be used to review changes in hair shape, and we encourage the reader to use this information as a foundation for innovative thinking in this field.

3.3.4.3 CHANGES IN HAIR SHAPE BY WATER-SETTING

a. The process

Water-setting, or the process of changing hair's shape with water, is by far, among all the temporary setting processes, the easiest to produce in hair. It only entails the use of water and a relatively low-temperature drying process. In order to understand it mechanistically using concepts from the science of SMPs, let us first review the key generic steps necessary to produce shape changes in semi-crystalline polymers (6), and then apply them to hair. Upon thoughtful comparison, polymers also require the same three key steps described above for changes in hair shape:

- 1)making fluid or softer at least one of the polymer phases,
- 2)imposing mechanically a new shape followed by stress relaxation in the soft/fluid phase, and
- 3)immobilizing the soft/fluid phase to lock-in the new shape. In general, temporary shape changes in SMPs also require keeping the polymer structure intact where the permanent shape memory is located (11).

Thus, when we want to impose a temporary shape on a semi-crystalline polymer, or by analogy, in hair, the first requirement is to keep the main polymer architecture intact. To be more specific, in the case of water-setting this implies that the disulfide bond cross-linked protein matrix and the protein crystalline regions should not be modified. Water-setting usually does not produce these modifications except when high temperatures, or large deformations are involved in the production of the temporary shape. High temperatures above 80°C may already start to produce disulfide bond breakage; while deformations higher than 5% already induce alpha to beta transitions in the crystalline phase which may be partially irreversible (23).

b. Physical processes taking place inside hair during water-setting

As outlined in the previous paragraph the imposition of temporary shapes, either

in polymers or in hair, requires three main steps (see [Figure 9](#)). For the water-setting of hair, step 1 consists of softening the gel structure of the amorphous phase by wetting the hair and allowing water to penetrate into the cortex. This first step will ensure that hydrogen bonds in the gel structure are broken by water. It is important to mention here that when water breaks hydrogen bonds in hair proteins, after step 1 the hair still has all of its covalent bonds intact, and therefore, the permanent shape memory is also unchanged (see [Figure 10](#)). As will be seen later, this contrasts with other processes where the hair becomes shapeless and completely loses its real permanent shape. In step 2, once the weak hydrogen bonds are broken, the hair is set into a new configuration with plastic rollers or with the fingers to create the desired new shape, which can be either that of light hair waves or tighter curls.

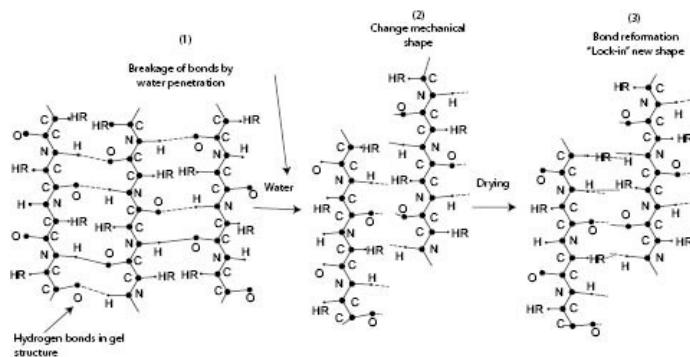


Figure 9) Schematic representation of three steps required to create a temporary shape by water-setting. The three-step process is essentially based on the breakage of hydrogen bonds in the gel structure of hair (step 1), imposition of temporary shape (step 2), and re-formation of hydrogen bonds to lock-in the new shape (step 3) (18).

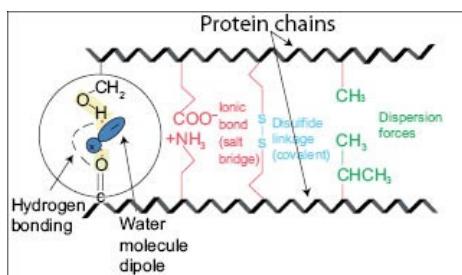


Figure 10) Schematic representation of hydrogen bond breakage by water dipoles during water-setting. Other covalent bonds are not affected.

Finally, in step 3, the hair has to be rapidly dried to extract water molecules in order to re-form the hydrogen bonds in the gel structure. This process will cause

hardening of the hair, thereby locking-in the temporary shape and imposing it over the primary hair shape. Drying can be achieved by just exposing hair to atmospheric conditions or it can be accelerated with the aid of blow-dryers. If the drying process is too slow and the hair rollers are removed, the crystalline micro-fibrils and amorphous matrix with its combined elastic force will push for hair to recover its permanent shape and the temporary shape will be lost. Fast drying with blow-dryers will ensure that water is rapidly removed from the gel phase before the hair can elastically recover its permanent shape. In this manner the new shape will be locked-in.

c. Temporary shapes induced by long-term deformations

Another form of water-setting is the one that takes place when hair is subjected to involuntary deformations for extended periods of time at room temperature and high moisture conditions. This situation occurs, for instance, when sleeping, using hats, etc. In these cases the assembly of hair fibers is held in a configuration for various hours, and after freeing the hair from the confined environment a temporary change in hair shape is produced. The phenomenon is caused by stress relaxation in the gel structure of the amorphous phase.

As was previously discussed, the gel phase of the hair is plasticized when the amorphous phase contains moisture. Water from the environment is absorbed in the gel structure and breaks a limited number of hydrogen bonds at protein neighboring sites. When there are no mechanical stresses applied to hair, the broken and unbroken bonds stay in equilibrium. However, when deformations appear as a consequence of imposed deformations, i.e., during sleep, use of hats, etc., broken and unbroken bonds undergo a bond interchange process and the protein chains move to relax internal stresses. This process of internal stress relaxation in the gel structure locks-in the temporary shape that is produced during sleep (bed hair), or with the use of hats, etc. (see [Figure 11](#)).



[Figure 11](#)) Caption showing typical appearance of bed hair (18)

The imposition of new temporary shapes on hair, either during sleep, use of

hats, etc., or during water-setting, will immediately produce internal elastic stresses in the structures where the real permanent shape resides. This occurs primarily in the crystalline regions and in the disulfide protein matrix. These internal stresses will remain there as long as the temporary shape is in place and are needed for the hair to recover its permanent shape. Temporary shapes imposed by water-setting will maintain their secondary character as long as the two following conditions are met. First, the elasticity of the crystalline and amorphous phases does not change, and second, that the gel structure of the amorphous phase remains unchanged. The first condition was previously reviewed and it was referred to as a requirement for superelasticity of the SMPs. This condition is required to ensure that the polymer will always remember its permanent shape. The second condition was also reviewed previously and it relates to the absence of relaxation in the soft phase; it is required to lock-in the temporary shape as long as it is necessary until it is released by an external stimulus.

Shape changes induced by water-setting can, rapidly or almost instantaneously, be reversed by water-wetting. The process of liquid water absorption by hair causes rapid hydrogen bond breakage, softening, and swelling of the gel structure, thereby releasing the temporary shape and allowing the permanent shape to be quickly recovered. If the hair didn't behave like a shape memory biopolymer, it would not remember and recover its permanent shape and would stay deformed like a viscous resin. Thus, water-wetting acts as an external stimulus to quickly release the temporary shape from hair.

d. Limitations of water-setting

Unfortunately, shape changes induced by water-setting in hair are temporary and cannot be maintained as long as we would like nor can they be dormant forever. *It is known, for instance, that shape changes induced by water are slowly recovered even when no water-wetting processes are applied.* Temporary shapes stored in the gel structure are gradually lost because, as has previously been explained by Max Feughelman (6), the gel structure of phase M in hair is viscoelastic, and even at very low moisture contents it will always relax to the Elastic Modulus of the crystalline phase. Thus, all types of temporary shapes induced by water-setting eventually revert back to the permanent shape by a stress-relaxation process and this occurs even under very dry conditions. The process may take months but the shapes will reverse! The memory shape properties of the gel structure in hair are transient, and temporary shapes induced

by water-setting cannot be maintained for very long periods.

Another important aspect of water-setting is that, depending on the type of hair, there is a limit on the type of initial shape that can be imposed. This fact becomes more clearly understood when we analyze the following questions:

- How tight can we induce a curl in hair that is very straight, or how straight can we render hair that is very kinky?
- Can this induced curl be achieved just by using a water-setting process? In other words, is it possible to put very tight curls in natural straight hair just by breaking and re-forming hydrogen bonds? Or is it possible to render very kinky hair straight just by breaking and re-forming hydrogen bonds?

Obviously, the answer to the above is no. Everyone who has tried to straighten extremely curly hair just by using water-setting will fail.

This failure can be explained by the fact that the gel structure has only limited mechanical stiffness, and therefore, cannot completely counter the strong elastic recovery forces of permanent shape coming from the crystalline and disulfide cross-linked protein phases. In other words, the gel structure is a weak physical memory and cannot store large changes in hair shape. Thus, the strength and degree of hair curliness is very important in temporary shape changes induced by water-setting. The straightening of extremely curly hair may require additional physical modifications of other, stronger protein structures that store temporary shape. Likewise, this type of reasoning can be applied for the imposition of tight curls in straight hair with water-setting.

According to the ongoing discussion, there appears to be a rule that relates strength of shape change, and number of memory sites required to achieve the changes in shape. This rule *always* prevails when imposing temporary changes in hair shape. In the case of straightening curly hair, the rule is: **the tighter the curl, the more the elements in the architectural sites in hair's memory need to be modified to make it straight.** This means that for any particular type of hair, there is a limit in the degree of shape changes that can be imposed by water-setting. In fact, this is the reason for the use of harsher temporary hair-shaping techniques, other than water-setting, for the straightening of curly hair, such as the use of hot irons and other treatments. As will be seen later in the following paragraphs, the rule of changing shape has important consequences not only for the imposition of temporary shapes but also for engendering those of permanent nature.

3.3.4.4 CHANGES IN HAIR SHAPE BY HOT IRON TREATMENTS

a. Introduction

Changing the hair shape with hot irons is a very old practice. Museums of history already report drafts of hot iron designs made in the 18th century (see [Figure 12](#)). Hot irons became popular in the early 1900s for the straightening of extremely curly hair of African origin (24). Later, over the years the application gradually extended to other types of hair to create/enhance stronger curls or to create straight patterns in hair. Currently, there are two main types of hot irons, namely, those of cylindrical shape and those with flat plates (see [Figure 13](#)). The use of hot irons with flat plates has become very popular for the straightening of wavy hair. The wanted effects from those who use it nowadays range, in terms of achieving long-lasting behavior—from totally irreversible, or permanent types, to those of long duration and reversible behavior. Most hot iron treatments used today leave only a short temporary change in hair shape. As a result, cosmetic formulators are perennially looking for new hot iron technologies that can extend the persistence and long-lastingness of heat treatments.



Figure 12) Caption displaying draft describing hot irons used in 1858 (24)

Most of the progress made in the treatment of hair with hot irons has produced innovations in their design and in the use of new ingredients or formulations to aid in the hot ironing process. Innovations in hot iron design comprise, to mention a few: more efficient temperature control, new ceramic materials to improve surface smoothness, better and effective hot iron/hair contact area, use of steam, and the incorporation of infrared heating elements (25). To this end, progress in the area of ingredients and formulations has led to the development

of two types of products; namely,

- 1) those based on the use of polymers geared to enhance shape effects and also to reduce hair damage (26), and
- 2) those using ingredients that modify or introduce new bonds in hair.

The latter type of products in most cases requires the incorporation of heat to modify the permanent shape memory of hair; either by introducing new cross-links, or by changing the disulfide cross-linking structure of the proteins. Examples of these are the formaldehyde (Brazilian) and sodium bisulfite (Japanese) treatments; both will be reviewed later in another paragraph.



Figure 13) Main modern types of hot irons, flat hot iron (top), and rod hot iron (bottom)

b. The process

A typical hair-straightening process with hot irons consists of the following steps: 1) pre-wash and condition hair with the shampoo and conditioner of choice, 2) dry hair until it keeps about 80% of its moisture content, 2) divide hair into thin swatch sections and then apply the hot iron. The temperature of the hot iron, depending on the type of desired effects, is usually selected between 160 and 230°C. The hot iron is passed two or three times over the hair swatches from root to tip at a speed of approximately one inch/sec. During application the hot iron is held firmly but gently with the hand, so the resulting force of friction will allow smooth sliding over the hair surface, and also, will enhance heat transfer at the interface of hot iron/hair. Excessive force application with the hand should be avoided since this will unnecessarily increase friction at the hot iron/surface interface causing the hot iron to slow down or completely to stop. If this happens the hot iron will damage the hair swatch. If properly used, flat hot irons produce immediate straightening effects in hair (see [Figure 14](#)).

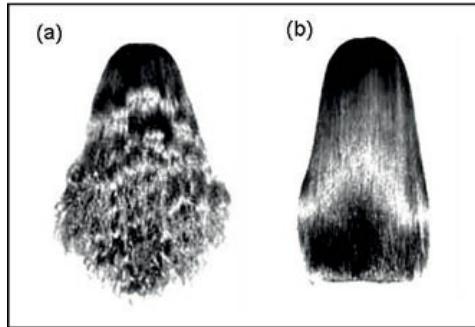


Figure 14) Changes in hair shape before (a) and after (b) treatment with a flat hot iron. The hair shape has been changed from permanent semi-curly to temporary straight.

Good contact between the hot iron and hair is always necessary for efficient heat transfer. It is important to recognize that heat transfer from the hot iron plates to the hair swatch is always poor because hair, like wool, is a poor heat conductor. Furthermore, because of hair irregularities at the cuticle sheath surface and in view of the formation of air pockets located between hair fibers in the swatch, there is always poor thermal contact between them. This condition causes uneven temperature gradients between hot iron surface and hair swatch. Measurements carried out by placing a thermocouple inside a hair swatch have shown that hair fibers placed in a row 100 micrometers below the hot iron surface always sense at all times an average of 20 to 25°C less than those at the row surface contacting the flat iron plate (27).

c. Mechanical action of hot irons

The mechanical action of hot irons on hair is different depending on whether we try to impose a curl on straight hair or whether we try to straighten curly or wavy hair. For instance, if we place a swatch of straight hair in a hot iron of cylindrical shape at 210°C, and press on the hair statically without motion, the hair will acquire a curled shape. In contrast, if we place a swatch of wavy or curled hair between the plates of a flat hot iron at 210°C and let it be there statically without motion, it will not become straight; the action of flattening alone will not lead to changes in hair shape from wavy/curly to straight. For hair to become straight it needs to be in motion so its length can be stretched at least until the curl opens and the hair becomes straight. Thus, there is a big difference in the way the hot iron acts mechanically. This difference depends on whether the change in shape is from straight to curly or from curly to straight. Very curly hair needs a relatively high friction imposed at the interface between the hot iron and the hair

surface so the iron plates can stretch and open the curl. In contrast, the process of changing straight hair to curly is not dependent on whether the hair is in motion or not.

Other factors that influence the friction process during the straightening of wavy hair with flat irons are the type of products applied to hair before hot ironing. Hair swatches to be ironed may contain polymers, surfactants, and oils on the surface prior to the ironing process. These ingredients, in particular, include polymers that have been placed there to aid in the styling process. The polymers appear to have an effect in preventing hair damage by eliminating uneven harsh temperature gradients that may create hot spots on the hair swatch (28). Polymers and oils can have, however, a negative effect on the hot ironing process as they can excessively increase or lower the interfacial friction between the iron and the hair surface (34). High levels of friction may cause the hot iron to stop or to produce lifting of cuticle cells and tear them away from the hair (see [Figures 15](#) and [16](#)), while very low friction may reduce the uncoiling of the curl compromising the efficiency of a hair-straightening process (27).

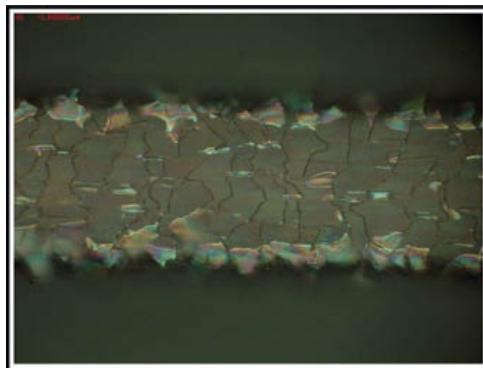


Fig. 15) Micrograph of a single hair fiber showing cuticle cell deformation and lifting after treatment with a hot iron at 210°C. The colored sections represent lifted cuticle cells showing patterns of light interference (27).

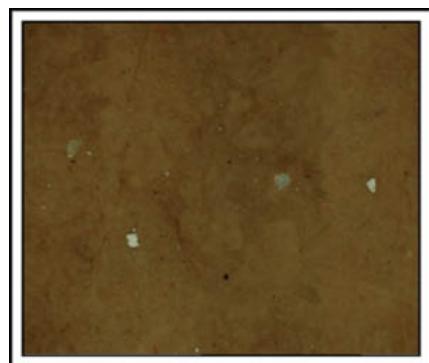


Figure 16) Micrograph showing fragments on cuticle cells left on plate of hot iron after a hair bundle was treated with a hot iron. Cuticle cell removal was produced by excessive friction at hot iron/hair interface (27).

3.3.4.5 PHYSICAL PROCESSES TAKING PLACE INSIDE HAIR DURING HOT IRONING

a. Water evaporation

Anybody who has witnessed for the first time the application of hot irons to hair is struck by the hissing sound of water vapor or steam coming out of the hair as the hot iron is passed over the hair swatches. At the microscopic level this means that a large quantity of “free” and “bound” water molecules are being rapidly extracted from the protein sites in the amorphous phase. Water-absorbing groups such as amino, carboxyl, and other functional groups capable of forming hydrogen bonds lose their water in this process. As a result, the gel structure of the amorphous protein phase changes from a soft and highly plasticized gel to a hardened amorphous mass.

Experimental observations indicate that rapid water evaporation from hair starts to take place when the hot iron reaches temperatures slightly higher than 100°C. Moreover, if the temperature of the hot iron is maintained at 120°C for 15 seconds while it is in contact with the hair swatch, the hair will be fully dehydrated. This is not surprising since bulk water starts to evaporate rapidly at 100°C. Yet, observations made by scientists in the laboratory and by many stylists confirm that when the temperature of the hot iron is set between 100 and 120°C, it is difficult or almost impossible to efficiently impose a temporary shape on hair, either straight or curly (27).

Furthermore, work carried out in this area indicates that the shape of a wavy hair swatch does not change efficiently from wavy to straight even when the temperature of the hot iron is increased to 150°C. These experiments also indicate that it is not until the hot iron reaches temperatures between 150 and 230°C, that a noticeable and efficient change in shape from wavy to straight starts to appear in hair (27). These observations indicate that the physicochemical processes that are responsible for inducing changes in the shape of hair occur at temperatures ranging between 165 and 230°C, and when the hair is almost fully dehydrated. Incidentally, the only protein processes that take place inside hair within this temperature range are the melting of crystalline

proteins and the softening of the amorphous matrix. The relation of these two processes with shape changes in hair will be discussed below.

b. Phase changes and transitions in hair

The thermodynamic transitions taking place in hair when its temperature is increased from 25 to 240°C have been the subject of numerous studies (29–31). Most hair types have been shown to undergo two main thermodynamic transitions, namely a first-order transition associated to the denaturation and melting of its crystalline structure, and a second-order transition associated to the softening or glass transition of its amorphous protein structure. The melting of the crystalline structure in virgin hair occurs at T_m 156°C, and the softening of the amorphous disulfide cross-linked protein phase occurs at its glass transition temperature, T_g 144°C.

The occurrence of these two transitions in most types of hair can be detected and confirmed by Differential Scanning Calorimetry. For instance, in [Figure 17](#) can be seen a typical DSC thermogram showing the endothermic peak related to the melting of the hair crystalline phase. Thus, since the working temperature of a typical hot iron treatment ranges between 150 and 230°C, it is straightforward that both of these transitions take place in the hot iron straightening process. In fact, as will be seen later, the better efficiency in changing hair shape temporarily with hot irons is due precisely to the occurrence of these transitions, as they are the sites with temporary memory properties.

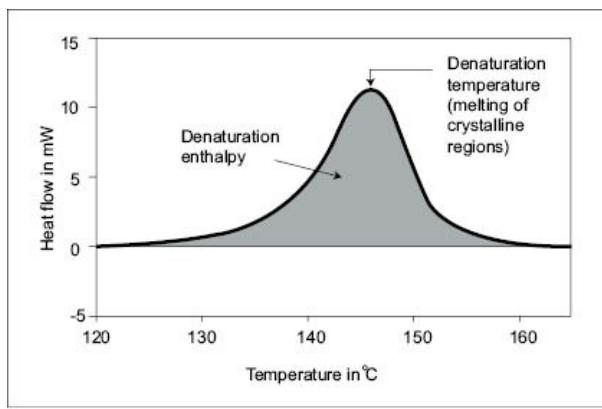


Figure 17) Caption of a typical thermogram obtained by DSC from hair (32).

c. The mechanisms of water and hot iron setting are different.

With the material presented thus far, it is not difficult to anticipate that the mechanism by which hot irons impose a temporary shape in hair is very different

from that occurring in water-setting. Setting hair with hot irons does not involve the key and crucial typical step of water-setting, namely that consisting of the breakage of a high quantity of hydrogen bonds to soften the gel structure followed by imposition of the new hair shape.

Setting hair with hot irons involves the formation of hydrogen bonds, but they do not contribute to imposing a new shape. In order to better understand the role of hydrogen bonding during hot iron treatments, let us start by analyzing the role of water on the properties of the gel structure in hair. When water molecules penetrate into the hair structure they adsorb and intercalate on protein-functional sites breaking hydrogen bonds between them. The breakage of these hydrogen bonds renders the gel structure of the amorphous phase softer (6). When the water molecules are removed by evaporation, the internal hydrogen bonds between neighboring protein chains re-form and transform the soft gel structure into a hard viscous gel mass.

Hair swatches to be treated with a hot iron are, however, not required to be wet, for they already contain small amounts of moisture in their gel structure. However, this limited amount of water only breaks a limited number of hydrogen bonds, and therefore, it produces a semi-soft gel structure. When hair tresses with such limited amounts of moisture enter into contact with a flat hot iron at high temperatures, there will be a rapid process of heat transfer into the hair cortical regions. For instance, if after heat absorption, the temperature of the amorphous protein and crystalline phases rises rapidly to levels higher than 160°C, there will be violent evaporation of this limited amount of water, thereby producing the hissing sound typical of hot iron treatments.

At temperatures higher than 160°C the hair will have lost most of its moisture, causing all broken hydrogen bonds to re-form with the consequent hardening of the gel structure. Systematic hair-straightening experiments carried out with Mulatto hair using hot iron temperatures ranging between 110°C and 130°C have shown that hair swatches already become fully dehydrated at these temperatures. Even in the face of full dehydration the process leads to very poor or almost no hair straightening. These observations clearly indicate that rapid hardening of the gel structure attained during hot iron treatments does not allow for an efficient change in hair shape by hydrogen bond re-formation. *In other words, hot iron treatments harden the gel structure of hair before the temporary shape is imposed on it (27).*

d. Hot iron setting: partial denaturation of micro-fibrils and vitrification of

the amorphous phase

According to the ongoing discussions, hydrogen bonding does not contribute much to changes in hair shape by hot irons. There are, however, two other important physical phenomena occurring in hair between 130 and 230°C capable of inducing temporary shapes. These are the partial melting or denaturation of the crystalline protein phase at T_m and the softening of the disulfide protein matrix at T_g . Incidentally, both types of phase transitions occur also in cross-linked semi-crystalline polymers with shape memory properties (SMPs) and are commonly used to impose temporary shapes (8). In [Figure 18](#) a schematic diagram explains the mechanism of temporary shape storage by semi-crystalline SMPs. The mechanism involves three key steps:

- a) heating the polymer above its crystalline melting and glass transition temperatures to soften the polymer,
- b) deforming the polymer to impose the temporary shape, and then,
- c) cooling down to lock-in the temporary shape.

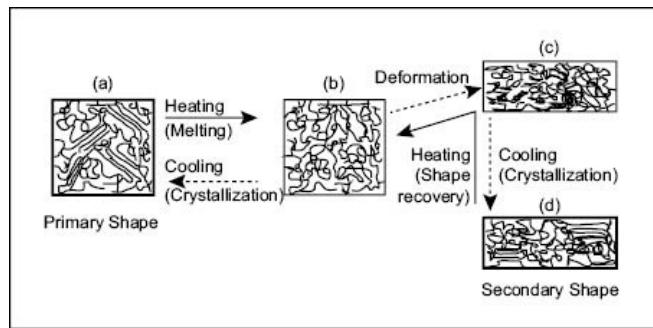
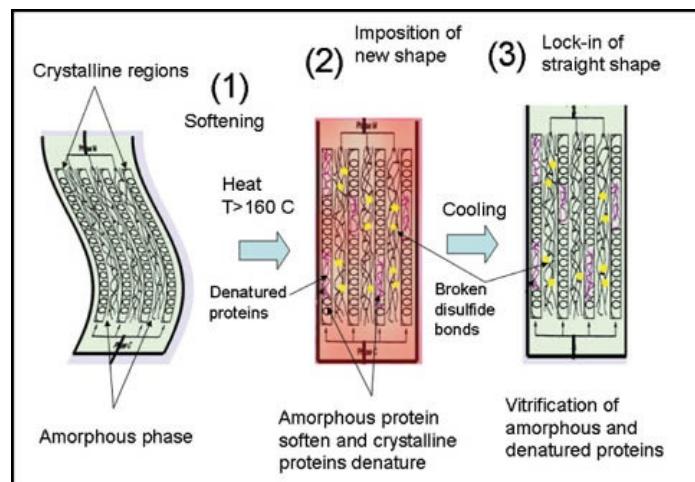


Figure 18) Schematic depiction of three steps needed for temporary shape fixing in cross-linked semi-crystalline shape memory polymers according to C.Liu *et al.* (8). Step 1 comprises melting of crystalline regions (a), step 2 requires the imposition of deformations to create the temporary shape (b), and step 3 involves cooling of the polymer for the recrystallization to lock-in the new shape (c).

Since hair is also a cross-linked semi-crystalline polymer, it is straightforward that hot irons must store temporary shapes in the hair structure by a similar process. Namely, when the hot iron is set at temperatures above 160°C and passes over the hair, it melts/denatures the crystalline regions and softens the amorphous phase. Then, as the hair is in motion the hot iron plates open the curl by friction and impose the straight shape by mechanical pressure. Once the hair moves out of the hot plates with its straight shape, it cools down. Cooling at

room temperature induces a vitrification process of the amorphous phase and also a recrystallization process or a reorientation of crystalline regions (see [Figure 19](#)). In this process the molten or denatured crystalline regions and the softened disulfide cross-linked proteins vitrify; while the non-denatured crystalline proteins reorient to comply with the new shape. This process locks-in the new straight shape in hair. Because hot irons use vitrification of amorphous and reorientation of crystalline proteins as processes to store temporary shapes, the stability and durability of the shape is longer than that obtained with water-setting.

It should be mentioned here that the denaturation of crystalline regions at high temperatures in hair is always an irreversible process and, therefore upon cooling, recrystallization of denatured proteins does not occur. Experiments have shown, for instance, that as the temperature of the hot iron rises between 160 and 220°C, the level of irreversible protein melting/denaturation also increases (27, 33, 34). Furthermore, it has been observed that as the level of denaturation increased in this temperature range, and while the hair curl was maintained open under the hot iron, the character of hair straightening shifted from temporary to more permanent (27). These observations clearly indicate that hot irons induce temporary shape changes in hair by a softening and vitrification process of its two protein phases. The amorphous phase locks-in the new shape by a pure vitrification process, while the crystalline phase, by either a reorientation process of the non-denatured crystalline regions or by vitrification of the denatured ones. The straightened shape becomes more permanent in character when the level of denatured crystalline regions that vitrify increases.



[Figure 19](#)) Diagrammatic representation of three steps required for the creation

of a temporary shape in hair with hot irons. In step 1 the hair is heated to its Tg so the crystalline regions partially melt irreversibly and the amorphous protein matrix softens, in step 2 a straight shape is imposed on hair, and in step 3 the hair is cooled down to vitrify the amorphous protein regions to lock-in straight shape (27).

The hot iron process could also be detailed as follows: hot irons increase the temperature of hair above its Tm and Tg. This causes the disulfide amorphous protein matrix to soften and become fluid-like while the crystalline regions start to undergo a kinetic melting or denaturation process. The degree of crystalline protein melting will depend, however, on the thermal stability of the amorphous matrix (29). If provisions are taken to reduce disulfide bond scission, then, most of the shape change induced by the hot iron will be temporary and due to vitrification of the amorphous phase and to a reorientation of crystalline regions. However, if disulfide bond breakage occurs readily in the amorphous proteins, the changes in shape will be due to a vitrification of amorphous and denatured proteins and the changes in shape will take a more permanent character (27).

For demonstration purposes [Figure 20](#) shows three hair fibers whose semi-elliptical shape underwent a softening and vitrification process with a hot iron at 220°C. Fiber (a) in Figure 20 shows a pronounced flattening effect on its semi-elliptical shape as a consequence of the softening/vitrification process during hot iron treatment, fiber (b) shows imprints of neighboring fibers, and finally, fiber (c) shows shape flattening due to a similar vitrification process. All these effects indicate that at a certain point during the hot ironing process at 220°C the hair proteins became very soft, viscous, and fluid-like and were able to change their geometrical configuration to comply with the hot iron geometry. Then, upon cooling, the proteins vitrified and reoriented into the new shape. The experiments also showed that these effects are only temporary, i.e., when the fibers are immersed in water they recover their original shape (34).

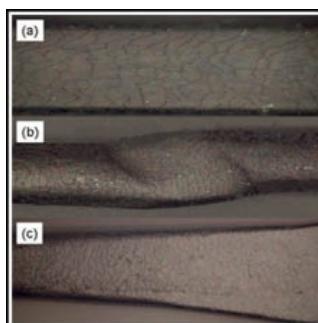


Figure 20) Micrographs of shape distortion produced by 4 cycles of hot irons at 210°C on hair fibers of Caucasian origin as follows: (a) flat shape distortion obtained on a single fiber with a 40 g compression force, (b) imprint left on fiber by neighboring fibers in a hair tress, and (c) deformation left in a fiber selected from a hair tress after treatment with hot iron (34)

Since temporary shape changes induced by hot irons involve only physical mechanisms, the conditions for their recovery also entail physical phenomena. These conditions are met when the protein chains in the vitrified amorphous phase enter into faster motion again. This could be done by increasing again the hair temperature above Tg or when the hair is wet. Thermal motion of main chains in polymers and proteins always occurs at room temperature, and their degree of motion is relative to their Tg. The higher the Tg in a polymer, the slower is the thermal motion of its main chains at room temperature (2). Thus, in theory, temporary shapes induced with hot irons will be very long lasting but unfortunately, the principle of superposition in polymers (2–4) also dictates that the Tg of a polymer can be lowered by a plasticizer, thereby increasing chain mobility.

Let us also remember that water is the main plasticizer of proteins, and it is also the main plasticizer of the gel structure in the hair amorphous phase (5). Even when water cannot penetrate and plasticize the crystalline regions, the effects of water in the amorphous phase have an impact on the orientation and arrangement of the crystalline phase. When the hydrophilic groups of the amorphous phase absorb water the amorphous phase relaxes, and this may result in a reorientation of the crystalline regions storing the temporary shape. The final result is a loss of temporary shape. Thus, even when temporary shapes induced by hot irons are of longer duration than those attained by water-setting, their shape is also lost when the hair absorbs water.

e. Heat transfer from hot iron to hair

It should be mentioned here that because of poor hair thermal conductivity, nonhomogeneity of cuticle cell surface, and packing irregularity of hair fibers, not all hair fibers in a hair swatch exposed to a hot iron surface will experience the same temperature. Those hair fibers in contact with the hot iron will certainly be expected to equilibrate rapidly to the hot iron surface temperature. However, those a few millimeters away from the surface will experience a much lower temperature. Systematic experiments using a thermocouple embedded in a hair

tress have shown that hair fibers in the middle of a 2 mm hair tress experience \sim 25°C less than those in contact with the hot iron (see [Figure 21](#)). Furthermore, additional experiments carried out with hair swatches \sim 1 mm thick and placed on a hot plate at 200°C showed that it takes time for heat to be transferred across a hair swatch into a metallic heat sink to equilibrate temperatures. Once equilibrium conditions are established, the temperature at the hair swatch surface not in contact with the hot plate is always \sim 60°C less than that in contact with the hot plate (see [Figure 22](#)).

The simple experiment described above does not represent realistic conditions of hot iron treatments because only one surface of the hair tress is exposed to high temperature. However, the experiment helps us to visualize the time delays needed for temperature to equilibrate across the hair tress. Therefore, we will expect that when a hot iron is passed at 160°C over a hair swatch only those fibers in contact will rapidly equilibrate temperatures with the hot iron; and these fibers will experience melting of crystalline regions and softening of the disulfide cross-linked matrix. Those fibers underneath may not experience this phenomena.

Furthermore, occurrences of the above-described transitions in hair are not only dependent on the position of the hair fibers relative to the hot iron surface but also dependent on the heating rate. For instance, it has been shown that the faster the rate of heating, the higher is the melting temperature of the crystalline regions (29). Since the hot iron is passed swiftly along the hair fibers we would expect, therefore, that if the hot iron temperature is set to 160°C, large numbers of hair fibers will not undergo these transitions. Thus, if we want all hair fibers in a swatch to experience the thermodynamic transitions required, two actions must be taken, namely, 1) to increase substantially the hot iron temperature above 160°C, or 2) to improve heat transfer across fibers. From the practical point of view, only the former action can be easily done and this can be accomplished just by setting the hot iron temperature to higher levels. This analysis helps explain why setting the hot iron temperature in the range of 170–210°C improves substantially the efficiency in hair straightening (27).

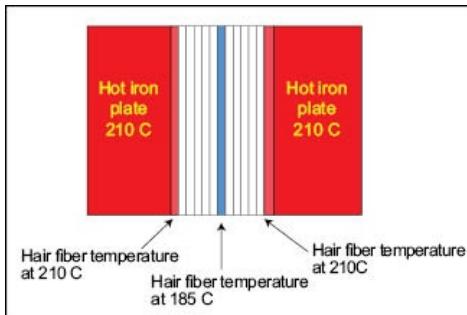


Figure 21) Schematic representation of hair swatch between hot iron plates. The experiments show that hair fibers in the middle section of the hair stress sense temperatures $\sim 20\text{--}25^\circ\text{C}$ lower than those at the periphery (27).

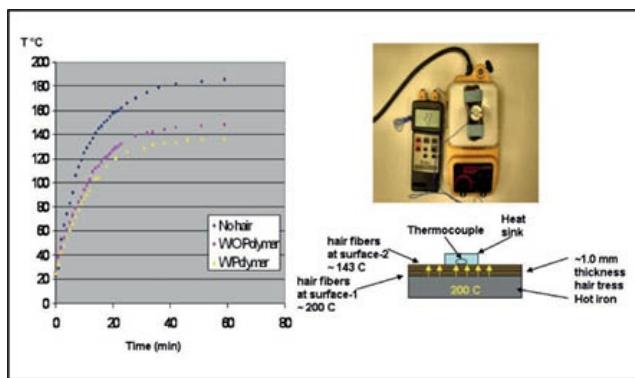


Figure 22) Graph showing variation in temperature as function of time (a) in a thermocouple placed at the top of a hair tress placed on a hot plate (b) an (c). The temperature of the hot plate was preset at 200 C and, then, at $t=0$ the hair and thermocouple were place on it (27).

f. Unwanted consequences of friction and rising hair temperature above T_m and T_g

Setting temporary shapes in hair with hot irons has advantages over water-setting or any other types of temporary setting. This is true, first, because hot irons introduce stronger changes in shape, and second, because the shapes last longer. In fact, an ideal hot ironing process would occur if the duration of the temporary shape could be controlled at will and the mechanism of shape storage would only involve reversible changes to the amorphous and crystalline phases. Unfortunately, it is very difficult to control water absorption by hair and also to prevent irreversible disulfide bond breakage and other forms of damage that occur in hair at high temperatures. Scientists studying heat damage in hair have reported the occurrence of protein oxidation, cuticle cell lifting/bulging,

formation of pores inside the hair (see [Figures 23](#) and [24](#)), degradation of tryptophan, and disulfide bond scission (35–37). All these effects make the process of flat ironing far from ideal.

The first cause of damage in hair treated with hot irons comes from the fact that the thermodynamic transitions responsible for storing the temporary shape in hair lead to processes that are not fully reversible. For instance, upon cooling, the softened disulfide cross-linked protein matrix does not return to its original native vitrified state; nor do the denatured crystalline regions recover their original crystalline structure (29). Extensive research carried out in various laboratories has already shown that when hair is heated above its T_m a process of partial irreversible protein denaturation takes place. Lack of full recovery of both amorphous and crystalline phases has been ascribed to various thermal damaging factors. Among these thermal damaging factors the most important one is irreversible disulfide bond breakage. This process causes covalent cross-linking points in the amorphous phase to decrease, thereby producing a different state of vitrification and a process of irreversible destabilization of the crystalline regions. Both processes contribute to a gradual loss of permanent shape and undesirable reduction in hair strength.

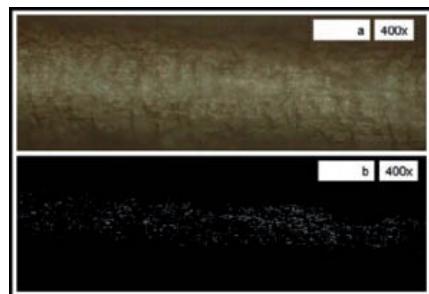


Figure 23) Micrograph of a hair fiber ($\phi \sim 76 \mu\text{m}$) subjected to five treatments of hot ironing showing the presence of pores at a depth of $10 \mu\text{m}$ inside the cortex (a). By using digital filters in the image, the pores were separated and the remainder of the picture was transformed into a black background (b) (34).

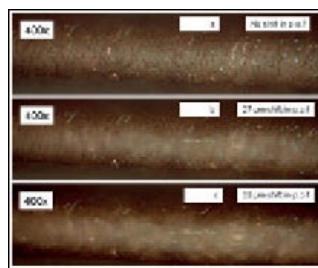


Figure 24) Micrographs of a hair fiber ($\phi \sim 64 \mu\text{m}$) showing image of surface

obtained with no shift in plane of focus (11a). Later the plane of focus (p.o.f.) was shifted in 27 and 32 µm, respectively; the white spots in the image are voids with sizes ranging between 3 and 10 µm. The hair fiber was subjected to five hot iron treatments with the hot iron at 190°C and a speed of 0.2 in/s (34).

Cosmetic scientists and formulators are continually working in this area to alleviate these damaging effects and to improve the widely used hot ironing process. Efforts geared into this direction have already resulted in the use of polymers that enhance straightening and decrease damage. Some groups have introduced the use of formaldehyde in hot iron applications. The use of this ingredient in combination with hot irons is very effective in almost permanently changing the shape of hair. However, because of its toxic properties its use has been banned in the U.S. The mode of action of this ingredient will be reviewed later.

3.3.4.6 HAIR SHAPE CHANGES INDUCED BY PERMANENT WAVING SOLUTIONS

a. The Process

The use of thioglycolate salt solutions to chemically reduce or break disulfide bonds in hair forms the basis for one of the most effective methods for permanently changing the shape of hair from straight into a more wavy and curly configuration. Essentially, the process consists of three basic steps that are similar to those already described for other forms of hair setting:

- 1)breakage of bonds to soften the hair, i.e., in this case the disulfide bonds,
- 2)imposition of a new shape, and
- 3)re-formation of bonds to lock-in the new permanent shape.

Breakage of disulfide bonds in hair is carried out by treating hair in a 7–9% solution of ammonium or sodium thioglycolate at a pH of ~ 9.5. Imposition of the new shape is made by winding hair swatches on plastic rollers. Finally, re-formation of the broken disulfide bonds is accomplished by applying a solution or cream containing 3% hydrogen peroxide to reoxidize broken bonds (see [Figure 25](#)).

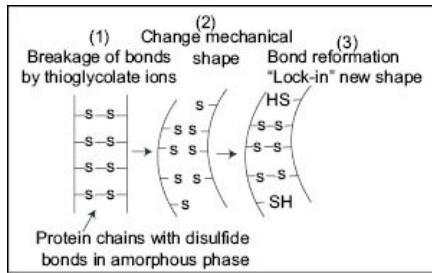


Figure 25) Diagrammatic representation of three steps required for the creation of a permanent shape in hair by permanent waving treatments. In step 1 disulfide bonds are unzipped to erase memory in hair, in step 2 a curly shape is imposed, and in step 3 the broken disulfide bonds are re-formed to lock-in the curly shape (18).

b. Physical and chemical processes taking place inside hair during permanent waving

The role of step one described above in permanently changing the hair shape with ammonium thioglycolate is not just to soften the hair, but mainly to delete the permanent shape in the hair architecture. This process is carried out by the thiolate ion which unzips disulfide bonds in the amorphous structure of hair and produces a certain degree of destabilization in the crystalline regions. Then, once the old shape is partially removed and when the hair is somewhat shapeless, a new shape is introduced in the protein chains by mechanical deformation of plastic rollers. Finally, the new shape is stored in the amorphous protein phase by re-zipping the same bonds in a different configuration by using hydrogen peroxide. Unfortunately, the disulfide breakage and re-formation process modifies only one part of the memory where the permanent shape of hair is stored. Namely, it only modifies part of the tertiary structure of the amorphous proteins. However, it does not modify the crystalline phase nor it does modify the primary and secondary structure of the proteins in the amorphous phase. This means that after the ammonium thioglycolate treatment, there are still shape recovery internal stresses coming from these structures. These stresses will push gradually for the recovery of the original permanent shape in the hair fibers.

Changes in the hair shape memory with ammonium thioglycolate are consequently partial, and will result in a very slow shape reversion process. At the molecular level this process is driven by the recovery of protein conformations to their native state. The forces driving this phenomenon stem from the primary and secondary structures of hair proteins in the crystalline and

amorphous phases. It should be mentioned here that the phenomenon of shape reversion is common in a host of denatured proteins and enzymes and, in particular, when it is related to disulfide bonds.

In a famous experiment, C. Afinsen showed that disulfide bond breakage/re-formation in ribonuclease leads to a mixture of denatured and scrambled protein conformations with no enzymatic activity (38). He also observed that the process of protein scrambling by disulfide bond breakage/re-formation was not sufficient for the enzyme to completely lose its shape memory or ability to revert to its native shape. For instance, Afinsen found that traces of mercaptoethanol/urea in combination with the scrambled enzymes in solution were sufficient for the enzyme to reverse and recover its native shape (see [Figure 26](#)).

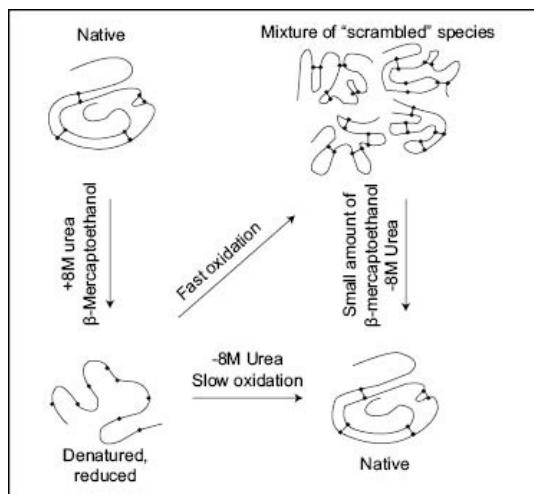


Figure 26) Schematic representation of C. Afinsen's experiment with ribonuclease showing that this enzyme is able to recover its shape even after its disulfide bonds were re-formed with the proteins in a scrambled configuration (38).

It would thus appear that the ability of proteins to recover their native shape is not completely lost when their disulfide bonds are arranged in a different configuration. This particular phenomenon exhibited by proteins helps to explain why the application of treatments that produce only disulfide bond breakage/re-formation in hair are not sufficient to produce irreversible changes in its protein architecture, so that they will support the desired permanent shape changes. Disulfide bond interchange, in combination with recovery stresses coming from the residual memory in the protein architecture of the amorphous and crystalline phases, will be able to drive the reversion process.

From the practical point of view, however, thioglycolate systems are

probably the best compromise that can be made between hair damage and persistence in shape changes. Ammonium thioglycolate systems have been shown to produce shape changes that are quasi-permanent and last for months. Certainly, more effective treatments could be designed, but they will probably have to include permanent changes to the primary, secondary, and tertiary protein structures of both the amorphous and crystalline phases. Such a process could be devised but will introduce irreversible damage into other regions of the hair fiber, not to mention the practical loss of recurring business and socialization phenomena that are embedded in the perennial trek to the local cosmetologist. In summary, the process of disulfide bond breakage/re-formation for storing permanent shape changes is not 100% efficient, but it is in a good position as a system because it is very selective to disulfide bonds. It is for these reasons that many scientists working in this field have rather opted for improving the system.

In fact, there is still a great deal of improvement that can be done with these systems. For instance, the disulfide bond breaking/re-formation process is usually assumed to be fully reversible, i.e., it is commonly believed that the amount of disulfide bonds that break can be completely re-formed. Scientists have found that this is not the case and that a small percentage of these bonds always remain permanently broken (39, 40). Much effort has also been dedicated to understanding the effects of temperature, thiolate ions diffusion, and reaction kinetics inside the hair (41, 46). The process of disulfide bond re-formation by means of oxidizing agents such as hydrogen peroxide and other reagents has also been the subject of numerous studies. Effect of fiber diameter on shape changes with these systems has also been the object of concern (43–46). All these studies represent efforts geared to improving the efficiency and effects of the disulfide bond breakage process.

Surprisingly, changes in the crystalline regions after permanent waving have not received much attention and only a few studies have appeared in the literature (47). These studies, however, do not seem to correlate efficiency of shape change with levels of irreversible change in crystalline regions. Certain substances such as urea in combination with ammonium thioglycolate salt solutions appear to enhance destabilization and breakage of hydrogen bonds, apparently improving the efficiency of permanent shape changes, yet their effect on hair's crystallinity has not been reported (48).

3.3.4.7 HAIR SHAPE CHANGES BY ALKALINE RELAXERS

a. The process

The straightening of hair with alkaline cream relaxers belongs to the category of permanent changes in hair shape. It is the most damaging process of all known hair permanent reshaping cosmetic treatments. This is so because during the alkaline straightening process not only are disulfide bonds broken, but also a series of other processes occur that damage the hair architecture. Among these processes are: permanent denaturation of hair proteins, irreversible breakage of disulfide bonds, protein alkaline hydrolysis, protein loses, and excessive swelling of hair (49–51). All alkaline straightening processes are based on the chemical reactivity of the OH ions towards the groups in hair proteins that store the permanent curly shape. The pH of these creams depends on the tightness of the curl, and it ranges between 11.5 and 14.0. As a result, the concentrations of alkaline ingredients in these creams often vary between 1.5 and 3%. Strong chemical bases are used for this purpose and these include sodium hydroxide, lithium hydroxide, combinations of lithium/sodium hydroxide, and guanidine hydroxide produced *in situ* by the combination of calcium hydroxide and guanidine carbonate.

There are various classifications of cream relaxers in the market. These are known as lye, no-lye, base, no-base, and mix, no-mix, etc. Regardless of their classification, the key active chemical in all of them is always the OH ion. Lye relaxers are those based on sodium hydroxide; no-lye relaxers are those containing any other ingredient than sodium hydroxide. A base relaxer refers to one that needs a layer of petrolatum on the scalp prior the straightening process to protect it from irritation; the no-base relaxer does not need petrolatum separately as it is already formulated in the cream; and finally, a mix relaxer refers to one needing the mixture of two ingredients to produce the OH ions, as is the case of the guanidine carbonate/calcium hydroxide relaxer.

Other types of chemicals used in the straightening of curly hair comprise the use of sodium bisulfite and thioglycolate salts; these systems are referred to as the Japanese and Thio relaxers, respectively. The action of these two ingredients is not based on the action of the OH ions but rather on the reactivity of the sulfite and thio groups. They are more selective to the breakage of disulfide bonds, and as will be seen later, they have advantages and disadvantages over alkaline cream relaxers. A typical salon application of a relaxer consists of three main steps: relaxer cream application, neutralization, and hair conditioning. During

the application step the cream is evenly distributed over the selected hair swatches. Almost immediately after application a diffusion of OH ions is triggered from the cream into the hair cortex via the endocuticle and cell membrane complex. As the ions diffuse they start to react with the hair proteins in the amorphous and crystalline phases. Experience shows that 15 or 20 minutes is sufficient for the cream to produce desired hair-relaxing effects.

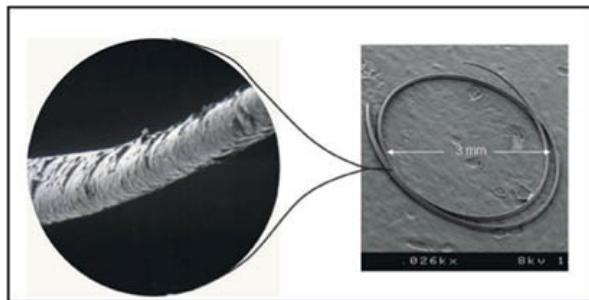
Relaxer creams in many cases are not designed to reach reaction equilibrium conditions with the proteins in hair. Usually, their OH ion concentrations, which depend on the pK_b of the base, are above that required for the hair-straightening process. Consequently, if they are left for longer periods of time on the hair, the effects can be partially depilatory. Thus, as soon as the beautician considers that the hair has been properly relaxed, the cream is rinsed out with water. At this point the OH ions are still reacting with the hair internal proteins; therefore, a neutralization process must be immediately introduced to stop the OH ion/protein reactions. The neutralization process is usually carried out with a shampoo containing citric acid. During this process a strong odor is perceived coming from liberated sulfur. Also, experiments show that the neutralizing solution turns turbid, indicating extraction of protein fragments from the hair structure (52). Because of the high level of damage to the whole hair architecture, a conditioning process is immediately introduced, mainly, to lessen fiber entanglement due to friction effects.

b. Physical and chemical processes taking place inside hair during alkaline straightening

The alkaline reshaping of very curly hair also starts by deleting the hair's permanent shape from the memory located in its architectural structure. In the case of permanent waving this could be done partially by using thio or sulfite compounds to unzip all disulfide cross-linking bonds and allow reorientation of the crystalline regions. The use of these ingredients for the straightening of very tight African-type hair is usually not recommended as it leads to a gradual shape reversion. This happens because disulfide bond breakage alone is not sufficient to delete enough shape memory from the curly hair so the straight shape will resist reversion. The degree of natural curliness in African hair is very tight and strong, and therefore, making it straight requires more changes in its protein chemical structure. This is true, in particular, on sites where the curly shape is imprinted. In other words, African hair was casted from its inception at the hair follicle with a primary, secondary, and tertiary protein architecture complying

and supporting very small radius of curvature and also high degrees of fiber elliptical shape (see [Figure 27](#)). This means that at the microscopic level the proteins in the amorphous and crystalline phases in these fibers are oriented and chemically bonded to accommodate for these geometries (see also Figure 8).

Thus, in order to attain a complete straightening effect in these curls a sizable amount of shape change or shape deletion is required. If the change in shape were from very tight curly to open curly, the amount of shape deletion in the hair architecture will be less, and this type of mild shape change would not even require the formation of new bonds to lock-in the new shape. However, complete straightening of very tight curly hair requires a higher degree of structure modification; first, because the tight curly shape has to be erased and, second, because the new straight shape needs to be locked-in. It should be recognized that the complete removal of curly shape from a curly hair fiber implies the breakage of chemical bonds that stay irreversibly broken, thus weakening the overall structure of hair. Therefore, the process of introducing a new permanent shape such as in the case of straightening curly hair may require either full reversibility of bond breakage/re-formation, or the introduction of new bonds to stabilize the new shape and also to strengthen the fiber.



[Figure 27\)](#) Micrographs of a single fiber of African origin showing tight coil radius of curvature and high degree of ellipticity (18)

As was previously stated, there is a rule relating shape changes and number of sites in memory for curly hair, the rule is: **the tighter the curl, the more the elements in the architectural sites of hair's memory need to be modified to make it straight.** Sizable permanent changes in hair shape always require the annealing of stresses in the internal structure of hair. Otherwise, they will produce strong internal fiber stresses that will drive a shape reversion process. Consequently, large changes in hair shape always need the modification of a higher number of memory sites to eliminate strong reversion forces. The degree of memory deletion process can be outlined in the following manner:

- Weak curls require the breakage of a few disulfide bonds.
- Medium strong curls require a larger amount of disulfide bond breakage.
- Tighter curls require, in addition, denaturation of crystalline regions.
- Very tight curls require all of the above, plus breakage of amide linkages in the proteins as well.

By the same token, the level of chemical and physical stabilization required by the new straight shape increases in the same order.

However, regardless of degree of initial hair curliness, the steps needed to straighten hair with any type of cream relaxer are all the same and almost identical to those required for other changes in hair shape:

- 1)the softening of hair, which may involve breakage of physical bonds, breakage of covalent bonds, and softening of the crystalline and amorphous regions,
- 2)imposition of a new shape, and
- 3)fixing or locking the new shape by re-forming broken chemical or physical bonds, and by rearranging crystalline and amorphous regions.

The role of the first step is not only to chemically soften the hair, but in doing so, also to delete the permanent shape from the hair architecture or memory. In the second step, a new shape is introduced in the shapeless hair, and in the third step, the new shape is stored and imprinted in the memory structure of hair. If we pay close attention to these steps we will immediately realize that the efficiency of changing hair shape, from curly to straight, does not depend only on how much memory is erased but also on how efficient the process of imprinting and storing the new shape is.

In the scientific literature it has already been shown that alkaline creams lead to substantial chemical and physical modifications to the hair structure (53–57). Among the main chemical reactions and physical processes are breakage of weak and strong hydrogen bonds, breakage of salt linkages, and the formation of the following products: lanthionine, cysteic acid, sulfur, and protein fragments. These reactions proceed according to the paths shown in [Figures 28](#) and [29](#).

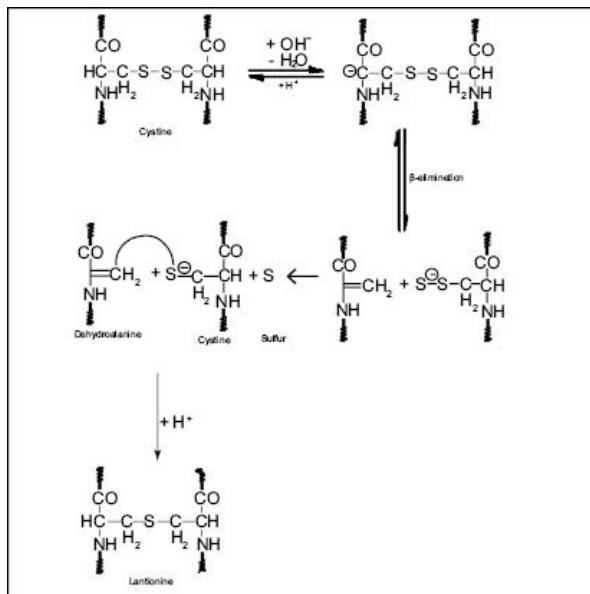


Figure 28) Diagram showing reaction of cystine and OH ions. The reaction takes place during the alkaline straightening of African hair at pHs ranging between 13.5 and 14.0, and it leads to the production of sulfur and lanthionine (32).

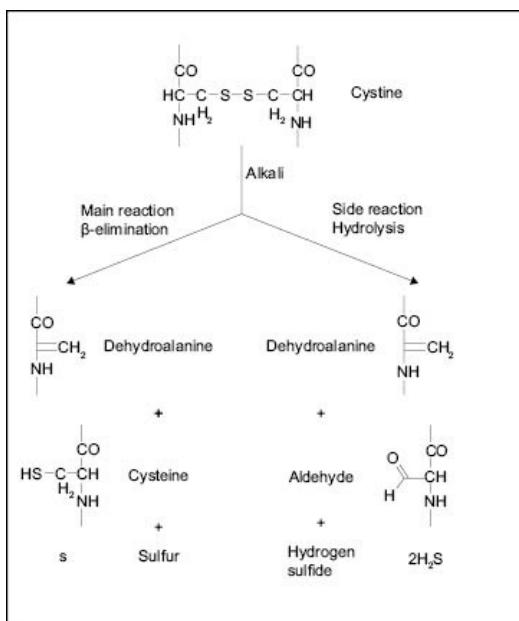
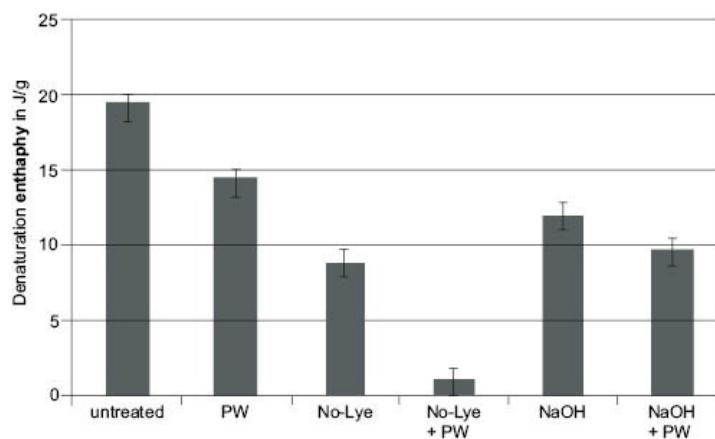


Figure 29) Diagram showing additional reactions of cystine and OH ions occurring in the alkaline straightening of African hair at pHs ranging between 13.5 and 14.0 (32).

Because of the high level of bond breakage, alkaline relaxers also produce

high levels of protein denaturation in both the amorphous and crystalline regions. [Figure 30](#) shows typical denaturation enthalpy reductions of various hair fibers subjected to a combination of alkaline and permanent waving treatments. The smaller the bar size in the graph, the higher is the degree of denaturation or disarray in the crystalline regions. All treatments in these experiments showed a decrease in the enthalpy of denaturation and some of them were more pronounced than others (32). Those treated with the alkaline relaxers alone, i.e., with NaOH and No-Lye are more real representations of the irreversible denaturation process taking place in real straightening hair practices, and they show reductions that range between 30 and 50% in their denaturation enthalpies.



[Figure 30](#)) Denaturation enthalpies of hair fibers after various treatments as follows: Untreated = fiber with no treatment, PW = fiber with permanent waving treatment, No-Lye = fiber treated with No-Lye relaxer, No-Lye+ PW = fiber treated with a combination of No-Lye straightening + permanent waving, NaOH = fiber treated with a relaxer based on NaOH, and finally, NaOH+PW = fiber treated with a combination of a relaxer and permanent waving (32).

This is the reason why alkaline relaxers are very efficient in deleting the permanent shape from the hair architecture—namely, because they modify a large number of hair's physical and chemical features in the protein structures containing the permanent shape memory; *i.e.* they break disulfide bonds, strong and weak hydrogen bonds, salt linkages, and a certain amount of amide bonds. By breaking all these bonds the OH ions render the amorphous phase extremely soft; in this process the crystalline regions also become scrambled and highly plasticized as well. The hair becomes, therefore, extremely soft and shapeless. The memory deletion process obtained by these effects does not guarantee however the imposition of a new shape, nor the process of locking-in the new

shape. For this to happen, new chemical bonds have to be introduced in combination with a process of reconstitution of the hair architecture.

c. Shapeless hair and a new straight shape

As previously discussed, when the alkaline straightening process starts to delete the tight curly shape from the hair memory, the hair fibers gradually become soft and shapeless. The new straight shape is also gradually introduced during the alkaline straightening process by the rubbing of the cream into the hair. The process of new bond formation also appears to occur as the cream is deleting the old curly shape. Experiments have shown, for instance, that if the process of hair straightening is carried out while the hair maintains its coiled shape, the straightening efficiency is less than if the curl is uncoiled and extended (52). Furthermore, if the acid neutralization step is done while the hair is kept in a more straighten configuration the efficiency in straightening also increases (52). These observations indicate, thus, that a certain amount of new bonds, most certainly lanthionine (see Figure 28) (53–59), are gradually introduced while the hair is still reacting with the cream. The straightening efficiency observed during neutralization, on the other hand, is certainly due to a vitrification process of the amorphous phase, and also to a partial reconstitution of the crystalline regions as salt linkages and strong hydrogen bonds re-form upon elimination of the OH ions.

The phenomena of fiber supercontraction and denaturation observed to occur in alkaline treated hair fibers, and reported by many scientists (60–62), cannot explain the straightening process when considered alone. These phenomena are at most manifestations of disulfide and hydrogen bond breakage and also of changes in protein conformation in the crystalline regions. Like the phenomenon of excessive water swelling, they do not impose by themselves the new straight shape or cause the locking-in of the new shape in the hair architecture. Furthermore, in terms of shape changes there are substantial differences between the processes of protein denaturation, supercontraction, and hair swelling. Protein denaturation does contribute to the removal of permanent shape from the hair architecture; while supercontraction and swelling do not, they are physical phenomena associated to destabilization of the protein structure. Protein denaturation per se, however, does not impose a straight shape; nor does it lock-in a new shape.

Currently, there are three main mechanisms that have been proposed to explain the straightening of very curly hair by alkaline creams. The oldest one

centers its arguments on the formation of lanthionine as the new chemical bond that is formed during the reaction of OH ions and hair. This bond is proposed to lock-in the straight shape (59–61).

Another mechanism introduced later bases the explanation on the phenomenon of supercontraction. This mechanism essentially proposes that the high level of contraction observed to occur in hair and wool when treated with high pH solutions is responsible for the imposition of the straight shape (60).

Yet, a more recently proposed mechanism suggests that the occurrence of protein denaturation is crucial and that lanthionine and the formation of any other products are just secondary to the straightening process (62). However, when we use concepts from the field of shape memory materials, we realize that the permanent straightening of hair must include the following steps: 1) removal of protein configurations that support the curly shape, 2) imposition of straight shape, and 3) locking-in of the straight shape, either physically or by forming new bonds.

It is quite possible, thus, that the straightening of curly hair by alkaline relaxers occurs by a combination of all three of the proposed mechanisms. Namely, 1) breakage of disulfide and protein denaturation are needed to delete the curly shape from the memory of hair, 2) a certain amount of fiber supercontraction aids during the cream rubbing process to create the straight shape, and 3) lanthionine formation is needed to stabilize the new straight shape.

Finally, it is obvious that the introduction of a straight shape in curly hair needs to be cosmetically acceptable. This means that both the new straight shape and the hair fibers need to have strength so the shape can be maintained to resist external forces without further unwanted deformation or breakage. The hair with the new straight shape has to be able to resist stresses coming from the grooming of hair, i.e., from its own weight and from hair movement produced by combing or styling, either when wet or dried. Experience tells us, however, that relaxer technology still is far from achieving this objective satisfactorily. Currently, many scientists are working to bridge this gap, and it has already been shown that for certain cases, i.e., for medium curly hair, it is possible to impose a straight shape without causing much damage.

These processes involve the introduction of new strong bonds in the protein chains, bonds that are capable of overcoming internal recovery stresses from the old permanent shape. Unfortunately, most of these new technologies are based on formaldehyde. This chemical is known to cause protein cross-linking in

proteins and therefore when used in hair it introduces new cross-links in the hair structure (63). Formaldehyde is, however, a potential carcinogen and its use has been banned in cosmetic applications.

Other hair-straightening technologies involving sodium sulfite or thioglycolate salts are more specific to disulfide bonds, but by their nature they are not efficient in fully removing the very tight curly shape from the hair memory. Consequently, later on the hair undergoes total or partial reversion to its curly shape. In summary, it can be said that the alkaline straightening of curly hair by OH ions is characterized by an efficient shape memory deletion process. It introduces a new straight shape, but it falls short of forming new bonds to lock-in the new straight shape so the final hair shape and hair fibers stay strong. We would be remiss if we did not point out that one other area needing research and improvement is the undesirable occurrence of chemical burns on the skin from the highly alkaline processes described above.

3.3.4.8 HAIR SHAPE CHANGES OCCURRING DURING GROOMING PRACTICES

a. Static vs. dynamic changes in hair shape

The imposition of temporary changes in hair shape is always carried out by external means, and depending on the way hair is handled they can be classified into two types: 1) shape changes imposed while hair is under static conditions, and 2) shape changes imposed while hair is under movement. In the first category we find water-setting, permanent waving, and alkaline straightening. All these shape changes are imposed with external devices that force the hair to stay statically or quasi-statically with almost no movement while the new shape is being imposed. By contrast, in the second category, shape changes are also imposed externally, but the temporary shape is imposed while the hair fibers are in a dynamic free or forced intensive movement. Thus, the final shape attained by the fibers is an average shape, and depends on fiber motion in the assembly. These are, for instance, shape changes produced by blow-dryers, combing devices, and by drying towels. In shape changes of the first type, the interaction of fiber to fiber is not important, while in those of the second type this interaction is extremely important as inter-fiber motion in the assembly determines the final average shape. In the following paragraphs we will focus our attention on the latter types of shape change.

b. Friction vs. bending forces in dynamic changes in hair shape

Movement of hair fibers in hair assemblies always occurs by currents of air, head motion, grooming devices, and even when wet hair is allowed to air dry. Because of hair flexibility, length, and motion intensity, the hair fibers bend, flex, and rotate inside the hair assembly. This flexion and rotation imposes instantaneous transient deformations on the fibers and ultimately leads to the formation of transient temporary shapes in the hair assembly. The transient deformations are, however, immediately countered by internal stresses coming from the permanent shape of hair. The hair assembly will stay deformed in a transient state or it will recover its shape depending on whether inter-fiber slippage occurs between fibers.

Ultimately, the degree of fiber inter-slippage will depend on the balance between single fiber bending and its frictional forces. For instance, slippage among fibers can be prevented if the inter-fiber frictional forces are larger than the bending forces. Under these conditions the hair fibers will behave as semi-solid ropes and the contact points between fibers will serve as strong welding joints that restrict inter-fiber movement. This condition occurs for low bending curvatures and high surface frictional forces, or when styling polymers are used. Polymer spot welding and high friction sites restrict fibers from moving and prevent the fibers from slippage when the hair assembly is being deformed.

Conversely, if a high degree of slippage occurs between hair fibers, then, the low surface frictional forces will not restrict inter-fiber movement. Consequently, slippage among fibers will occur and the permanent shape of both single fibers and hair assembly will be rapidly recovered while in motion. This occurs at sufficiently large bending curvatures or when there are relatively low frictional forces at the hair surfaces. These conditions correspond to the case when silicones or efficient conditioners are applied to the hair surface. The final average shape attained by an assembly is dependent on the balance between fiber slippage and friction forces. [Figure 31](#) displays two hair tresses (a) and (b) washed with different shampoos, and showing the effects of these two conditions. Both hair tresses were cut from the same hair batch and were allowed to air dry at room temperature conditions after shampooing. Hair tress (a) was washed with a simple cleansing shampoo, while hair tress (b) was washed with a “2 in 1” conditioning shampoo containing silicone. In Figure 31 it can be seen that hair tress (b) dries with its fibers in a wavy fashion or with a shape that resembles its permanent shape, while hair tress (a) dries into a straight lump. The reason for this is that during fiber movement and drying, fiber inter-slippage in

hair tress (b) was dominant and the final hair shape was driven by natural recovery forces.



Figure 31) Effect of silicone on the temporary shape of hair after drying. Hair tress (a) was washed with a cleansing shampoo while hair tress (b) was washed with a shampoo containing silicone (64).

Thus, the effect of fiber-to-fiber interaction via surface effects has a strong influence on the acquisition of a temporary shape when the hair is in motion.

Fiber-to-fiber interaction has, therefore, a strong impact on the final shape of the hair assembly. This means that if water-setting is taking place during hair movement, the final shape of the hair assembly will be determined by the interplay between hair inter-fiber friction forces, hair drying rates, hydrogen bond formation in gel-structure, and ability of hair to recover its permanent shape. The role that these processes play in setting dynamic temporary shapes is so important that in the following sections of this chapter their interplay will be analyzed in relation to various grooming practices.

3.3.4.9 HAIR VOLUME AND CHANGES IN HAIR SHAPE

a. Definition of volume

Hair volume can be defined as the effect obtained when a bundle of hair fibers, aligned in synchrony, occupy as a whole or individually a physical space larger than their real nominal volume. Volume in hair is more about the space occupied by the hair bundle than about hair mass or fiber density. The larger the space between fibers or the larger the total volume occupied by the hair bundle, the

more volume is perceived (see [Figure 32](#)). The definition of volume also includes the ability of the hair fibers to maintain their separate space and shape in synchrony when the assembly of hair fibers is in motion. It should also be perceived when the hair bundle is at rest. These conditions imply that temporary hair shape changes, producing volume should have the strength and elasticity to recover rapidly and in synchrony with shape distortions created during movement. As will be seen later, if this does not happen, the undesirable cosmetic effect will be that of frizzy hair.



Figure 32) Caption of hair assemblies showing hair volume. The assembly of hair occupies a larger space than it would if each single fiber were shaped straight. Observe also that hair fibers are aligned in synchrony with a particular bundle.

Temporary shape changes in hair that create volume effects can be introduced dynamically while the hair assembly is in movement or also statically. The former is the focus of this paragraph, since the latter belongs to the types of static shape changes already described in previous paragraphs. There are two ways of creating volume by a dynamic process of hair in motion:

- 1) by imposing a wavy or curved shape in a collective assembly of hair fibers in a synchronized manner while the fibers are in motion. This process usually involves rapid water-setting; and/or
- 2) by increasing the distance between individual hair fibers, either by creating inter-fiber friction or by using styling polymer films to produce light wavy shapes on smaller hair bundles.

b. Main challenges in creating volume

The creation of hair volume in hair assemblies by any of the procedures described previously always entails hydrogen bond formation, even when polymers or styling resins are used. This means that for all practical purposes, the process of dynamic volume creation has many similarities to the process of

water-setting. In water-setting the temporary shape is created statically with plastic rollers, while in the production of dynamic volume the temporary shape is created usually when the hair fibers are in movement. The reason for this is that temporary shapes with volumizing or styling characteristics are commonly created in hair by people after taking a shower, and they are rapidly and conveniently created when the hair is wet and being dried. Therefore, volumizing temporary shapes created by motion are better stored in the hair gel structure of the amorphous phase while the hair is dried. In the following paragraphs of this section, attention will mainly be given to the process of creating hair volume without the use of resins or styling polymers, while the use of styling polymers will be reviewed in a later section.

The main challenge in creating temporary shapes that will result in an increase in hair volume is to coordinate the process of hydrogen bond formation with hair movement in order that an average temporary shape can be captured. Implicit in achieving this is the creation of a temporary shape whose instantaneous strength will be able to resist later shape-recovery forces arising from the permanent shape. While daunting, we note that beauticians have been producing volume in hair just by manipulating hair in motion as it dries from its wet state. As was previously discussed, temporary shapes created by hair fiber assemblies in motion are determined by the interplay between hair inter-fiber friction forces, hair drying rates, hydrogen bond formation in gel-structure, and ability of hair to recover its permanent shape.

In order to better understand the role of hydrogen bond formation and hair movement, let us imagine an ideal and imaginary case of hair volume creation, where the volumizing shapes introduced during hair movement are ideal and will be frozen and stored instantaneously by an extremely rapid process of hydrogen bond formation.

In this ideal process, hydrogen bond formation would be so fast and intense that the instantaneous hair shape acquired during movement would be fixed on hair like a photographic process that freezes a hair shape image while it is in motion. This ideal process would produce a very strong and temporary, rigid pattern of hair volume, somewhat similar to that obtained with the use of styling gels. Such a temporary shape would also resist very well the internal recovery stresses from the permanent shape. Unfortunately, this ideal process does not exist. First, because the rate of hydrogen bond formation by evaporation is slower than the instantaneous shape movement and second, because the instantaneous formation of hair shape during movement depends on the balance

between inter-fiber surface friction and shape-recovery forces. Third, and finally, such an ideal process would create a very stiff and static hair pattern that for all practical purposes contradicts one of the main characteristics of hair volume, namely, that of a synchronized hair movement as well. In layman's terms, it is a beautiful thing to watch a woman with long flowing hair shake her head and all of the long tresses move together from side to side. We state this here to bring the reader back, temporarily, to why we have spent such a significant amount of time and scientific research: in the end, all of this technical understanding is intended to bring beauty to hair!

Let's analyze then, a more realistic case; a good example might be that of a head hair assembly with straight shape fibers that are six inches long and belong to a hypothetical subject. After the hair has been shampooed, conditioned, and towel dried, the subject intends to introduce volume by blow-drying. For the sake of simplicity in the analysis, the effect of conditioning ingredients on the hair surface will be omitted. When the straight hair has been wet and towel dried, it still contains about 80% of its internal moisture and, therefore, its gel structure still is very soft because most of its hydrogen bonds are broken.

If at this point the subject introduces motion into the hair fibers, they will be bent and flexed elastically, and the whole hair assembly will be turned into an elastic scrambled irregular pattern. Then, as the subject dries the hair with a blow dryer, he/she forces the hair to move with a brush or a comb to create wavy patterns. As the hair moves and dries, simultaneously hydrogen bonds are formed in the gel structure of each hair fiber, thereby trapping a synchronized wavy temporary shape into their temporary memory. This temporary shape will increase hair volume because the wavy shape of the hair fibers will occupy more space than when the fibers were in a straight shape.

In this simplistic example only two factors affecting the formation of shape were considered. These were the effects of hair drying and hydrogen bond formation. The effects of hair inter-fiber friction, permanent shape strength, and effects of products on rates of drying were not considered. It is easy to foresee, however, that when the fibers enter in motion, the type of shape that will be produced depends on whether the surface is well lubricated or not. It will also depend on the integrity of the hair fibers. It may be that the hair assembly was previously treated with an alkaline relaxer or with other harsh chemical ingredients and thus has a low content of disulfide bonds. In such a case, the instantaneous shape that would form during movement would be very different from that which would have formed had the hair been virgin. Thus, the

instantaneous shape that forms during hair movement is also dependent on how strong the real permanent shape is. If the fiber strength and permanent shape are weak, the hair fibers will behave more like viscous fibers and will not be able to recover from instantaneous deformations created by hair movement.

Another factor that was not considered in the prior simplistic analysis is the rate of drying. If the hair contains ingredients on its surface that will retard water evaporation, or if for some other reasons water evaporation is slow, then the process of hydrogen bond re-formation to lock-in the shape in motion will be slow as well. This means that the amount of hydrogen bonds that form at each instant will be so slow that the gel structure will not have the strength to counter the internal shape-recovery forces coming from the real permanent shape. Consequently, the hair wavy pattern produced by hair movement will not be locked-in. For the temporary shape to be locked-in, the numbers of hydrogen bonds that form per second during drying have to be enough to build a hardened mass in the gel structure, so that its strength would be able to counter at any time the internal recovery forces coming from the permanent shape.

By the same token, if the ingredients favor faster drying, then the gel phase in the hair fibers will harden more rapidly, and the wavy shape in motion will be entrapped more efficiently. This analysis explains what cosmetic formulations and beauticians already know, namely, that the types of ingredients applied to hair and the rate of hair drying both have an effect on hair volume. The design of formulations that increase volume thus contain ingredients that in some way or another modify inter-fiber friction, hair drying rates, hydrogen bond formation, or permanent shape.

c. Back-combing and “static fly away”

Back-combing and “static fly away” are two different processes also often used to increase volume. They do not involve the creation of temporary hair shapes by free fiber movement and are based on a different mechanism. Back-combing essentially consists of increasing inter-fiber friction to very high levels by lifting cuticle cells (see [Figure 34](#)). The protruding cuticle cells created in this manner interlock the hair fibers at various points. The idea is to totally restrict inter-fiber slippage and movement so the bending deformations introduced during back-combing are maintained. This technique is effective in creating volume; however, it is very damaging to hair. Another way to produce volume in hair is by creating electrostatic repulsion between each hair fiber. Electrostatic repulsion in hair, also referred to as “static fly away” by the cosmetic

community, is very common in dried hair, and is due to the buildup of an excessive electric charge in hair. This charge is usually of negative polarity and can also be introduced artificially onto the hair fibers.



Figure 33) Caption showing process of back-combing (“teasing”) to produce hair volume



Figure 34) Captions of hair swatches showing effects of “static fly away.” There is an attraction between hair fibers of each swatch due to the relative amounts of negative charge in each one, i.e., the hair tress on the right contains a higher level of negative charge buildup (64).

The process of creating an excess of electrical charge in hair fibers is not a difficult task since hair is an electrical insulator, having very poor electrical conducting characteristics. Therefore, hair, like other electric insulators, is prone to accumulate charges on its surface. Charges are easily transferred from one electrical insulator to another when their surfaces enter into intimate contact. The direction of electric current, or flow of electrons, from one material to another has been studied and categorized for a host of insulating materials and put into a table usually referred to as the triboelectric series (see [Table I](#)). Materials at the top of the chart have an excess of negative charges trapped into their structures, and therefore tend to transfer electrons to those at the bottom of the chart. In

Table I, it can be seen that hair is below rubber, which is the material often used to make combs. When hair is combed, its surface enters into intimate contact with the rubber in the comb. Electrons are transferred from comb to hair, and thus the hair acquires a negative charge.

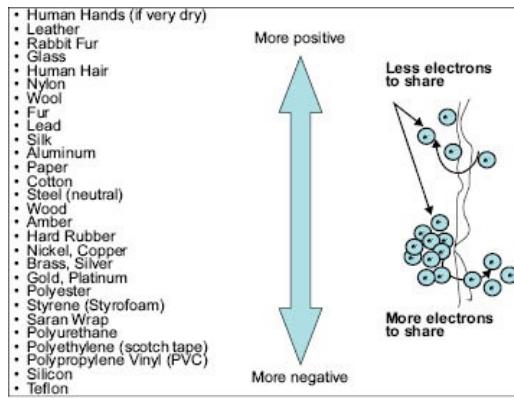


Table I) List of various insulating materials and their position in the triboloelectric series (65).

Since all combed hair fibers acquire the same type of charge, they repel each other, thereby creating a larger distance between them and, ultimately, increasing the hair volume of the assembly. Unfortunately, negative charge in hair does not last long since it escapes via moisture in the hair. Also, the presence of moisturizing ingredients on the hair surface reduces its ability to build up charge. Thus, when hair is highly moisturized, it cannot be charged no matter how many times it is rubbed against the comb. The deposition of certain polymers on the hair surface, however, increases charge buildup and can thus create an apparent effect of volume. The production of “static fly away” to increase volume in hair assemblies is, however, difficult to control as it depends on the rubbing intensity against the comb, i.e., excessive rubbing gives rise to excessive “static fly away.” Furthermore, “static fly away” also causes unwanted repulsion or attraction of hair assemblies in proximity, leading to frizzy hair and other unwanted effects (see Figure 34).

3.3.4.10 FRIZZ IN HAIR AND CHANGES IN SHAPE

Frizzy hair can be defined as the visual aspect of a hair assembly resulting from hair fibers that are positioned geometrically out of synchrony with the remainder of the bundle. The out-of-synchrony effect can be very small or large and can come from a few hair fibers, or almost the whole hair assembly (see [Figure 35](#)). The maximum radius of curvature of a frizzy hair fiber separating it from the

bundle can be of a few mm or larger (i.e., 1 or 2 cm). Out-of-synchrony effects appear in hair usually immediately after hair drying, and they are more pronounced in hair that has been chemically treated or in hair whose surface has been damaged. In non-frizzy hair, either curly or straight, all hair fibers are aligned in synchrony with their respective neighboring hair fibers. However, in hair with frizz, multiple hair fibers in various sections of the hair assembly separate randomly away from it, forming irregular-shaped curls or waves that are out of synchrony with the rest of the fibers in the bundle (see Figure 35).



Figure 35) Caption of frizzy hair produced by excess bleaching (left), and by repetitive hot ironing (right). Observe in both cases the large number of single hair fibers that separate out of synchrony from the bundles.

The causes of frizz arise mainly from the inability of hair fibers to recover their permanent shape during or after grooming deformations; i.e., after combing, drying, either by towel, blow-drying, or by hair movement during washing. The inability of hair to recover its permanent shape is caused by damage to the hair structure where the shape memory resides. When this happens, the hair partially or totally loses its permanent shape. Frizz can also be caused by excessive damage to the hair surface. Virgin and healthy hair has its permanent shape memory and also its surface intact. As a result, when the hair fibers are deformed, all of them respond in the same manner. In other words, when they are deformed by motion during grooming practices, they rapidly recover their shape and form an aligned bundle.

In contrast, damaged hair has its memory structures or surface damaged. This damage can be in the form of a partial destruction of disulfide networks in the amorphous phase, or in the form of irreversible denaturation of the crystalline phase. It could be said that hair fibers that present frizz do not remember their permanent shape, and therefore, are partially shapeless. Thus, when damaged hair fibers are deformed, depending on their degree of damage, they respond with shapes that are random and different. For instance, if the various damaged hair fibers undergo excessive stretching or bending during washing, combing, or

towel drying, their stretched or bent shape may stay like that or transform into a random shape. The result is that after the hair fibers are dried, their shape is out of synchrony with the remainder of the bundle.

Frizz caused by excessive damage in the cuticle cells of hair fibers is due to very high inter-fiber friction. As was previously discussed, the recovery and final shape in hair fibers during movement is determined by the interplay of the following factors: inter-fiber friction forces, hair drying rates, hydrogen bond formation in gel-structure, and ability of hair to recover its permanent shape. Those hair fibers in a bundle that present high levels of cuticle lifting will not be able to recover their permanent shape after the hair bundle is washed and dried. The reason for this is that high degrees of cuticle cell lifting causes high inter-fiber friction during fiber motion and this phenomenon results in a poor recovery of their permanent shape. Thus, in this case, the hair fibers separate from the bundle, making it appear frizzy. Another cause of frizz in hair comes from excessive static fly away. Typical examples of hair that develop frizz are excessively bleached hair, excessively dried hair, hair often treated with hot irons, and hair damaged during combing/tangling because of a lack of proper conditioning.

Frizz in hair can be reduced or alleviated by temporarily or permanently reconstructing the shape of frizzy hair. This can be done with hot irons, water-setting treatments, or even with treatments that introduce a new shape by reforming disulfide bonds. When frizz is corrected with temporary treatments such as water-setting or hot irons, the problem is not solved, as the hair fibers will become frizzy again after washing. If it is caused by excessive surface damage, then, frizz can be reduced with treatments that smooth cuticle cells or reduce excessive inter-fiber friction. In many cases, frizz is caused by a combination of damage to the disulfide bond network, damage to the hair surface, and by the appearance of static fly away. In this case, frizz reduction can only be accomplished by a combination of various treatments.

3.3.4.11 BODY IN HAIR AND CHANGES IN HAIR SHAPE

The subjective perception of Body is one of abundant hair that is strong and healthy. It manifests itself during hair movement as a combination of hair mass and hair shape. The closest mechanical descriptor for body is the flexural rigidity of a bundle of fibers. Unlike other mechanical properties that do not take into account shape, flexural rigidity combines Bending Modulus and shape of

materials (66). This property recognizes the fact that the same mass of any material is easier or more difficult to bend depending on the way that it is shaped. For instance, it is difficult to make a sheet of paper stand up straight on a surface because it flexes and bends down easily. However, if we fold the same sheet of paper in half it will be able to stand without bending. Thus, just by changing its shape we have changed its flexural rigidity (see [Figure 36](#)).

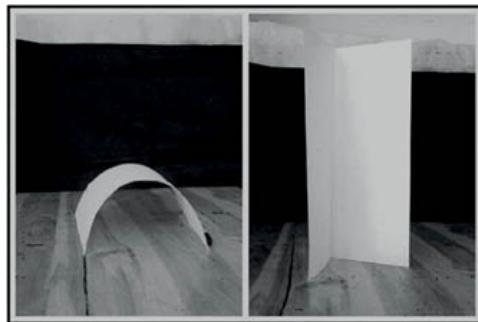


Figure 36) Effect of shape on the flexural rigidity of a sheet of paper. The sheet of paper cannot stand up straight on a surface because it flexes and bends down easily. Observe that after folding it in half the sheet of paper is capable of remaining straight.



Figure 37) Effect of hair curliness on the flexural rigidity of hair. The bundle in the left with curly hair is able to maintain almost a straight inclination in cantilever without flexing, while the bundle in right with straight hair fibers bends more easily (64).

When we apply these concepts to hair we realize that it is easier to flex a hair bundle of very fine hair when its fibers are in a straight fashion, than when a bundle of similar mass has its fibers tightly coiled (see [Figure 37](#)). Thus, shaping the fine hair fibers into a coiled configuration allows them to present higher flexural rigidity and higher body. The tighter the coil in hair fibers, the more body the hair assembly has. Furthermore, flexural rigidity in a solid body does not change with movement. However, in a hair bundle, since the hair fibers are

not tightly attached to each other, this property may change if the interplay of inter-fiber friction, hydrogen bond breakage with moisture, and drying rates are not properly set for that purpose. For instance, [Figure 38](#) shows two hair tresses with their half-lengths standing up and their tips pointing upwards. The bundle at the left was treated with a solution of cationic particles that increased inter-fiber friction, while the bundle at the right does not contain any product on its surface. The bundle at the left is stiffer than that on the right, and it shows higher flexural rigidity and more body because its pre-formed shape was not allowed to change by the high inter-fiber friction in the bundle.



[Figure 38](#)) Effect of inter-fiber hair friction on the flexural rigidity of hair. The hair swatch at the left was surface treated with cationic particles and is able to maintain an upward position vertically without any support, while the hair swatch at the right with no treatment bends more easily (64).

Body in hair is, therefore, dependent on the following three properties:

- a) hair fiber density, i.e., number of fibers per unit area,
- b) intrinsic protein density,
- c) inter-fiber friction, and
- d) temporary shapes created in hair fibers.

Since cosmetic treatments cannot change properties (a) and (b), in order to increase body, formulators always try to maintain temporary shapes with high flexural rigidity by changing properties (c) and (d). Products that increase body are, therefore, those that can influence and create the formation of temporary

shapes with high flexural rigidity, i.e., strong wavy patterns, high inter-fiber friction, inter-fiber spot welding, high drying rates, etc.

3.3.4.12 HAIR SHAPE CHANGES INDUCED BY STYLING POLYMERS

Styling polymers have been used for many years to produce or enhance temporary changes in hair shape and to create fashionable styles that are difficult to achieve by water-setting. The ways to produce these styling shapes vary from beautician to beautician but they can be divided into static and dynamic processes.

Styling static processes involve the following steps:

- 1) applying a new shape to hair with the help of rollers or other devices while the hair still contains ~ 60–80% of its moisture,
- 2) applying the formulation containing the polymer,
- 3) letting the polymer and hair dry, and finally,
- 4) removing the rollers or devices.

Styling dynamic processes involve, on the other hand, the following general steps:

- 1) inducing movement in hair fibers with blow-dryers or fingers while the style is being created,
- 2) applying the polymer as the hair moves to entrap an average shape, and
- 3) stopping movement and polymer application and letting the hair dry.

From the mechanistic point of view, both polymer styling processes are similar to the static and dynamic water-setting described in previous sections. The only difference is that when a styling polymer is used, these materials are applied to hair via various formulations to produce temporary shapes and/or to enhance their durability. In most cases the mode of action of styling polymers in changing hair shape is external and limited to the production of seam and spot-welding junctions between hair fibers to restrict inter-fiber movement, thereby giving rigidity to the hair assembly (see [Figure 39](#)). The polymer by itself does not affect the memory shape or intrinsic structure of hair. In other words, the polymer molecules do not interact with hydrogen bonds, disulfide bonds, or the crystalline structure of hair.



Figure 39) Micrographs of a bundle of hair fibers showing formation of seam and spot welding by polymers (64)

However, formulations used as polymer vehicles may contain water or solvents that penetrate into the cortical cells and enhance or inhibit plasticization of the hair gel structure by breaking or re-forming hydrogen bonds. As will be seen later, these effects are not due to the polymer per se and have an influence on how rapidly the temporary shape is entrapped and locked-in. In the following paragraphs an analysis is made of the influence that polymers have in assisting changes in hair shape. Special attention will be given to how the polymers modify the interplay of hair inter-fiber friction, hair drying rates, hydrogen bond formation in gel-structure, and hair strength to recover its permanent shape.

The effects that formulations have on these processes will also be reviewed but to a lesser extent. Properties and composition of the various polymers used in hair styling applications will not be reviewed here, but for information purposes a list of the most common is given in [Table II](#).

3.3.4.13 ROLE OF STYLING POLYMERS IN CHANGING HAIR SHAPE

As was previously discussed, the water-setting of hair, or the entrapment of a hair shape in movement without polymers, is very simple and controlled mainly by the interplay of hair inter-fiber friction, hair drying rates, hydrogen bond formation in gel-structure, and hair strength to recover its permanent shape. Hair shape entrapment with styling polymers adds, however, various levels of complexity to these factors not only because the polymers play externally a role on hair shape formation but also because the formulations influence the shape.

The added levels of complexity brought about by polymers and formulations are due to the following parameters:

- 1) rates of solvent evaporation; this will determine how rapid the polymer welding seams and spots dry and harden,
- 2) hair plasticization by the solvent; mainly when water is used as the solvent,
- 3) polymer glass transition temperature (T_g); this parameter determines how hard/brittle or soft/ductile is the spot welding, and
- 4) strength of adhesive joints at the hair/polymer interface. In the following paragraphs a discussion of all these factors will be made in relation to each one of the main formulations used in the styling of hair.

a. Temporary setting of hair with aerosols

In terms of entrapping a hair shape in motion, aerosol formulations are the simplest to analyze because the polymer is usually dissolved in ethanol or in combinations of this solvent with water. The reasons for the simplicity stem from the following facts: 1) the ethanol/water solution produces a very low level of hair plasticization; 2) the solvent mixtures have high rates of evaporation and, therefore, the polymer “welding spots” harden very rapidly, entrapping transient hair shapes efficiently. Thus, provided that the polymer or resin has the appropriate T_g , and that it is properly formulated and delivered from the aerosol bottle, and that the concentration of cationic charge along the polymer chain is sufficient, it will rapidly form welding seams and spots across hair fibers, restricting their motion and thereby entrapping the transient hair shape produced by movement (see [Figures 40](#) and [41](#)).

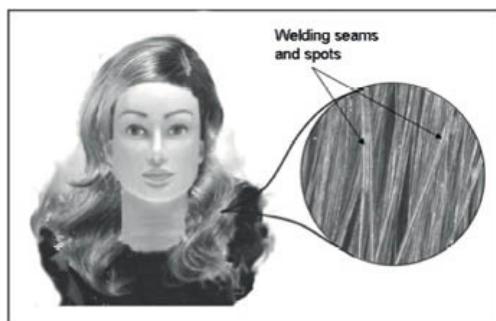


Figure 40) Illustration of hair in mannequin with styling polymer applied (left) and caption showing welding seams and spots (right)



Figure 41) Picture showing action of spraying a styling polymer to entrap shape of hair in motion

Aerosol formulations are also used to enhance temporary shapes produced statically, i.e., when the hair is not in motion. These types of applications do not have the complexities of entrapping an average shape produced by hair motion. The phenomena of fiber-to-fiber slippage, fiber bending, and fiber recovery from instantaneous shape deformations are absent in these cases. Temporary shapes produced under these conditions are preset by any of the methods described before for water-setting or hot irons under static conditions. In these cases the polymers are not used to entrap or capture a temporary shape from hair assemblies that are in motion, but rather are used to enhance long-lastingness of the preset temporary shape.

b. Temporary setting of hair with gels and mousses

Styling formulations like gels and mousses are based on water-soluble polymers and contain high amounts of water. Therefore, they have a double influence on shape formation while the hair is in motion. The water in these formulations diffuses into the hair and partially plasticizes the hair's amorphous phase; meanwhile the polymer/water system is able to provide a certain amount of hold to entrap a temporary shape. With polymer gel or mousse formulations the hair is put into motion with the fingers or a comb to create a temporary shape. During the application step, these formulations are administered to the hair surface with a very high shear rate. Thus, such pseudoplastic behavior results in a low enough viscosity at the high rates of shear to apply easily and effectively, but on stopping the application, the rate of shear goes to zero and the apparent viscosity rapidly increases to a very high value, thereby preventing the hair from recovering its permanent shape. Higher viscosities at zero shear rate (i.e.,

polymers that form a high-yield stress) are optimal for this approach.

Even when these formulations still contain high amounts of water, they are able to suppress internal hair recovery forces and inter-fiber slippage, providing a transient hold. Once the desired shape is attained the polymer/water system is allowed to dry, and subsequently the polymer hardens, forming “welding spots” and also seams, i.e., points of attachment across multiple hair strands, both of which restrict inter-fiber motion and give flexural rigidity to the temporary shape.

c. Mechanical properties of welding seams and spots

The complexity of hairstyle and hold ultimately depend on the mechanical properties, size, and distribution of welding seams and spots. Properties such as the glass transition temperature (T_g), susceptibility of the polymer to be plasticized by moisture, and adhesive strength at the polymer/hair interface are crucial in the final retention of temporary shape or style hold. Aerosol or sprays produce an irregular distribution of spot welding while gels and mousses yield more uniform welding patterns (see [Figure 42](#)). Furthermore, the welding patterns left by gels are, in particular, thicker and more uniform, and this explains why gel formulations, which create high-yield stresses at zero shear rate, are more suitable to create extreme styles. The distribution and amount of polymer that remains after gel application is such that the hair undergoes a transformation from a pure hair assembly to a polymer/hair composite with very different mechanical properties (68–71). The formed polymer/hair composite, depending on the polymer properties, is able to create shapes with very high flexural rigidity that can withstand high levels of relative humidity (see [Figure 43](#)).



[Figure 42](#)) Micrograph of a bundle of hair fibers showing seam welding by a gel formulation.



Figure 43) Effect of two gel formulations on the temporary hold of hair after five seconds of water immersion. The hair of the mannequin at the left was treated with a gel containing a polymer with a high Tg and low resistance to moisture, while the hair of the mannequin at the right was treated with a polymer with a high Tg but with high resistance to moisture (67).

d. Effect of polymer glass transition temperature (Tg) on styling

The glass transition temperature of a polymer is a parameter that relates to its viscoelastic behavior. Once the style is set and hair shape has been changed, the polymer welding seams and spots must withstand stress/deformations in the hair assembly arising from head movement and from the hair's own weight. The polymer also has to efficiently counter recovery stresses coming from the memory shape of hair in the amorphous and crystalline phases. These stresses will push the hair fibers for recovery of their permanent shape.

If the Tg of the polymer is too low or if its value decreases because of water plasticization, the spot welding will flow like a viscous "chewing gum" and will not be able to retain the temporary shape (see [Figure 44](#)). In contrast, if the Tg is too high the polymer will be very hard and brittle and the seams and spots will break by stress/deformations arising from hair movement. The final effect will be losses in the hairstyle and the production of "flakes" in the hair (see [Figure 45](#)).



Figure 44) Micrograph showing effects of low Tg in the seam welding of a styling polymer (65).



Figure 45) Micrograph showing flaking of a polymer due to a very high glass transition and lack of plasticizer (64).

These two effects are better visualized in [Figure 46](#), where three typical graphs depicting changes in hair Elastic Modulus as a function of temperature are shown. The graphs correspond to three hypothetical polymers (A), (B), and (C) with different glass transition temperatures. In these graphs, the solid line (a) represents the location of room temperature, the dotted line (b) the glass transition temperature (T_g) of the polymer, and the curved line (c) represents the variation of Elastic Modulus with temperature.

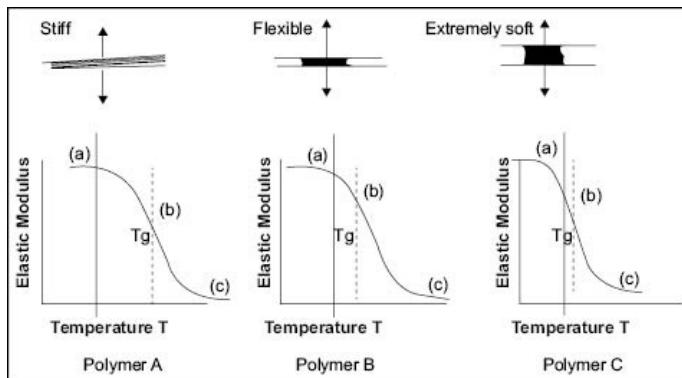


Figure 46) Graphs showing the relationship between Elastic Modulus and Temperature for three polymers with different glass transition temperatures (64).

Observe in this graph that when the glass transition temperature (T_g) of the polymer is way above room temperature, i.e., polymer A, the polymer is very stiff, and we will expect that the welding spots and seams will also be very hard and stiff. In the graph at the center with polymer B, the T_g is close to room temperature, and therefore, the seams and spots will be flexible. Finally, in the graph at the right with polymer C, the T_g is below room temperature and the seams and spots will behave extremely soft and will flow viscously. The glass transition of hair therefore has to be properly adjusted so it can provide the necessary hold without having the inconveniences of being very soft or extremely hard.

e. Adhesive strength at the polymer/hair interface

The adhesive strength at the polymer/hair interface of welding seams and spots is another important parameter in maintaining hairstyle (68, 69). The reason for this is that the polymer must be able to counter permanent shape-recovery stresses coming from the disulfide bonds and crystalline regions, and these stresses are transferred from hair into polymer via the adhesive joints. Further, the polymer needs to comply with the strong forces of hair contraction, which are produced during the de-swelling of hair. If the bonding strength of the polymer with the hair surface is weak and breaks, either because of contraction or recovery stresses, then there is no resistance to recovery, and the hairstyle will be lost soon.

In summary, styling polymers help in locking more elaborate hairstyles and temporary shapes, but they also have limitations in terms of the temporary shapes they can induce. For instance, styling polymers, no matter how high their T_g is, or how they are formulated, may not be able to straighten extremely curly

hair, or vice versa, they may not be able to produce tight curls in very straight hair. The reason for this is, again, that the polymer cannot counter the strong recovery forces coming from the permanent shape stored in the hair's memory. *Styling polymers can however contribute substantially to attaining these changes when combined with other treatments, i.e., with hot irons or other chemical treatments.*

The styling polymers used currently also have limitations in terms of complying with increasing demands of performance and with restrictions in the use of volatile organic compounds in aerosol formulations. These issues have put a lot of pressure on polymer designers and formulators to create novel ingredients that can meet performance and regulatory issues. New polymers are required to be completely soluble in water and/or at least to be partially soluble in it. This task represents a major challenge because in terms of performance, styling polymers are required to resist plasticization by moisture. Polymer synthesis scientists have responded to these challenges by creating polymers with particular pendant groups and domains that appear to provide water solubility and yet are able to transform into hydrophobic materials once they cast into dried films. The field of polymer innovations is vast and most certainly in the years to come there will be a solution to all these issues. Examples of some of these styling polymers are shown in Table II below.

Gels | **Styling Polymers – Sprays**

PVP Polyvinylphrrolidone	Octylacrylamide/Acrylates/Butylaminoethyl Methacrylate
VP/Dimethylaminoethylmethacrylate Copolymer	

Copolyme		
PVP/ DMAPA Acrylates Copolymer		VA/Crotonates/Vinyl Neodecanoate Copolymer
Hydrolyzed Crosspolymer	Wheat	Protein/PVP

Acrylates Copolymer	
VP/VA Copolymer	Acrylates/Dimethicone Copolymer (Elasteesse)
Vinyl Caprolactam/VP/	Digylcol/CHDM/Isophthalates/SIP Copolymer
Dimethylaminoethylmethacrylate Copolymer	Octylacrylamide/Acrylates Copolymer

Acrylates Copolymer	Ethyl Ester of PCM/MA Copolymer
PVM/MA Copolymer	
Polyquaternium-4	
Polyquaternium-11	

Chitosan

Chitosan PCA
Polyacrylate-3

Mousses
Polyquaternium-4
Polyquaternium-11

Hydrophilic Polyester Polyurethane

PVP
VP/VA Copolymer
VP/Dimethylaminoethylmethacrylate Copolymer
PVP/ Acrylates/Lauryl Methacrylate Copolyme
Hydrolyzed Wheat Protein/PVP Crosspolymer
Acrylates/ Allyl Methacrylate Copolymer?

Acrylates Copolymer
Polyquaternium-37

([**Table II**](#)) List of main hair styling polymers

Acknowledgments

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PART 3.3.5

EYELASHES: ANATOMY AND CONDITIONERS FOR INCREASING LENGTH AND FULLNESS/THICKNESS

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ABSTRACT

This chapter describes the anatomy of eyelash hair and conditioners as well as methodology to beautify one of the most beguiling substrates related to beauty.

The history of eyelash beautification is a long one. It was first documented in Egypt with the use of a mixture of donkey feces and kohl pigmentation to paint on lashes. Lash transplant was developed about a decade ago. Commercial lash conditioners first arose in early 2000 and were composed of oil and vitamins. They only produced modest results. A flurry of prostaglandin-containing lash conditioners were developed in 2007 with one published study described below. Subsequent second-generation bioactive peptide lash conditioners and recent third-generation adipose stem-cell secreted peptide are currently on the market. In view of the vast changes in the technology, and the importance consumers place upon beautiful eyelashes as an essential part of their overall approach to looking good leading to feeling good, this chapter is worth the read.

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3.3.5.1 LASH ANATOMY:

Eyelash hair is cosmetically unique and different than the rest of the hair on our body. Eyelashes are much shorter than scalp hair. Lashes are approximately 4–14 mm in length, depending on location, race, and age. Eyelash follicles extend about 2 mm deep into the dermis. Due to thinness of the eyelid, sectioning reveals a thin epidermis with the absence of hypodermis.

The upper eyelid contains approximately 300–400 eyelashes arranged in multiple rows in triangular or pyramid shape. Lower eyelashes have shorter and fewer lashes, with generally about 100–150 lashes on the lower lid.

All eyelashes are characterized by a tendency to bend from the bulb to the tip of the shaft. The degree of curvature is dependent on the asymmetric distribution of keratin 38 in the hair bulb, resulting in a curved fiber. Curvature is noted most in the African race, followed by Caucasian and least in Asians.

In contrast to scalp hair, the eyelash is free of arrector pili muscle. There are roughly 50 glands in the upper eyelids and 25 glands in the lower eyelids. Two types of glands, known as Zeis and Moll glands, surround the eyelash follicles. Zeis glands are unilobular sebaceous glands. They are found at the margin of the eyelids, producing an oily substance into the mid-portion of lash follicles. Moll glands are modified apocrine sweat glands. Meibomian glands are sebaceous glands secreting lipid to form a tear film. Melanocytes are found in very high density in three areas: the basal layer of the eyelid epidermis, the ORS, and the eyelash follicle.

3.3.5.2 LASH GROWTH CYCLE:

Length of eyelash cycle is much shorter than scalp hair, with anagen one to four months and telogen four to eight months. By comparison, scalp hair anagen phase is two to six years and the telogen phase (the transition from resting into the active growth phase) is three months. During the anagen (growth) phase, average daily growth rate is 0.12 mm with range ± 0.05 mm. Approximately 60–85% of eyelash follicles are in the telogen phase, in comparison to scalp which is only 15%. Thus duration of the eyelash anagen phase is strikingly shorter, with

growth rate lower than that of the scalp hair.

It has been observed that the use of lash conditioners increases the percentage of lashes in anaphase from 20 to 90% and extends the duration from four months to two years. Although the exact cellular mechanism is not well defined, it has been suggested to be through the regulation of intracellular c-AMP levels but has not yet been proven (16).

3.3.5.3 LASH CONDITIONERS:

a. History:

Synthetic prostaglandin analogues, used for the treatment of glaucoma in the form of ophthalmic drops, have been shown to result in the desired cosmetic side effect of increased eyelash length, number, and thickness (2–6). This is likely due to follicles entering the telogen phase prior to entering the anagen phase.

b. Safety Assessment:

To achieve the desired cosmetic goal of eyelash beautification by means of increasing length, number, and thickness, the novel synthetic compound dechloro ethylcloprostenolamide was developed in 2007 and its safety was assessed.

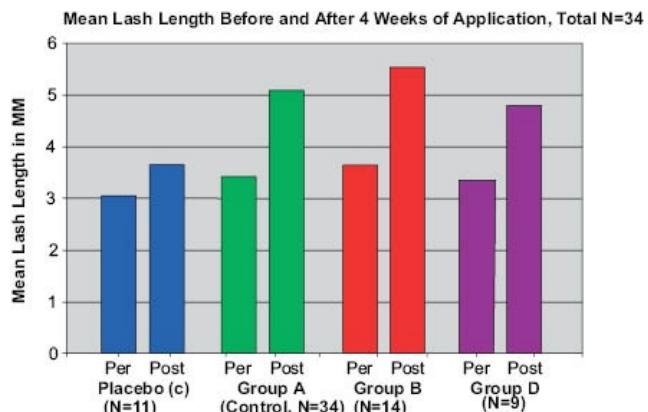
Human exposure with Human RIPT (Repeat Insult Patch Test) in 209 individuals confirmed no dermal irritation or sensitization. *In vitro* toxicity in mammalian cell was assessed and demonstrated to be non-cytotoxic. Local lymph node assays (LLNA) indicated negative for evidence of delayed dermal sensitization. Ames test evaluation by independent clinical testing (unpublished) in five salmonella strains indicated dechloro ethylcloprostenolamide is nonmutagenic. Ocular safety testing at an independent IRB-approved testing site by an ophthalmologist was conducted with four-week use in contact lens wearers, noncontact lens wearers, and individuals with self-assessed sensitive eyes. Individuals underwent serial ophthalmologic exams and questionnaires. No ophthalmic or ocular adverse effects were observed. There was no measurable change in intraocular pressure. It was concluded that the test material did not demonstrate a potential for eliciting ophthalmic irritation and is clinically safe. Safety study revealed no change in intraocular pressure, lacrimal duct, and conjunctiva, and was deemed safe for contact lens wearers and those with sensitive eyes.

c. Study:

In Choy et al., subjective reports by users of MD Lash Factor to improve the appearance of eyelashes—especially in regard to increasing length—was assessed (1). A randomized, double-blind, clinical trial was conducted with 34 healthy volunteers randomly assigned to one of three different treatment groups. These groups consisted of varying concentrations of the key ingredient (dechloro ethylcloprostenolamide, a novel synthetic topical prostaglandin analogue) and a placebo group. **Results:** All treatment groups demonstrated a statistically significant increase in eyelash length after four weeks of daily use. Group A: 1.69 mm, Group B: 1.94 mm, Group D: 1.44 mm and Group C (placebo): 0.63 mm. Twenty-four percent subjectively reported an increase in the thickness or fullness of their eyelashes. There were no complications. Fifteen patients reported minimal initial transient irritation. No discoloration was noted. **Conclusion:** Subjective reports by users of improved appearance in their eyelashes were confirmed. Use of dechloro ethylcloprostenolamide had minimal side effects.

Table I. Summary of results.

	Pre-application		After 4 weeks of application		
	Mean lash length±STD	Mean lash length±STD	Change in lash length (range)	% Change (range)	p-Value
Control group A, n = 34	3.46 mm±1.03	5.15 mm±1.13	1.69 mm (0–4.3)	49% (0–145)	8.78×10^{-8}
Group C (Placebo), n = 11	3.10 mm±0.70	3.73 mm±0.64	0.63 mm (0.1–1.5)	20% (3–51)	1.0×10^{-2}
Group B, n = 14	3.69 mm±1.15	5.63 mm±1.25	1.94 mm (0.2–4.2)	53% (3–156)	9.50×10^{-6}
Group D, n = 9	3.42 mm±1.12	4.87 mm±0.95	1.44 mm (0.4–2.0)	42% (10–87)	1.32×10^{-5}



d. Discussion:

The hypertrichotic properties of topical prostaglandin analogues used in the treatment of glaucoma have been well described in the literature (2–6). Elgin *et al.* (6) describe a 0.67 mm mean increase of eyelash length in 20 adults after six months of latanoprost therapy. Similarly, Sugimoto *et al.* (2) report a significant quantitative increase in eyelash length (0.7–0.8 mm) after two to ten weeks in 17 patients treated with latanoprost for glaucoma. Johnstone and Albert (3) also describe a greater thickness of eyelashes and additional lash rows as well as an increase in the length of lashes with topical latanoprost. Prostaglandin analogues used in these studies were in the form of ophthalmic drops. In Choy *et al.* study (1), the novel topical prostaglandin analogue, dechloro ethylcloprostenolamide, was applied directly to the eyelash roots, resulting in a significant increase in eyelash length at different concentrations of the active product as well as a subjective enhancement of eyelash thickness in 24% of the patients. The absolute and relative increase in eyelash length observed with dechloro ethylcloprostenolamide was greater than that reported in the literature with ophthalmic preparations.

Iris pigmentation and darkening of the periocular skin are reported as rare side

effects of topical ophthalmic prostaglandin (7–10). Neither of these side effects was observed in patients using dechloro ethylcloprostenolamide. Abelson *et al.* (11) describe hyperemia with topical ophthalmic bimatoprost peaking one day after the first instillation and returning to near-baseline levels with continued use. Only one patient reported moderate irritation and discontinued the product, whereas 15 patients reported mild irritation with the use of dechloro ethylcloprostenolamide. However, this mild irritation was transient and in most cases resolved within a few day of use.

Latanoprost has been described in the treatment of alopecia areata for cutaneous use and eyelash formation (12, 13). One of the study patients had alopecia areata. This patient was in a prior study for four weeks using topical ophthalmic bimatoprost on lashes with minimal eyelash enhancement. After four weeks of topical dechloro ethylcloprostenolamide, a significant increase in eyelash length (100%) was observed. Furthermore, four months of follow-up data were obtained in 21 patients who continued to use the active product daily and exhibited a mean increase in eyelash length approaching an 80% increase from baseline. During first year of use of dechloro ethylcloprostenolamide in MD Lash Factor in 70,000 patients, no confirmed serious adverse reaction or iris discoloration was noted. The mechanism of action is unknown.

e. Newer-Generation Lash Conditioners:

Second-generation prostaglandin-free formulations have produced similar effects to an earlier prostaglandin formulation, with consumers generally noting longer usage duration needed to achieve noticeable efficacy. Active ingredients are cytokines/bioactive peptides, antioxidants, prostaglandin precursors, and vitamins. Recently, a novel class of peptides derived from adipose-derived stem cells has been developed with no published data yet available on efficacy.

f. Observations with lash conditioner usage:

Users of lash conditioners will note new hair emerging from areas where one typically does not have lashes, such as the medial and lateral corners of eyes folds. Users with light lash colors will first note increase in length and thickness of lashes followed by color within the lashes. In our monitoring of 70,000 patients during the first year of use in all ethnicities, three cases of Hispanic users noted lashes falling out during first two months of use with a gradual return of lashes over a two-month period. This is likely due to catagen follicles entering the anagen phase. No confirmed iris pigmentation changes were

reported in 70,000 patients in 2009 by this author.

Similar hair enhancement has been noted from lash conditioner on brows and scalp. An independent clinical testing facility conducted open enrollment of 30 healthy subjects (50% male and 50% female) ages 18–45 with men with grade II–III Hamilton and women with grade I–2 to I–4 Ludwig classification. Dermatologists ruled out chronic skin issues, concurrent hair loss treatment, or treatment known to cause hair loss within six months. Follow-up was made at 42, 84, and 121 days. Data analysis of self-evaluation and photos was taken. Data analysis of self-evaluation involves establishing frequency tables to each qualitative question. Two percentages Z1 and Z2 are calculated as follows: Z1 = favorable opinion (Ex: “Completely agree” + “Somewhat agree”); Z2 = unfavorable opinion (Ex: “Completely disagree” + “Somewhat disagree”). The statistical difference in frequencies between favorable and unfavorable opinion is evaluated using the Chi-squared test at 5%.

g. Results:

1. 88% agreed that there were little to minimal discomfort while using the product.
2. 96% felt product was easy to apply.
3. 71% agreed product helped with hair growth with 17% noted improvement at four weeks, 38% noted at eight weeks and 8 % noted at 12 weeks.
4. 50% agreed product helped with thickness of hair.
5. 63% agreed improved volume of hair.
6. 46% agreed improved softness of hair.
7. 75% would recommend product.

CONCLUSION:

MD® Follicle Energizer showed subjective findings of improved hair appearance and user acceptance. This material may be considered as an adjuvant treatment to concurrent hair loss therapy.

FUTURE DEVELOPMENT:

Usage of proteins secreted by adipose-derived stem cells

A Question for Thought and New Directions in Hair Growth Products:

“Given the rich stem cells residing in hair follicles, is it feasible to

generate new hair follicles?"

A first attempt at arriving at an answer to this provocative question lies in the following quote from the American Journal of Cosmetic Surgery by Hirotaro Fukuoka, MD, PhD; Hirotaka Suga, MD, PhD; Keigo Narita, MD; Rei Watanabe, MD; Satoru Shintani, DDS, PhD:

"Scalp hair was noted to regenerate after topical application of such a protein."

The author's preliminary data on injection of stem cell-derived purified growth factors yielded interesting results after one treatment in four weeks in scalp hair. Such a regimen may be feasible for lashes and eyebrows as well.

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PART 3.4

SUBSTRATE: THE NAILS

**Part 3.4
Substrate:
The Nails**

PART 3.4

THE NAILS

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ABSTRACT

This chapter discusses the structure of nails and describes in detail various abnormalities that may occur. Since nails and their appearance are a significant

part of beauty/cosmetic care, it is important to understand the various issues that make nails unsightly and what can be done about them. The discussion also focuses on the potential effects of various chemicals used in nail beautification and provides critical thinking directions for improving nail appearance.

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3.4.1 INTRODUCTION -TOENAILS AND FINGERNAILS

The primary function of toenails and fingernails is to protect the distal aspect of the digits. They protect the bones and surrounding soft tissues from injuries.

They enhance tactile sensation to the digits. Nails are also used as a tool to increase precision gripping of small objects by human beings.

a. Fashion

Originally, polishing of the nails dates back to 3000 BC in China and later in Egypt. The color of the nail polish symbolized social classes [1]. The modern history of polishing the fingernails and toenails as a fashion statement dates back to the beginning of the 19th century. Oils, powders, and creams were applied to the nails and the nails were buffed, leaving them shiny [2]. Today, most nail polishes are derivatives of pigments in solvents.

b. Function

The nails are located at the distal ends of both the fingers and the toes on the dorsal aspect. Their maintained function is to protect the digits from trauma [3]. The curvature of the nail helps to define the shape of the distal end of the digits. Although there is a complete absence of nerve endings inside the nail, nails enhance the tactile sensation of the digits with pressure on the surrounding soft tissues and their nerve endings. This property, along with their useful ability to grip small objects, allows humans to have a more precise use of the fingers.

c. Anatomy

The “Nail Unit” consists of the following parts:

- Nail plate (*Corpus Unghis*)
- Nailbed
- Medial nail groove
- Lateral nail groove
- Distal nail groove (*Hyponychium*)
- *Epionychium*
- Posterior nail groove (Cuticle)
- Nail root (lunula, Matrix, Matrix Unguis)

The nail plate is the actual hard part of the nail. It is made of the tough protein keratin. *Unlike the keratin of skin*, the nails do not peel off in the form of scales. The keratin of nails also contains a much lower lipid content than skin, which allows much less water flux across the nail.

The nailbed in the skin below the nail plate contains living dermal tissue, which includes capillaries and glands. This vascular area gives the nail plate a pink appearance where it is adhered. At the distal end, the nail plate appears

whiter in color where it is separated from the vascular nailbed.

The medial and lateral nail grooves form the indentation on the lateral borders of the nail plate. They are sometimes called the nail folds.

The distal nail groove, called the hyponychium, is at the end of the digit where the epithelium of the skin meets the nail plate. It forms a seal that protects the nailbed from infection and trauma.

The epionychium is a thin band of epithelium that connects the nail plate to the proximal skin.

The posterior nail groove, called the cuticle, is a thin layer of epithelium that extends from the skin over the epionychium and the proximal end of the nail plate.

The nail root, also call the nail matrix, is the germinal tissue that is responsible for producing cells that become the hard nail plate. The nail root extends 5–10 mm proximal to the posterior nail groove. The visible part of the nail root beyond the skin is called the lunula and appears as a half-moon-shaped, lighter-colored region at the proximal part of the nail.

d. Development and Formation

The earliest signs of fingernail development appear in the ninth week in utero. Fingernails and toenails develop by the same process; however, toenail development is slightly slower and about four weeks behind that of fingernails.

Skin folds and grooves begin to form at the distal end of the fingers and will eventually define the structure of the nail unit. The first structure to appear is the matrix at week 11. By week 20, matrix cells exhibit adult keratinization.

At the week 32 a hard nail plate of the finger is formed. At birth, a long thin nail plate is present, which overhangs and curls over the distal digit.

In the discussion of nail formation, one must look at each component of the nail unit and what it contributes. The proximal nail fold is the wedge-shaped structure on the proximal edge of the nail unit. Its dorsal side differentiates to normal epidermis, while its ventral side differentiates to form the cuticle, which adheres to the dorsal surface of the nail plate.

The hyponychium is similar in that the distal portion contributes to the normal epidermis of the volar skin, while the proximal portion helps in adhering to the nail plate.

The matrix differentiates to form the nail plate as we know it. It occurs by specialized tissue kinetics, as described by Zaias [4], who showed this in

monkeys and rats by introducing a radio-labeled marker; this was later confirmed by Norton [5] in humans. These studies materially advanced our understanding of matrix kinetics.

The nailbed epithelium differentiates both in an upward and a lateral motion. The upward growth produces specialized epithelium, which adheres in a special interconnecting manner to the developing nail plate, thus aiding in the adherence of the nail plate to the nailbed.

The lateral growth of the nailbed epithelium is toward the distal edge and moves at the same rate as the nail plate formation. Other authors have suggested different theories of nail plate formation, included here primarily for historical reasons [6, 7].

The matrix lies flat in the proximal nail unit. It produces a sheet of onychocytes (corneocytes), which grow diagonally along its entire length and move distally in a contiguous fashion. This development of multiple sheets of onychocytes eventually forms the nail and produces the dorsal surface of the nail plate; the midportion of the matrix produces the midnail plate; and the distal portion produces the ventral surface of the nail plate. Based on this anatomical arrangement, it can be concluded that the thickness of the nail plate is directly related to the length of the matrix.

A disruption of any of the processes discussed earlier will produce specific diseases of the nail at specific sites, which will be discussed later in this chapter.

3.4.2 HISTOLOGY, ULTRASTRUCTURE, AND COMPOSITION

The epidermis of the proximal nail fold is similar to the normal epidermis dorsally except that it is devoid of adnexa (sebaceous glands, eccrine glands, hair, arrector pili muscle), while ventrally it produces the cuticle. The epidermis of the nailbed is similar to that of the skin, but has no stratum lucidum or stratum granulosum layers and no hair follicles or sweat glands. There is a unique arrangement of the dermal papilla and rete ridges, which form a parallel pattern in the nailbed and interconnect in a tight fingerprintlike pattern to the nail plate. The nailbed moves forward with the growth of the nail plate [4, 5]. The epidermis of the matrix is thick and passes into the substance of the plate, which is formed by changes similar to keratinization in the epidermis; however, the end product is nail plate instead of stratum corneum.

The appearance of the lunula probably reflects incomplete keratinization in the

matrix just before incorporation into the nail plate [8]. The nail plate is made up of impacted and adhering layers of flattened cornified cells (dead onychocytes) that have lost their nuclei. The cells contain hard keratin, similar to that of hair with a high sulfur content mainly in the form of cystine, which comprises about 9–12% of the weight of the nail.

The keratin fibrils are primarily oriented parallel to the nail surface from side to side. The nail typically contains about 7–12% of moisture and 0.15–0.76% of fat, a little more (1.38%) in infants. The composition of the nail plate varies slightly from the early *in utero* plate to that in adults. Baden [9] showed that water content of the nail plate is related to environmental relative humidity. Calcium constitutes about 0.02–0.04% of the weight and does not contribute to the hardness.

Other trace elements have been detected and measured in nail clippings. The nailbed and matrix have a rich supply of blood from two arteries that run laterally along the digits and form oxygen-rich capillary beds, which lie below the nail plate [3, 8].

3.4.3 RATE OF NAIL GROWTH

Nails, unlike hairs, grow continuously throughout life. The rate of growth of the nail plate is determined by the turnover rate of the matrix cells. Early measurement of the growth rate in undergraduates and schoolchildren were made by notching the nail about 2 mm from the margin of the lunula and recording its progressive displacement in each succeeding month. No differences were found within the age range of 19 to 23 years, no sexual difference, and only a very slight difference between hands, with growth on the right being faster. However, there were differences between fingers, growth being fastest on the third digit and least on the fifth finger. Bean [10] studied his own thumb for over 30 years and found growth to be faster during the second to third decades, followed by a slowing-down trend and then a leveling-out over time. The rate of fingernail growth varies between 0.5 and 1.2 mm per week. Fingernails grow faster than toenails which vary between .2 and .4 mm per week. Family tendencies favoring similar growth rates have been noted, as well as increased growth during the summer and diminished growth in cold climates. Certain conditions affect the rate at which the nail plate grows. On average, it is higher in psoriasis, pregnancy, and nail-biting trauma. Regrowth is temporarily depressed in infective diseases, particularly in viral conditions such as measles

or mumps, yellow nail syndrome, and starvation [3]. To replace a fingernail completely takes about five to six months; toenails require 12 to 18 months.

3.4.4 NAIL PATHOLOGIES

Abnormalities of structure and appearance of the nails are more than cosmetic. Many work hours are lost each year due to absenteeism of patients who suffer from nail disease. There are many causes of nail disease: genetic factors, hereditary factors, infection, inflammatory, environmental, traumatic, secondary to systemic disease, biomechanical considerations, and idiopathic as well as the effects of aging. Skin and nail changes begin to occur as people age. During the aging process, the fingernails and toenails have been shown to thicken and show a slowing ingrowth while becoming more susceptible to diseases [11]. Nail problems also occur during pregnancy and consist of transverse grooves, increased brittleness, softening, and no separation [12].

Diseases and disorders of the nails can be classified into conditions according to: maladies peculiar to the nails themselves, onychodystrophies, nail manifestations of dermatitis, nail manifestations of systemic disease, and congenital condition of the nails. Disease also can be grouped into different classifications according to the type of disease, including: infection, psoriasis, contact dermatitis, eczematous dermatitis, hypo-vitaminosis, tumor, trauma, and genetic disease.

Only the most common conditions are discussed here, but readers may consult complete texts such as Scher and Daniel [8] for a comprehensive account of the various conditions that affect the nail unit.

a. Absence of Nails (Anonychia)

Complete absence of nails from birth, anonychia, is rare and appears to be associated in several ways with other congenital, hereditary defects. Absence of nails may be individual, multiple, or total in some congenital conditions. Loss of nail plate may occur with some inflammatory conditions such as lichen planus and irritant dermatitis to artificial nails, or secondary to trauma.

b. Nail Shedding (Onychomadesis)

Nails can be lost either by loosening at the proximal end or by separation from the nailbed. Shedding can follow trauma or severe illness or can be caused by drugs. More serious loss with scarring sometimes follows trauma, defective

circulation, lichen planus, epidermolysis bullosa, or drug eruptions.

c. Nail Separation from the Nailbed (Onycholysis)

The separation of the nail from its bed is fairly common. There are many causes, but quite often none can be found. Nail separation may result from external damage both traumatic and self-induced, that is, overaggressive cleaning, fungal and yeast infections, acute and chronic dermatitis, or drug eruptions. Various conditions such as psoriasis, tumors, and many genetic disorders can also cause onycholysis [3, 8]. Of particular interest are reports of onycholysis caused by cosmetic products [13, 14] and the use of acrylic [15] or artificial nails that cause a contact or irritant dermatitis leading to nail separation [16]. Nail hardeners containing formaldehyde [13], nail varnish containing phenol, ultraviolet nail dryers [17], and similar agents have all been implicated. Once onycholysis is present it is not unusual for colonization by either a *Candida* sp. or *Pseudomonas* sp., giving the nail a yellowish or green color, respectively. Often onycholysis is treated by removing the distal part of the nail that is not attached to the nailbed. This is usually a painless procedure. Once the separated nail is removed, emollients are applied to the nailbed and distal end of the nail plate daily in order to soften the skin and nail and to get the nail to reattach to the skin as it grows distally.

d. Brittleness

Brittle nails are among the most common cosmetic complaints. The phenomenon tends to occur in advancing age. While sometimes they may be associated with some underlying conditions, no good data are available, and the causes are mostly not known but are known to be related to nutritional disturbances, thyroid disorders, and skin conditions. Environmental factors are certainly important. Nails are kept pliable by their moisture content, and long thin nails are especially susceptible to very dry climates. Frequent immersion of the hands in surfactant solution is conducive and some time ago it was believed that soaps and detergents removed the protective lipids from the keratin. The continual use of nail varnishes and varnish removers have also been blamed [8]. Almost 60 years ago the reactions of 25 businesswomen to the same brand of nail lacquer were studied. It was found that nearly half of them showed brittleness and splitting of the nails while the rest were unaffected. Thus, there is no support for attributing nail brittleness to the use of conventional nail lacquers. Some investigators have reported improvement with gelatin and application of essential oils, but no

consistently effective therapy is known. Other researchers recommend avoidance of frequent immersion and nightly use of emollients.

e. Striations (Onychorrhexis)

Longitudinal striations are common in healthy nails and become more prominent with aging, and other clinical or occupational conditions such as lichen planus, psoriasis, and trauma. More prominent longitudinal ridging is seen in median nail dystrophy, which is attributed to habit tics (self-induced behavior). Transverse, horizontal striations can occur as a developmental anomaly. Severe depressions, known as Beau's lines, indicate a period of severe systemic disease, such as measles, mumps, pneumonia, rheumatoid arthritis, myocardial infarction, pulmonary embolism, high fever, or coronary thrombosis. Drugs may also induce inhibition of normal matrix production of nail plate. They can also be caused by trauma. Thinning of the nail plate is seen as secondary to decreased peripheral circulation, lichen planus, iron deficiency anemia, and epidermolysis bullosa.

f. Spoon-Shaped Nails (Koilonychia)

In koilonychia or spoon nails, the nails are thin, soft, and concave in the center. The condition results from iron-deficiency, and is usually, but not invariably, associated with anemia. It can also be seen in onychomycosis and has been noted in motor mechanics, where, perhaps, it is due to softening as a result of the use of oils or soaps. This condition may also be seen as a temporary disorder in young children and is also a cemented congenital anomaly. Plummer-Vinson syndrome (a combination of koilonychia, dysphagia, and glossitis) is primarily seen in middle-aged women and is also called Plummer's nails.

g. Splitting (Onychoschizia)

The splitting of nails horizontally, parallel to the nail plate, so that pieces of the surface break away, is very common in women and in advanced age. The main causes are probably repeated immersion in water and the use of nail polish and polish removers. The suggested treatment is to rub emollients into the nail plate after bathing and avoid frequent use of nail polish and removers.

h. Pitting

Pitting of the nails is found most commonly in psoriasis. Pits represent punctuated or depressed sites in the nail plate secondary to irregular matrix

production. They can vary in size, shape, depth, and number. Pitting is the most common manifestation of nail psoriasis [21] but can also be seen in chronic dermatitis, fungal infections, alopecia areata, Reiter's syndrome, lichen nitidus [22], and lichen planus. Minor degrees of pitting may be seen in healthy nails, and when no other skin complaints are present, they are considered to be normal variants.

i. Leukonychia

Leukonychia is complete or partial whitening of the nail plate and can be divided into acquired and congenital types. Complete leukonychia occurs very rarely as an inherited abnormality; often, epidermal cysts on the glabrous skin are associated with it. Partial leukonychia is very common and may take the form of white spots or white transverse streaks. It may be associated with many types of illnesses, exposure to chemicals, and traumatic events to the matrix. A white appearance of the nails is not necessarily due to leukonychia; it may result from changes in the nailbed, not in the nail plate.

j. Onychomycosis

Fungal infections of the nail unit are the most common conditions affecting nails. There are four distinct types of clinical presentation: distal subungual, superficial white, proximal subungual, and candida albicans onychomycosis. Distal subungual onychomycosis is the most common type and has been shown to be an autosomal dominant inherited condition in the chronic dermatophyte syndrome. The discoloration begins at the distal edge of the nail and spreads proximally. Onycholysis begins with thickening and irregularities of the nail plate and causes it to separate from the nailbed; subungual debris begin to accumulate in secondary bacterial infections. Proximal subungual onychomycosis starts at the proximal nail fold. It often begins as a small white area located proximally on the nail plate and subsequently enlarges and spreads. Superficial white onychomycosis produces a white powdery appearance. Candidal onychomycosis is usually seen in patients with mucocutaneous candidiasis syndrome. It begins by directly invading the nail plate itself rather than extending onto the nail from the adjacent skin. It is much more prevalent in the toenail than the fingernails.

k. Paronychia

Paronychia is defined as inflammation of the proximal or lateral nail folds or a

combination of the two. Paronychia occurs after loss of the cuticle either by trauma or by aggressive cuticle trimming or cuticle pulling. It can be acute or chronic. Acute paronychia is caused by introduction of an infectious agent into the nail folds, usually through trauma, ingrown nails, cuticle pulling, or exposure to an irritant agent. Paronychia with bacterial infections often needs medical attention to drain the infection and remove the ingrown nail as well as treating with antibiotics. Chronic paronychia occurs over time due to continual exposure to an irritative behavior, chronic ingrown nails, constant exposure to water from hand washing, or a specific contactant such as food items or chemical irritants.

I. Discoloration

Nails may be discolored for a wide variety of reasons. External causes include hair and other chemical dyes, smoking, and chemical compounds such as mercury salts, dithranol, and picric acid. Tints may leak out of nail varnish and after repeated use may penetrate the nail [17–20]. The use of artificial nails and either acrylic or pre-formed plates can cause discoloration. Abnormal formation or very slow growth of the nail can also produce color changes. Psoriasis and onychomycosis, among other abnormalities, cause opaqueness and a yellowish discoloration. In the “yellow nail syndrome” the nails almost cease to grow and several months later become yellow or greenish. They may also be thickened and curved from side to side. Yellowish, green, or gray nails can also occur as growth of nails slows down in old age. Infection by *Pseudomonas aeruginosa* under the nail may cause a greenish, black, or blue discoloration. Systemic drugs can alter the color in many ways. Prolonged administration of tetracycline may occasionally turn nails yellow. Mepacrine or Zidovudine (AZT) makes them bluish black, and chloroquine may produce blue-black pigmentation of the nailbeds.

The pigmented discoloration or pigmented bands are the most important types to distinguish. Although most pigmented bands seen in nails represent benign lesions, such as nevi or traumatic or splinter hemorrhages, one must always be suspicious of pigmented bands that are malignant, such as malignant melanomas. Any new onset or rapidly changing pigmented band in the nail should be evaluated by a dermatologist for possible biopsy. Early diagnosis can save serious sequelae of malignant melanoma.

m. Subungual hematoma

Subungual hematomas may cause the collection of blood underneath the nail

plates. It is often associated with acute trauma. When associated with acute trauma, there is often severe pain from the pressure of the blood underneath the nail. Subungual hematomas can also occur more slowly with repetitive trauma. Often these are not painful at all. This is often seen in runners and dancers.

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PART 3.5

SUBSTRATE: THE NOSE

**Accessing the Biology of
Human Olfaction: New,
All-Natural Fragrance
Ingredients; Novel
Consumer Fragrance
Experiences and Applications**

**Part 3.5
Substrate:
The Nose**

THE NOSE:
Accessing the Biology of Human Olfaction:
New, All-Natural Fragrance Ingredients;
Novel Consumer Fragrance Experiences and Applications

by

Kambiz Shekdar, Visiting Scientist, Rockefeller University

ABSTRACT

Contemporary fragrance creation

Contemporary fragrance ingredient discovery and fragrance creation relies on sensory testing and evaluation methods by humans. Perfumers or experts discover new fragrance ingredients and they create new fragrance formulations while human sensory studies comprising panels of nonexpert or naïve human subjects are used for further evaluation. Whereas contemporary fragrance creation methods have been developed over decades and are considered the gold standard precisely because of their central reliance on humans, recent trends suggest that this process alone may soon become obsolete due to its lack of speed and throughput. First, consumer demand for products that are all-natural, economic, and sustainable is growing. In some consumer applications, synthetic ingredients have become a mainstay in the fragrance industry. At the same time, it is only becoming harder to identify natural fragrance ingredients that can be sourced reliably. Many natural ingredients are sourced from equatorial regions that are forecasted to suffer most severely from global warming and political instability. As a result, pressure for the rapid and reliable discovery of new, all-natural fragrance ingredients is increasing. In order to discover new fragrance ingredients for all-natural and sustainable products, new discovery methods that enable rapid and reliable testing of hundreds of millions of ingredients and extracts are required.

Next-generation fragrance creation

Borrowing from the fields of cell biology and drug discovery, the application of

cell-based high-throughput screening (HTS) methods stand to enable the rapid and reliable testing of hundreds of millions of ingredients and extracts to aid in new fragrance ingredient discovery. Biologically, the sense of smell in humans is mediated by a family of ~350 odorant receptors. In principle, laboratory cells can be created to comprise odorant receptors. These cells could then be used as miniaturized detectors for “turbo smell testing” in cell-based assays to detect new fragrance ingredients. Ingredients identified as active in the cell-based assays could then be evaluated in human sensory studies or by perfumers. Importantly, the success or reliability of any cell-based assay depends on how closely it is produced to mimic the natural or native biology of the receptor it is designed to comprise. Recent advances in the science of cell-based assay creation reported across receptor classes—including drug, taste and odorant receptors—suggest that it is now possible, for the first time, to produce these assays such that they mimic the physiology of their intended receptors to a much greater degree than had been previously possible. In the context of olfaction in which such an especially large number of receptors are implicated, the advent of physiologically relevant cell-based assays in combination with expert and naïve human sensory methods will allow more rapid and reliable fragrance creation.

In this chapter we review:

- (i) the background on the biology of olfaction and the sense of smell
- (ii) the basic design and principles of miniaturized cell-based assays for high-throughput odorant ingredient discovery
- (iii) novel classes of previously inaccessible natural fragrance ingredients that stand to alleviate the emerging bottlenecks, including natural ingredients that augment, diminish, or otherwise modulate the sensory profile of fragrances, and the forward-looking and previously unknown applications that these enable, and
- (iv) recent understanding of the biology of odorant receptors as it relates to the creation of a fragrance-discovery engine with unprecedented high fidelity to the natural sense of smell.

In addition to facilitating the discovery of new natural ingredients and combinations of ingredients with desired aromatic properties, a systematic cell-based discovery engine could produce new fragrance ingredients for more precise further investigation of the biology and basis of human olfaction as well as create novel consumer products and applications. All in all, the advent of a rapid and reliable odorant ingredient discovery engine could open the gates to a whole new world of aroma research and product development.

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The Biology of Olfaction and Fragrance Ingredient Discovery and Creation

3.5.1 ODORANT RECEPTORS

Odorant receptors (ORs) are the biological entities that mediate the detection of scent. In humans, a family of at least ~ 350 genes encoding ORs has been identified. (1) This family or group of ORs are G-protein coupled receptors, or GPCRs. GPCRs are a classification of protein molecules localized in the outer membranes of cells. The ~350 ORs are integral membrane proteins; they traverse the cell membrane and comprise domains that are intracellular, transmembrane, or extracellular. While it is thought that certain odorant molecules may penetrate the cell membrane to impart their activity by interacting with transmembrane domains of the ORs, it is generally thought that it is the extracellular domains of ORs that mediate the detection of odorant molecules, as these domains would be most accessible to volatile compounds in the nasal cavity.

The ~350 GPCRs that have been described as odorant receptors are defined as such based both on their sequence homology and relatedness as well as their expression in cells that line the nasal epithelium. However, not each of these ORs has been confirmed as an odorant receptor. In addition, it is possible that other receptors in the nasal cavity and elsewhere may also play a role in human olfaction.

It may be helpful to think about the large family of ORs as the keys of a piano keyboard, where some odorant molecules will hit or activate one single receptor, or key, essentially sounding off one single aromatic note, whereas other more complex ingredients or substances may hit an entire chord, or several odorant receptors, all at once. Depending on which ORs are activated and how strongly,

a different smell may be experienced or perceived in the brain.

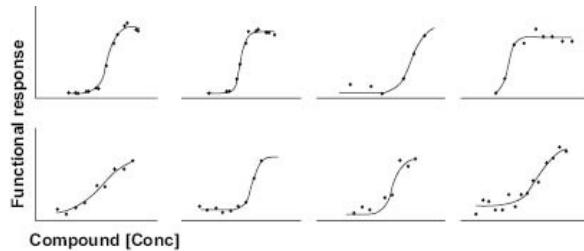
3.5.2 CELL-BASED HIGH-THROUGHPUT ODORANT DISCOVERY'PRINCIPLES AND BASIC DESIGN

Cell-based high-throughput screening of ORs

High-throughput cell-based screening of ORs requires laboratory cells that are treated to comprise ORs. Typically, for any given OR, a gene encoding the OR must first be introduced into cells, and the OR that is encoded must be synthesized by the cell and transported to its outer (plasma) cell membrane, or cell surface. The preparation of cells used may be transiently transfected cells or they may be stable cell lines. Transiently transfected cells refer to populations of cells that are freshly introduced with the gene encoding the OR, where a new batch of cells is produced each time, as required for testing. Stable cell lines refer to clonal populations of cells where a single cell introduced with the OR gene is selected and used to create a clonally prepared population of genetically identical cells. The advantage of using transiently transfected cells includes speed, as the time required to produce a stable cell line is not necessitated, whereas the advantage of using stable cell lines includes identification of a population of cells with desired and well-characterized properties. There are many methods available to achieve either transiently transected cells or to produce stable cell lines, and these must be carefully considered given the host cell type used and the gene to be expressed in order to achieve the most physiologically relevant cells and assays. In this chapter, data for the use of stable cell lines are presented.

In addition, creation of laboratory cells that comprise ORs that function as they do in their natural setting *in vivo* is challenging. The cells of the nasal epithelium that typically express ORs *in vivo* are highly specialized, polarized cells that are not approximated by typical laboratory cell cultures, which comprise cells derived from various tumors or are otherwise immortalized. Current methods for the production of cells suitable for HTS of ORs are described in the last section of this chapter. Once cells comprising the OR are produced, a cell-based assay that can be used to monitor the activity of the OR must also be produced. The activation of many classes of GPCRs, including ORs, results in an influx of calcium into the cytoplasm or interior of the cell. Therefore, the use of fluorogenic dyes that can be used to report the presence

and concentration of calcium is common. [Figure 1](#) shows cell-based assay responses for a panel of eight cell lines, each comprising a different native and full-length OR (See [Figure 1](#)).



[Figure 1](#) A cell based assay was used to test the activity of a set of 8 different ORs in response to an activating compounds. Dose response curves are shown. For each OR, a compound with an EC₅₀ value of less than 100 μM is shown. The assay response (Y-axis) over time (X-axis) is shown.

High-throughput screening and discovery of new fragrance ingredients

Once a cell-based assay is created, it must be miniaturized to high-throughput format, where typically 384-well plate or 1536-well plate microtiter well plates are used to interrogate the activity of many different samples on the OR at any given time. As the testing of a plate requires on the order of one to five minutes only, hundreds of millions of extracts and ingredients may be tested on a 24-hour basis using standard automated and scalable screening instrumentation.

In HTS assays, it is important that the assay response is sufficient and consistent for reliable discovery of modulators. That is, in each well tested with an activator of the receptor, the signal-to-noise ratio of the response generated in the cell-based assay compared to buffer-only control must be great enough to enable reliable detection of either a modulator or blocker. In addition, this assay response must be consistent from well to well, to enable comparison of testing results for different compounds and ingredients across a plate. One common measure of the suitability of a miniaturized cell-based assay for HTS is the value for the Z' Factor of the assay. The Z' Factor of an assay is derived using an equation that takes both the signal-to-noise ratio and the well-to-well consistency of the assay response into account, with values ranging from 0 to 1. (2)

Typically, a cell-based assay with a Z' Factor value of 0.4 or greater is considered suitable for HTS. [Figure 2](#) shows OR cell-based assays with Z' Factor values suitable for the HTS for the same set of eight receptors as depicted

in [Figure 1](#).

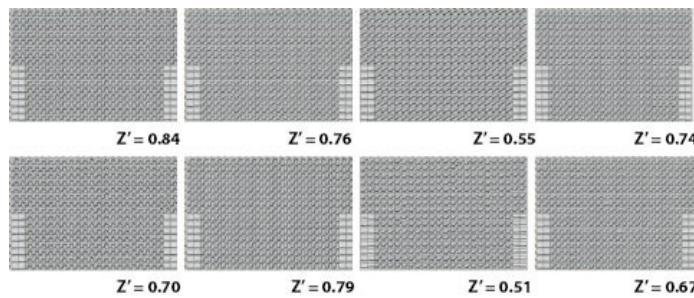


Figure 2 The same set of ORs as shown in [Figure 1](#) were miniaturized to 384-well plate HTS assay format. Responses for the testing of the 384-well plate assays with an activating compound (dark shaded) or buffer-only control (light shaded) are shown. Z' values of at least 0.4 were generated for each OR.

Once cell-based assays with a suitable Z' Factor are established, the first step in discovering new odorant ingredients for a given application is to determine which of the panel of ~350 known ORs are relevant for the desired application. To do this, a panel of cells representing the entire family of ORs is exposed to the aromatic substance and the ORs that are activated in response to it are identified. To identify the ORs that correspond to the aroma of a rose extract, for instance, the receptors that respond upon exposure to the extract are selected. Aromatic substances like rose extract often comprise hundreds or thousands of aromatic ingredients. It is the combination of the aromatic or sensory profile of each ingredient that produces the net or perceived sensory experience or fragrance. Often, the key aromatic notes of a composite fragrance are derived from one or a few of these ingredients. Careful testing and analysis must be used to select the most meaningful set of ORs for HTS for any given aromatic substance of interest. [Figure 3](#) shows the response of 320 compounds and extracts tested across the same set of ORs as shown in [Figure 1](#) using the same HTS assay as shown in [Figure 2](#).

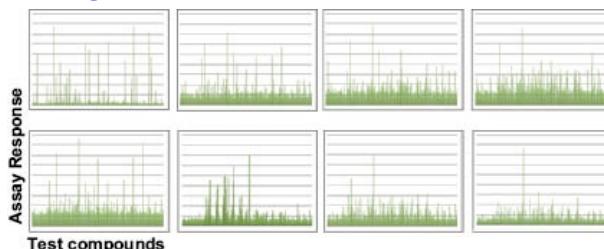


Figure 3 320 test compounds were tested against the same set of ORs

as shown in [Figure 1](#) using the same 384-well HTS assay as shown in [Figure 2](#). Responses of the ORs to each compound are shown. **The assay response (Y-axis) over time (X-axis) is shown.**

An important consideration for fragrance discovery is that certain closely related natural extracts may be differentiated from each other by one or a few ingredients or notes. For instance, the extract from a particular subspecies of rose that is especially desirable compared to other rose extracts may be differentiated by the presence of one or a few particular aromatic constituents. By profiling the OR activity that is elicited across the entire panel of ORs for various rose extracts, those ORs that correspond to the unique aromatic notes of the preferred rose extract may be identified. In a next step, high-throughput screening using cell-based assays comprising these ORs may be used to identify new, natural ingredients that can be used to help replicate the aroma of the preferred extract. It may be that a number of different ingredients are required to activate the entire complement of relevant ORs.

Typically, a high-throughput screen identifies hundreds of prospective “hits,” or compounds of interest. Further testing and evaluation are then required to confirm which of these impart the desired fragrance experience in human sensory studies.

Sensory confirmation, iterative screening, and optimization

Once initial compounds of interest are identified in a high-throughput screen, human sensory studies are required to characterize the sensory properties of the compounds. For instance, in a screen designed to identify compounds that mimic the scent of a rose, the identified compounds would be tested compared to the gold standard using human subjects. Typically, compounds identified directly in a first round of screening must be improved upon to meet desired final characteristics. Ideally, a high-throughput screen yields compounds a significant percentage of which are confirmed in such downstream testing as having the desired or expected properties that would be predicted by the screen. The correlation between cell-based high-throughput assays and downstream assays is greatly dependent on the quality of the cell-based assays and the degree to which they are physiologically relevant detectors. Reliance on OR cell-based assays that comprise only full-length, native, and unmodified receptors is critical for this reason; any alteration of the native ORs could greatly diminish the physiological relevance of the resulting assay.

Regardless of the quality of the assay, more often than not additional testing and work are required to result in compounds that meet all technical success criteria. Even given a fully physiologically relevant screen, the compounds that are identified may have additional off-target activity or effects, such as activity on untested receptors or side aromatic notes. Sensory testing is therefore an important tool in prioritizing the hit compounds. In order to improve upon the compounds, additional rounds of screening may be necessary. Here, a library of compounds that includes compounds structurally or chemically related to the initial hit compounds is assembled and screened. Similarly, in the case that the initial hits are extracts, a second round of testing can include extracts related to the initial extracts. Iterative rounds of testing and downstream sensory evaluation can be used to find optimized derivatives of the original hits that most closely or exactly produce the desired sensory effect.

By designing the screening library to include extracts and ingredients derived from natural sources of unlimited supply or ones that are renewable, a limitless number of new compounds can be made available for iterative cell-based and human sensory testing. Each aromatic plant or substance often comprises thousands of volatile ingredients. Various preparations of these can be used to amplify the number of test compounds in the library. For instance, a library can contain extracts derived from a plant species where the plant species is cultivated under multiple different conditions or in various locations or geographies, or different parts of the plant can be extracted separately. In addition, the extracts can be treated in a variety of ways, including by heating, exposure to acids or bases, or incubation with enzymes to create even further chemical diversity. We can begin by limiting the library to natural ingredients that have a past history of human consumption and limiting its treatment to steps that are not industrial in nature. In a manner similar to cooking, or any other reaction that can take place in a common kitchen, we can provide access to the advantage that the included test substances will be generally regarded as safe, based on existing protocols for the assessment of safety. This, in turn, facilitates downstream human sensory studies, compared to novel or human-made compounds that must be extensively tested before they can be studied using human subjects.

The Extra Challenge

First and foremost, consider that in the case of odorant receptor biology and discovery, no fewer than ~350 receptors are implicated, where most cell-based

drug discovery relates to one or a handful of receptors. To navigate this space effectively, there must exist not only a robust cell-based discovery platform that is as comprehensive and physiologically relevant as possible, but expert sensory testing and input must be used to as a guide to calibrate, or “tune,” the cell-based platform. In this connection, the analogy to the complement of ORs as keys of a piano keyboard is helpful. Imagine a complex aromatic substance or extract, for instance jasmine or mint extract. These substances may well interact with a large number of odorant receptors. In addition, some of the individual compounds contained in each extract may hit a cognate receptor hard and punctually, whereas other ingredients may exert their effect over time and more softly. A complete mapping of these substances across the entire known complement of ORs in addition to their known sensory attributes will be key to effectively understanding how to interpret the cell-based data generated during any discovery process. Access to the entire human olfactory system is required for effective discovery. Any effort at cell-based discovery that aims to rely on a representative subset instead of on all the known receptors carries the risk of missing important interactions.

3.5.3 NEW INGREDIENTS AND APPLICATIONS

Natural substitutes

In some fragrance applications, reliance is made on natural extracts that are of limited supply and high demand. In some instances, this has necessitated a shift towards the use of synthetic compounds and is in direct opposition to the expanding trend of sustainability and responsibility described elsewhere in this book. Often, it is challenging to exactly replicate the aromatic profile of the natural aromatic using the mixture of synthetics. In some cases there is a need for improvement, as consumer demand for the natural sensory experience is high and remains unmet. Furthermore, for natural fragrances that are in demand but of limited supply, development of new related fragrances is often impeded.

In this connection, the OR discovery engine may be used to identify new natural ingredients that can substitute for the original ingredients. In a first step, ORs corresponding to the desired aroma would be identified by exposing the panel to the aroma or to its signature components, if these are known.

Next, this subset of cell-based OR assays would be used in high-throughput screening to test millions of natural extracts, ingredients, and combinations of

these, in order to identify additional new ingredients that mimic the gold standard. Those ingredients that are identified may also have undesired characteristics. For example, such characteristics may have activity on additional ORs or undesired aromatic notes that are detected in downstream sensory testing. In these cases, additional rounds of iterative screening can be used. In each successive round, one would include prior hits in the library to be screened as well as a new set of test ingredients related to the original hits. For instance, if a substance in wild lily is found to partially mimic the desired scent of a rose but there is an undesired aromatic characteristic also, then the same wild lily, but cultivated in a variety of ways, may be used to prepare new libraries of extracts for testing. Additionally, a desired scent may be replicated, for instance, by combining two or more identified ingredients—each of which acts to impart a part of the aromatic profile of the gold standard.

Fragrance enhancers and fragrance blockers

Perhaps the most intriguing ingredients that stand to be discovered using a cell-based odorant discovery engine are fragrance enhancers and blockers. By definition, these ingredients work to modulate the sensory profile of other fragrance ingredients.

Using the analogy of music, a fragrance enhancer is defined as an ingredient that may or may not have its own aroma, which at certain concentrations, works to amplify or “turn up” the volume of a given desired fragrance or aromatic note. Similarly, a fragrance blocker is defined as an ingredient that can decrease or “mute” the smell of an undesired fragrance or malodor. Anecdotal examples of such ingredients have been known, often discovered randomly when a perfumer finds that one ingredient alters the sensory profile of other ingredients to which it is added. However, systematic discovery of such ingredients using perfumery alone has been challenging, as it is difficult to test any large number of random ingredients in combination with aromatic ingredients in order to identify those rare ingredients that can work as fragrance enhancers or fragrance blockers. The difficulty of accomplishing this is compounded by the fact that *any* molecule active on the odorant receptors can likely be expected to have an intrinsic odor of its own, as it is likely to interact with one of the large number of known odorant receptors. As such, classical methods would require thousands of test ingredients to be assessed, one by one, and at various doses against a key aromatic sample the sensory experience of which is desired to be amplified or muted. While skilled perfumers work to create fragrances, they are not machines

that can be used to test thousands of samples. Thus, the cell-based platforms for high-throughput cell-based discovery provide an approach to accelerating the effectiveness of perfumers in the design of a virtually endless supply of new fragrances.

In order to rapidly discover fragrance enhancers or blockers of a given aromatic substance, first the odorant receptors that correspond to that substance must be identified. In a next step, these odorant receptors would be exposed to the aromatic substance to record the response of the receptor. Next, test compounds would be added to monitor changes in this response. Because of the nature of high-throughput screening, hundreds of thousands of test ingredients could be tested at different doses per day. Such a process would allow the identification of those ingredients that work to either amplify or diminish the response of odorant receptors to the aromatic ingredient. These initial hits would then need to be further evaluated as described.

This approach can include a second round of testing using the high-throughput cell-based platform, for instance, to run dose-response studies. An ideal enhancer or blocker would be identified as one that increases or decreases the receptor activity at increasing doses of the test ingredient. [Figures 4](#) and [5](#) show dose-response curves for fragrance enhancers and blockers, respectively, discovered using cell-based HTS.

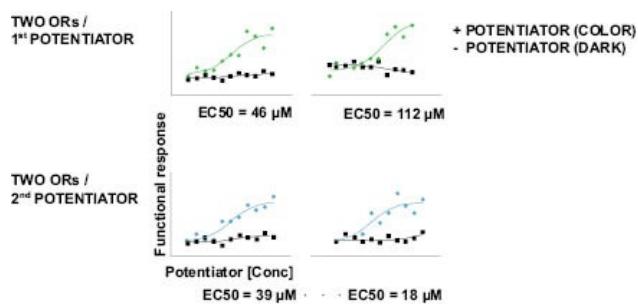


Figure 4 Dose response curves for two potentiators each active on at least two ORs are shown. To generate the dose response curves, increasing concentrations of the potentiating compound (color plot) or no compound (dark plot) were tested on the OR in the presence of the same concentration of activating compound. EC50 values for the potentiators were calculated.

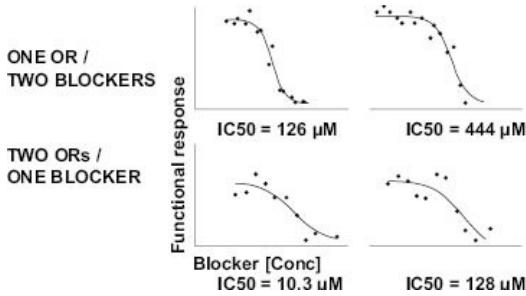


Figure 5 Dose response curves for compounds with blocking activity are shown. In the top panel, two different blockers each of which blocks the activity of one OR are shown. In the bottom panel, two ORs each blocked by the same compound are shown. Dose response curves are shown. IC₅₀ values for the blockers were calculated.

Next, sensory studies would be needed to confirm the activity of the ingredients identified in the screen in human sensory studies. Sensory testing would also work to identify any unintended or undesired intrinsic sensory characteristics of these ingredients, or doses at which none are registered. Finally, once ingredients that are confirmed as active in human sensory studies are identified, the process is repeated. In each round, this is done by sourcing additional ingredients that are structurally or chemically related to the initial positive hits for an iterative round of testing and are designed to identify related ingredients with improved sensory profiles and characteristics.

Even with a high-throughput discovery engine, the systematic discovery of fragrance enhancers and blockers is still expected to be challenging. This is because of the large number of odorant receptors. Any ingredient must have activity across the entire subset of target receptors such that the desired sensory profile is experienced by a human subject, and no activity on any unintended receptors produces an undesired sensory profile or off-note. To discover those rare ingredients that act across the array of odorant receptors in exactly the manner that results in the desired sensory profile is no small order. Because the nature of high-throughput screening is such that it allows testing of millions of ingredients and combinations of ingredients, it is possible to identify hundreds of initial hits such that a systematic process for the discovery of final ingredients that meet all required criteria can be envisioned.

Again, the likelihood of success in any such attempt is predicated on the fidelity of the cell-based discovery process; that is, the odorant receptors that are provided for testing using cell-based assays must be accurate in *in vitro*

correlates of native odorant receptor function and physiology *in vivo*. This had been a challenge to achieve, until now.

Novel applications

The advent of methods for the systematic and reliable discovery of fragrance enhancers and blockers ushers in a new era for fragrance creation and a set of novel applications. A number of these are highlighted for illustrative purposes.

First and most basic, a fragrance enhancer can work to decrease the amount of a fragrance ingredient that is used in order to impart a desired aromatic profile. If a certain aromatic is limiting, less of it may be used. In cases where synthetic chemicals have been relied upon to mimic the sensory profile of a rare or prohibitively expensive natural extract or essence, often these fail to exactly replicate the gold standard. Fragrance enhancers, which can be limited to natural ingredients themselves, could be used in this case to enable reliance on the actual natural extract or essence only. This solution not only satisfies consumer demand for the use of all-natural ingredients, it also accomplishes a cost-saving as less aromatic is required.

Second, the discovery of fragrance blockers that can be used to block malodors including body or bathroom odor can be contemplated. Such ingredients can displace the use of strong, human-made chemicals presently used to mask such odors. Simultaneously, a consumer-friendly goal is reached in that the use of excessive chemicals may be curtailed. Perhaps of greatest interest to the perfumer, blockers of the aroma of body odor and of ingredients such as alcohol can eliminate these smells such that perfumes can be appreciated unadulterated by malodor or alcohol solvents.

Future potential

Perhaps most intriguing, the advent of reliable high-throughput discovery of aroma ingredients opens up entirely new applications for the cosmetic and personal care industries as well as many others. It is difficult to even predict what these may be. To capture the imagination, a number of possible future applications are described, many of which depend on progress in unrelated fields. First, many experts and laypersons alike have wondered whether emotions or moods that can be triggered by a particular scent have a biological basis. Is the smell of chamomile soothing and calming, or are we accustomed to thinking that it is? It is possible that using ever-advancing methods of brain imaging, more data can be generated to help us address these questions: Do we

see the same brain activity patterns in response to smelling a soothing aromatic as we do when in a state of calm? Do multiple calming aromas all result in the same brain activity? Does the smell of a forest of greenery evoke the same brain activity as images that we associate with freshness?

Diverse studies will need to begin to explore these questions, including brain imaging and also physiological and sensory studies. Methodologies for each of these will need to be refined and developed. However, the advent of high-throughput discovery of odorant ingredients provides one previously missing key element: for the first time, it could be possible to identify aromatic ingredients that are characterized as activating just a single or a very small subset of odorant receptors. The availability of such ingredients where the biological basis for their activity is defined provides a toolkit of reagents to probe the human response. Theoretically, all those receptors activated by chamomile could be identified, as well as aromatic substances that selectively activate each of these. Use of such aromatics in brain-imaging studies could be used to test whether any of these produce the calming patterns described. The availability of a cell-based HTS platform could allow the identification of such aromatic ingredients that could aid in the understanding of the biology and physiology of human olfaction in this and other applications. Imagine if it would be possible to find aromas that make us feel fresh and in a state of wellness and well-being.

At a more practical level, consider how unavailable or unreachable the sense of smell has been to the average person: Whereas, until now, we can play notes and music, or paint brush strokes and paintings, it has only been the trained perfumer who can truly “touch smell.” The advent of fragrance enhancers could be used to enable consumer access to the art of fragrance creation. A home stereo system, if you will, could be imagined in every home, where at the touch of a button the consumer would call to order various notes, like mint or sea breeze, all contained in miniature chambers. Small amounts only of hundreds of aromatics would be needed, as enhancers could be used to increase the activity of tiny doses. One could paint the mood of their home or room. If one creates a new fragrance, one could email the recipe to a friend for them to conjure it up in their own home, or a new fragrance that is released could be downloaded from a perfume website for easy sampling at home.

3.5.4 METHODS

In the preceding sections, the basic principles and design for high-throughput odorant discovery were introduced, and novel classes of aromatic ingredients that could be discovered have been outlined. For the first time, cell-based access to odorant receptors in their physiologically relevant form exactly as they exist in nature has been shown. The figures presented demonstrate the utility of this technology to access a subset of odorant receptors that represent the broad diversity of odorant receptors *in vivo*. Most significantly, these data point to the need of working with cell-based assays that exactly mimic native receptor biology and physiology as the bedrock and fundament for any cell-based discovery engine that utilizes cell-based assays. Taken together, the following were demonstrated:

- 1) Cell-based assays comprising native full-length odorant receptors can now be created.
- 2) This has been done for a panel of odorant receptors representing the various classes.
- 3) Cell-based assays suitable for high-throughput screening were developed.
- 4) Fragrance enhancers were identified.
- 5) Fragrance blockers were identified.

In this section, the state-of-the-art will be reviewed. Briefly, the odorant receptor biology field is rich and diverse, with hundreds of laboratories both academic and commercial exploring various aspects of odorant receptor biology. The purpose of this section is to outline the one key bottleneck that had existed, and to review recent advances that have overcome this limitation: Until very recently, it had not been possible to express odorant receptors in their native and intact form, exactly as they exist in nature, in the context of laboratory cells required for use in HTS. It has been reported that this is because in these cells, any OR that is expressed becomes trapped in the ER compartment of the cell. (3,4)

To by-pass the difficulties that were encountered, most laboratories have largely relied on one of two methods to enable expression of odorant receptors in cells: Either the receptor is truncated, for instance by cleaving off residues from one or both ends, or the receptor is extended, by adding additional residues that facilitate its expression in laboratory cells.(5, 6)

Though never desirable, these alterations were necessitated to achieve any cell-surface expression at all. It had been hoped that these alterations were otherwise benign; however, absent native odorant receptor cell-based assays, the testing to confirm this was lacking. Moreover, it is not unreasonable to question

that alterations that make the gross difference of enabling a receptor to be expressed at the cell-surface in the first place have only this desired effect—to facilitate laboratory practices and no unintended consequences. The risk had always been that though accessible, these altered receptors represent mutants that have little if anything to do with native odorant receptor biology or function.

Conversely, consider that odorant receptors are normally expressed in highly specialized cells in the nasal epithelium. These cells are not the same as the ordinary laboratory cells that are commonly used in the creation of cell-based assays. Attempts to express odorant receptors in average laboratory cells may have been challenging because of the fact that while these cells are often cancerous cells of various tissues that are used because they are hardy and grow well in the lab, they may not provide important but as yet unknown factors for proper expression and function of odorant receptors *in vivo*. As laboratory cells are populations of genetically divergent and not genetically identical cells, however, it may be possible that they include rare and unique genetics that enable expression of receptors that are otherwise inaccessible in their native and full-length form.

Indeed, a technology was reported that enabled the rapid testing of millions of cells for the isolation of rare cells capable of expressing full-length and native receptors, including odorant receptors and other receptors that had been previously out of reach in their native and full-length form. (7)

The platform technology has been applied in a diverse range of fields spanning flavor ingredient discovery to drug discovery. In a number of these cases, the cell-based discovery systems that had been developed have successfully been employed to discover candidate molecules that work in downstream studies, including in some cases human studies and evaluations for molecules ranging from salt taste enhancers to blockers of pain perception.(8,9)

Looking Towards an Exciting Future

While these data are promising, much work remains to be done. These data demonstrate that for the first time, methods are coming online to access odorant receptors in their native form. This advance by-passes the need to rely on modified receptors, and can be expected to increase the reliability of the discovery platform and the likelihood of success in its use as part of a high-throughput aromatic ingredient discovery engine. Sensory confirmation and perfumery will always be required to identify aromatics that meet desired criteria in sensory applications; however, the ability to rapidly “turbo smell test”

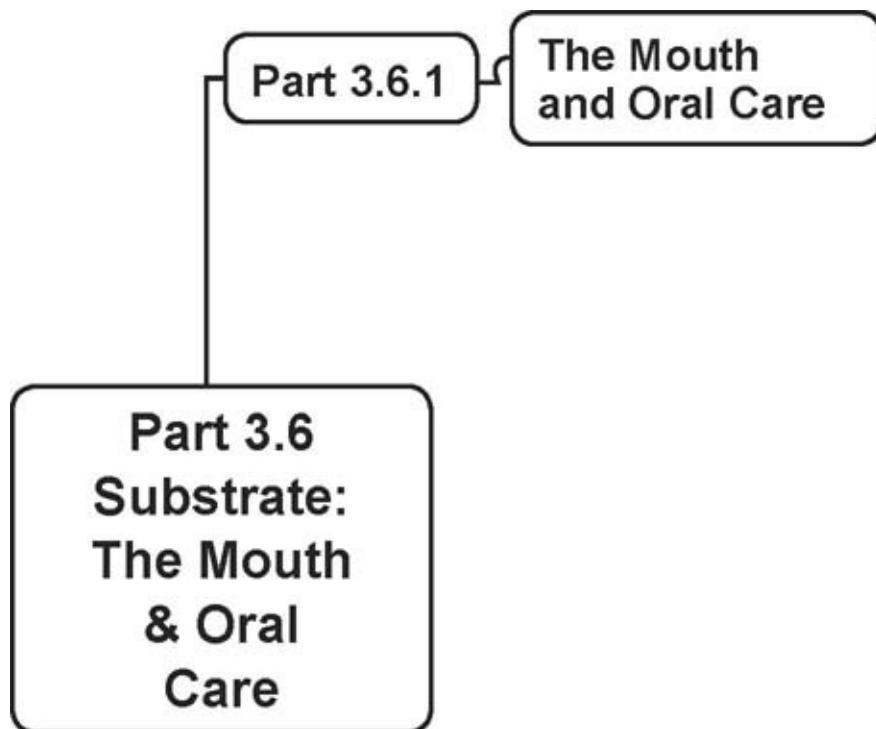
millions of natural ingredients against natural odorant receptors adds a previously unavailable discovery tool.

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PART 3.6

SUBSTRATE: THE MOUTH AND ORAL CARE



PART 3.6.1

THE MOUTH AND ORAL CARE

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ABSTRACT

While much of cosmetic chemistry and formulation is directed at maintaining or enhancing the appearance of the external surface of the body, the formulator of products for use in the human mouth must consider a more complex set of issues. Many oral care products address multiple consumer concerns at once, and it is common for an individual product to deliver both drug and cosmetic benefits. Understanding critical elements of oral biochemistry and microbiology will greatly help the formulator design products to deliver these benefits.

In addition to presenting some fundamentals of oral anatomy, physiology, and microbiology, this chapter will focus on the biology behind a variety of oral care consumer needs, the health and cosmetic problems behind these needs, and some approaches to solving these problems that can be introduced into oral care cosmetic or over-the-counter (OTC) drug products. Some of these concerns are summarized in [Table 1](#).

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References

3.6.1 THE TEETH AND THEIR SURROUNDINGS

The oral cavity contains the teeth, tongue, cheeks, palates, and gums (gingival tissues), and is bathed in saliva. The teeth and gums are major sources of consumer concerns, many of them related to dental plaque and other tooth deposits. However, oral care needs arise in other parts of the mouth as well. The following provides an overview of the biology of the oral cavity.

3.6.2 THE TEETH

The normal human dentition consists of 20 primary (“baby”) teeth, which erupt from six months to four years of age, starting with the incisors, and are replaced by 32 secondary (adult) teeth, starting with incisors at about six years of age and culminating with the third molars (“wisdom teeth”), which erupt (in people who have them) around 20–21 years of age. Tooth makeup and form are influenced by genetics, the preeruptive environment (notably nutrition), and the posteruptive environment. Dental anatomy and alignment as well as individual oral care habits and salivary chemistry can have a profound effect upon the quantity, quality, and pathogenicity of the dental deposits that form on the teeth. Different parts of the tooth exhibit markedly different structure and characteristics, which make them prone to different types of problems.

Consumer needs	Underlying problems	Functional solutions
	<i>“Cosmetic” Indications</i>	
• Attractive teeth/”, bright smile”	• Dental stain/discoloration • Potentially unsightly dental deposits	• Tooth stain removal/prevention • Plaque/calculus reduction
• Fresh breath	• Intrinsic/ extrinsic oral malodor	• Antimicrobial malodor prevention • Malodor masking/neutralization
• Fresh/clean mouth feeling	• Food/microbial residues (?)	• Flavorants, astringents, remove dental deposits (?)
• Clean mouth • Uncomfortable dental visits	• Dental plaque, calculus, stain • Uncomfortable dental cleaning to remove calculus or stain	• Plaque/calculus reduction • Stain reduction/prevention
• Dry mouth	• Insufficient saliva flow or altered saliva composition	• Lubricating/moisturizing rinses or gels • Artificial salivas
	<i>“Drug” Indications</i>	
• Healthy teeth/strong teeth	• Dental decay	• Decay prevention • Fluorides • Aid salivary function • Remineralization • Saliva stimulation

		<ul style="list-style-type: none"> • Plaque acid reduction
<ul style="list-style-type: none"> • Healthy gums 	<ul style="list-style-type: none"> • Gingivitis • Periodontitis (<i>not</i> an OTC indication) 	<ul style="list-style-type: none"> • Plaque/gingivitis reduction and prevention
<ul style="list-style-type: none"> • Sensitive teeth 	<ul style="list-style-type: none"> • Exposed, hypersensitive dentin 	<ul style="list-style-type: none"> • Tooth desensitization
<ul style="list-style-type: none"> • Oral comfort 	<ul style="list-style-type: none"> • Ulcers of the oral mucosa (e.g., canker sores) 	<ul style="list-style-type: none"> • Topical anesthetics • Antimicrobial agents

Table 1

a. Tooth Anatomy and Structure

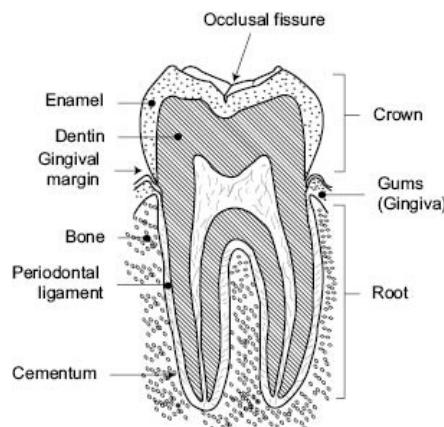


Figure 1. Simplified sketch of tooth anatomy. The tooth is divided into two areas: the crown (the enamel-covered part of the tooth that extends into the mouth) and the root, which is normally held firmly in place by the bone of the upper and lower jaws (Figure 1). Tooth crowns are covered by dense, hard dental enamel, the hardest tissue in the body. Enamel is thickest at the tip of the tooth and tapers to its thinnest point at the cemento-enamel junction (CEJ), where the crown gives way to the tooth root, which is covered by a thin mineralized layer called cementum. Underlying the enamel and cementum is a less dense mineralized tissue (dentin) that surrounds the dental pulp, which is rich in nerves and blood vessels.

In most individuals, the teeth are closely spaced and often touch side-to-side; the spaces between the teeth are referred to as interproximal spaces. The tooth

roots are held firmly in place in the jaws through periodontal ligaments. In normal healthy teeth, the gums surround the teeth and come to an apex just above the CEJ, so that the roots are not exposed to the oral cavity. The physical shape of teeth varies with tooth type, from the chisel-shaped incisors to the blocky molars, which often have pits and fissures on their biting surfaces. As discussed later, tooth shape can have a profound effect on processes that can lead to various dental problems.

b. Dental Enamel

Dental enamel is composed mainly of a partially carbonated form of the calcium phosphate mineral hydroxyapatite [$3\text{Ca}_3(\text{PO}_4)_2 \cdot \text{Ca}(\text{OH})_2$], which accounts for about 98% of its composition. Minor amounts of collagen-like protein, remnants of the organic matrix mineralized during tooth development, also remain in the enamel structure. Hydroxyapatite is capable of ion exchange, and anions such as F^- and CO_3^{2-} may replace the OH^- group, while cations such as Zn^{2+} , Sr^{2+} , and Mg^{2+} may replace Ca^{2+} . Caries susceptibility may be influenced by this ion exchange; for example, the extent to which OH has been replaced by F⁻ has a protective effect on the vulnerability of the enamel to acid demineralization. The hydroxyapatite, of which enamel is largely composed, is present in the form of crystallites that make up rods oriented at right angles to the surface. Water and selected ions can penetrate a small distance into the dental enamel surface along the plane of the crystallites, which can facilitate ion exchange.

As with all calcium phosphate minerals, dental enamel is soluble in acid. A substantial body of data indicates that tooth mineral is highly stable above a pH level of approximately 5.5, referred to as the “critical pH,” and that tooth demineralization occurs when the pH falls below this critical level. The extent of enamel solubility is determined largely by the amount of acid present (measured as pH), the duration of exposure to acid, the amount of soluble calcium and phosphate ions in the fluid bathing the enamel, and the presence of other ions that influence the balance between mineral dissolution and crystallization. Thus the concept of “critical pH,” while useful, should not be considered to be an absolute value.

c. Dentin

The layer of material beneath tooth enamel and cementum is the dentin. It contains approximately 70% hydroxyapatite, with the balance of its composition being collagen. Unlike the structure of enamel, which is characterized by

densely packed crystals, the dentinal matrix is perforated by a number of tiny, fluid-filled canals (dentinal tubules) that radiate from the pulp cavity to the surface. Dentin can be exposed to the oral cavity through damage to the enamel or gingival recession and wearing of the cementum (the mineral layer covering the dentin of the tooth root). The presence of open dentinal tubules can lead to hypersensitivity, in which the exposed dentin may be painfully sensitive to various stimuli, including heat, cold, pressure, sugar, acid, and so forth. This is discussed in more detail later in this chapter.

Dentin, like enamel, is also acid-soluble. Because it has less mineral content, and possibly because its structure more readily permits penetration of bacteria and acid, exposed dentin is generally believed to be more susceptible to decay than enamel. This explains the rapid advancement of dental decay after it penetrates the enamel layer and the vulnerability of exposed tooth roots to decay.

3.6.3 THE GUMS

The gums (gingival tissue) surround the teeth and overlie the bony structure in which the teeth are anchored. In health, the gum tissues directly adjacent to the teeth (the gingival margin) are firm and pink, extend just above the CEJ, and form a crevice that is not more than 3 mm deep. If dental plaque is permitted to accumulate adjacent to the gingival margin and within the gingival crevice, the gum tissue can become irritated, inflamed, and liable to bleeding. In some cases this inflammatory state stabilizes as chronic gingivitis, while in other cases the inflammation can advance to the destructive disease periodontitis.

3.6.4 ORAL FLUIDS

a. Saliva

Saliva is a major factor in the maintenance of a healthy mouth. Saliva is continuously being produced, bathing teeth and oral tissues in a dynamic environment that serves to lubricate the mouth, remove harmful materials from the oral environment, and maintain the mineral balance of the teeth.

Saliva is produced by three pairs of major glands and the smaller glands of the oral mucosa (labial, lingual, buccal, and palatal). The secretions differ from one another in composition and may also differ according to the rate of flow, time of

day, and so forth. For instance, it is well known that salivary flow is reduced at night; this makes it extremely important to cleanse the teeth between that previous midnight snack and going to bed, as the protective effects of saliva are substantially reduced during sleep.

Saliva contains mucopolysaccharides, proteins, enzymes, and inorganic materials such as calcium, sodium, potassium, chloride, bicarbonate, and phosphate ions, and bacteria shed from the oral surfaces and a variety of their constituents. The organic constituents of saliva are thought to be responsible for the development of the acquired dental pellicle (as described later). The presence of calcium and phosphate ions and the bicarbonate buffer system are believed to be significant both in the control of dental caries and in calculus formation. The pH of resting saliva ranges from about 6.5–7.2, while that of stimulated saliva can range up to about 8.0; the pH increase reflects a higher bicarbonate content in simulated supply.

The importance of saliva in preventing tooth decay following eating was demonstrated in the 1940s by R.M. Stephan. He devised a miniature pH electrode from antimony and used it to determine the pH of dental plaque at various sites on the teeth. His experiments showed that the pH of dental plaque decreased rapidly from about pH 6.82 to about pH 5 within a few minutes after consuming a sugar challenge and slowly returned toward neutrality over the next 20 to 60 minutes. If saliva flow to the teeth was restricted, the pH would remain depressed below the critical pH for enamel demineralization for much longer periods. The importance of saliva in maintaining oral pH may partially explain why an inadequate flow of saliva resulting from head and neck irradiation, drug-induced hyposalivation, or pathology can result in increased caries susceptibility and other oral problems.

b. Gingival Crevicular Fluid (GCF)

GCF exudes from the epithelium within the gingival sulcus or pocket. It is more similar to blood plasma than to saliva and can serve as a source of nutrients to the bacteria growing within the sulcus and along the gingival margin; it can also carry a variety of specific and nonspecific host defense factors.

3.6.5 THE ORAL SOFT TISSUES

The oral soft tissues consist of the tongue, gums, cheeks, hard and soft palate,

and sublingual region. The oral soft tissues are covered by a variety of nonkeratinized and keratinized epithelia. The surface of the soft tissues, most notably the tongue, can harbor large populations of oral bacteria that can serve as a reservoir to recolonize the teeth after cleaning. The microbial flora of the tongue can also play a prominent role in the generation of oral malodor (bad breath). Additionally, certain oral soft tissues are prone in some individuals to develop painful sores or ulcers, for example, aphthous ulcers (canker sores), which are addressed at greater depth later in this chapter.

3.6.6 DENTAL DEPOSITS

As soon as a tooth erupts into the mouth, and subsequently after every cleaning, it is prone to coverage with a variety of dental deposits that can cause diverse conditions that concern consumers. The enamel of a freshly cleaned tooth is rapidly covered by a pellicle of proteins adsorbed from saliva, followed by salivary bacteria that form dental plaque. Dental plaque bacteria can, under certain conditions, release by-products that can be harmful to the teeth or gums. Dental pellicle and plaque are also liable to accumulation of stain and neutralized deposits (dental calculus or tartar).

a. Dental Pellicle

Immediately after cleaning, the enamel surface is covered very rapidly by a 1–3 μm thick film of proline-rich phosphoproteins, peptides, and glycoproteins adsorbed from the saliva; thus the presence of a truly “clean” enamel surface in the oral environment is a fleeting phenomenon. The adsorption of salivary peptides and proteins appears to be relatively specific, starting within minutes of a tooth cleaning and continuing for several hours. While the acquired enamel pellicle is free from bacteria, it has been shown to contain a variety of bacterial constituents including enzymes such as glucosyltransferase, soluble polysaccharides, and various bacterial cell wall constituents.

b. Dental Plaque

Dental plaque has been recognized for centuries as a sticky film that forms on the teeth and has been the subject of concern as far back as the ancient Greeks. Over the years, the composition of dental plaque was thought to consist of food debris, mucus, denatured saliva, or “evil humors.” It was Anthony van

Leeuwenhoek, the inventor of the microscope, who first observed “tiny animalcules” (bacteria) in dental plaque in 1677. Around 1895, W.D. Miller established quite convincingly that dental plaque contained bacteria that could form acids from foods and that the acids could dissolve tooth enamel. This chemoparasitic theory of dental disease evolved to the belief that the way to control dental disease was to eliminate dental plaque, if not all bacteria, from the mouth. However, it was not until the 1960s through the 1980s that oral microbiologists started to understand the complexity and sequence of dental plaque and how it contributes to oral health and disease. Understanding the ecology of dental plaque and its effect on the character and pathogenicity of the plaque flora can provide strong insight into how a healthy balance with our natural oral flora can be established.

A central concept in our understanding of dental plaque and its pathogenicity is that plaque is actually a complex and heterogeneous microbial biofilm that varies substantially from site to site within the mouth, changes in nature over time, and is sensitive to a number of ecological parameters that can affect both its quantity and its quality. Dental plaque grows in an ordered manner, starting with the attachment of certain classes of bacteria (Gram-positive cocci) to the salivary pellicle through a set of relatively specific binding reactions similar to the interaction of antigens and antibodies. In general, these early colonizers are not associated with dental disease. Further plaque accumulation is a process combining growth of the early colonizers, elaboration of a sticky extracellular matrix that helps the bacteria adhere to the teeth and each other, and attachment and proliferation of additional bacterial species. Just as a plowed field can over time undergo succession from bare soil to grasses and weeds to shrubs to various species of trees, culminating in a mature forest, dental plaque changes and becomes more complex over time, dental plaque changes and becomes more complex over time. While the initial colonizing bacteria on smooth tooth surfaces are generally tolerant of oxygen and relatively nonpathogenic, later colonizers (especially in the region along the gumline and between the teeth where plaque tends to be thickest) become much richer in oxygen-intolerant anaerobic bacteria that can be associated with gum disease. In contrast, the microbial flora of tooth sites with reduced access to saliva (e.g., in the pits and fissures of the molars or in the interproximal spaces where teeth are tightly pressed together) can become dominated by bacteria that are relatively resistant to acid and can continue to produce acid at levels that can lead to tooth decay. The importance of particular populations of oral bacteria to oral problems such

as dental decay, gum disease, and oral malodor will be discussed in more detail later.

For further reading on dental plaque formation and ecology, its relation to disease, and strategies for controlling its pathogenicity, see the reviews by Burne and by Marsh *et al.* [1, 2].

c. Dental Calculus

The term “tartar” was commonly used to describe the mineralized deposits formed on neglected teeth. The origin of the term was derived from the similarity of mineralized dental deposits to the crystalline deposits formed in wine. The most common term in use is *calculus*. It may occur both above (supragingival) and below (subgingival) the gumline. Although these two forms of calculus may differ somewhat in composition, they both appear to originate from the mineralization of dental plaque bacteria and extracellular matrix.

Dental calculus varies in composition but always consists of about 80% of inorganic material containing calcium, magnesium, phosphorus, and other elements. The calcium and phosphorus are present in early plaque as Brushite ($\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$) and octacalcium phosphate [$\text{Ca}_8(\text{HPO}_4)_2(\text{PO}_4)_4$]. Whitlockite [$\text{Ca}_3(\text{PO}_4)_2$] may also be found, but the ultimate stage is probably apatite [$3\text{Ca}_3(\text{PO}_4)_2 \cdot \text{Ca}(\text{OH})_2$]. This transition, which is also characterized by increasing mineral hardness and tenacity, is helped by the presence of fluorine, which is present in calculus to the extent of about 400 ppm. The remaining 20% of calculus is an organic matrix containing carbohydrate, protein, lipid, and bacteria and their constituents.

Calculus is first observed when the plaque on the enamel surface begins to mineralize. Today the most generally accepted explanation is that calculus is formed by a seeding mechanism, followed by calcium phosphate crystal growth and maturation. Rates and amounts of calculus formation can vary widely among people and within the mouth. This is probably due to a variety of factors, including salivary chemistry, local pH, plaque accumulation, and oral hygiene.

3.6.7 MAJOR ORAL PROBLEMS AND THEIR REMEDIES OVERVIEW

The major oral care problems that concern consumers can be broadly categorized as medical or cosmetic, although some conditions may fall within

both categories. Medical concerns for oral health include dental decay and gum disease (both associated with distinct pathogenic dental plaque floras), dental hypersensitivity, and dry mouth. Cosmetic concerns include dental staining and tooth whitening, oral malodor, and dental calculus. Some of these problems are widely distributed in the population worldwide, while others are less common.

a. Dental Plaque

Dental plaque is not actually a problem, as it is ubiquitous in individuals exhibiting both dental health and dental disease. However, as discussed earlier, certain populations of microorganisms in dental plaque exhibit characteristics that play a major role in the causation of dental decay and gum disease. It is important to remember that the pathogenicity of dental plaque is related not only to its mass but also to relatively specific classes of microorganisms. For instance, individuals with active dental decay tend to harbor higher populations of plaque bacteria that can produce and tolerate higher levels of acids, which are responsible for the destruction of tooth enamel. In contrast, dental plaque associated with gum disease tends to be highly populated with anaerobic Gram-negative bacteria that produce toxic substances such as volatile sulfur compounds, short-chain fatty acids, or bacterial toxins. These substances may directly damage or irritate gum tissues or might cause an inflammatory response that can lead to pathological changes in the gum tissue. In many cases the microbiological character and pathogenicity of dental plaque can be markedly modified by behaviors, such as oral hygiene or dietary habits, which can have a profound influence on plaque ecology and the rise or control of pathogenic microbial communities in the plaque.

A wide variety of methods are used to study dental plaque in the oral cavity. Some clinical indices are used to record quantity of plaque on individual tooth surfaces whilst others are used to characterise plaque accumulation on different regions of the tooth such as on the gum margin or between the teeth. This variety of methods reflects the range of claims made for oral care products. For example, a toothbrush study may want to demonstrate superior cleaning on the gum margin or between the teeth, while an antiplaque toothpaste might demonstrate reductions in overall plaque accumulation on the tooth.

b. Dental Calculus

It has been recognized for some time that there is an association between the presence of calculus and the incidence of periodontal disease, although

enthusiasm for this concept waned considerably in the middle part of the twentieth century. The commonly accepted theory is that calculus irritates the gingival tissues and encourages the formation of a pocket between tooth and gingivae, in which food debris and bacteria may lodge. However, dental calculus is considered a cosmetic issue. Even in the absence of concern regarding pathogenicity, consumers have reason to care about controlling calculus. Its accumulation can be unsightly, and it is a frequent cause for uncomfortable visits to the dentist that are required for its removal from the teeth.

While mechanical treatment by a dental professional is the only means of removing calculus, it is possible to prevent or slow its formation by a number of physical and chemical means. Mechanical plaque control (i.e., brushing and flossing) can be very effective in reducing calculus formation by removing the plaque matrix required to initiate calculus calcification. For those individuals who require additional assistance in calculus control, there are a variety of oral care products, primarily toothpastes, that contain agents that inhibit calcium phosphate crystal growth and thereby calculus formation. The most common agents in tartar control toothpastes are pyrophosphate and zinc salts such as zinc citrate, although there are a variety of other calculus-inhibiting compounds in products and in the patent literature. At present there is one tartar control mouthrinse on the market, which uses zinc chloride as the antitartar agent. The current mechanism of action of anticalculus agents is thought to lie in their ability to interfere with the initiation or maturation of calcium phosphate crystals, resulting in less mineral mass and a less mature mineral structure that is softer and more easily removed by mechanical scaling. For further reading on dental calculus formation and control, see the reviews by Ciancio [3], Mandel [4], and Jepsen *et al.* [5].

c. Dental Caries (Tooth Decay)

Dental caries, commonly known as tooth decay, is a disease that is widely distributed worldwide and is associated with more frequent consumption of foods containing sugars or refined starches. Tooth decay used to be considered a disease of children, especially in affluent societies with plentiful access to sugary foods, since most tooth surfaces became decayed by early adulthood. In recent years, preventive measures such as water fluoridation, the use of fluoride toothpastes, and the placement of occlusal sealants has resulted in very significant reductions of childhood caries. While these reductions in tooth decay may carry over into adulthood, an intact tooth surface is at risk throughout the

life of an individual, and adults must also pay attention to caries prevention.

Dental decay occurs as the results of tooth demineralization by acids produced during carbohydrate fermentation by dental plaque bacteria. It is interesting to note that consumption of carbohydrates always results in the production of plaque acids but does not always result in tooth decay. This is because saliva can often neutralize and wash away plaque acids and deliver calcium and phosphate salts to remineralize enamel before irreversible damage is done to the tooth structure. Tooth decay occurs only if the magnitude and frequency of carbohydrate consumption cause decreases in plaque pH of magnitude and duration sufficient to overcome the natural reparative power of saliva.

Enamel decay usually starts in small circumscribed areas and spreads beneath the intact tooth surface. Early carious lesions, frequently referred to as “white spot” lesions, are characterized by a thin layer of intact dental enamel overlying partially demineralized dental enamel. A critical feature of these incipient lesions is that under the right conditions they may be reversed or arrested by the process of remineralization. This process is significantly enhanced by the presence of fluoride and extra calcium and phosphate ions in saliva and plaque fluid.

If the acid challenge is great enough in both magnitude and duration, the demineralization penetrates deeper into the enamel and spreads within it, roughly assuming the shape of a cone with its apex orientated toward the dentino-enamel border and the base still below the enamel surface. The lesions may still be reversed by conscientious use of fluoride or other remineralizing strategies. At this stage of the process there still may be no visible break or cavitation evident on the enamel surface. It is generally accepted that the dentin begins to demineralize or soften before an actual cavity becomes visible. Gradually the tissue softens sufficiently so that it breaks. At this point, the carious lesion becomes a cavity and can no longer be repaired by natural means, requiring restoration by a dentist.

Control of Caries

Reduction of Fermentable Carbohydrate Intake and Removal of Fermentable Debris from the Mouth A very large body of clinical and experimental evidence implicates the consumption of fermentable carbohydrates, especially sucrose, as a major causative factor in tooth decay. Reducing the consumption of fermentable carbohydrates, most especially reducing the frequency of intake, can markedly lower caries risk. A major cause of this reduction is almost certainly a decrease in the magnitude and duration of acid formation by dental plaque,

permitting more effective remineralization by saliva. Any action that accelerates removal of fermentable food residue from the mouth, such as rinsing and brushing of the teeth, can also shorten the duration of acid attack. Furthermore, a substantial number of studies indicate that stimulation of salivary flow by chewing a nonacidogenic substance such as sugarless gum can also accelerate the removal of fermentable residue and increase the rate of salivary buffering in the mouth. For further reading on the impact of diet and eating patterns on dental caries, see the review by Geddes [6].

Reduction in Bacterial Activity and Plaque Pathogenicity Bacteria present in the mouths of most individuals will ferment carbohydrates, especially sugars, causing a pH drop to levels ranging from about 5.5 to under 4.5, below the critical pH for enamel demineralization. This fall in pH is represented by the classic Stephan curve, which can be reproduced *in vitro* and *in vivo*. Certain microorganisms, including *Lactobacillus acidophilus* and *Streptococcus mutans*, have been identified as specific caries-causative pathogens. These microbes are particularly effective at producing acid and are particularly resistant to acid environments, which can lead to their domination in pre-carious and carious dental lesions. Thus practices that reduce the amount and duration of plaque acid production, such as less frequent snacking, may also produce reductions in these particularly cariogenic plaque bacteria. There is also evidence suggesting that some substances such as fluoride, chlorhexidine, or the sugar substitute xylitol may inhibit the metabolism of cariogenic plaque bacteria.

Tooth decay tends to occur more frequently on regions of the tooth that have relatively restricted access to saliva (e.g., in the pits and fissures on the tops of the teeth, or between the teeth) or on areas of exposed dentin, such as exposed tooth roots, which are more acid-soluble than tooth enamel. Thus measures that decrease bacterial access or accumulation to these restricted areas, such as placing occlusal sealants or flossing between the teeth, can substantially reduce their susceptibility to dental decay. There is also some evidence that placing occlusal sealants not only reduces caries but also may help reduce overall risk of dental caries by eliminating sites that are the primary ecological niches for *S. mutans* and other acid-tolerant, potentially cariogenic bacteria.

Decrease of Susceptibility of Tooth to Acid Attack It is clear that fluoride ions decrease the acid solubility of hydroxyapatite; the two strongest hypotheses for this protective effect are that fluoride may ion-exchange with the hydroxyl group in hydroxyapatite to produce more resistant fluorapatite, and fluoride ions present during acid challenge may decrease solubility at the enamel surface. This

effect on enamel solubility may be a principal mechanism by which water fluoridation and fluoride-containing toothpastes and mouthwashes exert their effect. In 1952, it was established in the United States that there was an inverse relationship between the incidence of caries in children and the fluoride content of the local water supply, and subsequent clinical and public health studies have firmly established the value of water fluoridation as a safe and effective way of reducing dental decay.

In the 1950s, fluoride toothpastes were introduced in the United States and are now available worldwide. A substantial body of experimental and clinical data clearly supports the highly significant clinical value of fluoride dentifrices and mouthrinses in the prevention of dental decay. The most common sources of fluorides used in oral care products are sodium fluoride, stannous fluoride, monofluorophosphate, and amine fluoride (amine fluoride is not approved for use in the United States). Anticaries fluoride products are regulated as drugs; in the United States, requirements for active ingredients, specifications, and testing of anticaries drug products for OTC human use are detailed in a final monograph (21 CFR Parts 310, 355, and 369, and Docket No. 80N-0042).

Remineralization

An additional approach to reducing dental decay is promoting the remineralization of dental enamel. As stated earlier, dental plaque acids act to dissolve dental enamel, and this is counterbalanced by the remineralizing action of saliva. Increasing the oral concentration of ions that enhance this reparative process may substantially reduce dental decay. Fluoride is well known to increase the rate of remineralization and the resistance of dental enamel to subsequent acid attack. A growing body of work suggests that increasing levels of available calcium may also increase the rate of remineralization. Approaches to increasing orally available calcium include methods as diverse as using two-phase systems to promote the deposition of calcium fluoride on the tooth surface and the use of calcium-and phosphate-rich milk proteins to speed remineralization. Currently additional remineralizing strategies to reduce dental caries are still under development. One issue that is still unclear is the potential effect of aggressive remineralizing strategies on the rate of dental calculus formation, which would be an undesired side effect. For additional reading on dental caries prevention, see the reviews by Featherstone [7], Edgar *et al.* [8], and Pitts [9].

In clinical studies of anticaries products the number of Decayed (D), Missing

(M) or Filled (F) teeth (T) or surfaces (S) occurring during a clinical trial are compared using the DMFT or DMFS indices, representing the sum of the Decayed, Missing, and Filled surfaces or teeth. Uppercase letters are used to designate the permanent dentition and lowercase the primary dentition. Historically these indices have been used to measure dental caries when they reach the stage of dentine involvement (cavitation), and such studies often involve many thousands of subjects over two or three years. More recently there has been a move to methods that consider remineralization and demineralization of early caries lesions (white spots). New caries classification systems such as those proposed by the International Caries Detection and Assessment (ICDAS) group [Pitts, 9] have been developed to assist in this process. There has also been a wide range of detection methods developed that enable monitoring of lesions such as those using Quantitative Light Induced Fluorescence (QLF, Inspektor, Amsterdam, Netherlands), Electrical impedance (CarieScan, Dundee, Scotland), and Red Fluorescence (Diagnodent, Kavo, Biberach, Germany).

d. Dental Erosion

Dental erosion is the dissolution of enamel and dentine by acid of either intrinsic or external origin. It is distinguished from dental caries in that the acid does not originate from fermentation of sugars by bacteria in dental plaque. The rate and severity of dental erosion is a result of the interplay between several chemical, biological, and behavioral factors. The various chemical properties of the acid like its pH, titratable acidity or buffering capacity and calcium-chelation ability determine its erosive potential. Biological factors like the various characteristics of saliva and acquired pellicle have also been found to be important in the development of dental erosion. The acquired pellicle may protect against erosion by acting as a diffusion barrier or a perm-selective membrane preventing direct contact between the acids and the tooth surface. Behavioral factors like the frequency and timing of consuming acidic beverages and food and abnormal eating and drinking habits have also been attributed to the development of dental erosion. Swishing of the erosive agent in the mouth increases agitation of the erosive agent and hence enhances the dissolution process because the solution on the surface of the hard tissue is readily renewed.

In the mouth the erosive process is complicated by mechanical forces from abrasion and attrition of the vulnerable softened layer.

e. Periodontal Diseases (Gingivitis and Periodontitis)

Teeth are attached to the basal bones of the jaw through the periodontal tissues and are surrounded by gingival tissues. In health, the gingival tissues are firm and the gingival crevices are relatively shallow. However, the gingival and periodontal tissues are susceptible to a variety of inflammatory diseases, generally classified as periodontal diseases. The most common of these are gingivitis and periodontitis.

Gingivitis is a reversible inflammation of the gums that is not accompanied by irreversible destruction of the periodontal support tissues. Gingivitis is associated with increased dental plaque accumulation and/or high populations of Gram-negative anaerobic bacteria in the plaque and is characterized by inflamed and easily bleeding gums. Gingivitis appears to be caused by substances produced in plaque that irritate the gum tissues and induce inflammation. There is a considerable body of evidence suggesting that, while gingivitis is not associated with a single pathogenic species, different groups of organisms are clearly associated with higher or lower capacity to promote gingivitis. Dental plaques dominated by species associated with early plaque colonization (e.g., Gram-positive streptococci) are more highly associated with gingival health, while more mature dental plaques dominated by Gram-negative anaerobic species such as *Fusobacterium nucleatum* and *Prevotella intermedia* are more highly associated with gingivitis. These Gram-negative species produce a variety of toxic or irritating substances including volatile organic acids (e.g., butyric or propionic acid), volatile sulfur compounds (e.g., H₂S or methyl mercaptan), various antigens, and endotoxins. These irritating substances are hypothesized to penetrate the gingival epithelium and provoke the body's inflammatory defense systems, which ultimately leads to the swollen and bleeding gums characteristic of gingivitis.

Gingivitis prevention has focused on mechanical and chemotherapeutic measures that reduce plaque mass and inhibit the emergence of more pathogenic microbial species. Many clinical studies have demonstrated that gingivitis can be reduced or eliminated by improved mechanical oral hygiene (i.e., better brushing and flossing), which reduces the amount of dental plaque and also selects for a less pathogenic, Gram-positive dominated plaque flora. As adjuncts to mechanical oral hygiene, a number of oral hygiene products containing antimicrobial agents have been formulated to help prevent gingivitis by chemical means. These compositions apparently exert their therapeutic activity by killing and inhibiting the growth and metabolism of plaque bacteria (thereby reducing plaque mass), slowing the progression of the plaque microflora toward

enrichment for more pathogenic Gram-negative anaerobes, and reducing the production of microbial irritants. Agents that have been clinically demonstrated to reduce gingivitis include chlorhexidine, a fixed ratio of essential oils (thymol, menthol, methyl salicylate, and eucalyptol), triclosan, stannous fluoride, and cetylpyridinium chloride. In 1998, the United States Food and Drug Administration concluded panel hearings on antiplaque, antigingivitis drug compositions. The panel voted and published in 2003 a recommendation to accept some of these agents as antiplaque, antigingivitis agents.

A number of other chemical interventions for plaque and gingivitis control have been proposed, although these technologies are not yet available. These approaches include measures to prevent colonization or aggregation of one or more species of plaque bacteria, measures to disaggregate plaque to make it easier to remove, and measures to reduce the host inflammatory response.

Periodontitis is a more serious form of periodontal disease characterized by severe inflammation, increased pocket depth, and irreversible loss of alveolar bone supporting the teeth. Current thinking about the etiology of periodontitis suggests that it results from an interaction between certain classes of particularly pathogenic microorganisms and the patient's specific and nonspecific inflammatory defense mechanisms. The pathogenic bacterial species associated with gingivitis and periodontitis overlap to some extent, although periodontitis-associated dental plaques contain some particularly pathogenic species such as *Porphyromonas gingivalis* and *Bacteroides forsythus*, which are not generally associated with gingivitis. Periodontitis is frequently preceded by and accompanied by gingivitis, but not all dental sites with gingivitis will progress to periodontitis. Periodontitis appears to progress in an episodic fashion characterized by short periods of rapid bone loss (possibly caused by a severe acute inflammatory episode) followed by extended periods of chronic inflammation and relative clinical stability. Periodontitis can only be diagnosed and treated by dental professionals, and there are no OTC oral care products specifically directed to reduction or prevention of periodontitis. However, prescription and OTC products clinically proven to reduce plaque and gingivitis are often included in supportive oral hygiene regimens after periodontal treatment. For further reading on the pathogenesis and treatment of gingivitis and periodontitis, see the reviews by Ciancio [3].

f. Dentinal Hypersensitivity

In healthy teeth the dentin of the crown and tooth root is generally covered

completely by enamel or gum tissue. However, when dentin is exposed by enamel decay or fracture or gingival recession that exposes the tooth root, it becomes susceptible to a variety of problems. Gingival recession can occur for a variety of reasons, including periodontal disease, gingival injury from improper brushing of the teeth, and so forth. Once exposed, the cementum covering the root can be quickly worn away to expose the dentin. In a substantial number of individuals, exposure of dentin can lead to sensitive teeth (dentin hypersensitivity), in which a variety of stimuli (e.g., heat or cold, pressure, or high-sugar foods) can trigger discomfort ranging from a mild twinge to severe pain. It is estimated that one in seven people is affected by some degree of dentin hypersensitivity. In its more severe forms this condition can be debilitating, affecting the dietary and oral hygiene habits of the patient.

While several theories have been advanced to explain the mechanism of dentin hypersensitivity, the most accepted model is the hydrodynamic theory. This theory proposes that exposed, unblocked dentinal tubules provide a channel from the outer surface of the tooth to the nerves in the pulp. Physical and chemical stimuli are postulated to affect the pressure on the fluid within the dental tubules, and these changes in hydrodynamic pressure are transmitted to the pulp nerves and interpreted as pain signals. The elements of the hydrodynamic theory (i.e., open dentinal tubules and responsive pulpal nerves) suggest two possible approaches to the reduction of dentin hypersensitivity: blocking the tubules or reducing the stimulation of pulpal nerves.

There are a variety of currently available professional and self-applied treatments for dentin hypersensitivity. The OTC products are dentifrices that provide agents that either promote blockage of open dentinal tubules (e.g., strontium chloride, arginine—calcium carbonate or stannous fluoride) or reduce the sensitivity of pulpal nerves by depolarizing the nerve membranes (e.g., potassium nitrate, potassium chloride, or potassium citrate).

For further reading on the origin and treatment of dentin hypersensitivity, see the reviews by West *et al.* [11] and Ciancio [3].

g. Dental Staining

While it does not contribute to dental disease, dental staining is of considerable concern to a large number of consumers who believe that stained teeth detract from their overall appearance. This concern is possibly most acute in the United States, although there is evidence that the desire for “cleaner, brighter” teeth is increasing in other parts of the world. Dental stain or discoloration can be

roughly categorized as either intrinsic or extrinsic. Intrinsic discoloration exists within the tooth structure itself and may result from discoloration laid down during tooth formation, thinning of tooth enamel (permitting the color of the underlying dentin to show through), or loss of tooth vitality. Extrinsic discoloration results from deposition of exogenous colored material (chromogens) on the tooth surface. There are a variety of theories concerning the chemistry of extrinsic tooth staining, which probably results from a number of causes. These may include binding of ingested chromogens (e.g., pigments from tea, coffee, or tobacco) to the tooth surface either directly or through mediation of “bridging” molecules, binding of metal ions such as tin or iron (which can form a variety of pigmented complexes) to the tooth surface, or the binding to the tooth surface of colorless substances that later undergo a chemical reaction that transforms them to colored compounds. For further reading on the chemistry and mechanisms of dental staining, see the review by Nathoo [12].

There are a variety of measures to reduce or prevent dental staining, which can be grouped into measures that remove extrinsic stain by mechanical or chemical means, measures that prevent extrinsic stain buildup, and measures that chemically bleach extrinsic or intrinsic dental stain. Consumer products that mechanically remove dental stains are probably the most abundant and are dominated by dentifrices containing a variety of abrasive systems. Some of these products, most notably some targeted to smokers, are more abrasive than “normal” toothpastes; care must be taken while formulating or selecting these products that a safe range of abrasivity is not exceeded. There are also a number of OTC dentifrices or mouthrinses that claim to bind to complex staining compounds and prevent their accumulation.

There are a growing number of professional and self-applied products that contain bleaching agents that can reduce the color of extrinsic or intrinsic dental stain. Bleaching products contain a variety of peroxides (e.g., hydrogen peroxide, carbamide peroxide) and chlorine compounds (e.g., chlorine dioxide), and exhibit a range of clinical effectiveness. Because it is possible that some of these bleaching agents could be harmful to the teeth or oral soft tissues, especially if misused, this class of products has come under increasing scrutiny from regulatory and professional agencies. For instance, oral care products containing peroxide are not permitted in Canada. While it is possible to make a variety of safe and effective bleaching products to reduce dental staining, the formulator is well advised to become familiar with applicable standards and regulations to ensure product safety and regulatory compliance.

h. Oral Malodor

Another concern of consumers of cosmetic oral care products is oral malodor (also known as bad breath or halitosis). Bad breath has been of documented concern since ancient Greek and Roman times and continues to be of concern. Oral malodor can be classified into two basic categories, intrinsic and extrinsic. Intrinsic oral malodor has its origin within the individual and is most commonly caused by substances produced by bacteria in the oral cavity. Malodorous substances of bacterial origin are primarily produced by anaerobic species (some, but not all, of which may be associated with gingivitis or periodontitis) and include volatile sulfur compounds, volatile organic acids, and a variety of other volatile malodorous compounds. A smaller, but still significant, source of intrinsic oral malodor is a variety of systemic diseases or disorders that result in the exhalation of malodorous substances in lung air.

Extrinsic oral malodor originates from the ingestion of substances (such as garlic, cheese, tobacco, etc.) that contain malodorous compounds. The duration of extrinsic oral malodor is proportional to the amount and frequency of ingestion of the offending substance and the amount of time required to “wash out” the malodorous substances.

While there is relatively little that can be done to alleviate intrinsic oral malodor arising from systemic disease or disorder short of correcting the underlying condition, there are many approaches and products directed at reducing extrinsic and intrinsic malodor of oral origin. These approaches can be categorized into those that remove or wash away the malodorous substances, those that mask or cover up malodorous substances with more pleasant-smelling flavors or fragrances, those that chemically bind and neutralize the malodorous substances, and those that prevent or reduce the elaboration of intrinsic oral malodors.

Mechanical means of removing oral malodor include rinsing the mouth and brushing or scraping the teeth and tongue. In addition to washing away malodorous substances, improved oral hygiene can affect the ecology of the microbial communities in dental plaque and on the tongue, reducing the populations of anaerobic bacteria that are responsible for a large portion of intrinsic oral malodor and for gingivitis and other oral diseases.

Numerous products are employed to cover up bad breath; these include a variety of cosmetic mouthrinses, toothpastes, mints, chewing gums, and breath sprays whose principal function is to introduce flavors (such as mint) that can

overpower or mask unpleasant mouth odors. Note that many of these approaches also increase the wash-out of odorous substances either directly (e.g., mouthrinses) or indirectly through salivary stimulation (e.g., chewing gums or breath mints). For these products, a formulator's concern is primarily directed at improving the delivery and residence time of the "reodorant" flavor systems.

Another very popular approach to reducing oral malodor is to interfere with the ability of oral bacteria to produce malodorous substances. The most common means of achieving this is through the use of products containing antimicrobial systems that either kill or metabolically inhibit substantial populations of bacteria that produce the offending substances. Since bacteria that are dead or injured are incapable or less capable of metabolizing and producing malodorous substances, this approach can be highly effective in reducing malodor originating from bacteria growing in dental plaques or on the tongue. Many of the antibacterial products used to reduce plaque and gingivitis (e.g., a mixture of four essential oils, chlorhexidine, triclosan, or stannous fluoride) may also be useful in controlling intrinsic oral malodor.

A fourth approach to controlling oral malodor is to ingest materials capable of binding with volatile odorous compounds and either neutralizing them or rendering them nonvolatile. Examples of products embodying this approach include those that contain metal ions such as copper, zinc, or tin (i.e., stannous ion), all well known to bind with volatile sulfur compounds. Other products in this class contain baking soda, which is also claimed to neutralize a variety of volatile malodorous compounds. These anti-odor substances have been formulated into mouthrinses, toothpastes, chewing gums, mints, and other oral dosage forms. Another class of compounds that has been proposed to systemically inactivate malodor consists of herbs or herb extracts (such as parsley oil) that are consumed as powders, tablets, or capsules. These products, which claim to inactivate malodorous substances in the stomach and thereby lower oral malodor, have not been demonstrated to have any value in reducing oral malodor. For further reading on the causes and management of oral malodor, see the review by Scully *et al.* [13].

i. Dry Mouth (Xerostomia)

Reduced saliva flow leading to dry mouth syndrome (xerostomia) can originate from a number of sources, including systemic disease, destruction of salivary gland tissue by radiation or chemotherapeutic cancer treatment, and medicinal drug use. While systemic diseases (such as Sjogren's syndrome) that destroy

salivary function are relatively rare, induced dry mouth syndrome, especially that caused by drug use, occurs to some extent in a substantial percentage of the population. Total or near total destruction of salivary function such as that exhibited by patients with Sjogren's syndrome or head and neck irradiation for cancer treatment can have a devastating effect on the oral cavity. Rampant caries, severe fungal infections such as candidiasis (thrush), loss of ability to taste, discomfort due to a pronounced sense of dryness, and irritation of the oral soft tissues are common in patients affected by severe xerostomia. Dry mouth of lesser severity, for instance that caused by some drugs, can also result in some of these problems, although usually to a less severe degree. There are now over 200 prescription and nonprescription drugs known to reduce saliva flow, including antidepressants, antihypertensives, and antihistamines; therefore this is more than a minor problem, especially in older individuals taking multiple medications.

Management of patients with dry mouth generally involves first reducing their susceptibility to oral disease. This commonly includes daily use of fluoride mouthrinses or gels and the use of antifungal drugs when necessary. A number of other products generally classified as "artificial salivas" are also available to increase the patients' comfort by reducing the sensation of oral dryness. These products typically contain a variety of humectants and lubricants to increase the sensation of oral moistness, as well as buffers and sometimes fluoride.

For further reading on xerostomia evaluation and management, see the review by Eveson [14].

j. Aphthous Ulcers (Canker Sores)

While oral ulcers, most notably canker sores (aphthous ulcers), are not as widely distributed a problem as gingivitis, they still affect millions of patients yearly and can be very troublesome and painful to those unfortunate enough to suffer them. Canker sores most commonly arise on the tongue and buccal mucosa. Their cause is still a matter of some debate and has been variously attributed to bacterial, viral, and autoimmune factors, although the evidence for an autoimmune origin is currently the strongest. A variety of factors has been suggested to predispose susceptible individuals to a canker sore attack, including minor injuries or irritation to the tongue or oral mucosa, selected foods, sodium lauryl sulfate (in toothpaste), and the like. Canker sores are characterized by shallow crater-like lesions approximately 2–10 mm in diameter (although sometimes larger) with sunken, grayish centers and slightly raised borders. They

can occur singly or in multiple clusters. Once manifested, they last approximately five to ten days and can be quite painful. For review see Akintoye [15].

There is no known prevention for canker sores except to avoid things that the patient's experience suggest are predisposing factors. Treatment of existing canker sores is generally targeted at reducing discomfort or speeding healing. Several products aimed at reducing discomfort contain topical anesthetics such as benzocaine or lidocaine, while others include agents such as diphenhydramine or corticosteroids to reduce the inflammatory response. Antimicrobial mouthrinses, most notably mouthrinses containing essential oils or chlorhexidine, have been shown to speed healing or reduce the incidence of canker sores. Agents such as silver nitrate or sulfuric acid have also been suggested to cauterize canker sores; this approach is generally regarded as unsafe and not recommended. As additional information regarding aphthous ulcers becomes available, additional approaches to treatment and prevention can be expected.

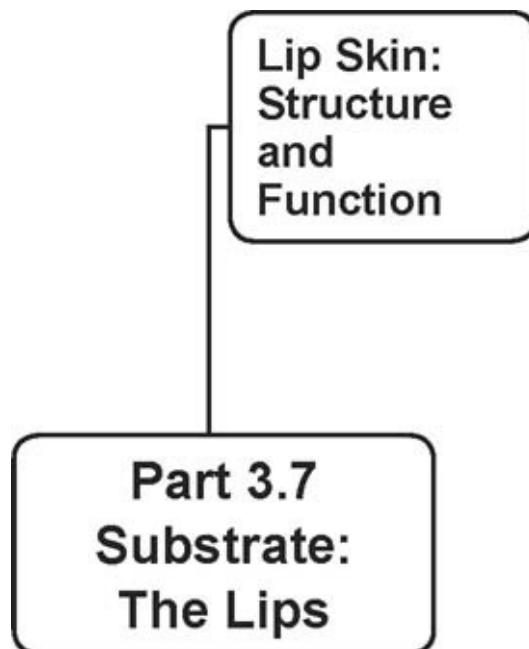
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PART 3.7

SUBSTRATE: THE LIPS



PART 3.7

LIP SKIN: STRUCTURE AND FUNCTION

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ABSTRACT

The lips are covered by a thin keratinized epithelium layer on a flexible, connective tissue with a rich blood supply. The *stratum corneum* on the vermillion zone of the lip contains the same types of ceramides, cholesterol, and fatty acids found in other regions of epidermis, but the barrier function is weak, permitting a relatively high TEWL. There are sebaceous follicles in the connective tissue that supply sebaceous lipids to the lip surface. The morphology of the lips changes abruptly with increasing age.

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3.7.1 VERMILION ZONE/VERMILION BORDER

The lips represent a specialized transitional region in going from the skin to the oral mucosa. The line where the facial skin meets the surrounding the red external lip is known as the vermillion border of the lip. This term has frequently been misused to refer to the entire external red portion of the lip. The entire external red portion of the lip is referred to as the vermillion or vermillion zone. This vermillion zone has an underlying connective tissue that is more flexible than the connective tissue of most regions of the skin and is covered by a thin

orthokeratinized epithelium [1]. The underlying connective tissue has a rich blood supply, which contributes to the red coloration [2].

3.7.2 TEWL/HYDRATION

The projected area of the corneocytes on the vermillion zone of the lip is slightly larger than that of corneocytes from the cheek, and the TEWL through the lip is greater than TEWL through the cheek [3]. The transition from the exterior keratinized epithelium of the vermillion zone and the internal nonkeratinized epithelium is abrupt; however, the portion of the vermillion zone closest to the oral mucosa is sometimes referred to as the submucosa. Surprisingly, the hydration of the submucosa is less than the rest of the external vermillion zone [4]. Hydration of the upper lip is significantly greater than hydration of the lower lip.

3.7.3 SEBACEOUS FOLLICLES

Within the connective tissue along the vermillion border of the lip are sebaceous follicles, which are sebaceous glands without associated terminal hairs. The composition of the lipid mixture produced by these sebaceous follicles is the same as that produced by the major sebaceous glands of the skin [5]. The composition of sebum in the lumen of the gland is 57% triglycerides, 25% wax monoesters, 15% squalene, 2% cholesterol esters, and 1% cholesterol [6]. As sebum flows to and over the skin surface, the triglycerides undergo partial hydrolysis to release fatty acids, some of which are antimicrobial [7]. The most notable antimicrobial fatty acids are lauric acid (C12:0) and sapienic acid (C16:1Δ6). Sebaceous follicles are not only found in the vermillion border of the lip and in the oral mucosa, they encircle every orifice of the human body [7]. This distribution strongly suggests a protective function. In general, sebum secretion rates in adults decreases with increasing age. TEWL through the vermillion zone also decreases with age, but hydration appears to be independent of age [8].

3.7.4 STRATUM CORNEUM LIPIDS

As with other regions of the skin, the major lipids found in the *stratum corneum* of the vermillion zone are ceramides, cholesterol, and fatty acids. These lipids are

important in that they determine the permeability barrier function of the lips. The ceramides found here, as in other regions, are a complex mixture of structural types. Ceramides consist of a fatty acid amide linked to a long-chain base. The amide-linked fatty acids can be normal fatty acids, α -hydroxyacids, or ω -hydroxyacids, and the long-chain bases can be sphingosine/dihydrosphingosine, phytosphingosine, or 6-hydroxysphingosine. When the amide-linked fatty acid is an ω -hydroxyacid, a normal fatty acid is ester-linked to the ω -hydroxyl group.

A ceramide nomenclature system has been proposed in which the amide-linked fatty acid and long-chain base are each indicated by a single letter (normal fatty acid, N; α -hydroxyacid, A; ω -hydroxyacid, O; sphingosine/dihydrosphingosine, S; phytosphingosine, P; 6-hydroxysphingosine, H) [9]. The presence of an ester-linked fatty acid is indicated by a prefix E. In this system, a ceramide containing an α -hydroxyacid amide-linked to phytosphingosine would be ceramide AP. All nine possible structural types of ceramides have been detected in *stratum corneum* from the vermillion zone, and their relative proportions are similar to those found in other regions of skin. Weight percents of the ceramides from the *stratum corneum* of the vermillion zone are: EOS, 4.4; NS, 10.5; EOP, 0.7; NP, 23.3; EOH, 2.6; AS, 11.3; NH, 20.9; AP, 8.8; AH, 17.4.

3.7.5 CHANGE WITH AGE

A series of studies have demonstrated that the upper lip measured from the bottom of the nose lengthens with increasing age, while the vermillion zone becomes narrower [10–12].

We note that the literature has been comprehensively reviewed in this brief chapter and that not much work has been done in this area. This will be of special interest to cosmetic and personal care scientists and formulators since products for lip care vary widely from moisturizers to lipstick. As such, we believe that lip care and beauty can be advanced considerably by providing a deeper understanding of the differentiation between “normal” skin and that of lip skin.

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PART 3.8

SUBSTRATE: FEMININE REJUVENATION

**Part 3.8
Substrate:
Feminine
Rejuvenation**

PART 3.8

FEMININE REJUVENATION

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ABSTRACT

In this chapter we address head-on this rarely discussed substrate, in the best *Harry's Cosmeticology* tradition.

As we have seen in many other chapters in this book, the perception of beauty and its relationship to well-being and skin health are intimately connected. Recent work in the cosmetic and personal care field has now extended beyond mere ingredients and formulations to other factors that provide an impact on beauty in the wider sense.

With the growing advent of dermatologists, OB-Gyn professionals, and other medical researchers formulating their own product lines, we deem it appropriate and timely for a detailed discussion of the anatomy, function, dysfunction, and approaches to improve the perception and well-being of individuals who want to feel and project beauty in their world.

While many may begin with the thought that this body substrate is more intended for medical/drug intervention, the reader will see there is much opportunity for the development of cosmetic-based over-the-counter, natural approaches that offer enhancement of this category and are both needed and wanted by the world's female population.

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3.8.1 THE ANATOMY

Vulva includes female genital regions that are externally visible in the perineal

region composed of mons veneris, labia majora, labia minora, and clitoris.

- **Mons veneris**, overlying the symphysis pubis, is the fatty prominence covered by curly hair.
- **Labia majora** are composed of two longitudinal folds of skin extending in elliptical fashion to enclose the vulvar cleft. Contain abundance of adipose tissue, sebaceous glands, and sweat glands. Embryologically analogous to penile shaft of male genitalia and the skin covered by hair on their upper outer surfaces.
- **Labia minora** are thin, firm, pigmented, redundant folds of skin, which split anteriorly to enclose the clitoris and meet posteriorly by the vestibules. Skin of labia minora does not contain hair follicles and is poor in sweat glands but rich in sebaceous glands.
- **Clitoris** is a small cylindrical erectile organ at lower border of symphysis. It is made up of two crura, body and glans. Crura lie deeply, in close apposition to peri-osteum of ischiopubic rami. They join to form the body of the clitoris, which extends downward beneath a loose prepuce to be capped by the acorn-shaped glans. Only the glans of the clitoris is visible externally between the two folds formed by bifurcation of labia minora. The visible clitoris is one to two inches in length. It is attached to the pubic bone and separates into two crura that are each about two to four inches in length. Clitoral hood acts as a shield for the clitoral glans. The role of the clitoral hood is to provide protection from too much stimulation.

3.8.2 VULVAR INNERVATIONS: PHYSIOLOGICAL AND ANALYTICAL

Vulva and perineum are innervated by femoral nerve posterior and lateral margins of vulva along leg crease. Genitofemoral and ilioinguinal nerve from L-1 and L-2 innervate the mons pubis upper labia majora to the level of urethra. Perineal branch of pudendal nerve (S-2–S-4) covers the lobes of labia majora. A rich plexus of over 8,000 nerve endings covers the dorsal aspect of the glans clitoris from the deeper pudendal nerve. The inferior cuneal nerve from S-1–S-3 also covers the vulva.

Assessing Sensory Perception on the Vulva and on Extragenital Sites

Sensory perception on the vulva as well as extragenital sites occurs by means

of detection of transient light touch by Meissner corpuscles and transient deeper pressure by Pacini corpuscles. Slowly adapting receptors, such as Merkel cells and Ruffini receptors, are responsible in responding to more sustained touch, such as sensing texture or shape.

While perception of burning or itching appears to be unaffected with aging, vulvar sensitivity to mechanical stimuli declines after menopause.

However, of the various demographics (age, gender, ethnicity) and anthropometric variables (height, weight, size of the body), *the most significant effect on sensory perception is advancing age*. This is also true for sensitivity to vibration, thermal threshold, and mechanical stimuli. Ethnic differences in sensory perception also have been reported. For example, Japanese subjects were more sensitive to touch and pain on the cheeks and palm of hands than Caucasians. However, Japanese subjects rated pain as less severe on a subjective scale than Caucasians—despite being more sensitive at perceiving it. Cultural stoicism may have contributed to higher tolerance to pain (1).

A Canadian study of 40 premenopausal women found that the labium minora and the mucosa of the vulvar vestibule were less sensitive than the forearm to filament touch and pressure. Although the labium minora was more sensitive to pain than forearm (2), similarly, a study of 13 premenopausal women found vulvar vestibule to be less sensitive to filament touch and pressure than deltoid muscle, forearm, or thigh (3).

A Swedish study found the clitoris to be less sensitive to perception of vibration than dorsum of hand but more sensitive than dorsum of the feet (4).

A Turkish study on vibratory threshold found vulvar sites (labia majora and minora, clitoris and vaginal introitus) were comparable in sensitivity to the first and second fingers and to the nipples. In this study, lips and ears were the least sensitive to vibratory stimuli (5).

A study conducted in the USA of both healthy and neurologically impaired women (6) noted significant loss of sensitivity to pressure/touch in postmenopausal women, hypoestrogenic women, women with vulvar atrophy, neurologically impaired women, and women with impaired sexual function, all of whom showed clear effect of estrogen on vulvar sensitivity. Although vulva has lower density of estrogen receptors than vagina, the effect of estrogen on touch sensitivity appears profound (7). Estradiol treatment significantly increased sensitivity of vestibule to pressure/touch relative to placebo at four to

six weeks. Although the mechanism of action is not known at this time, the potential sensor neural targets may be C fibers of Merkel cells (8).

In a Canadian study of premenopausal women aged 18–45, it was found that sexual arousal had no effect on extragenital sensations, suggesting dyspareunia is not due to lack of arousal (9).

Thresholds to touch averaged over all vulvar sites (clitoris, labium minus, perineum, and anal verge) were noted to be 4.6-fold lower in normoestrogenic compared to hypoestrogenic women (10).

Because of the importance of sensation to female sexual well-being and the perception and projection of beauty, in its broadest sense, an objective of an appropriate vulvar skin regimen should include the maintenance of vulvar sensorium by preserving nerve sensitivity functions.

3.8.3 VULVAR ATROPHY: CAUSES AND PHYSIOLOGY

In the U.S., half of the population is expected to be over the age of 50 in the next 20 years. There is a misconception that skin disorder is only cosmetic. *Because physiological changes occur with skin, if the causes are left untreated, such disorders can lead to a vicious cycle of chronic irritation and pruritis. These changes affect sexual function, comfort, and self-esteem.*

Common physiological changes with age include:

- lower elasticity and diminished tensile strength,
- reduced dermal thickness,
- reduced vascularity and cellularity of dermis,
- decreased sensory perception,
- reduced hydration,
- flattening of the dermal-epidermal junction,
- reduced epidermal thickness,
- increased permeability, and
- slower wound healing.

An awareness of these facts will bring a new pathway to individuals and companies looking for new products, new categories, and a common wish to enhance all aspects of individual beauty. The intention here is to expedite work

in this area beyond the medical/drug approaches already in existence and to include the development, use, and application of cosmeceuticals, natural products, etc. that are having a positive impact on beauty enhancement.

Skin changes can be slow and progressive with loss of subcutaneous fat beneath mons veneris and labia majora. Skin becomes pale, dry, and frail with tissue of low elasticity and pliability. Minimal lubrication results, due to decreased blood flow. Vulva becomes increasingly shrunken in appearance while pubic hair becomes thin, sparse, and brittle. There is reduction in size of labia minora, clitoris, and prepuce. Vulvar tissue can be diminished, obliterated, or even fused, with irritation and erythema evident. Skin becomes thin, shiny, and inelastic. Microscopically there are decreases in epithelial thickness and vascularity, and decreases in nerve endings but increases in dermal fibrosis.

An increase in permeability with thinning of the protective epithelium can predispose the vulva to chronic atrophic vulvitis with inflammation and abrasion/fissures from repeated irritation. If uncorrected, this process can lead to progressive sclerosing atrophy with stenosis of the vaginal orifice and effacement of the labia minora and clitoris (Kraurosis vulvae).

Causes of Vulvar Atrophy:

By 2010, over 64 million women in the U.S. were over the age of 45. It is estimated that vulvar atrophy is a silent epidemic affecting up to 50% of postmenopausal women. Women suffer in silence with this chronic and progressive medical condition and its impact on quality of life, sexual function, and urogynecologic health. Left untreated, vulvar atrophy can cause personal and marital distress and contribute to female sexual dysfunction (11). Many are reluctant to discuss their concern due to embarrassment, cultural taboo, and uncertainty about treatment options. In our opinion, women's quest for personal beauty goes far beyond how their face looks, what color and shape their hair is, and the degree of wrinkling they have.

Vulvar Changes:

These changes are the result of a loss of tissue elasticity and thinning as well as decreased vascularity and subdermal inflammation. After childbirth, darker pigmentation changes of vulvar region are also noted. Vulvar skin retracted with tissue demarcation between labia majora and minor becomes blunted. Fragile tissue becomes prone to cracking and splitting. Thinned vulvar skin loses much of its natural surface defense mechanism and is more likely to become and remain inflamed.

With chronic inflammation of the skin in this area, lichen sclerosis develops, involving skin and mucous membrane of vulvar and rectal area. With higher pro-inflammatory cytokines—interleukin-1, interleukin-6, and TNF alpha in older skin—an inflammatory response is likely. The body responds to new inflammation with repair accompanied by vulvar and perineal itching, thereby causing a vicious cycle. *Further thinning of keratin layer of labia majora and minora decreases protection against bacteria and fungus, leading to chronic infection.*

Common causes of hypoestrogenic atrophy are natural menopause and surgical/chemo-or radiation-induced ovarian failure. Usage of maintenance endocrine therapies can also cause ovarian dysfunction and vulvar atrophy. One common example is aromatase inhibitor used by breast cancer patients.

Other causes of such atrophy include young women undergoing a gonadotropin-releasing hormone agonist or antagonist, which has induced a hypoestrogenic state. Breastfeeding women have diminished estradiol level, causing atrophy and decreased libido. Furthermore, allergy sufferers with chronic antihistamine use and ones on parasympathomimetic or tricyclic antidepressant medications (12) will have similar symptoms. Still other causes include use of birth control pills, Sjorgen's syndrome, anorexia nervosa, bulimia, or infertility treatments.

3.8.4 TREATMENTS

American television is full of drugs designed to correct male erectile dysfunction. For women of such partners, there can be a significant downside if they suffer from vulvar atrophy. Coitus with vulvar atrophy can cause lower genital tract skin and mucous membrane laceration due to dryness and decreased elasticity. Pain will cause pelvic floor muscle contraction and reduced vaginal secretion. *Over-the-counter lubricants with propylene glycol and chemical preservatives can cause sensitivity to women with an inflamed mucous membrane, leading to more local inflammation and pain.* Other products can dry out over time with firm residual kernels to become an added source of irritation.

If progressive tissue changes of thinning and inflammation occur, effects of estrogen are less dramatic. Estrogen will accelerate healing and decrease inflammation but will not return the tissue to a premenopausal state. It is not a

fountain of youth but will halt further degeneration.

As developers of new cosmetic and personal care ingredients, formulations, and products, our goal is to alleviate symptoms/discomfort and improve the quality of life by minimizing or reversing physiological changes.

a. Over-the-Counter Treatments

A number of OTCs can be used by women with minimal symptoms who are concerned about hormone use or are not candidates for hormonal treatment. Definitive efficacy data are lacking for almost all of the OTCs. These products are vaginal moisturizers and lubricants, but none are designed for the vulva.

Some OTCs may contain irritating ingredients such as warming additives, dyes, perfumes, bactericides, spermicides, benzocaine, chlorhexidine, and preservatives (parabens and propylene glycol). Because petroleum-based products such as mineral oil and petroleum jelly can interfere with condom use, they are best avoided.

b. Pharmacologic Treatment:

Estrogen

Topical low-dose estrogen is considered first-line pharmacologic treatment. Progesterone is not indicated with low-dose topical estrogen. Although limited in data in randomized, controlled clinical trials, estrogen is noted to be effective. A position statement from North American Menopause Society (NAMS) provides an evidence-based conclusion and recommendation for local estrogen treatment. Furthermore, NAMS concludes that there are inadequate data to support annual endometrial sampling or transvaginal ultrasound in asymptomatic patients (13).

In a cohort study of 1500 previously treated patients with breast cancer, no statistically significant difference in disease-free interval between use and without usage of vaginal estrogen was noted (14).

Usage of vaginal ring and vaginal tablet estrogen showed no significant changes in endometrium (15). Similar results were confirmed by endometrial biopsy (16). Due to lack of long-term safety data, one needs to have well-documented informed consent to risk and benefits prior to prescribing local estrogen to a patient with a history of hormone-sensitive malignancy.

After publicity surrounding Women's Health Initiative Studies, there is less frequent use of Hormone Replacement Therapy (HRT) in the U.S. This NIH (National Institute of Health) sponsored study highlighted the potential danger of taking systemic HRT, with small increased risk of developing breast cancer in women taking HRT for five years or more. Greater chance of heart attack and stroke were also noted. This finding led to a precipitous drop in numbers American women using HRT. *Unfortunately, benefits were downplayed and risks were emphasized.*

NAMS recommendations for patients without hormonally sensitive tumors is that they should not be treated differently than routinely managed postmenopausal patients. To date, the FDA has not yet approved estrogen-based products for use in patients with hormonally sensitive malignancy.

c. Novel stem-cell-derived peptides

SERM (Selective Estrogen Receptor Modulator) Ospemifene

Recently, the FDA approved a non-estrogen oral medication known as ospemifene. Ospemifene is a selective estrogen agonist and antagonist depending on tissue type. It has been approved for dyspareunia. Common side effects reported include: hot flashes, muscle spasms, increased sweating, and increased vaginal discharge.

Compared with placebo in clinical trial, daily usage of ospemifene decreased incidence of dyspareunia by -1.55 from baseline standard deviation of 2.7 compared with decrease of -1.29 from baseline by placebo.

Because ospemifene can act as an estrogen in other organs, it carries a risk of endometrial cancer, stroke, and blood clots. It should not be used in breastfeeding or pregnancy or used together with coumadin, rifampin, ketokonazole, omeprazole, rifampin, or bupropion.

At higher dosages, ospemifene is associated with increase in thymoma, adrenal subcapsular cell adenoma, sex cord stromal tumors, granulose cell tumors, and luteoma tumors in animal studies.

Topical growth factors and peptides

As stated above, physiological changes occur with aging skin in this substrate as well. Issues we would like to address in formulating a cosmetic product include:

- Improved sensory perception
- Improved hydration

- Improved appearance in color and tone
- Improved elasticity and tone of skin
- Improved firmness and volume

Topical growth factors and peptides have played a role in reversing the aging process described above, although the main use of growth factors is in wound healing (56, 57). Kinetin was first isolated from Herring sperm DNA in 1955, with natural antioxidant properties.

Stem Cells: The New Frontier

In a manner similar to that described for Topical Growth Factors and Peptides, Adult Stem Cell Derived Cells have exerted in vivo and in vitro effects that address the issues mentioned above.

Stem cells are a population of immature tissue precursor cells capable of self-renewal and provision of multi-lineage differentiable cells for tissues. Adult stem cells have fewer issues compared with embryonic stem cells, such as difficulties in control of differentiation and issues relating to ethics. Stem cells provide a building-block function by moving to the injured area and differentiating into neighboring cells, and by replacing damaged cells. By secreting diverse growth factors, they activate the neighboring cells to exhibit diverse pharmacologic action (paracrine effect). Human subcutaneous adipose tissue is a promising source of adult stem cells that secrete various cytokines and growth factors. These in turn control and manage the damaged neighboring cells (18, 19). The figures below demonstrate these results, which include an increase in collagen and anti-wrinkle effects.

Anti-wrinkle effect:

Stem cells were injected subcutaneously into a UVB irradiation-induced - wrinkle. Reverse replica demonstrates wrinkle-diminishing effects.

- a. control, b. 1×10^3 , c. 1×10^4 , d. 1×10^5 cells (23)

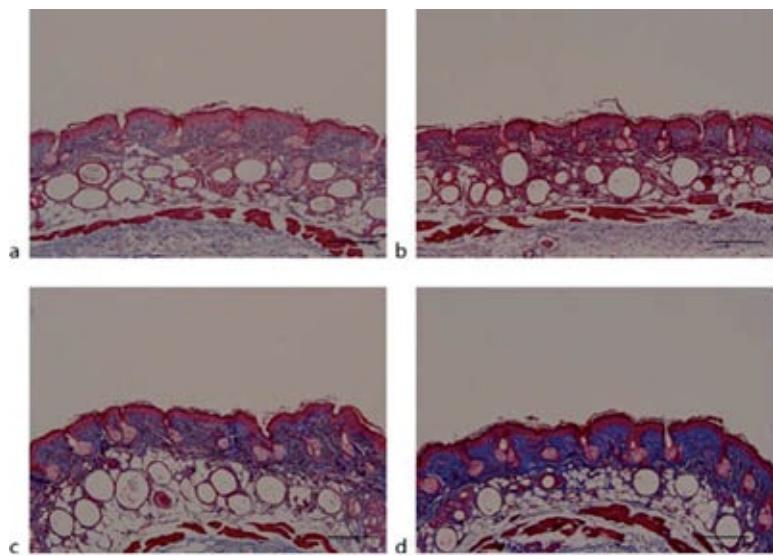


Figure 1 Stem Cells

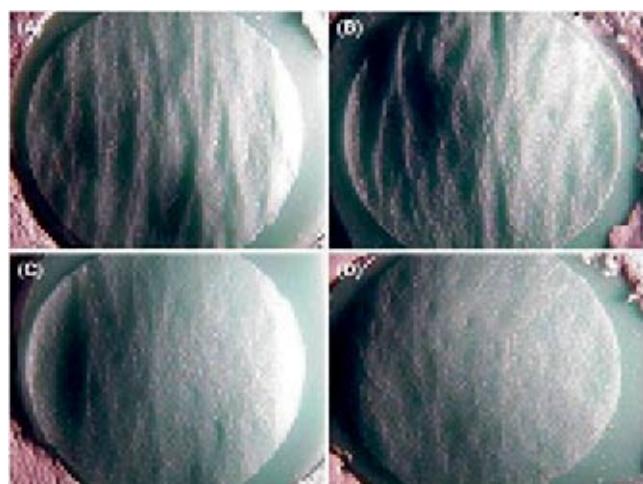


Figure 2 Collagen-stained blue, demonstrating increase in collagen content with adult-derived stem cell. A: control, B: 1×10^3 , C: 1×10^4 , D: 1×10^5 (23).

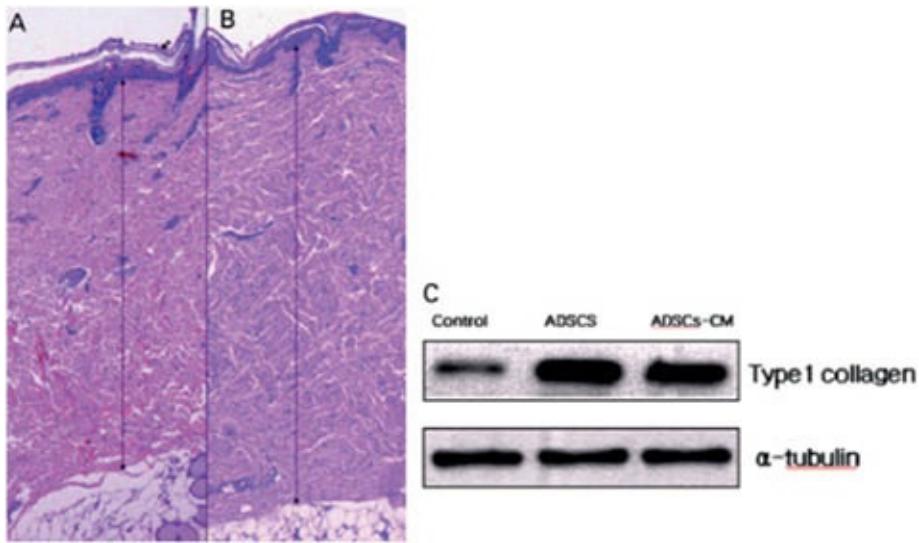


Figure 3 Histopathology of pig skin 14 days after injection of ADSC (1×10^6). A denotes control; B represents treatment arm. Western blot noted $40 \times$ increase in collagen expression (22).

Stem cells are limited because collection is required and only autologous transplantation is possible. Injection is mandatory, with short life span out of body and a long time to manufacture. Adult-Derived Stem Cell secreted factors have advantages over ADSC in that ADSC can be difficult to handle, transport, and commercialize, and they have a short shelf life. There is also the threat of passing on viruses, diseases from other animal-source nutrients to cultured stem cells in the laboratory, uncontrolled growth, and misdirected growth, especially of embryonic cells.

Recently, novel processing has enabled mass production of quality-controlled peptides from adult stem cell growth mediums. Protein extracts from conditioned media are available with following key ingredients in sufficient concentration for efficacy:

- TGF beta 1 (transforming growth factor)
- PDGF (platelet-derived growth factor)
- KGF (keratinocyte growth factor)
- Basic FGF (fibroblast growth factor)
- HGF (hepatocyte growth factor)
- VEGF (vascular endothelial growth factor)
- SOD (superoxide dismutase)

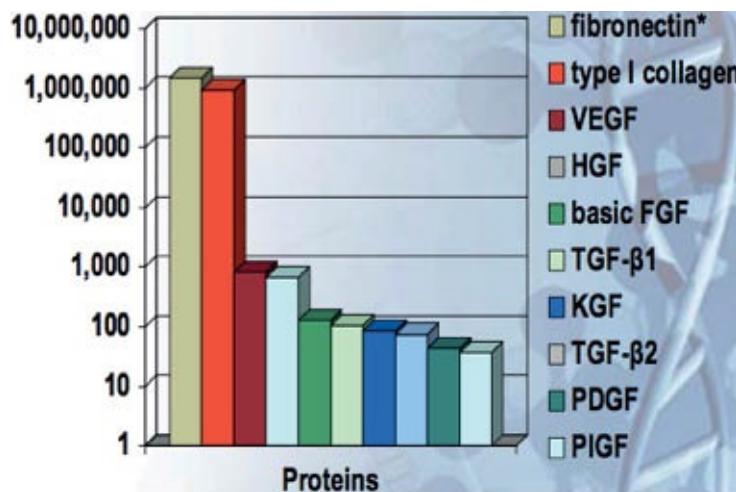


Figure 4

TABLE 1. Concentration of Cytokines and Extracellular Matrix Proteins

Proteins	Concentration
PDGF	44.41 ± 2.56 pg/mL
bFGF	131.35 ± 30.31 pg/mL
KGF	86.28 ± 20.33 pg/mL
TGF-β1	103.33 ± 1.70 pg/mL
HGF	670.94 ± 86.92 pg/mL
VEGF	809.53 ± 95.98 pg/mL
Type I collagen	921.47 ± 49.65 ng/mL
Fibronectin	1466.48 ± 460.21 ng/mL

Table 1, Reference (23)

These cytokines are needed in maintaining components of the dermis, promoting wound healing, stimulating collagen synthesis/secretion, regulating cell growth and division, promoting epithelial cell growth, promoting the formation of new blood vessels, stimulating and forming of new blood vessels, and providing protection of skin from oxidative damages.

In a clinical study using Adult Derived Stem Cell Protein Extract (AAPE; Proteomics Inc., Seoul, Korea) applied topically 12 times at two-week intervals for wrinkle indications, 47% of subjects showed good to excellent improvement, with 63% judged to have very good to excellent improvement. Cytokines were

injected intradermal into skin to evaluate efficacy in subsequent studies and exhibited confirmed efficacy.

One such peptide has been approved by U.S. CTFA as of September 2006 (Cosmetic Toiletry and Fragrance Association/ Personal Care Products Council) with the INCI name of Human Adipocyte Conditioned Media Extract (21343). Readers interested in learning more about peptides can consult another chapter on this subject in this text. Results for ADSC-derived factors are promising and might have potential in skin regeneration because they can be manufactured in large scale with long-term stability while relatively devoid of safety issues (20, 21).

3.8.5 A NEW FRONTIER:

Adipose-derived stem cells and their secretory factors are a promising therapy for skin aging as reported in *Dermatologic Surgery* (Oct. 2008). Such products have been used on the face with good results in the author's clinical practice. Based on extrapolations from patients' subjective reports, and observation from past gynecological experience that the vulvar skin's rapid healing is due to a rich vascular supply similar to the face, formulations specifically designed for the vulvar region have been prepared containing such stem cells. *Initial results have shown a promising, positive, subjective response in restoration of sensitivity and skin quality. As such, these results represent a new frontier and direction for product developers interested in addressing the issues discussed in this chapter.*

Topical usage of adult stem cell secreted factor for wrinkle reduction. Daily application photos were taken four weeks apart. See [Figures 5.](#)



Figure 5 Topical usage of adult stem cell results



Figure 6 Improved firmness in periocular skin four weeks after injection of adult stem cell derived factor.



Figure 7 Improved firmness and color via injected adult stem cell derived factor four weeks after treatment.

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