Review Article

The Diverse Roles of Hyaluronidase: Revealing Its Biochemical, Preclinical, and Clinical Applications

Mahdiyeh Safary[1,2], Mobina Tajdari[1,2], Amirreza Peyrovinasab[1,2], Seyyed Mohammad Hassan Modarressi[3], Sakineh Alijanpour[4], Mohammad Hossein Modarressi[5] , Amir Rezazadeh[6]*,

[1] *Department of Clinical Pharmacy, Faculty of Pharmacy, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran*
[2] *GI Pharmacology Interest Group (GPIG), Universal Scientific Education and Research Network (USERN), Tehran, Iran*
[3] *Research and Development Department, Hora Pharmed Company, Tehran Iran*
[4] *Department of Biology, Faculty of Science, Gonbad Kavous University, Gonbad, Iran*
[5] *Department of Medical Genetics, Tehran University of Medical Sciences, Tehran, Iran*
[6] *Department of Clinical Pharmacy, School of Pharmacy, Iran University of Medical Sciences, Tehran, Iran*

Article info:
Received: 14 March 2025
Revised: 29 April 2025
Accepted: 04 May 2025

* **Corresponding Author:**

Amir Rezazadeh
Department of Clinical Pharmacy, Faculty of Pharmacy, Iran University of Medical Sciences, Tehran, Iran

Email: Rezazadeh.am@iums.ac.ir

ABSTRACT

Hyaluronidase, a group of enzymes that catalyze the hydrolysis of hyaluronic acid, plays a pivotal role in modulating the extracellular matrix and enhancing tissue permeability. Hyaluronidase has gained widespread attention for its diverse biochemical properties and expanding therapeutic potential. This review provides a comprehensive overview of hyaluronidase, focusing on its biochemical characteristics, mechanisms of action, and regulatory pathways. We examine its utility in preclinical models, highlighting its role in drug delivery, tissue remodeling, and cancer research. Clinically, hyaluronidase has been employed in various domains, including ophthalmology, dermatology, oncology, and as an adjuvant in subcutaneous and intramuscular drug administration. Additionally, its role in reversing complications from dermal filler injections has led to increased use in aesthetic medicine. Despite its broad application, challenges such as immunogenicity, variability in enzyme sources, and potential adverse effects warrant continued investigation. Through an integrated analysis of current evidence, this review aims to elucidate the multifaceted roles of hyaluronidase and explore its emerging applications in modern medicine.

Keywords: Biomedical application, Biostructure, Hyaluronic acid, Hyaluronidase, Novel treatment

Use your device to scan and read the article online

Citation: Safary M, Modarressi SMH, Tajdari M, Peyrovinasab A, Rezazadeh A, Alijanpour S, Modarressi MH. The Diverse Roles of Hyaluronidase: Revealing Its Biochemical, Preclinical, and Clinical Applications. Acta Biochimica Iranica. 2025;3(2):73-82.

https://doi.org/***

Introduction

Hyaluronidase is an enzyme that breaks down hyaluronic acid and has gained significant attention in the medical field for its diverse clinical applications. Initially recognized for its role in tissue remodeling and wound healing, hyaluronidase is now utilized across various medical disciplines (1). Hyaluronidase is used in clinical settings to improve drug delivery by degrading hyaluronic acid, a component of the extracellular matrix that can obstruct therapeutic distribution. By degrading hyaluronic acid, hyaluronidase enhances drug penetration, especially in cancer treatment, and allows for faster and more consistent absorption when used with subcutaneous drug administration (2). Additionally, hyaluronidase improves the efficacy of local anesthetics by promoting their diffusion within tissues, thereby reducing onset time and enhancing pain management during procedures such as ophthalmic surgery and dental work (3). Hyaluronidase is essential for managing complications from hyaluronic acid-based dermal fillers, which are often used in cosmetic and reconstructive procedures (4). Ongoing research is exploring further applications of hyaluronidase, including its potential to enhance the delivery of macromolecular drugs like antibodies and nanoparticles, and to improve the efficiency of injectable vaccines by overcoming extracellular matrix barriers (5). This review aims to elucidate the multifaceted roles of hyaluronidase and explore its emerging applications in modern medicine.

Method of research

This narrative review gathered data from Google Scholar and PubMed, focusing on biochemical, preclinical, and clinical research published in English from 1994 to 2024. It used a broad set of keywords related to hyaluronidase, encompassing fields such as cardiovascular disease, pulmonary issues, skin treatments, infertility, cancer research, ophthalmology, cosmetic applications, angiogenesis, extravasation, orofacial cleft, cell culture, and tissue engineering.

Structure and Biochemical properties of hyaluronidase

Hyaluronidase is an enzyme that degrades hyaluronic acid, a vital component of connective tissues, and is considered the most important enzyme in the interstitial matrix. Its structure varies across species, with mammalian, bacterial, and insect hyaluronidases exhibiting distinct features, yet all share the common function of hyaluronic acid degradation (1). Crystallographic analysis of this enzyme reveals two connected domains: an epidermal growth factor-like region involved in protein interactions and a catalytic domain with a distorted $(\beta/\alpha)_8$-barrel structure similar to bee venom hyaluronidase. The catalytic domain facilitates enzymatic activity, while the non-catalytic domain may assist in substrate recognition (6). The enzymatic action of hyaluronidase involves several key steps. First, the enzyme binds to hyaluronic acid through its active site, which interacts with the glycosidic bonds in the hyaluronic acid molecule. Hyaluronic acid is composed of D-glucuronic acid and D-N-acetylglucosamine linked by β-1,4 and β-1,3 glycosidic bonds (1). Hyaluronidase specifically breaks the β-1,4 linkage between N-acetylglucosamine and glucuronic acid, lowering the activation energy for the reaction (7). After hydrolysis, smaller hyaluronic acid fragments are released, which can be further degraded or cleared from the body. The enzyme then continues to catalyze the breakdown of hyaluronic acid, promoting efficient turnover in tissues (8). Generally, the enzymatic mechanism of this enzyme's action involves the hydrolysis of the glycosidic bonds in hyaluronic acid. The human genome includes six genes resembling hyaluronidase. HYAL1, HYAL2, and HYAL3 are found on chromosome 3p21.3, while HYAL4, PH-20/SPAM1, and the pseudogene HYALP1 are located on chromosome 7q31.3. Due to limited expression, HYAL3, HYAL4, HYAL5, and the pseudogene HYAL6 likely do not contribute to hyaluronic acid breakdown (9). HYAL1 is the main enzyme for degrading hyaluronic acid in the extracellular matrix, expressed in various tissues, while HYAL2, located on the cell surface, also contributes to this process and plays a role in cell migration and adhesion. HYAL3 is less characterized and has limited tissue distribution compared to HYAL1 and HYAL2 (10). Enzymes with specificity include testicular-type hyaluronidase (hyaluronoglucosaminidase; hyaluronate 4-glycanohydrolase, EC 3.2.1.35), leech hyaluronidase (hyaluronate glycanohydrolase, EC 3.2.1.36), and bacterial hyaluronidase (hyaluronate lyase, EC 4.2) (11). Hyaluronidase has a short half-life of approximately 2–3 minutes due to rapid metabolism in the liver and kidneys and the presence of hyaluronidase inhibitors in the plasma (1).

The physiological role of hyaluronidase in each body organ

Hyaluronidase is a versatile enzyme with a wide range of bodily functions, including tissue remodeling, cell migration, reproductive biology, and immune response. As mentioned before, its ability to degrade hyaluronic acid and modulate the extracellular matrix makes it a key player in numerous biological processes essential for health and development. Fragmentation of hyaluronic acid through the action of hyaluronidase is believed to inspire the development of new blood vessels, a process known as angiogenesis. The process of fibrotic healing in wounds that occur in adults and

in the late stages of pregnancy is linked to increased hyaluronidase activity, leading to the breakdown and removal of hyaluronic acid (12). This enzyme is thought to play a crucial role in maintaining the balance of hyaluronic acid levels in the body, including the turnover of hyaluronic acid in the anterior chamber of the eye and the breakdown of highly concentrated hyaluronic acid used for its viscoelastic properties (13-14). Hyaluronidase is involved in the inflammatory response by promoting the migration of immune cells to the site of inflammation. It helps create channels through the extracellular matrix, allowing immune cells to reach the affected tissue quickly (15). Hyaluronidase is also involved in sperm penetration of the zona pellucida during fertilization, allowing sperm to reach and fertilize the egg (16). In cancer cells, hyaluronidase can promote tumor invasion and metastasis by degrading the extracellular matrix and facilitating cancer cell migration (17). Table 1 presents the various subtypes of hyaluronidase enzymes and their physiological functions in the body. Hyaluronidase enzymes from distinct generations exhibit diverse functions and exert multiple effects across various tissues. Any aberration in the expression of these genes can lead to dysfunction in multiple organs. The following section will define the function of hyaluronidase and demonstrate the impact of its dysfunction on each organ within the body:

Cardiovascular system

The relationship between hyaluronic acid and hyaluronidase is a key factor in the development and progression of heart disease. Different hyaluronidases exert a distinct influence on specific phenotypes. Preclinical studies have demonstrated that an excess of hyaluronic acid may lead to cardiopulmonary complications, including enlargement of cardiac valves, disruption of the extracellular matrix, and significant lung fibrosis (18). The presence of heart abnormalities lacking hyaluronidase 2 indicates that removing hyaluronic acid is necessary for proper heart formation. Disruption of hyaluronic acid breakdown by hyaluronidase 2 leads to increased levels of hyaluronic acid, which can trigger the transition from endothelial to mesenchymal cells and their proliferation. This process appears to be the primary factor contributing to the observed structural abnormality of the heart. These abnormalities progress to severe diastolic dysfunction, eventually leading to heart failure. In a murine model of myocardial infarction, decreased levels of hyaluronidase 3, which are controlled by interleukin 10 (IL-10), have been found to reduce hyaluronic acid degradation and subsequent collagen deposition. The suppression of hyaluronidase 3 promotes the healing of the left ventricle after a heart attack through an IL-10-mediated mechanism (19).

Pulmonary system

The critical role of hyaluronidases, specifically hyaluronidase 1 and 2, in lung repair and the development of pulmonary hypertension has been emphasized in previous studies. When the expression of hyaluronidase 2 is reduced, it results in elevated levels of hyaluronic acid and proteases in infected human lung fibroblasts, leading to binding with mast cells and an escalation in inflammation. Moreover, vessel-derived hyaluronidase 2 plays a pivotal role in the remodeling of the pulmonary vasculature and initiation of pulmonary hypertension, particularly in the absence of superoxide dismutase. Therefore, the inhibition of hyaluronidase 2 is an effective method for maintaining the homeostasis of high-molecular-weight hyaluronic acid, preventing proliferation caused by oxidation and hypoxia (10, 18).

Skin

Hyaluronidase, documented as KIAA1199 (cell migration-inducing and hyaluronan-binding protein), is thought to play a critical role in skin physiology, particularly in the degradation of hyaluronic acid by human skin fibroblasts (20). While IL-1β, tumor necrosis factor-alpha (TNF-α), and IL-6 are known to

Table 1: Different subtypes of hyaluronidase enzymes and their physiological roles in the body

Enzyme name	Amino acid length	Molecular weight	Tissue expression	Subcellular locations	Physiological roles	References
Hyaluronidase-1, Q12794-HYAL1_HUMAN,	435 amino acids	48368 Dalton	Kidney, Liver, Urine	Secreted, Lysosome	Extracellular matrix remodeling, Wound healing, immune response, drug penetration, skin physiology, kidney disease, and gastrointestinal health.	(11)
Hyaluronidase-2, Q12891-HYAL2_HUMAN,	473 amino acids	53860 Dalton	Heart, Lung, Placenta	Cell membrane; Lipid-anchor, GPI-anchor	Heart development, lung remodeling, pulmonary hypertension, and inflammation.	(10)
Hyaluronidase-3, O43820-HYAL3_HUMAN,	417 amino acids	46501 Dalton	Bone marrow, Nervous system, Lung	Cytoplasm	Heart development, wound healing, immune response, and cell migration.	(59)
Hyaluronidase-4, Q2M3T9-HYAL4_HUMAN,	481 amino acids	54249 Dalton	Cerebrospinal fluid, Breast, Pancreas	Membrane; Multi-pass membrane protein	Wound healing, inflammation, tissue repair, drug penetration, cancer invasion, and cell migration.	(60)

suppress KIAA1199 expression and promote hyaluronic acid synthesis, histamine has been found to upregulate KIAA1199, resulting in enhanced hyaluronic acid degradation (21). Notably, exposure to UVB radiation triggers an increase in hyaluronidases 1 and 2 in human skin, indicating a potential involvement of hyaluronidase metabolism in the inflammatory response after sunburn (22). Additionally, the preclinical investigation of hyaluronidase and its role in cutaneous wound healing has been a topic of significant interest within the scientific community. These studies have uncovered that reducing the expression of hyaluronidases 1 and 2 in wounds leads to lower levels of low-molecular-weight hyaluronic acid fragments and delayed wound healing (14).

Kidney

Degradation of hyaluronic acid by hyaluronidase 1 is linked to the development of conditions such as ischemia/reperfusion, acute kidney injury, and chronic kidney disease. Increased levels of hyaluronidase have been observed in patients undergoing dialysis, suggesting its potential as a biomarker and a target for inhibition to preserve the hyaluronic acid conformation of the endothelial glycocalyx (23). Similar to one of the most important cancer induction mechanisms, non-enzymatic hyaluronidase 2, which is present in alkaline environments and is distinct from the CD44 receptor, can also contribute to inflammation and fibrosis (24).

Gastrointestinal system

Studies have shown that the hepatic degradation of hyaluronic acid is performed by sinusoidal endothelial cells and is mainly facilitated by hyaluronidases 1 and 2. Clinical research involving the human liver has shown an elevation in hyaluronidases 1 and 2 in liver conditions like steatosis, steatohepatitis, and cirrhosis. Hyaluronan has long been regarded as a biomarker for both acute and chronic liver diseases (25). Elevated serum hyaluronidase levels are observed in patients with hepatitis C, whereas individuals with chronic hepatitis B exhibit decreased transmembrane protein 2 gene levels (26).

Biomedical application of hyaluronidase

Hyaluronidase's broad spectrum of applications in the medical field is attributed to its unique mechanism of action, making it a valuable tool in enhancing treatment across various disciplines. The following sections outline both preclinical and clinical uses of this remarkable enzyme. Furthermore, Table 2 shows the FDA-approved and potential uses of hyaluronidase.

Infertility

Hyaluronidase breaks down hyaluronic acid in the cumulus oophorus, which surrounds the oocyte in the female reproductive system and is vital for fertilization by providing structural support to the oocyte and facilitating sperm penetration. In some cases of infertility, abnormalities in this structure can hinder fertilization (27). Research has explored the use of hyaluronidase in assisted reproductive technologies (ART) to select viable sperm for fertilization. This treatment identifies sperm with intact DNA and functional maturity by examining their ability to bind to hyaluronic acid in the cumulus oophorus. The hyaluronan-binding assay is proposed as an effective method for selecting high-quality sperm for intracytoplasmic sperm injection (28). Using hyaluronidase to eliminate non-viable or immature sperm that do not bind to hyaluronic acid may enhance the chances of successful fertilization and embryo development in ART. It may also improve sperm motility and capacitation, offering additional benefits in infertility treatments (29).

Prevention of perineal trauma

Between the 1950s and 1960s, perineal hyaluronidase injections were commonly used to reduce perineal trauma, alleviate pain, and prevent episiotomies. Studies from that time suggested that this method was simple, safe, and cost-effective, with no significant adverse effects. However, recent research has shown conflicting results about its efficacy (30). A randomized study assessed hyaluronidase's effectiveness in reducing perineal trauma among 148 nulliparous women during vaginal delivery, comparing 5,000 units of hyaluronidase to a placebo. Results showed no significant differences in laceration or episiotomy rates, but less perineal edema was noted 24 hours post-delivery in the hyaluronidase group, with no major adverse events. In a separate trial with 160 first-time mothers, an injection of 20,000 turbidity-reducing units of hyaluronidase showed no significant improvement in perineal integrity compared to the control group, although severe trauma was slightly lower in the experimental group—a difference that was not statistically significant (31). The effectiveness of hyaluronidase injection in reducing perineal trauma is uncertain due to undefined dosage, limited follow-up data, and a lack of high-quality studies. More well-designed randomized controlled trials are needed to assess its safety and efficacy in vaginal deliveries.

Local anesthesia

Hyaluronidase is occasionally utilized as an adjunctive agent in combination with local anesthetics to enhance their diffusion and uptake, thereby accelerating the onset and prolonging the duration of anesthesia (2). The efficacy of hyaluronidase in combination with local anesthesia has been explored across different medical interventions. Through its ability to degrade the extracellular matrix and enhance tissue permeability, hyaluronidase facilitates the optimal diffusion of the local anesthetic agent within tissues, thereby promoting a wider and more uniform spread of anesthesia. A study in 2022 found that adding hyaluronidase to local anesthetic solutions in dental procedures led to a quicker onset of

Table 2: FDA-approved medicines and investigational applications of hyaluronidase in medicine

Application	Description	FDA-approved brands	References
Infertility	-Used in ART to select viable sperm -Enhancing sperm motility and capacitation	*Hyaluronidase 80 units* (*Cooper Surgical*)	(27-29)
Prevention of Perineal Trauma	-Historically used to reduce perineal trauma during childbirth	Not commonly branded	(30, 31)
Local Anesthesia	-Enhancing the secretion and absorption of local anesthetic agents, resulting in a faster onset and longer duration of anesthesia	*Hylenex, Amphadase, Vitrase, Hydase*	(2, 32)
Ophthalmology	-Managing periocular edema -Enhancing the delivery of intraocular injections -Aiding in cataract and vitreoretinal surgeries.	*Vitrase, Hylenex, Amphadase, Hydase*	(2, 33, 34)
Cosmetic	-Used to dissolve hyaluronic acid-based dermal fillers to manage complications and reverse undesirable outcomes from cosmetic procedures.	*Vitrase, Hylenex, Amphadase, Hyalase*	(4, 35-38)
Angiogenesis	-Modulating the extracellular matrix properties -Influencing the migration and organization of endothelial cells during new blood vessel formation.	Research-based; not commercialized	(39, 40)
Extravasation	-Treating extravasation injuries from intravenous therapy and chemotherapy drugs	*Vitrase, Amphadase, Hylenex, Hydase*	(41-43)
Orofacial Cleft	-Studied for its role in the genetic etiology and potential treatment of orofacial cleft.	Research-based; not commercialized	(44, 45)
Research (Cell Culture & Tissue Engineering)	-Facilitating cell detachment -Altering cell adhesion, migration, proliferation, and differentiation -Enhancing cell infiltration into tissue-engineered scaffolds	Research-grade hyaluronidase	(37, 46-48)
Cancer	-Enhancing drug delivery -Reducing tumor interstitial pressure -Modulating the tumor microenvironment to improve treatment outcomes	*Hylenex, HyQvia, Phesgo, Rituxan Hycela, Herceptin Hylecta*	(17, 49-54, 56, 57)
Cancer Biomarker	-A potential early and reliable biomarker for bladder, prostate, and ovarian cancer	Research-based; not commercialized	(49, 55)
Drug Delivery System	-Enhancing the penetration of medicine through tissues -Improving the bioavailability and efficacy of injected medicine	*Vitrase, Amphadase, Hylenex, HyQvia, Hydase*	(3, 58)

anesthesia and enhanced pain management during dental procedures (32). Generally, combining hyaluronidase with local anesthesia may enhance pain control and anesthesia effectiveness during medical procedures. Further research is needed to optimize its dosing and administration for various clinical applications.

Ophthalmology

One common use of hyaluronidase in ophthalmology is in the management of periocular edema from cosmetic procedures or trauma. It can be injected locally to disperse hyaluronic acid-based dermal fillers and reduce swelling by promoting their breakdown (33). Hyaluronidase has potential in enhancing the delivery of intraocular injections, like anti-vascular endothelial growth factor medications for retinal diseases such as age-related macular degeneration and diabetic retinopathy. By breaking down hyaluronic acid in the vitreous humor, it may improve the diffusion and distribution of these injections, potentially leading to better treatment outcomes (3). Hyaluronidase has been used for over 80 years as a supplementary agent in retrobulbar, peribulbar, and sub-tenon blocks. Hyaluronidase is used in cataract surgery to help disperse ophthalmic viscoelastic devices (OVDs) that maintain space and protect the cornea. It is also used in vitreoretinal surgery for the dispersion of OVDs to facilitate procedures in the posterior segment of the eye (2). Furthermore, hyaluronidase has been explored as an adjuvant therapy in ophthalmic surgery to reduce intraocular pressure and facilitate the dispersion of injected medications or viscoelastic substances during procedures like cataract and glaucoma surgery (34).

Cosmetic

Hyaluronic acid-based dermal fillers are widely used for facial rejuvenation and enhancement, as they help maintain hydration, volume, and elasticity in the skin. These fillers can restore lost volume, smooth wrinkles, and enhance facial contours. However, complications such as overcorrection, asymmetry, lumps, or vascular issues can occur. In these cases, hyaluronidase can effectively dissolve fillers and manage complications. Several studies indicate that hyaluronidase is effective and safe for managing complications from hyaluronic acid fillers in cosmetic procedures (4). Using hyaluronidase in cosmetic procedures requires skill and precision to achieve optimal results and minimize potential side effects. The correct dosing, technique, and timing are essential elements for the effective utilization of hyaluronidase in resolving issues associated with dermal fillers (35). The appropriate dose depends on factors such as the urgency of the issue, the location of the filler, the volume, and the physical quality of the hyaluronic acid gel, as well as the characteristics of the patient (36). The use of this enzyme frequently entails a titration method, allowing the practitioner to assess the allergic reaction after each injection. Administering hyaluronidase in incremental doses is recommended to minimize filler deterioration and enable fine-tuning (4). Hyaluronidase is used in cosmetic procedures to break down excess hyaluronic acid, reducing wrinkles and fine lines. This therapy is generally well tolerated, with minimal side effects such as temporary itching and allergic reactions, with reported incidences ranging from 0.05% to 0.69%. The risk of allergic reactions increases significantly when hyaluronidase exceeds 100,000 units intravenously, reaching 31.3% at 200,000 units (37). Most reactions are immediate hypersensitivity (type I), mediated by immunoglobulin E, appearing as erythematous swelling within 1 to 2 hours, unresponsive to antibiotics but relieved by systemic or topical steroids or antihistamines. Delayed hypersensitivity (type IV), mediated by T-cells, may occur after 24 hours, with a negative diagnosis confirmed by a non-reactive skin test within 20 minutes. Performing the skin test with 3 units of hyaluronidase is recommended (38)..

Angiogenesis

Hyaluronidase plays a significant role in angiogenesis by modulating the extracellular matrix and influencing the migration and organization of endothelial cells in new blood vessel formation (39). Hyaluronidase can interact with cell surface receptors and signaling pathways related to angiogenesis. For example, it may activate receptor tyrosine kinases like fibroblast growth factor receptor and vascular endothelial growth factor receptor, which are essential for angiogenesis. By modulating these signaling pathways, hyaluronidase can influence endothelial cell behavior and promote angiogenesis (40).

Extravasation

Extravasation is a commonly observed phenomenon in intravenous therapy and chemotherapy drugs, potentially causing tissue damage. It can result in four main types of injury: vasoconstriction, osmotic, pH-related, and cytotoxic (41). Medications with a higher risk of causing extravasation include antibiotics (like penicillin and vancomycin), vasopressors (such as dopamine and norepinephrine), anticonvulsives (phenytoin), injectable fluids (like calcium gluconate and potassium chloride), and chemotherapy drugs (e.g., anthracyclines, vinca alkaloids, taxanes, and oxaliplatin). Intradermal phentolamine has traditionally been the preferred treatment for vasopressor extravasations (42). Alternative antidotes and supportive care agents have become available to manage cases of extravasation, such as hyaluronidase, terbutaline, topical anesthetics (procaine, lidocaine), antimicrobials (chlorhexidine, silver sulfadiazine), debridement agents (collagenase ointment), steroids, and vasodilators (nitroglycerin) (43). Current guidelines recommend warm compresses and a vasodilator for managing vasopressor extravasation. For vesicants with issues related to pH, osmolarity, cytotoxic concentration-dependence, and absorption refractoriness, warm compresses and administration of hyaluronidase are recommended (42). Hyaluronidase is typically injected locally into the extravasation area subcutaneously, with a usual dose of 100–150 (42).

Orofacial cleft

Cleft lip and palate are prevalent birth defects caused by genetic and environmental factors. Despite extensive research on the genetic origins of this disease, there is still much to learn about this aspect (44). In 2017, a global study identified a new genetic cause of orofacial cleft in humans and mice and revealed the initial molecular cause of human cortriatriatum sinister, emphasizing the role of hyaluronidase 2 and hyaluronan turnover in development. A study on ten individuals with this disorder found nine new pathogenic mutations, correlating genotype with a distinct craniofacial phenotype, myopia, cleft lip/palate, and congenital heart defects. Computational modeling indicated potential harmful effects on protein folding, while functional analyses showed these mutations caused protein instability and a lack of hyaluronidase 2 on the cell surface (45).

Research (cell culture & tissue engineering)

Hyaluronidase is used in cell culture to promote cell detachment from dishes and assist in cell isolation, purification, and harvesting. It can also influence cell adhesion, migration, proliferation, and differentiation by affecting the extracellular matrix and cell signaling pathways. In tissue engineering, hyaluronidase is used to modify the extracellular matrix, enhancing tissue regeneration and cell migration. It cleaves glycosidic bonds in hyaluronic acid, lowering the matrix viscosity

and facilitating cell movement (46). Cell migration is essential for biological processes like wound healing, immune response, and embryogenesis. Studies indicate that hyaluronidase can enhance cell infiltration in tissue-engineered scaffolds by degrading hyaluronic acid barriers, thereby promoting cell migration and aiding vascularization and tissue integration (47). Hyaluronidase treatment of septal cartilage reduced sulfated glycosaminoglycan levels but allowed for continued growth of the constructs. This reduction led to higher collagen-to-sulfated glycosaminoglycan ratios, which improved tensile strength and stiffness. Over time, sulfated glycosaminoglycan levels slightly increased in deficient constructs, though initial tensile improvements were lessened. Adjusting the hyaluronidase dose during neocartilage synthesis may enhance biomechanical properties for later surgical implantation (46). Hyaluronidase enhances membrane permeability by degrading hyaluronic acid in the extracellular matrix, making tissues more receptive to injected fluids. Consequently, this reduces the viscosity of hyaluronic acid, improving tissue diffusion and the resorption rate of excess fluids (37). Hyaluronidase can be used to modulate antimicrobial release from hyaluronic acid systems. Ran et al. illustrated this activity. They developed a hyaluronidase-activated photothermal substance for eradicating microorganisms utilizing silver nanoparticles and graphene oxide. Silver nanoparticles and graphene oxide were incorporated into a hyaluronic acid polymer to create nanocomposites. The release of hyaluronic acid-coated silver nanoparticles, induced by hyaluronidase, exhibited antibacterial properties against Staphylococcus aureus (48).

Cancer

Hyaluronidase has been researched in cancer therapy for its ability to degrade hyaluronic acid, a component of the extracellular matrix that can promote the proliferation and metastasis of cancerous cells. It has potential as a therapeutic agent by enhancing drug delivery, reducing tumor interstitial pressure, and modulating the tumor microenvironment (49). Moreover, hyaluronidase has been studied alongside other cancer therapies, like immunotherapy and targeted treatments, to enhance outcomes. By modulating the tumor microenvironment and enhancing drug delivery, hyaluronidase may help overcome resistance mechanisms and improve the response to cancer treatments (50). Cancer cells often overexpress the CD44 receptor, which binds to hyaluronic acid, promoting their migration, spread, invasion, and metastasis. By breaking down hyaluronic acid, hyaluronidase effectively disrupts the CD44–hyaluronic acid interaction, positioning it as a viable treatment option for cancers associated with this pathway (51). Hyaluronidases 1 and 2 have distinct roles in breaking down hyaluronic acid—hyaluronidase 1 operates in tumor cells, while hyaluronidase 2 acts extracellularly. Both require CD44 for activity but function independently. Hyaluronidase 1 specifically generates angiogenic fragments that may affect tumor behavior (17). A significant adverse effect of cancer treatments, such as surgery and radiation, is acquired lymphedema, highlighting the need for careful management of treatment-related complications (52). Lymphatic system aberration is characterized by significant swelling, adipose tissue development, inflammation, and fibrosis in affected areas. Furthermore, the expansion of fibrotic tissue in the lymphedematous area causes persistent edema that cannot be alleviated spontaneously (53). Nevertheless, the absence of appropriate animal models has proven to be a significant challenge in the development of an effective therapeutic strategy for acquired lymphedema. A 2017 study in Korea successfully established such a model by surgically removing specific lymph nodes and lymphatic vessels, resulting in lymphedema in mice. The affected areas showed increased hyaluronic acid levels compared to normal mice. Treatment with subcutaneous hyaluronidase injections every two days led to a notable reduction in lymphedema volume by day seven post-operation (54). Histochemical analysis showed decreased collagen accumulation and myofibroblast differentiation in epidermal tissues after hyaluronidase injection. This treatment resulted in increased interferon-γ, more T-helper 1 cells, and reduced interleukin-4 and interleukin-6 in the lymphedematous region and spleen (52). Previous research has proposed that hyaluronidase could serve as an early and reliable biomarker for bladder, prostate, and ovarian cancer. A clinical trial investigated the use of hyaluronic acid and hyaluronidase as urine biomarkers for early prostate cancer detection. Urine samples from 118 high-risk patients were analyzed using ELISA assay, revealing that both biomarkers were independently linked to prostate cancer and showed significant predictive ability. Additionally, a study involving 111 patients suggested that hyaluronidase 1 could serve as a prognostic marker for predicting the progression to muscle invasion in prostate cancer (55). In 2013, a non-invasive method was developed to identify advancements in bladder and prostate cancer using a hyaluronic acid fluorescence resonance energy transfer (HA-FRET) probe. This method allowed for the measurement of hyaluronidase levels in synthetic urine, correlating the degree of FRET release with hyaluronidase concentration (56). A study found elevated hyaluronidase 1 levels in clear cell and mucinous epithelial ovarian cancers (EOCs) and noted that the hyaluronidase 1 gene is repressed by estrogen receptor alpha (ERα). This suggests that hyaluronidase 1 could be a potential biomarker and therapeutic target for EOC subtypes with low ERα levels, indicating its role in EOC pathogenesis. Previous studies in Cancer Research found that hyaluronidase combined with chemotherapy for pancreatic cancer reduced tumor

pressure and improved drug delivery, enhancing effectiveness in preclinical models. In breast cancer, hyaluronidase reduced tumor growth and metastasis by disrupting the tumor microenvironment and boosting the immune response (57). The FDA approved a pre-mixed vial containing trastuzumab, pertuzumab, and recombinant human hyaluronidase for treating HER2-positive early breast cancer (58). Research on hyaluronidase in cancer treatment shows promise in improving conventional therapies. Further clinical studies are needed to assess its safety and effectiveness across various cancer types.

Concluding Remarks

Hyaluronidase has evolved from a simple "spreading factor" into a versatile therapeutic and diagnostic tool with significant implications across a range of biomedical fields. From facilitating subcutaneous drug delivery and improving ophthalmic and dermatologic procedures to reversing dermal filler complications and supporting oncological and inflammatory disease models, hyaluronidase continues to demonstrate broad utility. However, challenges remain, including immunogenic responses, enzyme source variability, and dosage standardization. Future research should aim to refine recombinant enzyme formulations, develop targeted delivery systems, and explore novel therapeutic indications, particularly in oncology and regenerative medicine. A deeper understanding of hyaluronidase's molecular mechanisms and interactions within complex biological systems will be key to unlocking its full clinical potential.

Conflict of Interest

The authors declared that they have no conflict of interest.

Funding

There is no funding in this study.

Author's Contributions

MS, MT, and AP: Original draft writing.

MM, AR, SM and SA: literature review and the manuscript revision.

Abbreviations

ART: Assisted Reproductive Technology.
EC: Enzyme Commission.
FDA: Food and Drug Administration.
GPI-anchor: Glycosylphosphatidylinositol-anchor.
HA: Hyaluronic Acid.
HYAL: Hyaluronidase.
IL-1b: Interleukin-1 beta.
IL-10: Interleukin-10.
OVDs: Ophthalmic Viscoelastic Device
TNF-a: Tumor Necrosis Factor-Alpha

References

1. Jung H. Hyaluronidase: An overview of its properties, applications, and side effects. Arch Plast Surg. 2020;47(04):297-300. https://doi.org/10.5999/aps.2020.00752
2. Mohankumar A, Rajan M. Role of hyaluronidase as an adjuvant in local anesthesia for cataract surgery. Indian J Ophthalmol. 2023;71(7):2649-55. https://doi.org/10.4103/ijo.ijo_2515_22
3. Weber GC, Buhren BA, Schrumpf H, Wohlrab J, Gerber PA. Clinical applications of hyaluronidase. Therapeutic enzymes: function and clinical implications. 2019:255-77.
4. Kroumpouzos G, Treacy P. Hyaluronidase for dermal filler complications: review of applications and dosage recommendations. JMIR Dermatol. 2024;7(1):e50403. https://doi.org/10.2196/50403
5. Ahmadian E, Dizaj SM, Eftekhari A, Dalir E, Vahedi P, Hasanzadeh A, et al. The potential applications of hyaluronic acid hydrogels in biomedicine. Drug Res. 2020;70(01):6-11. https://doi.org/10.1055/a-0991-7585
6. Marković-Housley Z, Miglierini G, Soldatova L, Rizkallah PJ, Müller U, Schirmer T. Crystal structure of hyaluronidase, a major allergen of bee venom. Structure. 2000;8(10):1025-35. https://doi.org/10.1016/s0969-2126(00)00511-6
7. Kaul A, Short WD, Wang X, Keswani SG. Hyaluronidases in human diseases. Int J Mol Sci. 2021;22(6):3204. https://doi.org/10.3390/ijms22063204
8. Stern R, Jedrzejas MJ. Hyaluronidases: their genomics, structures, and mechanisms of action. Chem Rev. 2006;106(3):818-39. https://doi.org/10.1021/cr050247k
9. Csoka AB, Frost GI, Stern R. The six hyaluronidase-like genes in the human and mouse genomes. Matrix Biol. 2001;20(8):499-508. https://doi.org/10.1016/s0945-053x(01)00172-x
10. Chowdhury B, Hemming R, Hombach-Klonisch S, Flamion B, Triggs-Raine B. Murine hyaluronidase 2 deficiency results in extracellular hyaluronan accumulation and severe cardiopulmonary dysfunction. J Biolo Chem. 2013;288(1):520-8. https://doi.org/10.1074/jbc.m112.393629
11. Triggs-Raine B, Natowicz MR. Biology of hyaluronan: Insights from genetic disorders of hyaluronan metabolism. World J Biol Chem. 2015;6(3):110. https://doi.org/10.4331/wjbc.v6.i3.110
12. West DC, Shaw DM, Lorenz P, Adzick NS, Longaker MT. Fibrotic healing of adult and late gestation fetal wounds correlates with increased hyaluronidase activity and removal of hyaluronan. Int J Biochem Cell Biol. 1997;29(1):201-10. https://doi.org/10.1016/s1357-2725(96)00133-1
13. Schwartz DM, Jumper MD, Lui G-M, Dang S, Schuster S, Stern R. Corneal endothelial hyaluronidase: a role in anterior chamber hyaluronic acid catabolism. Cornea. 1997;16(2):188-91. https://doi.org/10.1097/00003226-199703000-00011
14. Reitinger S, Lepperdinger G. Hyaluronan, a ready choice to fuel regeneration: a mini-review. Gerontology. 2012;59(1):71-6. https://doi.org/10.1159/000342200
15. Fronza M, Caetano GF, Leite MN, Bitencourt CS, Paula-Silva FW, Andrade TA, et al. Hyaluronidase modulates inflammatory response and accelerates the cutaneous wound healing. PLoS One. 2014;9(11):e112297. https://doi.org/10.1371/journal.pone.0112297
16. Seol D-W, Joo SH, Kim Y-H, Song B-S, Sim B-W, Kim S-U, et al. Sperm hyaluronidase is critical to mammals' fertilization

for its ability to disperse cumulus–oocyte complex layer. Asian J Androl. 2022;24(4):411-5. https://doi.org/10.4103/aja202176
17. Lokeshwar VB, Selzer MG. Hyaluronidase: both a tumor promoter and suppressor. Hyaluronan Cancer Biol. 2009:189-206. https://doi.org/10.1016/b978-012374178-3.10011-0
18. Chowdhury B, Xiang B, Liu M, Hemming R, Dolinsky VW, Triggs-Raine B. Hyaluronidase 2 deficiency causes increased mesenchymal cells, congenital heart defects, and heart failure. Circ Cardiovasc Genet. 2017;10(1):e001598. https://doi.org/10.1161/circgenetics.116.001598
19. Jung M, Ma Y, Iyer RP, DeLeon-Pennell KY, Yabluchanskiy A, Garrett MR, et al. IL-10 improves cardiac remodeling after myocardial infarction by stimulating M2 macrophage polarization and fibroblast activation. Basic Res Cardiol. 2017;112:1-14. https://doi.org/10.1007/s00395-017-0622-5
20. Yoshino Y, Goto M, Hara H, Inoue S. The role and regulation of TMEM2 (transmembrane protein 2) in HYBID (hyaluronan (HA)-binding protein involved in HA depolymerization/KIAA1199/CEMIP)-mediated HA depolymerization in human skin fibroblasts. Biochem Biophys Res Commun. 2018;505(1):74-80. https://doi.org/10.1016/j.bbrc.2018.09.097
21. Chopra D, Brehm JE, Morrison B. Hyaluronidase as a successful treatment modality for scleroderma-induced microstomia. Dermatol Surg. 2022;48(9):1014-5. https://doi.org/10.1097/dss.0000000000003543
22. Averbeck M, Gebhardt CA, Voigt S, Beilharz S, Anderegg U, Termeer CC, et al. Differential regulation of hyaluronan metabolism in the epidermal and dermal compartments of human skin by UVB irradiation. J Invest Dermatol. 2007;127(3):687-97. https://doi.org/10.1038/sj.jid.5700614
23. Colombaro V, Jadot I, Declèves A-E, Voisin V, Giordano L, Habsch I, et al. Hyaluronidase 1 and hyaluronidase 2 are required for renal hyaluronan turnover. Acta Histochemica. 2015;117(1):83-91. https://doi.org/10.1016/j.acthis.2014.11.007
24. Harada H, Takahashi M. CD44-dependent intracellular and extracellular catabolism of hyaluronic acid by hyaluronidase-1 and-2. J Biol Chem. 2007;282(8):5597-607. https://doi.org/10.1074/jbc.m608358200
25. Kim J, Seki E. Hyaluronan in liver fibrosis: basic mechanisms, clinical implications, and therapeutic targets. Hepatol Commun. 2023;7(4):e0083. https://doi.org/10.1097/hc9.0000000000000083
26. Orăşan OH, Sava M, Iancu M, Cozma A, Saplonţai-Pop A, Sarlea Ţărmure S, et al. Serum hyaluronic acid in chronic viral hepatitis B and C: a biomarker for assessing liver fibrosis in chronic hemodialysis patients. Int Urol Nephrol. 2015;47:1209-17. https://doi.org/10.1007/s11255-015-1017-x
27. Rezaei-Agdam H, Moshari S, Nahari E, Minas A, Daliri Z, Hallaj M, et al. Zeta and hyaluronic acid assessments, novel sperm selection procedures, in animal model for male infertility. Andrologia. 2019;51(11):e13447. https://doi.org/10.1111/and.13447
28. Nasr-Esfahani M, Razavi S, Vahdati A, Fathi F, Tavalaee M. Evaluation of sperm selection procedure based on hyaluronic acid binding ability on ICSI outcome. J Assist Reprod Genet. 2008;25:197-203. https://doi.org/10.1007/s10815-008-9223-4
29. Nixon B, Schjenken JE, Burke ND, Skerrett-Byrne DA, Hart HM, De Iuliis GN, et al. New horizons in human sperm selection for assisted reproduction. Front Endocrinol 2023;14:1145533. https://doi.org/10.3389/fendo.2023.1145533
30. Sawan D, Hersant B. Therapeutic use of hyaluronidase in obstetrics. Open J Obstet Gynecol. 2021;11(11):1581-8. https://doi.org/10.4236/ojog.2021.1111147
31. Kwon H, Park HS, Shim J-Y, Lee KW, Choi S-J, Choi GY. Randomized, double-blind, placebo-controlled trial on the efficacy of hyaluronidase in preventing perineal trauma in nulliparous women. Yonsei Med J. 2020;61(1):79-84. https://doi.org/10.3349/ymj.2020.61.1.79
32. Gomes T-P, Palma L-F, Tornelli M-J, Tornelli H-R, Fukuoka C-Y, Borsatti M-A. Hyaluronidase following buccal infiltrations of articaine with epinephrine for anesthesia of mandibular first molars: a split-mouth, double-blind, placebo-controlled randomized clinical trial. J Clin Exp Dent. 2022;14(11):e938. https://doi.org/10.4317/jced.59809
33. Silverstein SM, Greenbaum S, Stern R. Hyaluronidase in ophthalmology. J Appl Res. 2012;12(1).
34. Benozzi J, Nahum LP, Campanelli JL, Rosenstein RE. Effect of hyaluronic acid on intraocular pressure in rats. Invest Ophthalmol Vis Sci. 2002;43(7):2196-200.
35. Signorini M, Liew S, Sundaram H, De Boulle KL, Goodman GJ, Monheit G, et al. Global aesthetics consensus: avoidance and management of complications from hyaluronic acid fillers—evidence-and opinion-based review and consensus recommendations. Plast Reconstr Surg. 2016;137(6):961e-71e. https://doi.org/10.1097/prs.0000000000002184
36. Borzabadi-Farahani A, Mosahebi A, Zargaran D. A scoping review of hyaluronidase use in managing the complications of aesthetic interventions. Aesthet Plast Surg. 2024;48(6):1193-209. https://doi.org/10.1007/s00266-022-03207-9
37. Wohlrab J, Finke R, Franke WG, Wohlrab A. Clinical trial for safety evaluation of hyaluronidase as diffusion enhancing adjuvant for infiltration analgesia of skin with lidocaine. Dermatol Surg. 2012;38(1):91-6. https://doi.org/10.1111/j.1524-4725.2011.02146.x
38. Feighery C, McCoy E, Johnston P, Armstrong D. Delayed hypersensitivity to hyaluronidase (Hyalase™) used during cataract surgery. Contact Dermatitis. 2007;57(5). https://doi.org/10.1111/j.1600-0536.2007.01038.x
39. Pardue EL, Ibrahim S, Ramamurthi A. Role of hyaluronan in angiogenesis and its utility to angiogenic tissue engineering. Organogenesis. 2008;4(4):203-14. https://doi.org/10.4161/org.4.4.6926
40. Spinelli FM, Vitale DL, Demarchi G, Cristina C, Alaniz L. The immunological effect of hyaluronan in tumor angiogenesis. Clin Transl Immunol. 2015;4(12):e52. https://doi.org/10.1038/cti.2015.35
41. Shibata Y, Taogoshi T, Matsuo H. Extravasation of noncytotoxic agents: skin injury and risk classification. Biol Pharm Bull. 2023;46(6):746-55. https://doi.org/10.1248/bpb.b22-00850
42. Ong J, Van Gerpen R. Recommendations for management of noncytotoxic vesicant extravasations. J Infus Nurs. 2020;43(6):319-43. https://doi.org/10.1097/nan.0000000000000392
43. Stefanos SS, Kiser TH, MacLaren R, Mueller SW, Reynolds PM. Management of noncytotoxic extravasation injuries: A focused update on medications, treatment strategies, and peripheral administration of vasopressors and hypertonic saline. Pharmacotherapy. 2023;43(4):321-37. https://doi.org/10.1002/phar.2794
44. Muggenthaler MM, Chowdhury B, Hasan SN, Cross HE,

Mark B, Harlalka GV, et al. Mutations in HYAL2, encoding hyaluronidase 2, cause a syndrome of orofacial clefting and cor triatriatum sinister in humans and mice. PLoS Genet. 2017;13(1):e1006470. https://doi.org/10.1371/journal.pgen.1006470
45. Zaaba MIS, Mokhtar KI, Rajion ZA. Revisiting Genetics of Cleft Lip with or without Cleft Palate and Cleft Palate Only: A Narrative Review. Arch Orofac Sci. 2023;18(2). https://doi.org/10.21315/aos2023.1802.rv01
46. Watson D, Reuther MS, Wong VW, Sah RL, Masuda K, Briggs KK. Effect of hyaluronidase on tissue-engineered human septal cartilage. Laryngoscope. 2016;126(9):1984-9. https://doi.org/10.1002/lary.25720
47. Collins MN, Birkinshaw C. Hyaluronic acid based scaffolds for tissue engineering—A review. Carbohydr Polym. 2013;92(2):1262-79. https://doi.org/10.1016/j.carbpol.2012.10.028
48. Ran X, Du Y, Wang Z, Wang H, Pu F, Ren J, et al. Hyaluronic acid-templated Ag nanoparticles/graphene oxide composites for synergistic therapy of bacteria infection. CS Appl Mater Interfaces. 2017;9(23):19717-24. https://doi.org/10.1021/acsami.7b05584
49. Velesiotis C, Vasileiou S, Vynios DH. A guide to hyaluronan and related enzymes in breast cancer: biological significance and diagnostic value. FEBS J. 2019;286(15):3057-74. https://doi.org/10.1111/febs.14860
50. Whatcott CJ, Han H, Posner RG, Hostetter G, Von Hoff DD. Targeting the tumor microenvironment in cancer: why hyaluronidase deserves a second look. Cancer Discov. 2011;1(4):291-6. https://doi.org/10.1158/2159-8290.cd-11-0136
51. Shakouri A, Parvan R, Adljouy N, Abdolalizadeh J. Purification of h yaluronidase as an anticancer agent inhibiting CD44. Biomed Chromatogr. 2020;34(1):e4709. https://doi.org/10.1002/bmc.4709
52. Roh K, Cho S, Park J-h, Yoo BC, Kim W-K, Kim S-k, et al. Therapeutic effects of hyaluronidase on acquired lymphedema using a newly developed mouse limb model. Exp Biol Med. 2017;242(6):584-92. https://doi.org/10.1177/1535370216688570
53. Cho S, Roh K, Park J, Park YS, Lee M, Cho S, et al. Hydrolysis of hyaluronic acid in lymphedematous tissue alleviates fibrogenesis via TH1 cell-mediated cytokine expression. Sci Rep. 2017;7(1):35. https://doi.org/10.1038/s41598-017-00085-z
54. Dalaei F, Bucan A, Wiinholt A, Jørgensen MG, Hansen CR, Baun C, et al. Short term treatment of secondary lymphedema with hyaluronidase injections reduces mouse hindlimb lymphedema. J Plast Surg Hand Surg. 2023;58:40-7. https://doi.org/10.2340/jphs.v58.7791
55. Kramer MW, Golshani R, Merseburger AS, Knapp J, Garcia A, Hennenlotter J, et al. HYAL-1 hyaluronidase: a potential prognostic indicator for progression to muscle invasion and recurrence in bladder cancer. Eur Urol. 2010;57(1):86-94. https://doi.org/10.1016/j.eururo.2009.03.058
56. Chib R, Raut S, Fudala R, Chang A, Mummert M, Rich R, et al. FRET based ratio-metric sensing of hyaluronidase in synthetic urine as a biomarker for bladder and prostate cancer. Curr Pharm Biotechnol. 2013;14(4):470-4. https://doi.org/10.2174/1389201013149990222
57. Provenzano PP, Cuevas C, Chang AE, Goel VK, Von Hoff DD, Hingorani SR. Enzymatic targeting of the stroma ablates physical barriers to treatment of pancreatic ductal adenocarcinoma. Cancer Cell. 2012;21(3):418-29. https://doi.org/10.1016/j.ccr.2012.01.007
58. Tan AR, Im S-A, Mattar A, Colomer R, Stroyakovskii D, Nowecki Z, et al. Fixed-dose combination of pertuzumab and trastuzumab for subcutaneous injection plus chemotherapy in HER2-positive early breast cancer (FeDeriCa): a randomised, open-label, multicentre, non-inferiority, phase 3 study. Lancet Oncol. 2021;22(1):85-97. https://doi.org/10.1016/s1470-2045(20)30536-2
59. Krupkova O, Greutert H, Boos N, Lemcke J, Liebscher T, Wuertz-Kozak K. Expression and activity of hyaluronidases HYAL-1, HYAL-2 and HYAL-3 in the human intervertebral disc. Eur Spine J. 2020;29:605-15. https://doi.org/10.1007/s00586-019-06227-3
60. Muto J, Sayama K, Gallo RL, Kimata K. Emerging evidence for the essential role of hyaluronan in cutaneous biology. J Dermatol Sci. 2019;94(1):190-5. https://doi.org/10.1016/j.jdermsci.2019.01.009