

Mitigating Glycation and Oxidative Stress in Aesthetic Medicine: Hyaluronic Acid and Trehalose Synergy for Anti-AGEs Action in Skin Aging Treatment

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Abstract: This comprehensive review explores the pivotal roles of glycation and oxidative stress in the aging process of the skin, emphasizing their targeted therapeutic applications in aesthetic and regenerative medicine, as well as anti-aging interventions. Glycation, a biochemical process involving the non-enzymatic attachment of sugars to proteins, lipids, or nucleic acids, culminates in the formation of Advanced Glycation End products (AGEs). These AGEs are significant contributors to aging and various chronic ailments, triggering oxidative stress and inflammatory pathways, thereby manifesting as wrinkles, diminished skin elasticity, and other age-related dermal alterations. A central focus of this review is the synergistic interplay between Hyaluronic Acid (HA) and Trehalose in combating these aging mechanisms. HA, renowned for its anti-inflammatory and antioxidative properties, assumes a pivotal role in modulating Reactive Oxygen Species (ROS) levels and safeguarding against oxidative damage. Concurrently, trehalose targets glycation and oxidative stress, exhibiting promising outcomes in augmenting skin health, providing Ultraviolet B (UVB) photoprotection, and manifesting notable anti-photoaging effects. The combined administration of HA and trehalose not only addresses existing skin damage but also confers preventive and reparative benefits, particularly in stabilizing HA and mitigating glycation-induced stress. Their synergistic action significantly enhances skin quality and mitigates inflammation. The implications of these findings are profound for the future of anti-aging therapeutics in aesthetic medicine, suggesting that the integration of HA and trehalose holds promise for revolutionary advancements in preserving skin vitality and health. Moreover this paper underscores the imperative for continued research into the combined efficacy of these compounds, advocating for innovative therapeutic modalities in aesthetic medicine and enhanced strategies for combating aging, glycation, and oxidative stress.

Keywords: hyaluronic acid, trehalose, skin aging, glycation, oxidative stress, anti-aging therapies

Introduction

Glycation and oxidative stress are critical factors in the aging of skin, making them important therapeutic targets in anti-aging treatments and regenerative medicine. Glycation, a biochemical reaction where sugars bind non-enzymatically to proteins, lipids, or nucleic acids, results in the formation of Advanced Glycation End products (AGEs). These AGEs, accumulating under conditions of oxidative stress, initiate a cascade of reactions that accelerate aging and exacerbate various chronic diseases. Significantly, in the realm of dermatology, these changes manifest visibly on the skin as wrinkles, decreased elasticity, and other age-related alterations, primarily due to the glycation of vital structural proteins like collagen.

In clinical practice, inhibiting glycation is essential, as the accumulation of AGEs in the skin contributes to visible signs of aging, such as wrinkles and reduced elasticity, which are exacerbated by external factors like ultraviolet (UV)

radiation. Collagen, a key structural protein in the skin, is particularly susceptible to glycation, which leads to its stiffening and impaired function. Additionally, glycation-induced modifications can disrupt the extracellular matrix and hinder processes like wound healing, further contributing to age-related skin issues.

Current treatments to counteract glycation focus primarily on lifestyle interventions, such as reducing sugar intake and limiting exogenous AGEs from diet and tobacco. While some treatments, such as antioxidants and compounds like aminoguanidine, show promise in mitigating glycation, their efficacy is often limited, particularly in advanced stages of aging where glycation damage has already accumulated. Moreover, these treatments do not fully address the oxidative stress and inflammatory pathways that also play a pivotal role in accelerating skin aging.

Given these limitations, there is a need for more comprehensive approaches that target both glycation and oxidative stress. This review explores the intricate roles of Hyaluronic Acid (HA) and trehalose in mitigating these detrimental processes. HA, a polysaccharide naturally present in the human body, possesses notable anti-inflammatory and antioxidant properties, effectively countering the damaging effects of oxidative stress and inflammation. Concurrently, trehalose, a naturally occurring disaccharide, targets glycation and oxidative stress, exhibiting promising outcomes in augmenting skin health, providing UVB photoprotection, and showing anti-photoaging effects. Together, HA and trehalose offer a synergistic approach in aesthetic medicine, as they not only mitigate existing damage but also provide preventive benefits. The combined administration of these compounds stabilizes HA and reduces glycation-induced stress, significantly enhancing skin quality and reducing inflammation.

The article aims to delve into the synergistic effects of HA and trehalose, highlighting their potential as innovative approaches in aesthetic and therapeutic anti-aging treatments. The convergence of these compounds in combatting the underlying biochemical pathways of aging offers a promising frontier in dermatology and regenerative medicine.

The Role of Glycation and AGEs in Skin Aging and Oxidative Stress

Glycation, also known as non-enzymatic glycosylation, is the process where sugars attach to molecules like proteins, lipids, or nucleic acids. This phenomenon is crucial for understanding the mechanisms underlying aging, particularly concerning skin health and oxidative stress, often referred to as “inflammaging”. AGEs are intricate molecules associated with aging and chronic diseases, which can either be ingested through food or synthesized within the body. In pathological conditions, such as diabetes mellitus, there is a notable acceleration in the metabolic pathways leading to AGE synthesis. It is theorized that the rate of AGE formation depends on various factors, including the availability of carbohydrate-derived precursors (particularly, intermediate products of glycation), the levels of reactive oxygen species within the organism, and the dynamic process of protein turnover.

The formation of endogenous AGEs occurs in three stages within the Maillard reaction (Figure 1). Initially, glucose binds to free amino groups in molecules such as proteins, forming Schiff bases, which then rearrange into Amadori products. These Amadori products subsequently break down into highly reactive dicarbonyls, which serve as precursors to AGEs. The final stage involves these dicarbonyls reacting to form stable, brownish AGEs. Additionally, other pathways such as the Hodge, Wolff, and acetyl alcohol pathways also contribute to AGE formation, often involving free radicals and metal ions. Exogenous AGEs, primarily derived from food and tobacco, are similar to those produced internally and are abundant in heat-treated Western diets. The body can absorb these dietary AGEs, contributing to inflammation and oxidative stress, which exacerbate various chronic diseases. Reducing dietary intake of AGEs can help manage these conditions by decreasing chronic inflammation.^{1–3}

AGEs, in high concentrations, are significant contributors to aging and the exacerbation of numerous degenerative diseases, including diabetes, atherosclerosis, chronic kidney disease, and Alzheimer’s disease.^{1,4} AGEs accumulate in tissues as a result of oxidative stress conditions. They can bind to the Receptor for AGE (RAGE) and initiate oxidative stress and inflammatory pathways in cells. This process leads to the modification of proteins, enzymes, lipids, and nucleic acids, altering their properties and functions. Key protein residues, like lysine and arginine, are involved in the active sites of enzymes, and their modification by AGEs can lead to enzyme inactivation. AGEs also serve as catalytic sites for free radical formation, exacerbating intracellular oxidative stress and increasing Reactive Oxygen Species (ROS) production through various mechanisms, including reduced activity of superoxide dismutase (SOD) and catalase, decreased glutathione storage, and activation of protein kinase C.⁵ Specific receptors for AGEs, most notably RAGE,

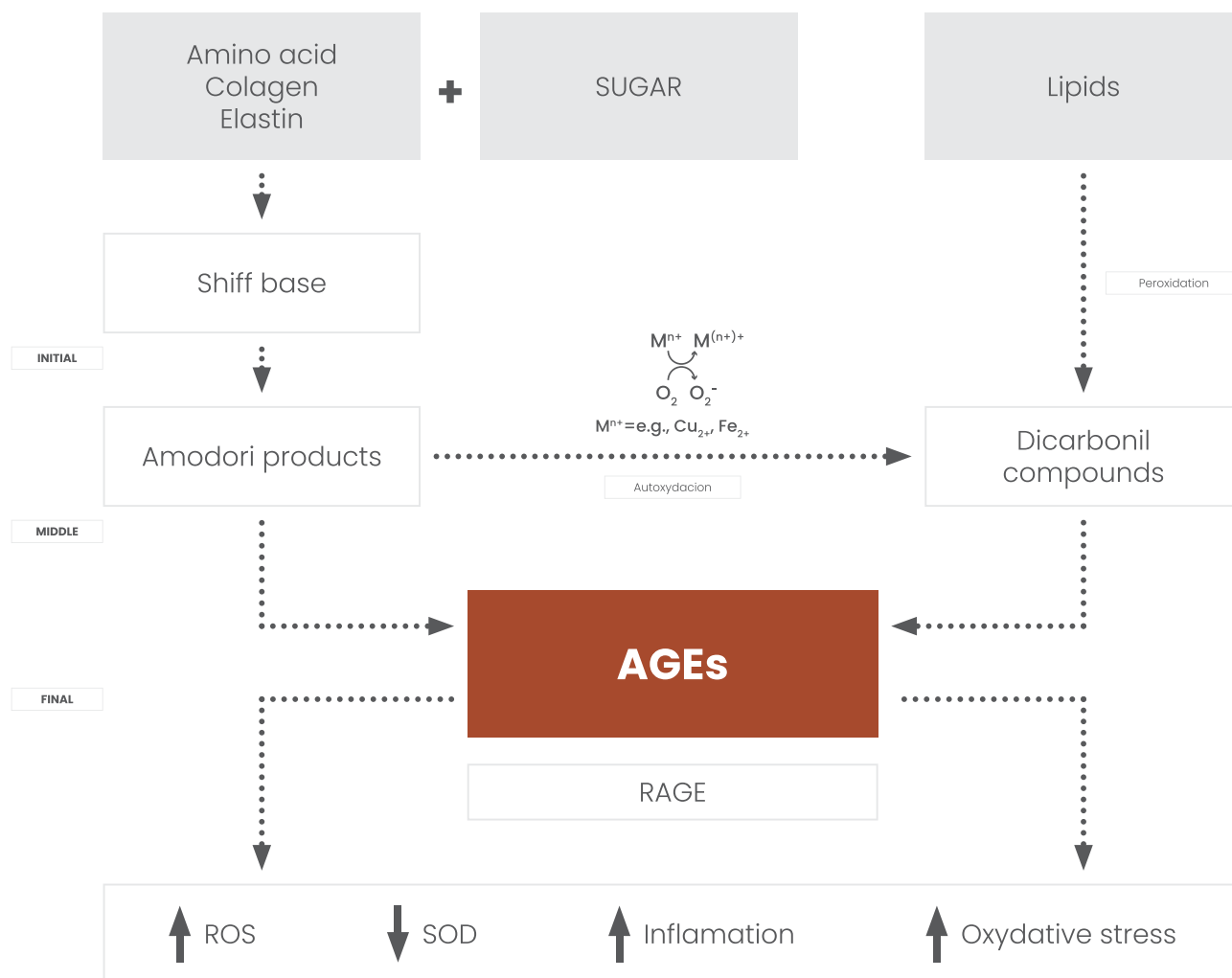


Figure 1 Schematic representation of AGEs formation, showing progression from initial sugar-protein interactions to final AGEs products and their role in oxidative stress induction.

are expressed on various cell surfaces. Upon binding AGEs, these receptors activate signaling pathways such as NF- κ B kinase (Nuclear Factor kappa-light-chain-enhancer of activated B cells) and Mitogen-Activated Protein (MAP), increasing the production of Matrix Metalloproteinases (MMPs) and inducing heightened intracellular ROS and cytokine production. This leads to a vicious cycle of chronic inflammation and oxidative stress, contributing to age-related and diabetic pathologies like atherosclerosis, asthma, arthritis, myocardial infarction, nephropathy, retinopathy, periodontitis, and neuropathy.^{6–8}

In the context of skin aging, the accumulation of AGEs intensifies with age and is exacerbated by exogenous factors such as ultraviolet (UV) radiation, leading to the development of wrinkles, loss of elasticity, dull yellowing, and other skin issues. UVB and UVA rays have distinct but significant effects on the skin. UVA exposure, in particular, is closely linked to glycation, oxidative stress, and collagen damage, all of which contribute to the aging process. UVA irradiation enhances the formation of superoxide anion radicals (O_2^-) and hydroxyl radicals ($OH^\bullet$), increasing oxidative stress in the dermal matrix. This results in damage to dermal fibroblasts, accelerated formation of glycation products, and a reduction in glyoxalase activity, which normally detoxifies harmful AGE precursors. Additionally, glycated collagen and elastin become resistant to degradation, leading to their accumulation and further contributing to skin aging and elastic tissue proliferation. UVA also induces melanin production via the RAGE signaling pathway, contributing to skin pigmentation. In contrast, UVB rays are more directly associated with DNA damage and inflammation in the epidermis, often leading to sunburn and increased risk of skin cancers.¹ Hyaluronic acid, due to its anti-inflammatory and

antioxidant properties, offers a level of protection against both UVB and UVA rays, mitigating oxidative stress and contributing to the skin's defense mechanisms in the aging process. Both the dermis and epidermis undergo aging, photoaging, and react to glycation, but these skin layers respond in distinct ways to external factors like UV exposure and AGEs. The epidermis primarily experiences oxidative stress and reduced production of enzymes like GLO-2 under UV exposure, leading to the accumulation of AGEs and associated skin issues such as pigmentation, dullness, and wrinkles. In contrast, the dermis is more affected by the glycation of structural proteins like collagen and elastin, which have slower turnover rates. Glycation in the dermis leads to stiffness, loss of elasticity, and macula formation due to the crosslinking of AGEs with these proteins (Figure 2).¹ Glycation, particularly of skin proteins like collagen, is a fundamental contributor to endogenous skin aging. Symptoms such as reduced mechanical stress resistance, impaired wound healing, and distorted vascularization can be partially attributed to glycation. AGEs alter both the physical and biological properties of extracellular matrix proteins, notably collagen, and their accrual results in significant modifications to skin structure and function.^{5,9}

Interventions to reduce AGE formation offer promising therapeutic options. These include limiting the intake of sugar and exogenous AGEs along with a well-functioning internal defense mechanism against glycation. Enzymatic degradation of AGEs, followed by renal excretion, is instrumental in this process. The glyoxalase system, serves as the foremost pathway for detoxifying reactive dicarbonyls and thus plays a critical role in AGEs detoxification. Additionally, the DJ-1/Park7 pathway is capable of repairing early-stage glycated intermediates and affected guanine residues in nucleic acids. The Ubiquitin-proteasome System (UPS) and the Autophagic Lysosomal Proteolytic System (ALPS) both facilitate the removal of AGEs, either independently or in tandem. Notably, the autophagy system is particularly relevant for decomposing polymer complexes unmanageable by the proteasome. The presence of Oxidized Protein Hydrolase (OPH) in human erythrocyte cytosol, which degrades glycated proteins, can be potentiated by certain herbal extracts. Receptors like AGER1 (AGE Receptor 1) mitigate oxidative stress and inflammation by binding and degrading AGEs, thus reducing RAGE expression, but excessive AGEs intake can deplete AGER1, worsening inflammation and insulin resistance. Soluble forms of RAGE receptor (sRAGE) act protectively by competitively inhibiting RAGE and removing AGEs, thereby preventing tissue damage and decelerating age-related chronic diseases. There are numerous therapeutic

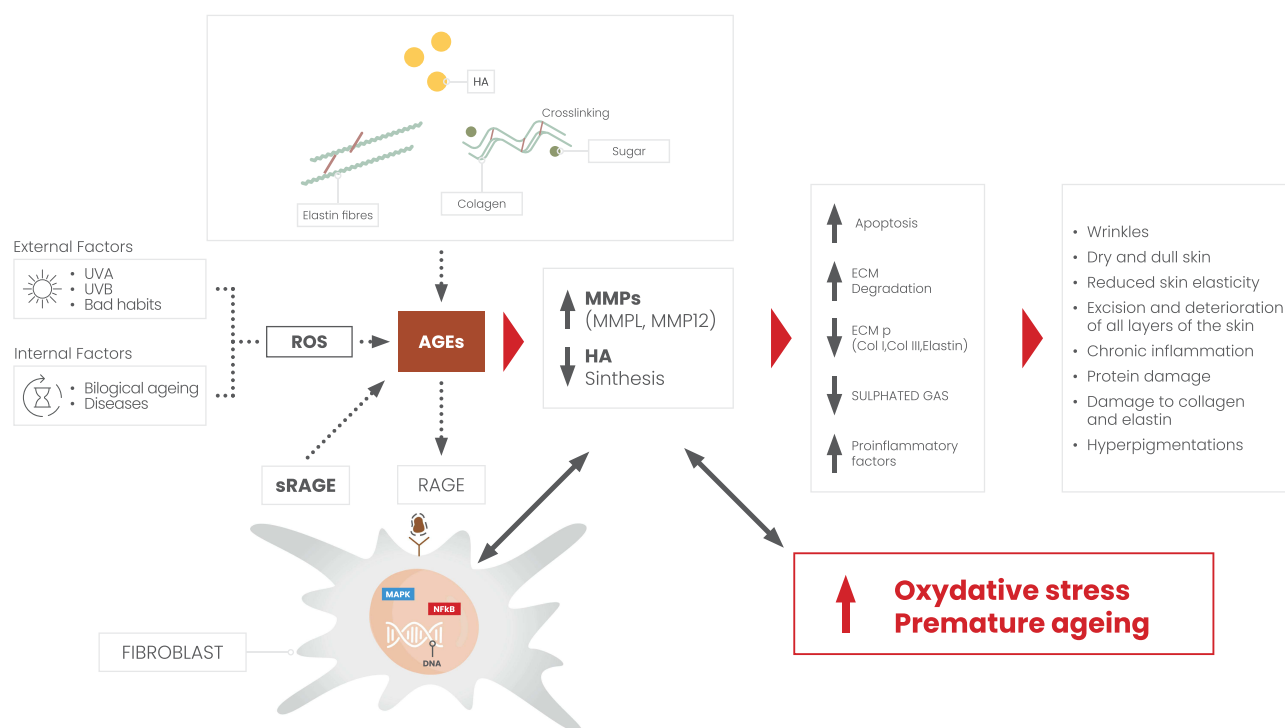


Figure 2 Molecular mechanisms of skin aging, showing the interplay between external and internal factors leading to oxidative stress, extracellular matrix degradation, and visible signs of aging through AGEs-mediated pathways.

agents targeting AGEs, encompassing synthetic compounds, natural substances, dietary components, and vitamins. These agents exert their effect by neutralizing free radicals generated during saccharification, trapping dicarbonyls, chelating metal ions, and disrupting the covalent crosslinks within AGEs.^{1,10,11} (Figure 1).

In summary, glycation and the consequent accumulation of AGEs play a central role in the process of oxidative stress and skin aging. This complex relationship underscores the importance of comprehending glycation mechanisms and developing targeted interventions to mitigate its impact on aging and associated diseases.

Hyaluronic Acid and Its Properties

Hyaluronic acid (HA), a naturally occurring polysaccharide, is a polymer composed of dimers of D-glucuronic acid and D-N-acetylglucosamine linked by β -1,4 and β -1,3 glycosidic bonds. Hyaluronic acid can be categorized by molecular weight: Low molecular weight HA (LMW HA) is less than 1000 kDa, while high molecular weight HA (HMW HA) is above 1000 kDa. HA is synthesized by three HA synthases: HAS1, HAS2, HAS3. These enzymes, embedded in the cellular membrane, produce HA polymers of various sizes. HAS1, being the least active, along with HAS2, regulates the synthesis of high molecular weight HA. HAS2 is more active than HAS1, and its upregulation is involved in wound healing, inflammation, and tissue growth. HAS3 is the most active and drives the synthesis of low molecular weight HA. These isozymes are under separate control of growth factors such as fibroblast growth factor, platelet-derived growth factor, EGF (Epidermal Growth Factor), and cytokines like TGF- α (Transforming Growth Factor alpha) and TGF- β (Transforming Growth Factor beta).^{12–15} In physiological conditions, HA is synthesized as a macromolecule exceeding 100 kDa.^{12,13}

HA degradation occurs through hyaluronidases such as HYAL1 and HYAL2 following internalization with the CD44 receptor. HYAL2 cleaves HA into fragments around 20 kDa in size, which are then endocytosed. HYAL1, located in intracellular organelles like lysosomes, further breaks down these fragments into tetrasaccharides. Additionally, exoglycosidases, β -glucuronidase, and β -N-acetyl-glucosaminidase degrade these fragments into monosaccharides. The breakdown can also involve receptor HARE; CEMIP for HMW HA and free radical-mediated degradation. Furthermore, areas of high oxidative stress can lead to the degradation of HA into LMW fragments, exacerbating inflammation and creating a cycle of chronic inflammation, oxidative stress, and HA degradation^{16–19} (Figure 3).

Hyaluronic acid is a significant molecule in the field of biomedical research, particularly due to its remarkable anti-inflammatory and antioxidant properties.^{12,13} These properties are pivotal in counteracting cellular damage induced by oxidative stress, which involves an imbalance of reactive oxygen species (ROS) and antioxidative defenses. The antioxidative action of HA is evident in numerous experimental models. For example, studies involving mechanical compression of cartilage have shown that HA application can substantially decrease ROS production.¹⁷ Similarly, animal studies have indicated a reduction in nitric oxide (NO) levels upon HA treatment, further corroborating its antioxidative effectiveness.²⁰ Moreover, HA actively modulates cellular responses, particularly influencing the expression of matrix metalloproteinases (MMPs) and aggrecanases involved in tissue remodeling. This modulation is significantly mediated through HA's interaction with CD44 receptors, with in vitro studies highlighting the critical role of these receptor-mediated pathways in HA's antioxidative action.

Advanced glycation end-products (AGEs) play a significant role in impaired wound healing by promoting chronic inflammation and disrupting the normal healing process. The binding of AGEs to their receptor, RAGE, activates the transcription factor NF- κ B, which in turn increases the production of inflammatory cytokines such as IL-6, IL-1 α , and TNF- α . This sustained inflammation prolongs the early stages of wound healing, preventing progression to later stages. Elevated levels of AGEs also lead to increased matrix metalloproteinase (MMP) activity, which breaks down collagen and further impairs wound healing. In diabetic wounds, AGEs disrupt the function of critical cells such as macrophages, fibroblasts, and neutrophils, impairing processes like collagen production, cell proliferation, and immune responses. This leads to delayed healing, increased risk of infection, and complications such as excessive oxidative stress. Additionally, AGEs induce apoptosis in fibroblasts, reducing their ability to contribute to wound repair. Overall, the accumulation of AGEs contributes to a prolonged inflammatory state, impaired tissue regeneration, and delayed wound closure, particularly in diabetic wounds.²¹

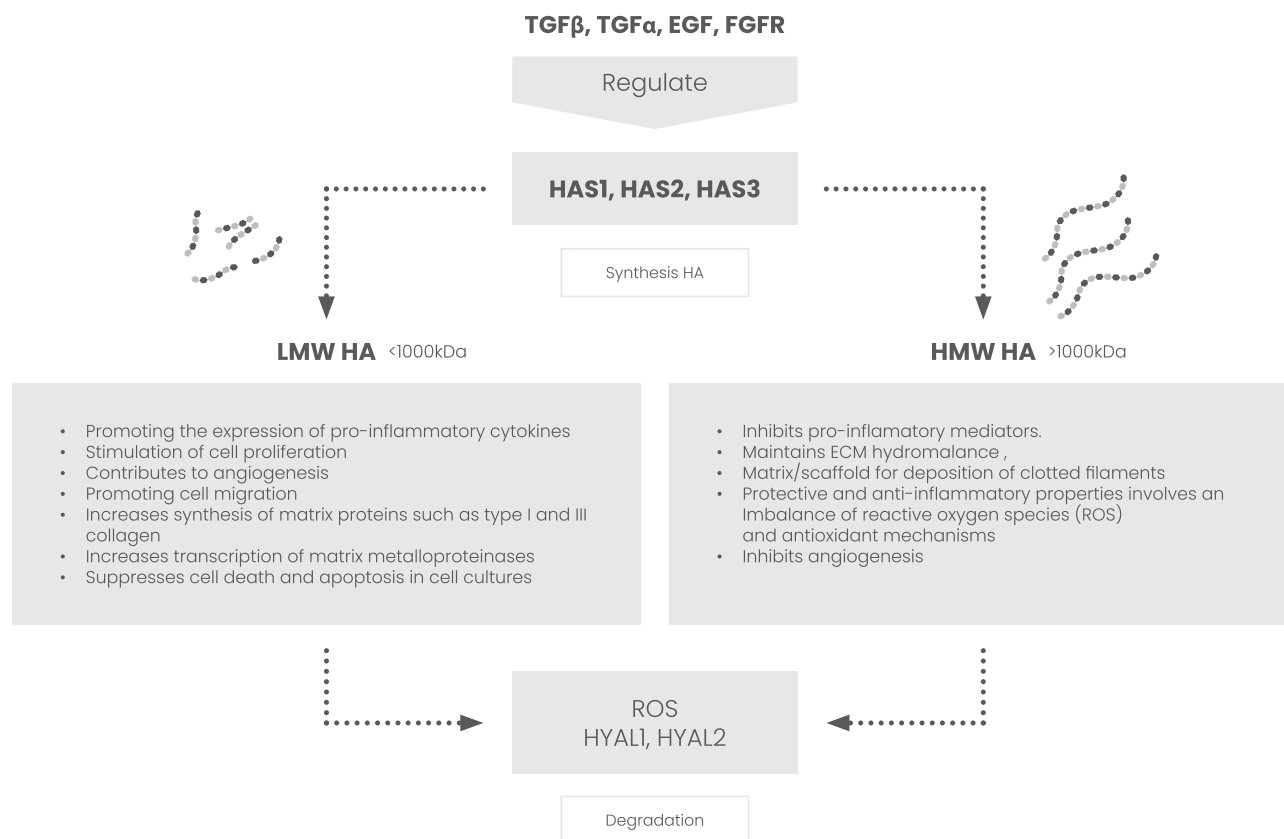


Figure 3 Schematic diagram showing HA key steps from its synthesis to its degradation. Differential effects of high molecular weight (HMW, >1000 kDa) and low molecular weight (LMW, <1000 kDa) hyaluronic acid on cellular processes, regulated by growth factors and hyaluronidases.

Hyaluronic acid (HA) effect in tissue differing significantly based on its molecular size. HMW-HA, particularly those above 1.2 MDa, exhibits protective and anti-inflammatory properties. It effectively inhibits the activation of NF- κ B induced by AGEs, reducing the subsequent production of pro-inflammatory cytokines, including interleukin-1 α , interleukin-6, and tumor necrosis factor- α . This is crucial as AGEs accumulate in long-lived proteins and amyloid deposits, prompting pro-inflammatory cytokine expression through NF- κ B dependent pathways. In contrast, LMW-HA exhibits pro-inflammatory effects by potentiating NF- κ B activation and stimulating cell proliferation. Through interactions with receptors like CD44 and factors such as CXCL-1, it contributes to angiogenesis and promotes matrix protein synthesis, including collagen type I. LMW-HA also induces nitric oxide synthase, upregulates matrix metalloproteinases transcription, and enhances cell migration while suppressing cell death and apoptosis in cell cultures.

The protective role of HMW-HA extends to macrophage studies, where it inhibits AGE-induced NF- κ B activation, in stark contrast to the pro-inflammatory effect observed with LMW-HA. Other glycosaminoglycans, like D-glucuronic acid and chondroitin sulfate, do not replicate HMW-HA's protective effect against NF- κ B activation by AGE. This specificity underscores the unique therapeutic potential of HMW-HA in conditions characterized by chronic inflammation and oxidative stress. HMW-HA is characterized by high viscoelasticity and low diffusivity, impacting its biological functions. It inhibits cell proliferation, exhibits anti-angiogenic properties, and suppresses the production of pro-inflammatory mediators, thus playing a crucial role in controlling cell growth, replication, and inflammatory responses. Additionally, HMW HA has immunosuppressive qualities, contributes to the organization of the extracellular matrix (ECM), and plays a vital role in maintaining water balance and regulating tissue hydration. It also protects against tissue damage by scavenging free radicals and guards against apoptosis. The protective role of HMW-HA becomes increasingly important considering the natural aging process and the presence of inflammatory states. As one ages, the molecular weight of HA polymers decreases, potentially leading to increased susceptibility to chronic inflammation in older individuals. Therefore, understanding the precise molecular mechanisms through which HMW-HA exerts its protective action

remains an important area of research.^{8,18,19,22–24} SAGEs, ie Semi-synthetic Glycosaminoglycan Ethers, have also attracted attention as a family of sulfated and metabolically stabilized anionic polysaccharide derivatives. As novel hyaluronic acid derivatives, SAGE exhibits broad anti-inflammatory effects by effectively binding to and inhibiting RAGE (receptor for AGEs) to reduce the inflammatory response. This interaction is crucial in conditions characterized by chronic inflammation and oxidative stress.¹⁵

The roles of HA in wound healing vary with its molecular weight. HA undergoes constant changes (synthesis and degradation) at different stages of wound healing, with a half-life of only 2–3 days. In the hemostasis phase, HMW-HA forms a matrix/scaffold for the deposition of clotted fibrin. During the inflammation phase, HMW-HA is a major component of the edematous fluid filling and expanding the tissue around a fresh wound. LMW-HA, resulting from the cleavage of HMW-HA by Hyal-2 in platelets, stimulates an inflammatory response by promoting cytokine production. In the proliferation phase, HMW-HA inhibits angiogenesis but rapidly degrades into strongly angiogenic LMW-HA, which is mitogenic for endothelial cells and stimulates tyrosine kinase cascades. It also promotes the production of collagen III by fibroblasts. In the remodeling phase, the hypothesis is that the concurrent presence of HA guides the substrate specificity of MMP activity (removing type III collagen while leaving type I). LMW-HA stimulates MMPs involved in remodeling. Exogenous LMW-HA shows potential for tissue regeneration without scars, reducing scarring.^{8,22,23,25–27} Treatment with native HA rejuvenates the skin by increasing hydration of the epidermis and dermis, creating a favorable environment for exchange and interactions between cells of the extracellular space. The HA products containing HMW-HA and LMW-HA in various formulations improve skin hydration and elasticity, increase HA levels in the skin, reduce signs of aging like skin roughness and wrinkles, and reduce scars.^{22,24,25}

In conclusion, the diverse roles and implications of hyaluronic acid (HA) in biomedical research and therapeutic applications cannot be overstated. The distinct properties of HA, varying with its molecular weight, offer a wide spectrum of biological activities that are crucial in the fields of wound healing, tissue regeneration, skin rejuvenation, and anti-inflammatory therapies. Future research endeavors should focus on further elucidating the molecular mechanisms behind the varying effects of different HA sizes, which will undoubtedly enhance our understanding and utilization of this remarkable molecule. The potential of HA-based treatments, especially in managing chronic inflammatory conditions and age-related degenerative diseases, represents a promising frontier in medical science. The continued exploration of HA's properties and applications could lead to groundbreaking advancements in clinical practices.

Trehalose: A Novel Agent in Skin Health and Anti-Aging Therapeutics

Trehalose, a naturally occurring disaccharide, has been identified as a key player in combating the effects of glycation, oxidative stress, and the aging process of the skin. This review synthesizes findings from various studies to provide a comprehensive understanding of trehalose's role and its potential therapeutic applications.

In the realm of skin health, trehalose has demonstrated effectiveness in enhancing the survival of random-pattern skin flaps, a common tool in reconstructive surgery. Its ability to activate autophagy, leading to increased angiogenesis, reduced apoptosis, and diminished oxidative stress, is particularly notable. Trehalose stimulates the expression of Vascular Endothelial Growth Factor (VEGF) and cadherin 5, alongside an increase in MMP9 expression, thereby augmenting the viability of skin flaps.²⁸

Beyond its reconstructive applications, trehalose has demonstrated photoprotective properties against UVB-induced damage in keratinocytes. It enhances the interaction between Tissue Inhibitor of Metalloproteinase (TIMP) 3 and Beclin1, while also increasing ATG9A localization in lysosomes. These actions contribute to reduced cell death and improved cell migration, highlighting trehalose's potential in preventing or treating UVB-induced skin diseases.²⁹

Moreover, trehalose exhibits significant anti-photoaging properties, particularly in response to UVB radiation. It effectively scavenges reactive oxygen species (ROS) and increases the content of endogenous antioxidants in human immortalized keratinocytes (HaCaT) cells. By suppressing UVB-induced MMP expression and boosting procollagen I synthesis, trehalose mitigates the detrimental photoaging effects of UV radiation.³⁰

The structural stability of trehalose is key to its function. Its non-reducing terminal hydroxyl group prevents it from undergoing glycation reactions, thereby enhancing its stability and preventing the formation of AGEs when interacting with Human Serum Albumin (HSA). This property underscores trehalose's potential in dietary and therapeutic applications to inhibit glycation.^{31,32}

In stress conditions, such as heat shock, trehalose is known for its role in protecting membranes and macromolecules. It slightly protects superoxide dismutases (SODs) activity and effectively scavenges hydrogen peroxide and superoxide anions, suggesting a direct role in eliminating ROS under stress.³³

In clinical settings, particularly in ophthalmology, trehalose has been adapted for stabilizing cell membranes and is increasingly recognized for its influence on cellular mechanisms foundational to oxidative stress and the inflammatory process. Trehalose acts directly at the cellular level, impacting fibroblast function and modulating pro-inflammatory cytokines. This interaction is crucial in preventing the degradation of collagen and the extracellular matrix, essential for maintaining water-electrolyte balance in intercellular spaces.³⁴

Trehalose emerges as a multifaceted agent with promising applications in skin health, particularly in inhibiting glycation, reducing oxidative stress, and preventing skin aging. Future research should explore its full therapeutic potential and potential applications in clinical practice. The convergence of these findings positions trehalose as a significant compound in the fields of dermatology and regenerative medicine, offering new avenues for the treatment and prevention of skin aging and related conditions.³⁵

Synergistic Effects of HA and Trehalose

In the field of biochemical research, the synergistic interaction between Hyaluronic Acid (HA) and trehalose is gaining attention due to its potential in stabilizing HA and mitigating glycation and oxidative stress. This chapter provides a comprehensive analysis of the mechanisms underlying this synergy and explores their collective impact on skin integrity and inflammation.

Hyaluronic Acid, a high-molecular-weight polysaccharide, is integral to tissue hydration and extracellular matrix composition. Despite its biological significance, HA is susceptible to rapid degradation by hyaluronidase and other environmental factors, which compromise its therapeutic efficacy.²³ The molecular weight of hyaluronic acid for biomedical applications significantly affects its properties and stability. Low molecular weight hyaluronic acid, is characterized by molecular dispersion and dispersed biopolymer particles in the solution. Such solutions show less pronounced cohesive and rheological properties, which indicates that they do not form strong polymer networks. Conversely, high molecular weight hyaluronic acid, forms distinct, three-dimensional polymer networks that are more cohesive. High molecular weight hyaluronic acid also has greater resistance to enzymatic hydrolysis, contributing to the longer durability of medicines based on it. Additionally, increasing the molecular weight of hyaluronic acid leads to an increase in the viscosity of solutions, which is important in biomedical and bioengineering applications.²⁴

Trehalose, a disaccharide with notable bioprotective properties, has been identified as an effective stabilizing agent for HA.^{16,36,37} Approved by the FDA for use in various forms and pharmaceuticals, trehalose's unique α,α -1,1 glycosidic bond gives it remarkable properties like heat and acid stability. Its low hygroscopic nature ensures stability even in high humidity environments. As a stabilizing agent, trehalose protects protein-based compounds like hyaluronic acid from degradation by harsh conditions and solvents. It forms hydrogen bonds with polar residues in these proteins, acting as a water substitute and preventing denaturation. This interaction is crucial for preserving the conformational integrity of HA, thereby reducing its susceptibility to enzymatic and non-enzymatic degradation pathways. Additionally, the desiccation property of trehalose impedes the chemical reactions responsible for HA breakdown, further enhancing its stability.³⁶ The enhanced stability of HA in the presence of trehalose extends its functional lifespan in tissues post-injection. This prolongs its antioxidative and anti-inflammatory effects and optimizes the water-electrolyte balance in the extracellular matrix, essential for maintaining cellular homeostasis and tissue hydration.³⁶ The HA-trehalose complex exhibits a significant protective effect against glycation, a process where irreversible bonds between sugar molecules and proteins lead to the formation of AGEs, implicated in skin aging. The presence of trehalose appears to mitigate the glycation process, thus preserving the mechanical and functional properties of HA within the skin matrix.³⁸

In terms of oxidative stress, a critical factor in cellular aging and tissue damage, the HA-trehalose synergy shows a considerable antioxidant effect. Trehalose enhances the intrinsic antioxidant properties of HA, providing a more effective defense against reactive oxygen species and external stressors. This combinatorial effect is not only preventive but also reparative, addressing existing oxidative damage within the skin.^{39,40} (Figure 4).

Additionally, trehalose's anti-inflammatory properties augment the benefits of HA. It activates autophagy via mTOR-independent pathways and upregulates BNP3, a key autophagy-related protein, thereby exerting anti-inflammatory effects

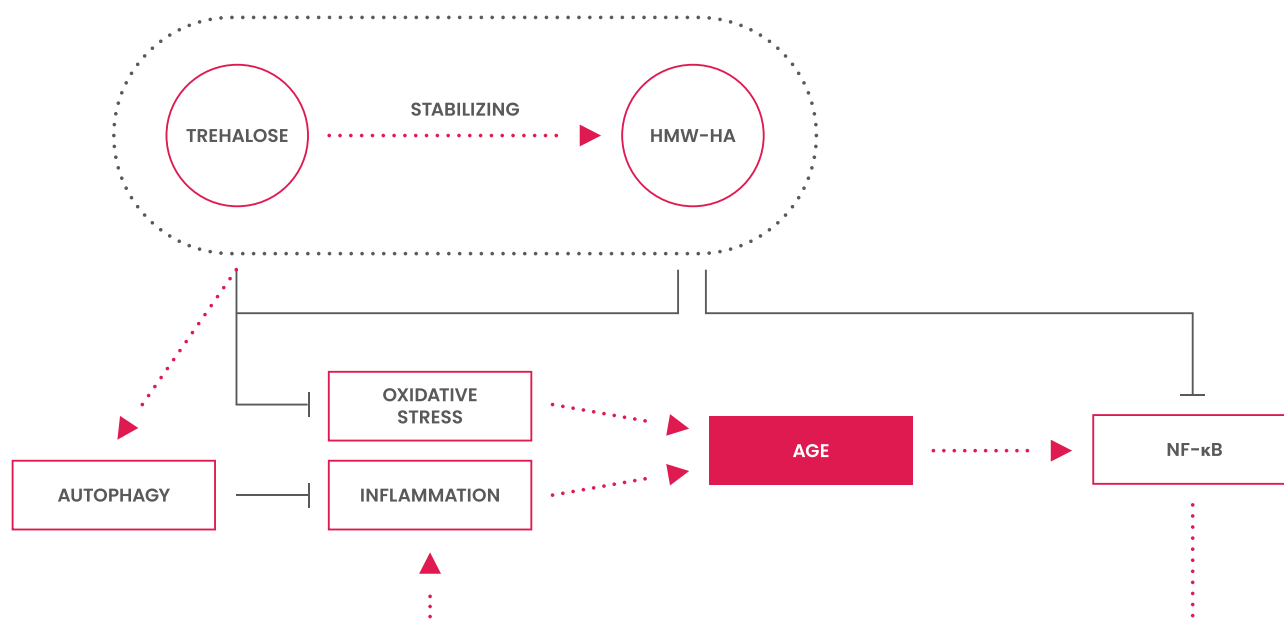


Figure 4 Schematic diagram showing synergistic action of Trehalose and HA.

at a cellular level. This property is especially beneficial in inflammatory disorders such as osteoarthritis (OA), where HA and trehalose may collectively decelerate disease progression.^{41,42}

In summary, the integration of HA and trehalose presents a promising approach in addressing the challenges of HA instability, glycation, and oxidative stress. Their synergistic effects in improving skin quality and reducing inflammation have significant implications for their use in dermatological and therapeutic applications, offering potential advancements in anti-aging and regenerative medicine.

Present and Potential Clinical Applications

Clinical trials have shown the efficacy of HA and trehalose, especially in the context of knee OA. In these studies, a novel trehalose hyaluronate formulation significantly improved clinical outcomes, maintaining effectiveness over six months. This contrasts with control treatments that showed improvements at three months but did not sustain these effects long-term.⁴² These findings are crucial as they underline the extended efficacy of trehalose hyaluronate over traditional HA treatments. Moreover, the stability of trehalose under various conditions, such as high temperatures and acidic environments, contributes to its effectiveness and safety in medical applications.^{43,44}

The research by Fariselli et al⁴⁵ shows the effectiveness of a trehalose/hyaluronic acid combination in treating Dry Eye Disease (DED). The study reported improvements in ocular discomfort, reduced ocular surface damage, and increased tear film stability. This suggests the synergistic potential of combining trehalose with HA, offering a multifaceted approach to addressing different aspects of DED. The association of these compounds could be particularly effective in breaking the cycle of tear film instability, inflammation, and epithelial changes that characterize DED.

Lastly, Astolfi et al⁴⁶ found that HA/trehalose-containing eye drops not only improved clinical signs and goblet cell recovery in a DED mouse model but also significantly reduced inflammatory markers. This highlights the potential of trehalose in breaking the DED cycle, offering an alternative to traditional therapies. The use of trehalose in this context demonstrates its effectiveness in mitigating inflammation, an important factor in DED pathogenesis. The protective effects of trehalose on cell membranes and its ability to improve the density of neutral mucins in goblet cells without adverse side effects make it a valuable component in long-term therapy for DED.

Trehalose itself, has been used in organ preservation and reducing postoperative adhesions, indicating its potential in managing inflammatory conditions in medicine. This aspect could be particularly beneficial in preventing further inflammatory damage in treatments involving HA.^{47,48}

The future of aesthetic medicine holds promising advancements, particularly in the utilization of hyaluronic acid (HA) and trehalose. Research has increasingly focused on these compounds due to their unique properties and benefits in various treatments. In the field of aesthetic medicine, the clinical applications of hyaluronic acid (HA) and trehalose are attracting increasing interest due to their unique properties and effectiveness. HA is known for its ability to induce collagen production, essential for maintaining skin plumpness and elasticity. The primary challenge is creating a product with HA at a molecular weight that ensures optimal therapeutic effects and greatest stability. This is where trehalose plays a pivotal role. In skin booster formulations, trehalose might be included to protect HA from complete enzymatic breakdown. This synergy not only prolongs hydration effects but also enhances collagen synthesis, leading to more effective and lasting results in aesthetic treatments.⁴⁹

In summary, the efficacy of hyaluronic acid (HA) and trehalose has been confirmed in treating knee osteoarthritis and dry eye disease, showing longer-term effectiveness than traditional HA treatments. The combination of trehalose with HA in DED therapies improves ocular comfort and reduces inflammation, suggesting its potential in alternative treatments. Additionally, this combination might enhance hydration and collagen synthesis, indicating promising applications in aesthetic medicine. The unique properties of these compounds are proving beneficial in various medical treatments, offering more effective and sustained results.

Conclusion

This review highlights the significant potential of hyaluronic acid (HA) and trehalose in anti-aging therapies, particularly in aesthetic medicine. HA's modulation of ROS and anti-inflammatory properties complement trehalose's ability to enhance skin viability, protect against UVB damage, and combat photoaging. Together, they offer promising solutions to glycation and oxidative stress in skin aging. Their collaborative use presents an innovative approach in aesthetic medicine, promising more effective solutions for preserving skin health and youthfulness. Further research into their synergistic capabilities holds promise for future advancements in anti-aging treatments.

Abbreviations

AGEs, Advanced Glycation End products; ROS, Reactive Oxygen Species; HA, Hyaluronic Acid; SOD, Superoxide Dismutase; NF- κ B, Nuclear Factor kappa-light-chain-enhancer of activated B cells; MAP, Mitogen-Activated Protein; MMPs, Matrix Metalloproteinases; RAGE, Receptor for Advanced Glycation End products; DED, Dry Eye Disease; TGF- α , Transforming Growth Factor alpha; TGF- β , Transforming Growth Factor beta; UVB, Ultraviolet B; VEGF, Vascular Endothelial Growth Factor; TIMP, Tissue Inhibitor of Metalloproteinases; HSA, Human Serum Albumin; OPH, Oxidized Protein Hydrolase; AGER1, AGE Receptor 1; sRAGE, Soluble form of RAGE receptor; LMW HA, Low Molecular Weight Hyaluronic Acid; HMW HA, High Molecular Weight Hyaluronic Acid; HAS1, HAS2, HAS3, Hyaluronic Acid Synthases 1, 2, and 3; HYAL1, HYAL2, Hyaluronidases 1 and 2; EGF, Epidermal Growth Factor; UPS, Ubiquitin-Proteasome System; ALPS, Autophagic Lysosomal Proteolytic System; SAGEs, Semi-Synthetic Glycosaminoglycan Ethers; OA, Osteoarthritis.

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Disclosure

The authors report no conflicts of interest in this work.

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