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A multifaceted anti-aging composition targeting the restoration of functional proteins in skin layers

Xiaoyun Liu[1,*] , Jianjun Dong[1] , Lei Wang[1] , Linhong Yang[1] , Tai'an Wang[1]

[1] Research and Development Centre, BLOOMAGE BIOTECHNOLOGY CORPORATION LIMITED., Jinan, China

1. Introduction

The skin serves as the primary interface between the human body and the external environment, with its diverse functions largely mediated or controlled by proteins. Transcriptomic analyses reveal that 71% (n=14,224) of human proteins (n=20,162) are expressed in the skin, and 602 of these genes show an elevated expression in the skin compared to other tissue types. In fact, the functional specialization of skin layers and cell types is not solely determined by the presence or absence of specific proteins but rather by their spatial distribution and quantitative abundance. A comprehensive quantitative proteomic atlas of healthy human skin demonstrated that more than 6000 proteins are expressed across all structural layers yet exhibit significant abundance variations between layers and cell types[1]. These findings align with the broader principle that tissue identity arises not from binary protein expression but from precise regulation of protein production levels, localization, and concentration dynamics[2].

A tissue-based human proteome atlas has identified skin-enriched genes that predominantly encode proteins associated with barrier function, pigmentation regulation, and hair follicle development[3]. The integrity of the epidermal barrier, which is essential for maintaining skin health, relies on the precise coordination of diverse molecular and immunoregulatory pathways[4]. Notably, CD44 has been shown to play a significant role in skin lipid barrier formation, tight junction (TJ) assembly, and keratinocyte differentiation by participating in hyaluronic acid (HA)-mediated signaling cascades, thereby modulating the structure of the stratum corneum (SC) and epidermal barrier function[5-7]. Furthermore, it has been demonstrated that the HA-CD44 signaling pathway can selectively induce different epidermal processes, such as epidermal proliferation, skin thickness, differentiation, and epidermal barrier formation; and more importantly, this pathway has the potential to improve the age-related decline in epidermal function[8]. In conclusion, these findings highlight the importance of CD44 and HA-CD44 signaling in skin barrier function and suggest that targeting this pathway may offer promising therapeutic strategies for skin-related conditions.

The epidermal function is highly dependent on the support of the dermal-epidermal junction (DEJ). DEJ not only provides structural support for keratinocytes but also creates a specific microenvironment for mediating signals that influence their behavior. By anchoring keratinocytes to the dermal compartment, the DEJ ensures mechanical cohesion between epidermal and dermal layers and regulates epithelial renewal and regeneration[9,10]. This specialized interface operates as a molecular filter, selectively governing the transport of biomolecules across epidermal-dermal boundaries based on size and charge, while simultaneously resisting mechanical stress through an elaborate network of intracellular,

transmembrane, and extracellular proteins[11]. The main structural elements of DEJ consist of two polymer networks made of laminins and collagen IV, which are primarily connected by nestin and basement membrane proteoglycans[12]. Among them, Collagen IV, the most abundant component in DEJ, accounts for over half of its mass and determines the tensile strength of the basement membrane[9,13]. Complementing this framework, type VII collagen forms anchoring fibrils that interweave with interstitial collagen fibers in the papillary dermis, functioning as structural "straps" that tether the lamina densa to the papillary dermis[14]. Overall, the DEJ plays a vital role in maintaining the normal structure and function of the skin by providing structural support, controlling biomolecular transport, and transmitting mechanical forces. Its complex network of proteins ensures the proper connection and interaction between the epidermis and dermis.

Both collagen type IV and collagen type VII mentioned above belong to the collagen family. Among the 28 identified collagen types, over 17 (including I, III, IV, V, VI, VII, VIII, XII, XIII, XIV, XV, XVI, XVII, XVIII, XIX, XXIII, XXIX) have been reported to be expressed in the skin[15-21]. Collagen proteins, vital constituents of the extracellular matrix (ECM), comprise approximately 30% of the human body's dry mass and are fundamental in preserving tissue architecture and functionality[22]. Collagens can be categorized into fiber-forming proteins (e.g., types I, II, III, V, and IX) and non-fiber-forming proteins based on their fibrous tissue structure. The collagens predominantly linked to skin structure and physiology are primarily fiber-forming types, specifically types I and III, which constitute approximately 80%-85% and 8%-11% of the dermal extracellular matrix, respectively[23,24]. These collagens assemble into robust extracellular fibers that confer tensile strength and mechanical resilience to the skin, enabling protection against external trauma. Notably, the age-related decline in the synthesis of types I and III collagen is a hallmark of skin aging, leading to structural deterioration and loss of functional integrity[25].

In this study, we use a multi-dimensional strategy to target core functional proteins across distinct cutaneous structural strata, aiming to comprehensively improve skin homeostasis and counteract aging manifestations through synergistic endogenous stimulation and exogenous supplementation. Utilizing a novel compound formulation comprised of fermented Gentiana lutea root extract, phytosterols, and recombinant type III collagen, we systematically validated its efficacy through multi-model approaches: in vitro cellular assays, 3D epidermal equivalents, and human clinical trials complemented by metaproteomic analysis. Different from other studies, our research focuses on the combined effects of functional proteins at the core of different structural levels of human skin, aiming to exert a comprehensive anti-aging effect from the changes/improvements at the protein level.

2. Materials and Methods

2.1 Quantitation of CD44 synthesis

Immunodetection and quantification of CD44 were performed on NHEK from 55-year-old donors treated for 48 hours with complex 0.5%, or nothing (control). Each condition was conducted in 6 replications, and variations of CD44 expression were expressed as a percentage of untreated cells (control). CD44 was detected by immunostaining using a specific primary monoclonal antibody and a secondary antibody coupled to fluorochrome. Quantification of fluorescence staining was performed on 2 distinct images per replicate by integration of the specific fluorescence signal normalized by the number of cells and then expressed as a percentage of relative fluorescent units normalized to untreated NHEK (control).

2.2 Measurement of Decorin and Tight Junction Markers

Gene expression analysis was performed by RT-qPCR on RNA samples from NHEK incubated for 24 h with complex 1%. Total RNAs of each condition were extracted and purified. cDNA synthesis was performed using reverse transcription (RT) before their amplification by quantitative polymerase chain reaction (qPCR). Relative mRNA expression of indicated genes was determined by the comparative $\Delta\Delta$Ct method, with 2 housekeeping genes used

for data normalization. Each experimental condition was performed in triplicate. Variations of mRNA expression were expressed as a percentage of the untreated condition (control).

2.3 Measurement of type I collagen and type III collagen

The in vitro dermal regenerative potential of the complex was assessed utilizing fibroblast cell cultures. Human dermal fibroblasts were incubated with a 1% concentration of the complex for a duration of 48 hours, with cells cultivated in the absence of the complex serving as controls. Subsequent quantification of type I and type III collagen levels was performed employing ELISA methodology.

2.4 Measurement of type IV collagen and type VII collagen

2.4.1 Preparation of 3D skin models

The 3D skin models were cultured in a special 12-well tray with inserts. First of all, fibroblasts were seeded at a density of 25×10^4 cells/cm^2 onto a dermal substrate made of chitosan-cross-linked collagen–GAG matrix prepared as previously described. Complex at 1% was added 7 days after fibroblasts were seeded (D7) and until the end (Day 35). At day 21, keratinocytes were seeded on the dermal equivalent at a density of 25×10^4 cells/cm^2. At day 28 (D28), the three-dimensional models were cultured at the air-liquid interface, leaving the epidermal compartment air-exposed to stimulate cellular differentiation. Finally, at D35, reconstructed skins were harvested and processed for histological and immunohistochemical analysis. They were fixed in neutral buffered formalin 4% for 24 h and embedded in paraffin or in optimal cutting temperature (OCT) compound and frozen at −20°C. Paraffin-embedded formalin-fixed samples were then cut into 5 µm sections. After dewaxing and rehydration, sections were stained with haematoxylin and eosin (H&E) for routine histology or immunohistochemistry.

2.4.2 Collagen expression assay

Fluorescence staining of type IV collagen and type VII collagen was performed, and the staining intensity was measured on a 3D skin model using a 1% complex, with the blank test group serving as a control group.

2.5 Clinical test

2.5.1 Metaproteomic analysis

A clinical study was conducted and followed by a metaproteomic analysis of skin swabs. This study allows the identification of more than 6000 proteins expressed by human skin, bacteria, and fungi.

The first group (group 1) is composed of 20 volunteers aged between 40 and 55 years old (mean age: 48 years). The second group (group 2) is composed of 20 healthy female volunteers aged between 25 and 35 years old. In this study, we compared the protein interactions between these two groups at baseline (D0) and after 56 days of applying the complex. By analyzing the metaproteomic changes before and after product application in group 1, we aimed to evaluate the complex's potential in reversing age-related changes in the function and composition of skin proteins.

2.5.2 Anti-wrinkles assay

30 subjects aged between 40 and 55 years old were involved in the test. The subjects have applied complex 2% or placebo twice a day, in the morning and evening, for 4 weeks. Measuring facial skin conditions with Visia®.

3. Results

3.1 Quantification of CD44 synthesis

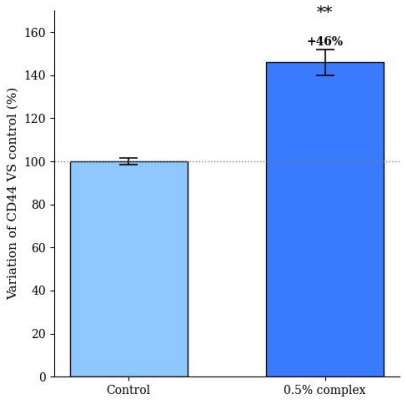

Figure 1. Quantification of CD44 synthesis in NHEK after treatment with 0.5% complex. Mean± SD, statistical significance: **p<0,01 vs. control

Upon exposure to a composition concentration of 0.5%, a 46% augmentation in CD44 content was observed. This alteration in CD44 levels suggests that the cutaneous barrier function, which encompasses processes such as skin lipid production, tight junction functionality, and keratinocyte differentiation, could potentially be enhanced following treatment with the composition.

3.2 Quantification of decorin and tight junction markers

Decorin, a small leucine-rich proteoglycan, plays a critical regulatory role in epidermal terminal differentiation. Concurrently, tight junctions — specialized intercellular junctions comprising structural components such as claudin-1 and occludin—are established between adjacent keratinocytes in the stratum granulosum. These junctional complexes form a paracellular barrier essential for maintaining epidermal permeability homeostasis and preventing transepidermal water loss.

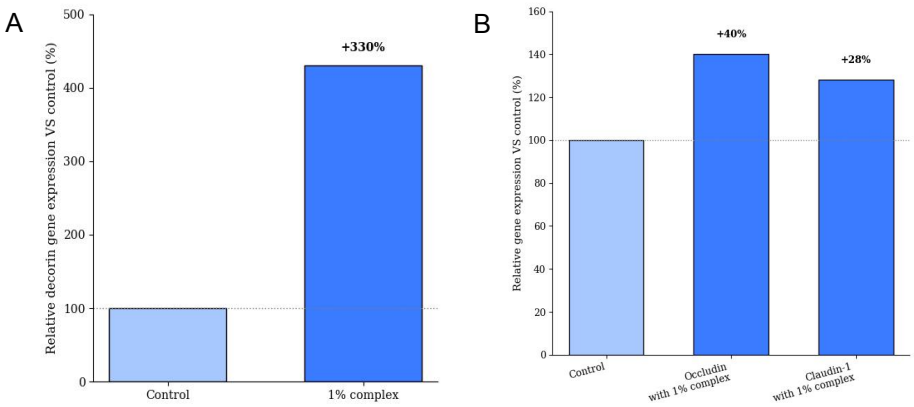

Figure 2. Relative expression of genes related to epidermal differentiation（A）and cell cohesion（B）in keratinocytes after the complex treatment.

The complex upregulates decorin gene expression by +330% in treated keratinocytes compared to untreated keratinocytes (control). Moreover, tight junction markers, occludin and claudin-1 gene expression were also increased by respectively +40% and +28% in keratinocytes treated with the complex compared to untreated NHEK (control). The complex

upregulates genes coding for epidermal differentiation and granular layer cohesion to reinforce the skin barrier.

3.3 Quantification of type I collagen and type III collagen

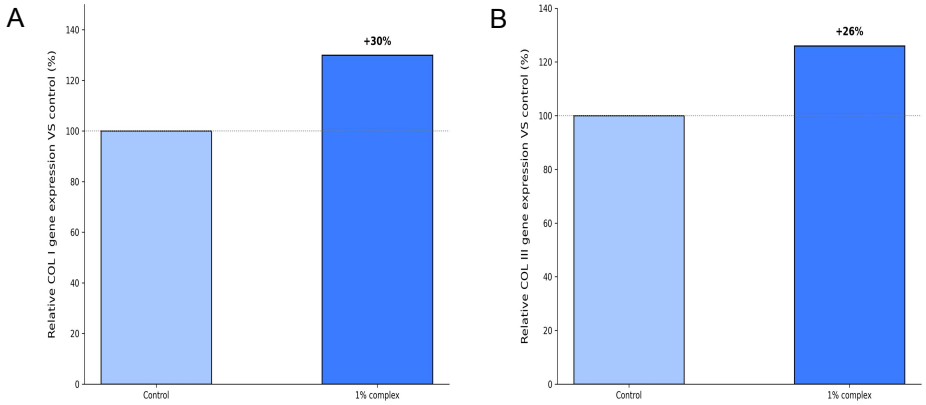

Figure 3. The complex increases type I collagen（A）and type III collagen（B） expression.

The significant upregulation of both Type I and Type III collagen following treatment with the 1% complex demonstrates compelling anti-aging efficacy through fundamental extracellular matrix (ECM) restoration.

3.4 Quantification of type IV collagen and type VII collagen

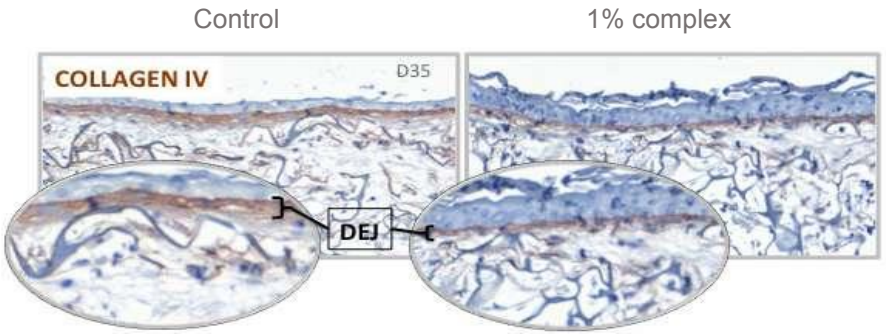

Figure 4. The complex improves deposition of collagen IV at the DEJ.

Although type IV collagen, a major component of the basement membrane composition, declines with age, the total thickness of this membrane increases as tissue renewal decreases[26]. Figure 4 shows that the complex ingredient improves collagen IV distribution showing a better organization and modelling of basement membrane.

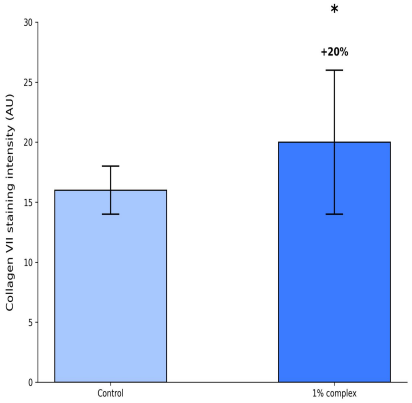

Figure 5. The complex increases collagen VII expression at the DEJ.

*p<0.05, Student's t-test

Figure 5 shows that the complex increases the expression of collagen VII, which is a component of anchoring fibrils essential for the cohesion of the dermal-epidermal junction. Consequently, the complex ingredient improves the morphogenesis of the DEJ and strongly suggests beneficial effects in aged skin from restoring BM components and DEJ integrity.

3.5 Clinical test

3.5.1 Metaproteomic analysis

	Dehydrated older D0	The complex D56
Skin Barrier Preservation*		
Microbial Ecological Balance*		
Skin Immune*		
ECM(Extracellular Matrix)Protein Synthesis*		
Inflammatory Stress*		
Cellular Metabolism*		

Downregulation Upregulation

Figure 6. Regulation and clustering by main functions of proteins significantly regulated by 56 days of treatment with the complex of 1% vs. D0.

Proteins from human, bacterial and fungal origin were clustered according to their functions and expression modulation (down-regulated: red, upregulated:blue), *p<0,05

The protein expression profile in the older group could show functional restoration toward a more youthful state.

Therefore, this complex ingredient restores the youthful holobiont protein functions. As shown in our study, with aging and dehydration, skin barrier function weakens. As indicated in Figure 10, after 56 days of treatment, the complex ingredient restores youthful protein functions by upregulating proteins involved in skin barrier protection and dermal matrix, while downregulating inflammation stress proteins. Moreover, treated skin also showed improvements in natural immune defenses and cellular metabolism, with the upregulation of related proteins from both human and microbial origins.

3.6.2 Anti-wrinkles

Following 28 days of topical application with a 2% complex formulation, a statistically significant reduction in periocular wrinkle severity was observed in volunteer subjects compared to baseline measurements (D0/T0) (Fig. 7).

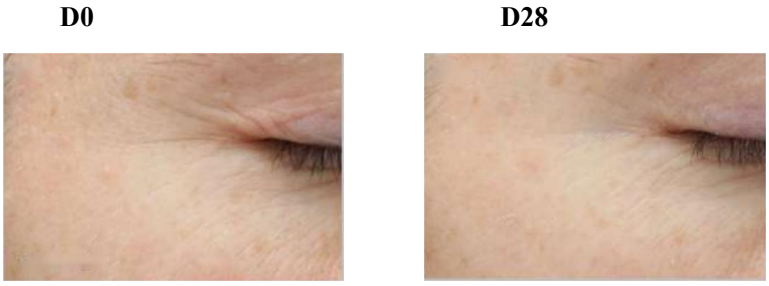

Figure 7. The complex of 2% visibly reduces the appearance of wrinkles, and existing fine lines.

4. Discussion

Skin aging is an inevitable biological process characterized by progressive degradation of extracellular matrix components, most notably collagen[27]. However, this phenomenon extends beyond collagen depletion—core functional proteins across all cutaneous layers undergo quantitative and qualitative alterations due to chronological aging and photoaging. These changes manifest as dysregulated protein production, aberrant localization, and disrupted concentration dynamics, collectively impairing skin homeostasis. With advancements in omics technologies, emerging multi-omics approaches now enable systematic investigation of these age-related proteomic perturbations. In this study, we employed metaproteomic analysis to elucidate the interplay between the test formulation, epidermal protein networks, and the skin microbiome, thereby providing a mechanistic understanding of its anti-aging effects.

The outcomes from both in vitro and in vivo experiments corroborate the complex's potential in alleviating signs of aging. Specifically, the complex increased the expression of key proteins, such as CD44 and TJ markers, which are critical for maintaining the skin barrier and its integrity. Moreover, the upregulated expression of collagen I and collagen III in fibroblasts, along with the organized expression or enhanced expression of collagen IV and collagen VII in 3D skin models, indicates a reinforcement of the dermis and DEJ. Metaproteomic analysis further emphasized the complex's role in restoring proteomic balance and bolstering skin barrier preservation, particularly in aged skin. The clinical trial results provided further confirmation of these findings, showing marked reductions in wrinkles and a more youthful skin appearance after just 28 days of treatment. These results indicate that the complex works in a synergistic manner by both supplying exogenous proteins and stimulating endogenous protein production, thus presenting a promising approach for anti-aging skincare.

5. Conclusion

In this study, we validated the efficacy of a complex that mitigates skin aging by enhancing epidermal barrier function and strengthening the structural integrity of the DEJ and dermis. In conclusion, enhancing the expression or structural organization of core functional proteins across different skin layers represents a promising strategy to counteract skin aging.

References.

[1] Dyring-Andersen B, Løvendorf MB, Coscia F, et al. Spatially and cell-type resolved quantitative proteomic atlas of healthy human skin. Nat Commun. 2020;11(1):5587. Published 2020 Nov 5. doi:10.1038/s41467-020-19383-8

[2] Pontén F, Gry M, Fagerberg L, et al. A global view of protein expression in human cells, tissues, and organs. Mol Syst Biol. 2009;5:337. doi:10.1038/msb.2009.93

[3] Uhlén M, Fagerberg L, Hallström BM, et al. Tissue-based map of the human proteome. Science. 2015;347(6220). doi:10.1126/science.1260419

[4] Baker P, Huang C, Radi R, Moll SB, Jules E, Arbiser JL. Skin Barrier Function: The Interplay of Physical, Chemical, and Immunologic Properties. Cells. 2023;12(23):2745. Published 2023 Nov 30. doi:10.3390/cells12232745

[5] Bourguignon LYW, Ramez M, Gilad E, et al. Hyaluronan–CD44 Interaction Stimulates Keratinocyte Differentiation, Lamellar Body Formation/Secretion, and Permeability Barrier Homeostasis. Journal of Investigative Dermatology. 2006;126(6):1356-1365. doi:10.1038/sj.jid.5700260

[6] Kirschner N, Haftek M, Niessen CM, et al. CD44 Regulates Tight-Junction Assembly and Barrier Function. Journal of Investigative Dermatology. 2010;131(4):932-943. doi:10.1038/jid.2010.390

[7] Bourguignon LY. Matrix hyaluronan-activated CD44 signaling promotes keratinocyte activities and improves abnormal epidermal functions. Am J Pathol. 2014;184(7):1912-1919. doi:10.1016/j.ajpath.2014.03.010

[8] Bourguignon LY, Wong G, Xia W, Man MQ, Holleran WM, Elias PM. Selective matrix (hyaluronan) interaction with CD44 and RhoGTPase signaling promotes keratinocyte functions and overcomes age-related epidermal dysfunction. J Dermatol Sci. 2013;72(1):32-44. doi:10.1016/j.jdermsci.2013.05.003

[9] Roig-Rosello E, Rousselle P. The Human Epidermal Basement Membrane: A Shaped and Cell Instructive Platform That Aging Slowly Alters. Biomolecules. 2020;10(12):1607. Published 2020 Nov 27. doi:10.3390/biom10121607

[10] Tsutsui K, Machida H, Nakagawa A, et al. Mapping the molecular and structural specialization of the skin basement membrane for inter-tissue interactions. Nat Commun. 2021;12(1):2577. Published 2021 May 10. doi:10.1038/s41467-021-22881-y

[11] Aleemardani M, Trikić MZ, Green NH, Claeyssens F. The Importance of Mimicking Dermal-Epidermal Junction for Skin Tissue Engineering: A Review. Bioengineering (Basel). 2021;8(11):148. Published 2021 Oct 20. doi:10.3390/bioengineering8110148

[12] Sekiguchi R, Yamada KM. Basement Membranes in Development and Disease. Curr Top Dev Biol. 2018;130:143-191. doi:10.1016/bs.ctdb.2018.02.005

[13] Hashmi S, Marinkovich MP. Molecular organization of the basement membrane zone. Clin Dermatol. 2011;29(4):398-411. doi:10.1016/j.clindermatol.2011.01.009

[14] Ko MS, Marinkovich MP. Role of dermal-epidermal basement membrane zone in skin, cancer, and developmental disorders. Dermatol Clin. 2010;28(1):1-16. doi:10.1016/j.det.2009.10.001

[15] Sorushanova A, Delgado LM, Wu Z, et al. The Collagen Suprafamily: From Biosynthesis to Advanced Biomaterial Development. Adv Mater. 2019;31(1):e1801651. doi:10.1002/adma.201801651

[16] Sun W, Shahrajabian MH, Ma K, Wang S. Advances in Molecular Function and Recombinant Expression of Human Collagen. Pharmaceuticals. 2025; 18(3):430. https://doi.org/10.3390/ph18030430

[17] Theocharidis G, Connelly JT. Minor collagens of the skin with not so minor functions. J Anat. 2019;235(2):418-429. doi:10.1111/joa.12584

[18] Shuttleworth CA. Type VIII collagen. Int J Biochem Cell Biol. 1997;29(10):1145-1148. doi:10.1016/s1357-2725(97)00033-2

[19] Bretaud S, Guillon E, Karppinen SM, Pihlajaniemi T, Ruggiero F. Collagen XV, a multifaceted multiplexin present across tissues and species. Matrix Biol Plus. 2020;6-7:100023. Published 2020 Jan 15. doi:10.1016/j.mbplus.2020.100023

[20] Bonnet I, Jobeili L, Cadau S, et al. Collagen XVIII: A key interfacial component of the skin architecture. J Cosmet Sci. 2017;68(1):35-41.

[21] Söderhäll C, Marenholz I, Kerscher T, et al. Variants in a novel epidermal collagen gene (COL29A1) are associated with atopic dermatitis. PLoS Biol. 2007;5(9):e242. doi:10.1371/journal.pbio.0050242

[22] Deshmukh SN, Dive AM, Moharil R, Munde P. Enigmatic insight into collagen. J Oral Maxillofac Pathol. 2016;20(2):276-283. doi:10.4103/0973-029X.185932

[23] Reilly DM, Lozano J. Skin collagen through the lifestages: importance for skin health and beauty. Plastic and Aesthetic Research. 2021;8:2. doi:10.20517/2347-9264.2020.153

[24] Davison-Kotler E, Marshall WS, García-Gareta E. Sources of Collagen for Biomaterials in Skin Wound Healing. Bioengineering (Basel). 2019;6(3):56. Published 2019 Jun 30. doi:10.3390/bioengineering6030056

[25] Varani J, Dame MK, Rittie L, et al. Decreased collagen production in chronologically aged skin: roles of age-dependent alteration in fibroblast function and defective mechanical stimulation. Am J Pathol. 2006;168(6):1861-1868. doi:10.2353/ajpath.2006.051302

[26] Vázquez F, Palacios S, Alemañ N, Guerrero F. Changes of the basement membrane and type IV collagen in human skin during aging. Maturitas. 1996;25(3):209-215. doi:10.1016/s0378-5122(96)01066-3

[27] Wang S, Li F, Feng X, et al. Promoting collagen synthesis: a viable strategy to combat skin ageing. J Enzyme Inhib Med Chem. 2025;40(1):2488821. doi:10.1080/14756366.2025.2488821