

# MEDICINE UPDATE

A Reprint of Medicine Update on Trichology

Pre-clinical study

## KERATIN, COLLAGEN & MICRONUTRIENTS TO UPREGULATE IGF-1: A NEW PARADIGM FOR HAIR GROWTH

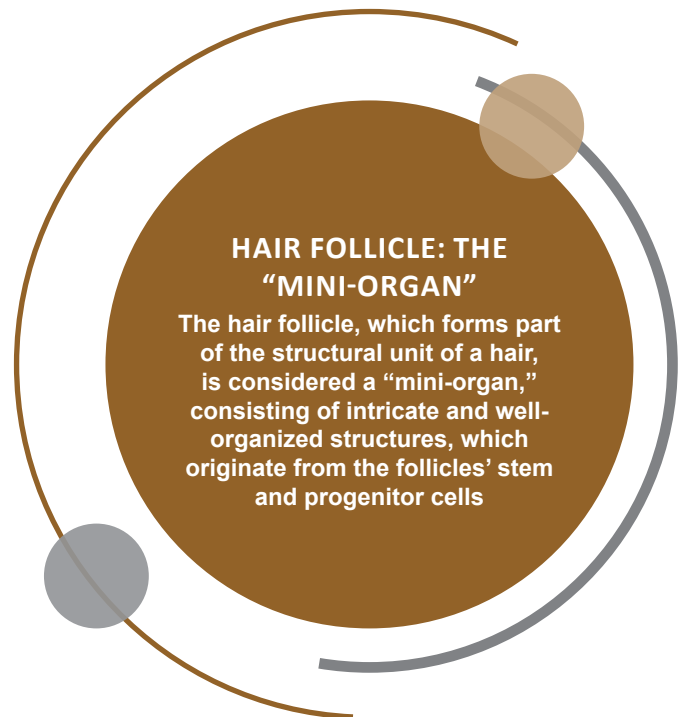
### IMPORTANCE OF HAIR IN HUMANS

Hairs represent an important defining feature of mammals and have several critical functions, including physical protection, production of sebum and apocrine sweat, social and sexual interactions, thermoregulation, and provision of stem cells for skin homeostasis.<sup>1-3</sup> And these clearly justify the reason why hair loss is one of the most common complaints for which both male and female patients seek treatment. This important feature in humans not only has physiological significance but plays a great role in their beauty, social acceptance, and self-esteem.

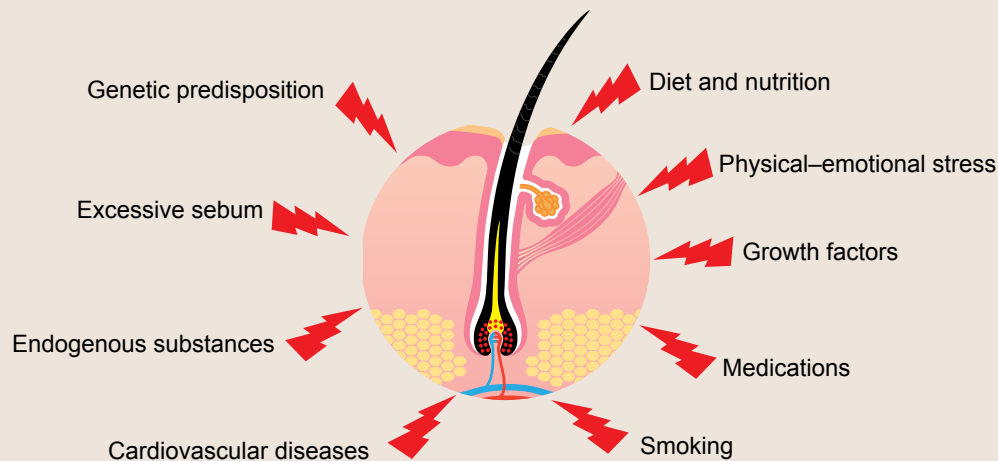
### Unmet need in hair loss therapy

A number of common treatments are available for hair loss, including the conventional chemical approaches like minoxidil, finasteride, herbal extracts, platelet-rich plasma (PRP), adipose-derived stem cells, keratinocyte-conditioned media, and hair transplantation. However, the fact is that none of these methods have been able to bring satisfactory results in the long term and/or have drastic side effects, limiting their clinical use.<sup>4,5</sup>

Therefore, there is a need to explore alternative therapeutic solutions capable of generating functional hair follicles. This could possibly be achieved through hair cycling regeneration, which is important for functional hair follicle regeneration. In fact, the activation, stability and sustainability of hair cycling are considered to be key factors in achieving the longevity



of hair follicle function.<sup>3,4</sup> Therapeutic manipulation of hair follicle cycling therefore represents not only a key challenge but opportunity in the management of hair loss.<sup>6</sup> Particularly, great interest has been attracted by strategies directed at inducing and maintaining the anagen phase.

**FIGURE 1****FACTORS AFFECTING HAIR GROWTH**

**Adapted from:** 1. Houshyar KS, Borrelli MR, Tapking C, et al. Molecular Mechanisms of Hair Growth and Regeneration: Current Understanding and Novel Paradigms. *Dermatology* 2020;236:271–280. 2. Semalty M, Semalty A, Joshi GP, et al. Hair growth and rejuvenation: An overview. *Journal of Dermatological Treatment*. 2010; Early Online, 1–10.

## FACTORS AFFECTING HAIR GROWTH AND DEVELOPMENT

Hair development is a continuous cyclic process, and is governed and influenced by a myriad of internal and external factors. The loss of hair – clinically termed as alopecia – can thus occur in a variety of patho-physiological states associated with hormonal imbalance, age, autoimmune conditions, medications, and genetics (Figure 1).<sup>2,6</sup>

## ENDOGENOUS GROWTH FACTORS INVOLVED IN DEVELOPMENT OF HAIR FOLLICLES

A number of growth factors and receptors have been identified that regulate the development of hair follicles. Some of the growth factors contributing to the hair follicle growth and cell cycle regulation include:

- Epidermal growth factor (EGF)
- Transforming growth factor- $\beta$  (TGF- $\beta$ )
- **Insulin-like growth factor-1 (IGF-1)**
- Hepatocyte growth factor (HGF)
- Keratinocyte growth factor (KGF)
- Vascular endothelial growth factor (VEGF).

Any changes occurring in the distribution of relevant growth factor receptors and their expression levels can therefore affect the growth and development of hair follicles.

## IGF-1: Why important for hair growth promotion

The IGF-1 is a multifunctional regulatory growth factor that is believed to control the cell proliferation and the survival of hair follicle cells. The dermal papilla cells (DPCs) can secrete IGF-1, IGFR-1, and IGFBPs. Results of experimental research have shown that the exogenous IGF-1 not only changes the level of protein kinase C (PKC) but also it is essential for maintaining the hair growth.<sup>4</sup>

IGF-1 has been shown to affect follicular proliferation, tissue remodelling, and the hair growth cycle, along with follicular differentiation, identifying IGF-1 signalling as an important mitogenic and morphogenetic regulator in the biology of hair follicles, i.e., hair growth cycle and the differentiation of the hair shafts.<sup>7</sup>

Amongst the various growth factors postulated to play a role in hair follicles, IGF-1 is known to be regulated by androgens. Accordingly, dermal papillary cells from balding scalp follicles were found to secrete significantly less IGF-1 as compared to their counterparts from non-balding scalp follicles.

## EFFECT OF DIET ON HAIR GROWTH

Diet seems to play an important role in hair biology, with evidence suggesting that nutritional deficiency may possibly impact both the structure and the growth of hair.<sup>8</sup> These effects on hair growth may encompass telogen effluvium as well as the diffuse alopecia; while studies have also reported potential

**TABLE 1: EFFECTS OF NUTRIENT DEFICIENCY ON HAIR LOSS**

Nutrient	Effect of deficiency
Iron	Chronic diffuse telogen hair loss with iron deficiency anaemia
Zinc	Statistically lower serum zinc concentrations found in patients with androgenic alopecia, male/female pattern hair loss, or telogen effluvium compared to healthy controls
Niacin (Vitamin B3)	Diffuse hair loss with pellagra due to severe deficiency
Fatty acids	Loss of scalp and eyebrow hair
Selenium	Experimental and anecdotal report on selenium deficiency and sparse hair growth
Vitamin D	Serum vitamin D2 levels in females with either telogen effluvium or pattern hair loss shown to be significantly lower than in age-matched controls, with decreased levels correlating to increased disease severity
Biotin	Deficiency can result in alopecia
Amino acids and proteins	Protein malnutrition can result in hair loss

**Adapted from:** Guo EL, Katta R. Diet and hair loss: effects of nutrient deficiency and supplement use. *Dermatol Pract Concept*. 2017;7(1):1–10.

associations between nutritional deficiency and different forms of pattern hair loss, including androgenetic alopecia, female pattern hair loss, and alopecia areata (Table 1).<sup>8</sup> Nutrient deficiencies may arise because of genetic disorders, medical conditions, or dietary practices, and therefore screening for such deficiencies must be guided by history and physical

examination. Supplementation would then be an appropriate strategy in individuals with proven deficiency of particular nutrients. An Indian Cross-sectional Study also showed that nutritional deficiency is a common problem in participants with hair loss, and suggested need for identification and correction of nutritional deficiencies in patients with hair loss.<sup>9</sup>

## IMPORTANCE OF PROTEIN SUPPLEMENTATION IN HAIR GROWTH

It is known that the states of protein energy malnutrition, such as in kwashiorkor and marasmus, can result in hair changes, which include hair thinning and hair loss.<sup>8</sup> The role of the essential amino acid, l-lysine in hair loss also appears to be important. Double-blind data confirmed findings of an open study in women with increased hair shedding, where a significant proportion responded to l-lysine and iron therapy.<sup>10</sup> Another double-blind, placebo-controlled study assessing the ability of an oral marine protein supplement to promote terminal hair growth in adult women with self-perceived thinning hair associated with poor diet, stress, hormonal influences, or abnormal menstrual cycles, reported that the protein supplemented patients had a significant increase in the number of terminal hairs within the target area, which was significantly greater than placebo. Protein supplementation also resulted in significantly less hair shedding and higher total Self-Assessment and Quality of Life Questionnaires scores.<sup>11</sup>

## KERATIN: AN INDISPENSABLE PROTEIN FOR HAIR GROWTH

Keratin is the protein from which the majority of hair are made, and that facilitates hair follicle repair.<sup>12</sup> During hair growth and keratinization process along the hair fiber, several keratins arrange into filaments to contribute to the production of the hair shaft. These multigene proteins are members of the “Intermediate Filament” family, including ‘soft keratins’ expressed mainly in epithelial tissues and ‘hard keratins’ involved in the formation of hard keratinized structures such as hairs and nails.<sup>5</sup> Supplying the body with supplementary keratin can therefore be highly effective for growing longer hair, and keep them thick and strong throughout the hair growth cycle.<sup>12</sup> Human hair keratins are majorly composed of proteins (65-96%), followed by lipids (1-9%), melanin (3%) and other minor compounds.<sup>13</sup>

In hair, the bulk of keratins are expressed in the middle cortex (“keratinizing zone”) of the ascending hair fiber. Other keratin expressions are restricted to the hair cuticle and are sequentially expressed during hair morphogenesis.<sup>14</sup> Overall, keratin is thus indispensable for the growth and structural integrity of the hair. Out of the 54 human keratin genes, at least 26 (~50%) are specifically expressed in the hair follicle.<sup>15</sup>

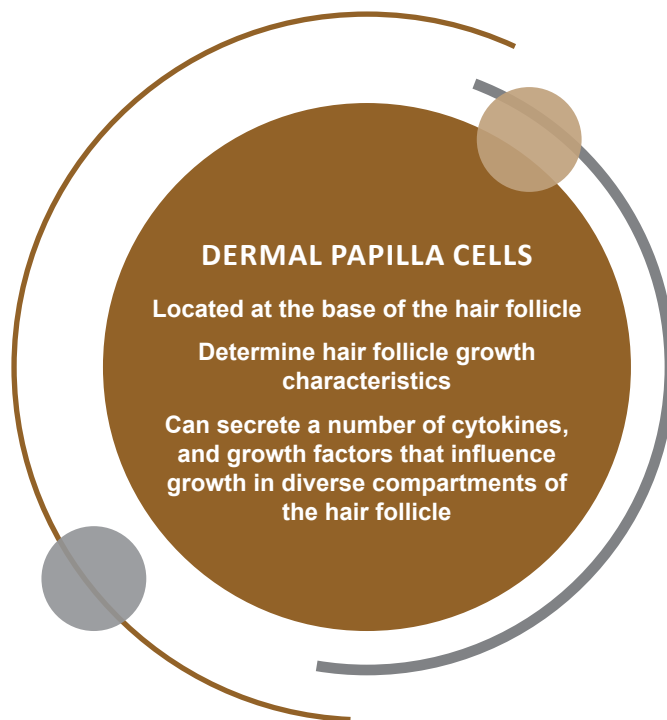
## HYDROLYZED COLLAGEN: A SAFE COSMETIC BIOMATERIAL WITH GOOD MOISTURIZING PROPERTIES

Antioxidants' use has significantly increased in recent years in the diet of people, with natural antioxidants replacing the synthetic antioxidant ingredients owing to their safety, and nutritional and therapeutic values. Hydrolyzed collagen – a low molecular weight protein – is a popular ingredient considered to be an antioxidant that has been widely utilized because of its excellent biocompatibility, easy biodegradability, and weak antigenicity. It is a safe cosmetic biomaterial with good moisturizing properties, oral ingestion of which improves the skin properties such as elasticity, skin moisture, and transepidermal water loss.

**Source:** Aguirre-Cruz G, León-López A, Cruz-Gómez V, *et al.* Collagen Hydrolysates for Skin Protection: Oral Administration and Topical Formulation. *Antioxidants (Basel)* 2020;9(2):181.

## ROLE OF VITAMINS AND MINERALS IN NORMALIZING THE HAIR GROWTH CYCLE

Micronutrients are major elements in the normal hair follicle cycle, playing a role in cellular turnover, a frequent occurrence in the rapidly dividing matrix cells in the follicle bulb; thus, pointing towards the important role these micronutrients – vitamins and minerals – play in the normal hair follicle development. Deficiency of such micronutrients may therefore represent a modifiable risk factor associated with the development, prevention, and treatment of hair-loss.<sup>16</sup>



In fact, vitamin deficiencies are thought to be one of the causes of alopecia, for which the treatment would be dietary supplementation. Likewise, minerals are also important for proper hair growth. Relating this, an analysis of the hair shows that it is composed of iron, oxygen, hydrogen, nitrogen and sulphur. The blood must therefore be supplied with these minerals so that nourishment will be carried to the scalp.<sup>6</sup> Table 2 depicts the role of various nutrients and minerals and their possible mechanisms in preventing hair loss.<sup>6</sup>

**TABLE 2: NUTRIENTS AND MINERALS: MECHANISMS OF PREVENTING HAIR LOSS**

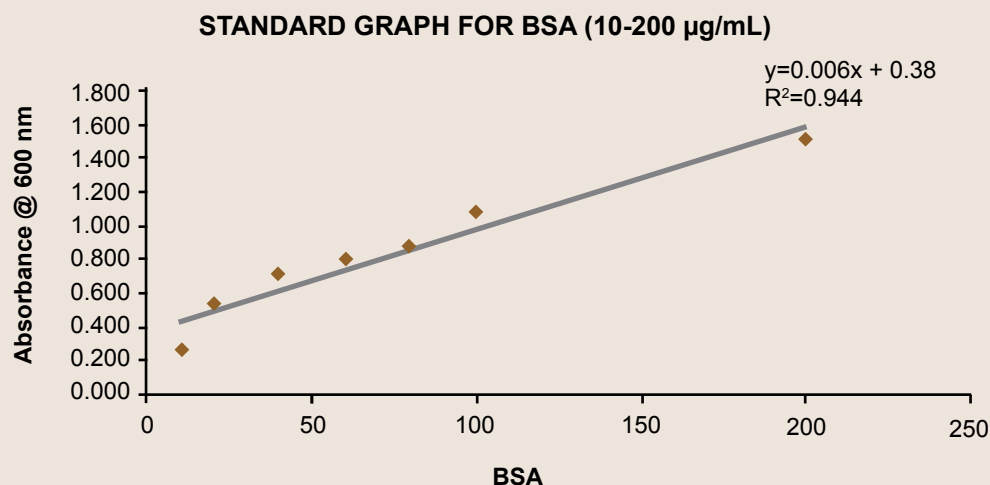
Nutrients and minerals	Mechanism of preventing hair loss
Niacin (vitamin B3)	<ul style="list-style-type: none"> <li>Enhances blood flow to scalp through vasodilatory effects</li> <li>Reduces the level of cholesterol and hence the level of 5-alpha reductase on scalp</li> </ul>
Vitamin B complex	<ul style="list-style-type: none"> <li>Improves blood flow to scalp</li> <li>Decreases cholesterol accumulation to scalp</li> <li>Protects hair and scalp from free radical damage</li> </ul>
Ascorbic acid (vitamin C)	<ul style="list-style-type: none"> <li>Improves blood flow to scalp and maintains capillaries carrying blood to follicles</li> </ul>
Tocopherol (vitamin E)	<ul style="list-style-type: none"> <li>Enhances oxygen uptake and thus improves blood flow to scalp</li> </ul>
Zinc	<ul style="list-style-type: none"> <li>Enhances immune function and thus stimulates hair growth</li> </ul>
Essential fatty acids	<ul style="list-style-type: none"> <li>Improves hair texture</li> <li>Prevents loss of dry brittle hairs</li> </ul>
Amino acids	<ul style="list-style-type: none"> <li>Improves quality of hair texture</li> </ul>

**Adapted from:** Semalty M, Semalty A, Joshi GP, *et al.* Hair growth and rejuvenation: An overview. *Journal of Dermatological Treatment*. 2010; Early Online, 1–10.

## PRE-CLINICAL STUDY: EFFECT OF PROTEIN ON MARKER FOR HAIR GROWTH PROMOTION

IGF-1, the well-studied marker for hair growth promotion, was found to be upregulated in human hair DPCs upon treatment with dissolved compounds of Keratin, collagen and micronutrients formula (Azkera tablets) in a Preclinical study (unpublished data).<sup>17</sup> The study – In-vitro Hair growth studies for Azkera tablets – used the following type of assay: Total protein estimation/Bradford assay, and Hair growth study in human hair dermal papilla cells. The product was sourced from the Apex Laboratories. There were two parts of the study:

**FIGURE 2**



Adapted from: Preclinical study (Data on file, Apex Laboratories)

### Part-I

- **Aim:** To determine the total protein content in Azkera tablets
- **Methods:** Assay: Bradford assay; Standard: Bovine serum albumin (BSA)

#### Procedure:

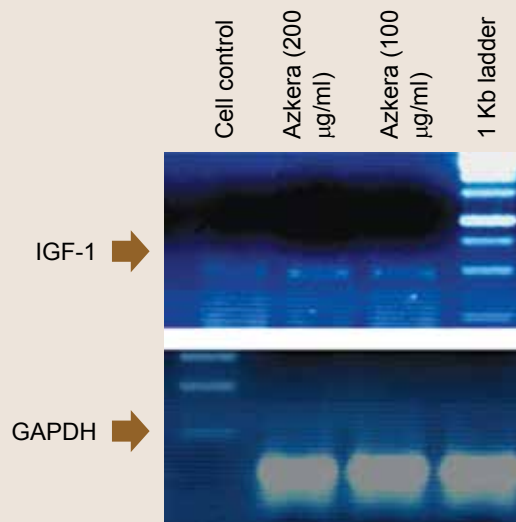
- Various concentrations of standard protein BSA (10-200 µg/mL) were made to plot a standard graph.
- The Bradford reagent (200 µL) was added to all the samples and monitored at 600 nm.
- The protein concentration in dissolved Azkera tablets was deduced from the standard graph (Figure 2).

#### Results:

- **Total Protein content in Azkera tablets** = 196.66 µg/10 mg.
- » The total protein content was 196.66 µg/10 mg, indicating the presence of total protein in dissolved fraction of Azkera tablets.

**FIGURE 3**

### GENE EXPRESSION PROFILE OF TEST SAMPLES



Adapted from: Preclinical study (Data on file, Apex Laboratories)

## Part-II

- **Aim:** Gene Expression study of dissolved fraction of Azkera test samples in human hair dermal papilla cells.
- **Methods:** Assay: Gene Expression by semi quantitative PCR; Time of incubation: 24 h; Cell line used for bioassay: Human hair dermal papilla cells ( $10^5$  cells/ml); Concentrations Tested: 200, 100 $\mu$ g/ml
  - » Human hair dermal papilla cells were treated with 200, 100 $\mu$ g/ml concentration of test samples and incubated at 37°C for 24 hours.
  - » RNA was isolated from the samples and subjected to cDNA synthesis (reverse transcription) and semiquantitative PCR amplification using gene specific primers.
  - » The glyceraldehyde 3-phospho dehydrogenase (GAPDH) used as internal control for gene expression studies.

### INFERENCE AND IMPRESSION

- Azkera tablet has high protein content per tablet.
- Azkera contents cause upregulation of the gene for IGF-1 (a well-studied biomarker for hair growth promotion) in human hair dermal papilla cells.

### Results:

- The results showed that the test samples at 200, 100 $\mu$ g concentration up-regulated the IGF-1 gene expression when compared to the cell control (untreated cells); Figure 3.

**Study Supervisor:** Dr. Sunil Prabhu

**Study Done at:** SARANI BIO-INTEGRA PRIVATE LIMITED

**Study Sponsor and Source of the Formulation:** Apex Laboratories

## HAIR CYCLE & POTENTIAL ROLE OF KERATIN, COLLAGEN AND MICRONUTRIENTS FORMULA (AZKERA)

As mentioned earlier, hair growth and development follow a continuous cyclic process. All mature follicles go through a growth cycle, which consists of four main phases: growth (anagen), regression (catagen), rest (telogen), and shedding (exogen).<sup>2</sup> The hair follicle undergoes continuous cycling throughout adult life and its elements are regenerated with each hair cycle.

At the onset of the anagen, i.e., the growth phase, the stem cells present within the bulge region begin to proliferate to produce a new lower hair follicle. These hair follicle bulge cells in humans are keratin 15 (K15) positive. The “bulge activation hypothesis” proposes that bulge stem cells maintain hair follicle homeostasis through periodic cycling. Proliferation of bulge stem cells marks the initiation of anagen, resulting in daughter cells from asymmetric division. The stem cell progeny cells migrate to the follicle base and become highly proliferative “transiently activated matrix cells” capable of giving rise to all cell lineages of the mature hair follicle.<sup>2</sup>

Molecular mechanisms mediating the hair follicle cycling remain poorly understood. Nevertheless, it is important to note that the maintenance of anagen is controlled by several growth factors including the insulin-like growth factor-1 (IGF-1).<sup>2</sup> IGF-1 is involved in the positive regulation of the

dermal papilla cell (DPC) hair follicle-inductive capacity; DPC is a kind of differentiated dermal cell at the base of hair follicle, important for hair-induction, and plays the main role in developing human hair follicles. The interaction between hair follicle stem cells and DPCs plays a significant role in the regulation of hair cycling.<sup>3</sup> Keratin, collagen and micronutrients formula (Azkera), with its high protein content, affects the IGF-1 to exert its potential hair growth promoting effect (Figure 4), and therefore could approach the therapeutic solution capable of generating functional hair follicles.

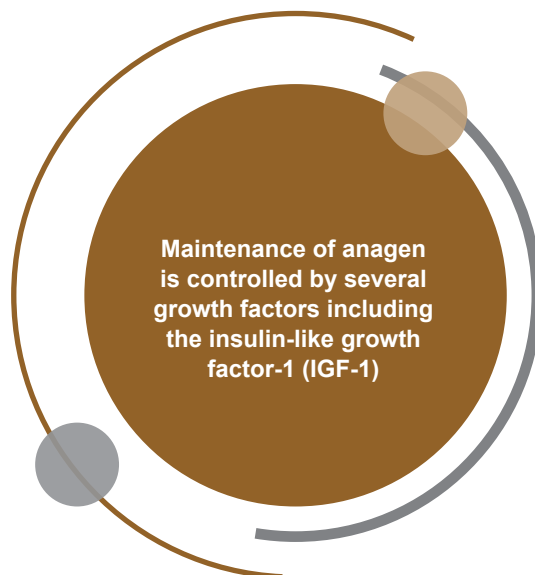
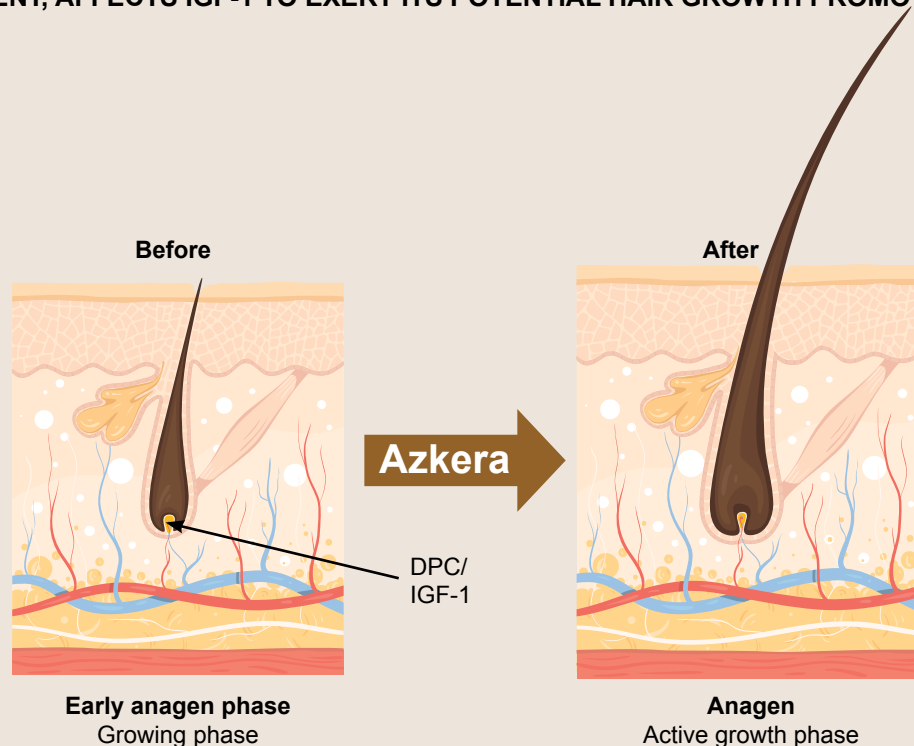




FIGURE 4

**KERATIN, COLLAGEN AND MICRONUTRIENTS FORMULA (AZKERA), WITH ITS HIGH PROTEIN CONTENT, AFFECTS IGF-1 TO EXERT ITS POTENTIAL HAIR GROWTH PROMOTING EFFECT**



**Adapted from:** 1. Houshyar KS, Borrelli MR, Tapking C, *et al.* Molecular Mechanisms of Hair Growth and Regeneration: Current Understanding and Novel Paradigms. *Dermatology* 2020;236:271–280. 2. Ji S, Zhu Z, Sun X, *et al.* Functional hair follicle regeneration: an updated review. *Signal Transduction and Targeted Therapy* 2021;6:66. 3. Preclinical study (Data on file, Apex Laboratories)

## CONCLUSION

Therapeutic manipulation of hair follicle cycling represents not only a key challenge but opportunity in the management of hair loss. A product like Azkera, with its high protein content, affects IGF-1 expression in dermal papilla cells, to exert its potential hair growth promoting effect, and therefore could approach the therapeutic solution capable of generating functional hair follicles. The paradigm for management of hair loss could thus transition from preventing hairs from falling to hair promotion and regeneration.

## REFERENCES

- Martel JL, Miao JH, Badri T. Anatomy, Hair Follicle. Treasure Island (FL): StatPearls Publishing; 2021 Jan-. Available at: <https://www.ncbi.nlm.nih.gov/books/NBK470321/> [Accessed on: 22/12/2021].
- Houshyar KS, Borrelli MR, Tapking C, *et al.* Molecular Mechanisms of Hair Growth and Regeneration: Current Understanding and Novel Paradigms. *Dermatology* 2020;236:271–280.
- Ji S, Zhu Z, Sun X, *et al.* Functional hair follicle regeneration: an updated review. *Signal Transduction and Targeted Therapy* 2021;6:66.
- Taghiabadi E, Nilforoushzadeh MA, Aghdami N. Maintaining Hair Inductivity in Human Dermal Papilla Cells: A Review of Effective Methods. *Skin Pharmacol Physiol* 2020;33:280–292.
- Tenore GC, Caruso D, Buonomo G, *et al.* Annurca Apple Nutraceutical Formulation Enhances Keratin Expression in a Human Model of Skin and Promotes Hair Growth and Tropism in a Randomized Clinical Trial. *J Med Food* 2018;21(1):90–103.
- Semalty M, Semalty A, Joshi GP, *et al.* Hair growth and rejuvenation: An overview. *Journal of Dermatological Treatment*. 2010; Early Online, 1–10.
- Trüeb RM. Further Clinical Evidence for the Effect of IGF-1 on Hair Growth and Alopecia. *Skin Appendage Disord* 2018;4:90–95.
- Guo EL, Katta R. Diet and hair loss: effects of nutrient deficiency and supplement use. *Dermatol Pract Concept*. 2017;7(1):1–10.
- Gowda D, Premalatha V, Imtiyaz DB. Prevalence of Nutritional Deficiencies in Hair Loss among Indian Participants: Results of a Cross-sectional Study. *Int J Trichology*. 2017;9(3):101–104.
- Rushton DH. Nutritional factors and hair loss. *Clin Exp Dermatol*. 2002;27(5):396–404.
- Ablon G. A 3-month, randomized, double-blind, placebo-controlled study evaluating the ability of an extra-strength marine protein supplement to promote hair growth and decrease shedding in women with self-perceived thinning hair. *Dermatol Res Pract*. 2015;2015:841570.
- MPB research. Making Hair Loss Optional. Available at: <https://www.hairloss-research.org/keratin-for-hair-growth-how-it-works-2/> [Accessed on: 23/12/2021].
- Lee H, Noh K, Lee SC, *et al.* Human Hair Keratin and Its-Based Biomaterials for Biomedical Applications. *Tissue Engineering and Regenerative Medicine* 2014;11(4):255–265.
- Rouse JG, Van Dyke ME. A Review of Keratin-Based Biomaterials for Biomedical Applications. *Materials* 2010;3:999–1014.
- Moll R, Divo M, Langbein L. The human keratins: biology and pathology. *Histochem Cell Biol* 2008;129:705–733.
- Almohanna HM, Ahmed AA, Tsatalis JP, *et al.* The Role of Vitamins and Minerals in Hair Loss: A Review. *Dermatol Ther (Heidelb)*. 2019; 9(1):51–70.
- Preclinical study (Data on file, Apex Laboratories)

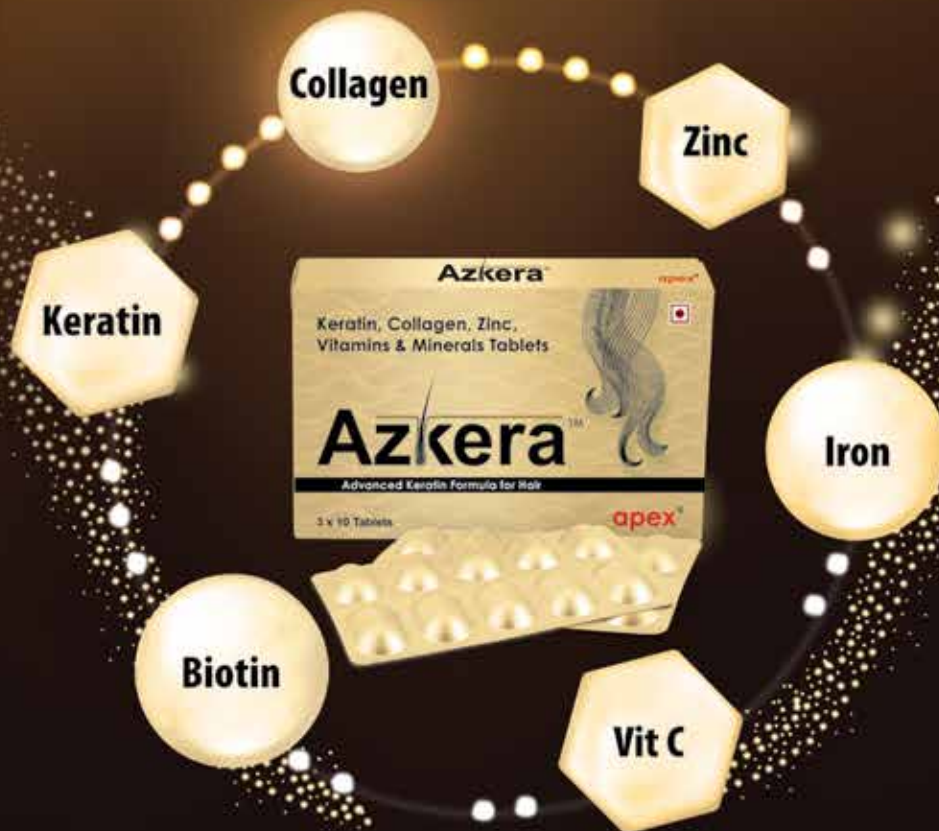
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