Module 1

Hyaluronic Acid: Advent in Dentistry!

Contents

Hyaluronic acid: A key molecule

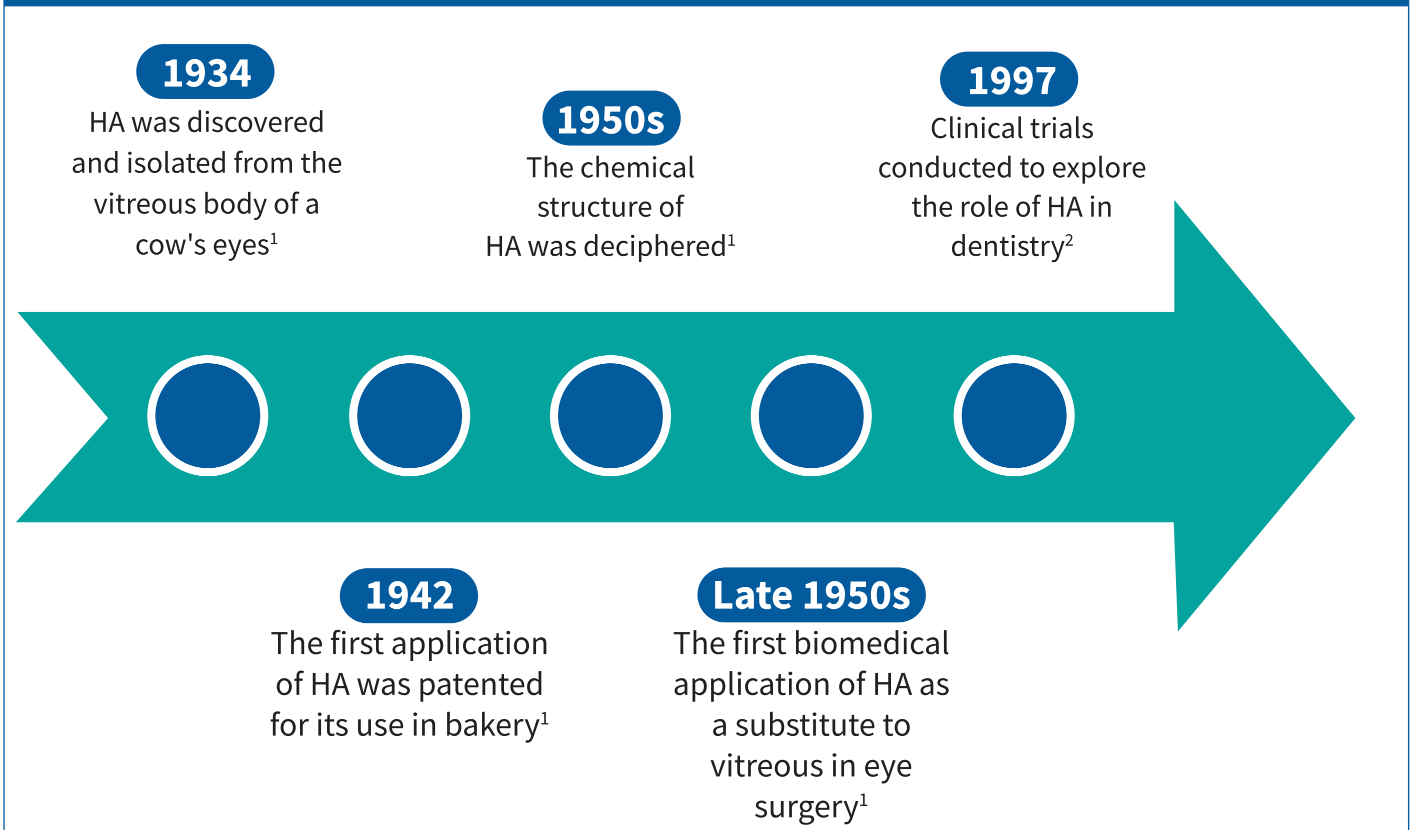
What is hyaluronic acid?

Hyaluronic acid (HA) is a linear non-sulfated glycosaminoglycan that naturally occurs in the extracellular matrix (ECM) of connective tissues, other tissues, and the synovial fluid.[1,2] Hyaluronic acid is derived from ‘hyalos’ (the Greek word for glass) + uronic acid.[1] It plays a vital role in cell signaling, regeneration of tissues, healing of wounds, morphogenesis, matrix organization, and pathobiology.[3] Interactions with growth factors, osmotic pressure regulation, intra- and extracellular interactions, and lubrication of tissues are some of the physiological and structural functions of HA, which enable to maintain integrity of the tissues.[2]

Historical background

The historical background of HA is summarized below in Figure 1.

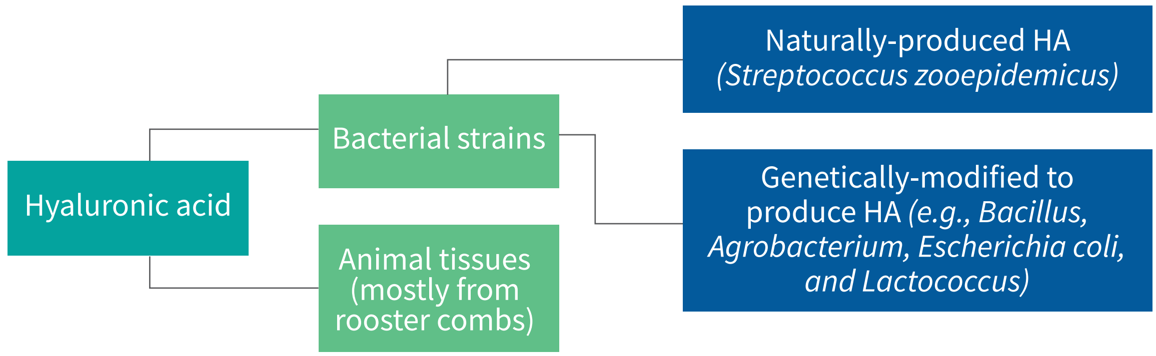
Figure 1: Hyaluronic acid historical background.[1,2]



What is the source of hyaluronic acid?

Hyaluronic acid can be extracted from animal tissues or bacterial strains as depicted below in Figure 2. However, there is always an associated risk of contamination with viruses or animal proteins (when extracted from animal sources), and toxins along with bacterial proteins (when extracted from bacteria), which may trigger an immune response.[4]

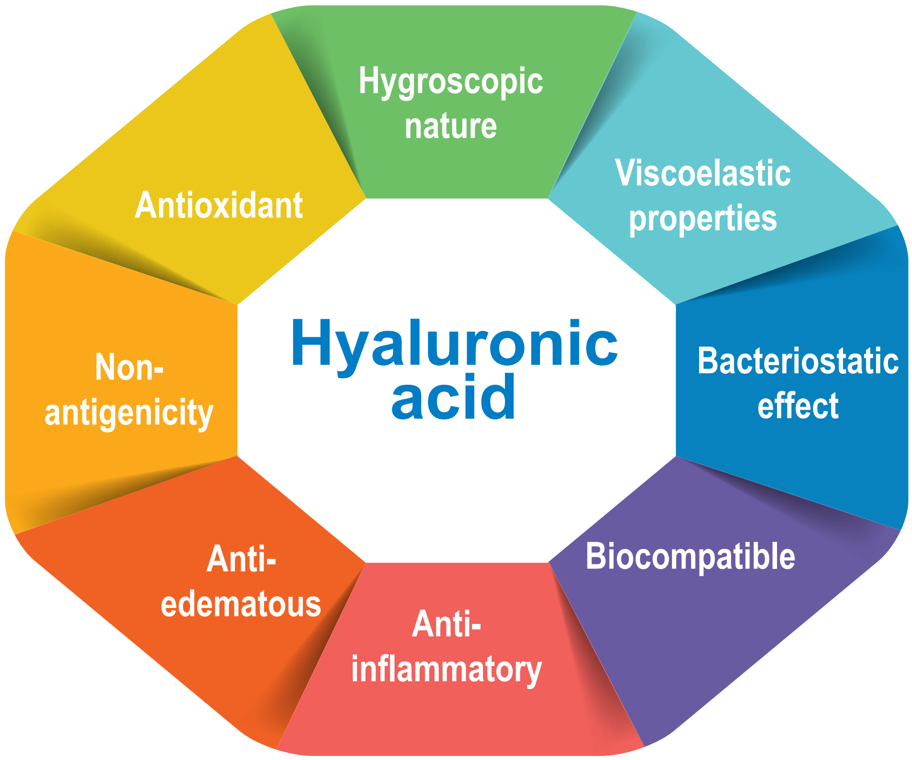
Figure 2: Sources of hyaluronic acid.[4]



Physiochemical and biological properties of hyaluronic acid

Some of the properties of HA make it a potent therapeutic agent in the treatment of inflammation in the areas of orthopedics, dermatology, and ophthalmology (Figure 3).[2] Exogenous HA is being explored for use as a drug delivery system for oncological treatments, and as a treatment option for rhinology, urology, aesthetic medicine, pneumology, arthrology, and cosmetics.[5]

Figure 3: Physiochemical and biological properties of hyaluronic acid.[2]



Hygroscopicity

The hygroscopic nature of HA enables it to maintain conformational stiffness and retain water. It plays a vital role in space-filling, shock absorption, lubrication, and protein exclusion when used as physical background material.[2]

Viscoelasticity

In various periodontal regenerative procedures, HA functions as a surface protectant and helps to maintain spaces. Additionally, the viscoelasticity of HA delays viral and bacterial penetration.[2]

Bacteriostatic effect

A potent bacteriostatic effect is exhibited by medium-and low-molecular–weight HA on strains of *Prevotella oris, Aggregatibacter actinomycetemcomitans,* and *Staphylococcus aureus* that are often found in gingival and periodontal wounds. Reduction of bacterial burden at the wound site may improve the clinical outcome of regenerative therapy.[2]

Biocompatibility and non-antigenicity

The biocompatibility and non-immunogenic nature of HA enables its use in bone regeneration and wound and periodontal tissue healing.[2]

Anti-inflammatory effect

The anti-inflammatory effect of HA can be attributed to the scavenging action of exogenous HA.[2]

Anti-edematous

The anti-edematous effect of HA might be linked to its osmotic activity. The tissue healing ability of HA enables its use as adjunctive treatment to mechanical therapy.[2]

Antioxidative properties

Hyaluronic acid scavenges the reactive oxygen species and functions as an antioxidant

thus, stabilizing the granulation tissue matrix.[2]

Mechanism of action of hyaluronic acid

Hyaluronic acid performs its biological actions by two basic mechanisms:[5]

- By acting as a passive structural molecule
- By acting as a signaling molecule

Passive structural molecule

The passive mechanism is associated with the physicochemical properties of high-molecular–weight HA. Hyaluronic acid can modulate hydration of tissues, osmotic balance, and the physical properties of ECM owing to its macromolecular size, and hygroscopic and viscoelastic nature.[5]

Signaling molecule

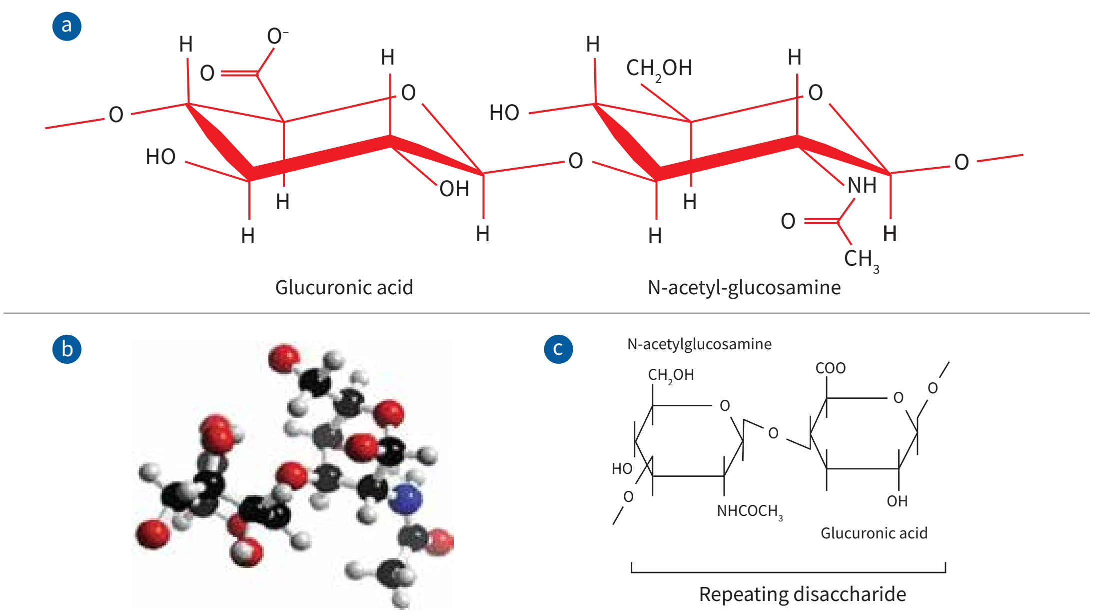
Hyaluronic acid functions as a signaling molecule by interacting with its binding proteins. Multiple factors like the molecular weight of HA, location, cell-specific factors, and the bond between HA and its proteins regulate the inflammatory activities, cell migration, and cell division and differentiation.[5]

Biology and chemistry of hyaluronic acid

Chemical structure of hyaluronic acid

Hyaluronic acid is a polysaccharide made up of D-glucuronic acid and N-acetyl-d-glucosamine (Figure 4). It is an unbranched linear chain of monosaccharide units linked by alternating β1,3 and β1,4 glycosidic bonds. The secondary structure of HA is made by the axial hydrogen atoms of the eight carbon-hydrogen (CH) groups on alternate sides. The intermolecular hydrogen bonding stabilizes the tertiary structure of the molecule.[2]

Figure 4: Hyaluronic acid chemical structure representation.[2]



a) Chemical structure of HA (b) 3D model of hyaluronic acid (c) Repeating units of D-glucuronic acid and N-acetyl-d-glucosamine.

Occurrence of hyaluronic acid

Hyaluronic acid is present in all the organs in vertebrates; however, it is found in abundance in the ECM of the soft connective tissues. Hyaluronic acid functions as protective, structure stabilizer and shock-absorber in the skin. About 5 g is the estimated total amount of HA present in the skin, which is a third of the total HA content in the human body. Soft connective tissues such as the umbilical cord, synovial fluid, and the skin contain high concentrations of HA, while the blood serum contains the lowest.[2]

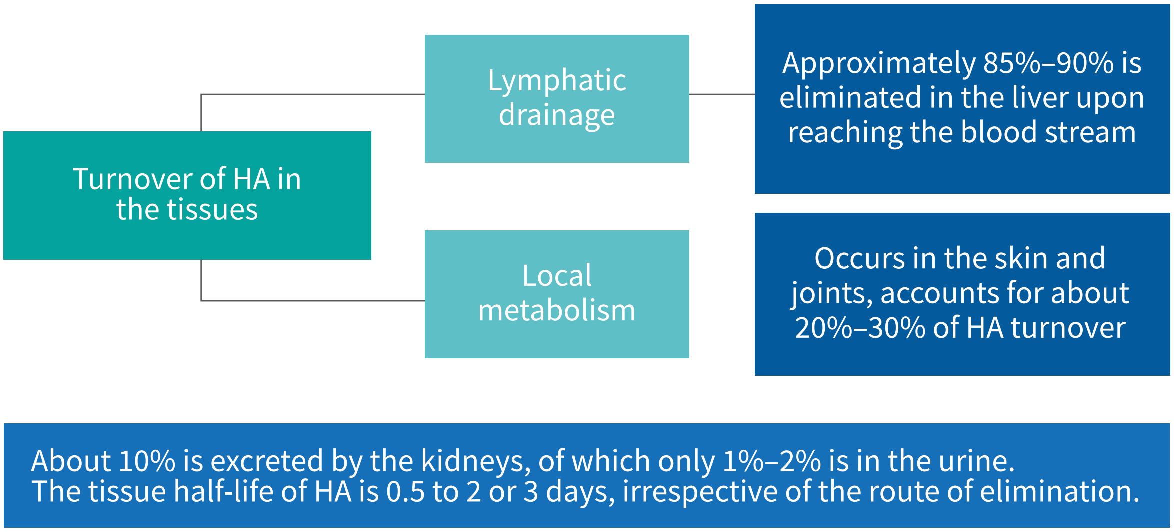
Almost all the cells of the body are capable of synthesizing HA and the synthesis occurs in the cell membrane. The membrane-bound protein synthesizes HA in the plasma membrane and is secreted directly into the extracellular space. Fibroblasts, in the presence of endotoxins, also produce HA.[2]

Hyaluronic acid is found in periodontal tissues, predominantly in gingiva and periodontal ligament (non-mineralized tissues) and in small amounts in cementum and alveolar bone (mineralized tissues). High-molecular–weight HA occurring in periodontal tissues is synthesized by the hyaluronan synthase (HAS) enzymes (HAS1, HAS2, and HAS3).[2]

Metabolism of hyaluronic acid

The metabolism of HA is summarized below (Figure 5).

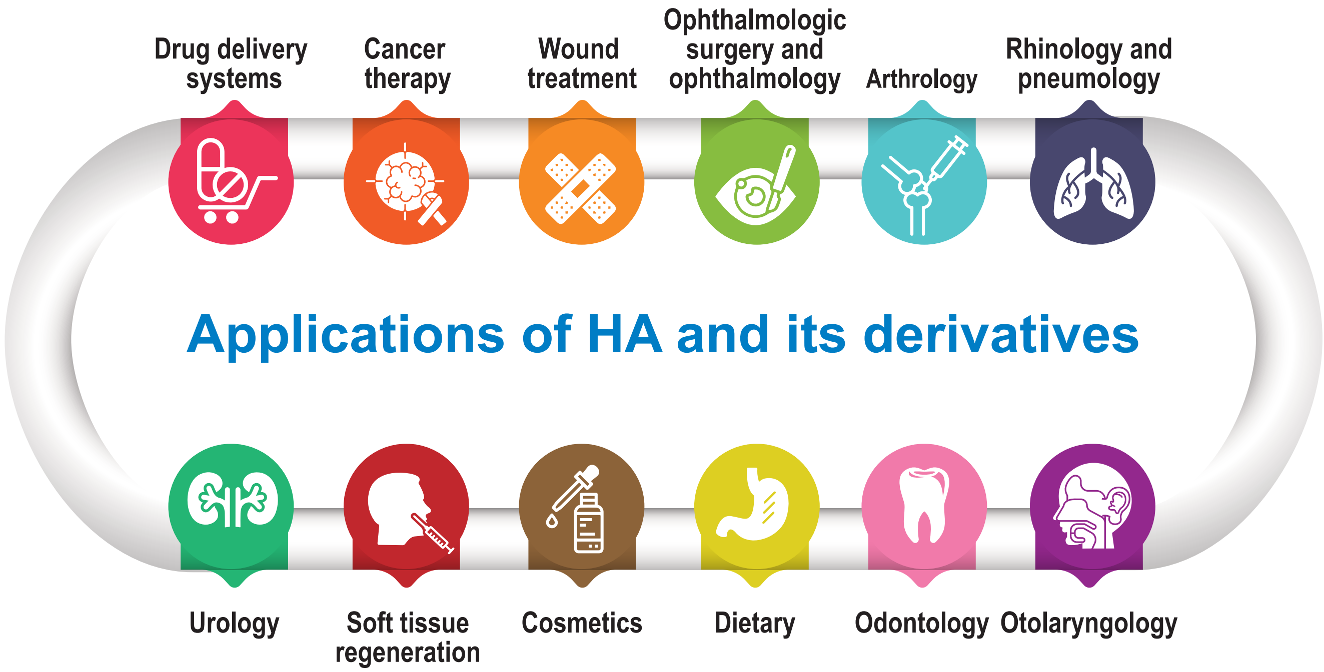
Figure 5: Hyaluronic acid metabolism.[5]



What are the applications of hyaluronic acid and its derivatives?

Native HA and its derivatives have multiple applications in the fields of medicine, pharmaceuticals, cosmetics, and food. Some of the applications of HA and its derivatives are summarized below (Figure 6).[5]

Figure 6: Applications of hyaluronic acid.[5]



Drug delivery

Hyaluronic acid and its derivatives aid to improve drug delivery. They are used as single agents or in combination. Conjugation of active ingredients with HA enables the development of pro-drugs with improvised stability, efficacy, and physicochemical properties.[5]

Oncology

Hyaluronic acid functions simultaneously as both drug carrier and targeting agent, in the form of polymer-antitumoral conjugates or delivery systems, containing anti-cancer therapeutics.[5]

Treatment of wounds

Hyaluronic acid finds potent application in wound healing as it promotes the proliferation, migration, and adhesion of fibroblasts to the wound and stimulates collagen production.[5]

Ophthalmic application

Since HA is a component of the human eye, HA-based ophthalmic products are biocompatible and do not trigger any adverse reactions. Hyaluronic acid-based solutions find application in devices used in surgeries to protect and lubricate tissues of the eyes or replace the vitreous fluid.[5]

Arthrology

Intra-articular HA injection is widely used, as it promotes the synthesis of new HA in the synovial cells, stimulates the proliferation of chondrocytes, and reduces the degradation of cartilage.[5]

Pneumology and rhinology

Being a part of the normal airway secretions, high-molecular–weight endogenous HA plays a role in the homeostasis of the upper airways and the lower airways. Hyaluronic acid carries out the following functions:

- Exhibits potent anti-inflammatory and anti-angiogenic activities
- Promotes survival of the cell and mucociliary clearance
- Enables the organization of ECM and stabilization of the connective tissues
- Sustains the process of healing
- Regulates hydration of the tissues

The high-molecular–weight exogenous HA finds potent application in disorders involving inflammation, oxidative stress, and remodeling of the epithelium such as asthma, cystic fibrosis chronic obstructive pulmonary disease, and allergic/non-allergic rhinitis.[5]

Urogenital application

Intravesical HA demonstrates the potential to reduce the recurrence of urinary tract infections like bacterial cystitis, reduce symptoms, and confer protection to the mucosa when administered alone or as a combination therapy with chondroitin sulfate or alpha-blockers.[5]

Regeneration of soft tissues

The content of HA in the skin decreases with age, causing a visible loss of hydration, elasticity, and volume of the facial skin, resulting in wrinkles. Dermal fillers with HA are being developed to rectify facial imperfections like wrinkles or scars and to help restore the lost volume.[5]

Cosmetology

Hyaluronic acid serves as a moisturizing active ingredient in cosmetics like gels, serums, and emulsions for youthful skin.[5]

Diet

Hyaluronic acid is a vital ingredient in enriched foods and supplements. As it improves the appearance of the skin, it is acknowledged as a nutri-cosmetic.[5]

Utility of hyaluronic acid: Through the lens of a dentist

Hyaluronic acid: An apt biomaterial for dentistry

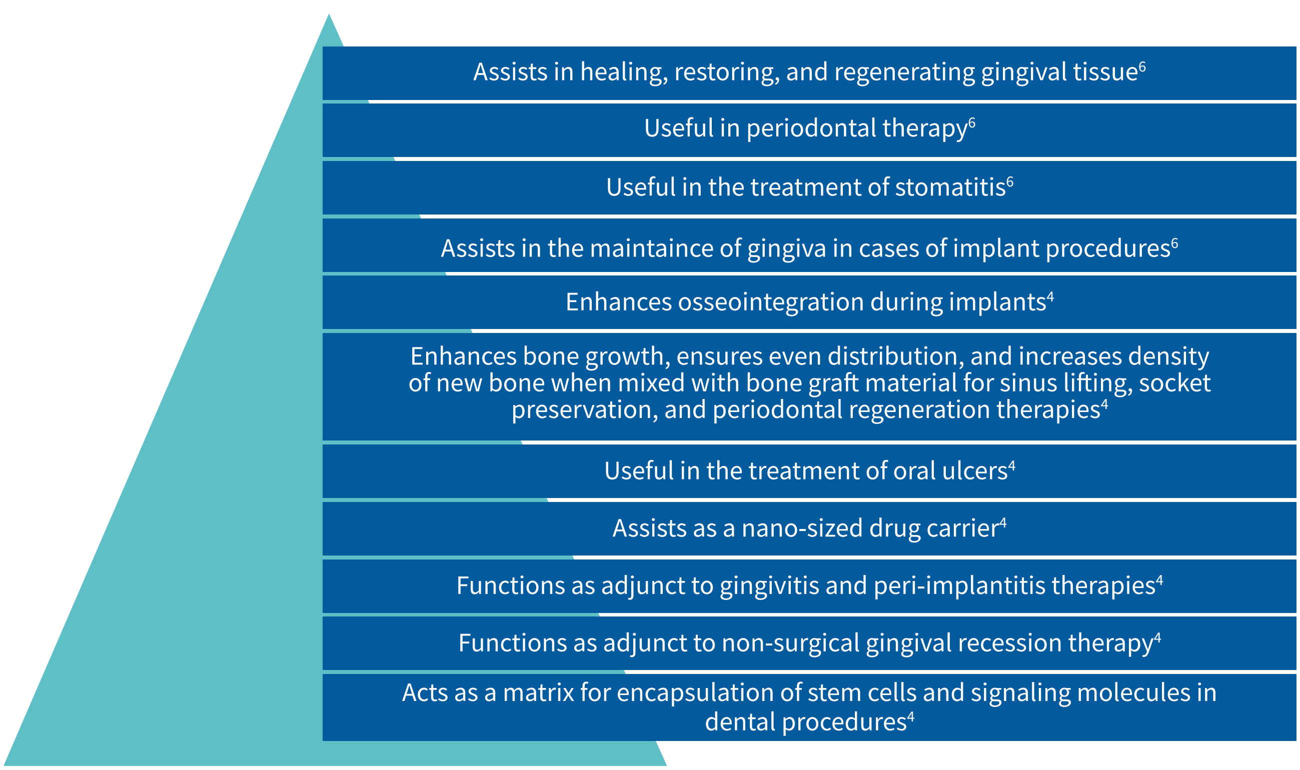
Biological materials like HA are used in dental applications to regenerate soft tissues, build a barrier between the soft and hard tissues (bone), promote the healing of wounds, and facilitate the regeneration of hard tissues.[4]

Modified HA can work as a gel scaffold, trigger, or reservoir for releasing chemical signals. These properties allow the use of HA, a companion biomaterial in dentistry, especially for tissue regeneration and healing of wounds. There is a lack of mechanical integrity when HA is used as a standalone material in dental procedures.[4]

Hyaluronic acid application in dentistry

Applications of hyaluronic acid in dentistry include the following (Figure 7).

Figure 7: Applications of HA in dentistry.[4,6]



Role of hyaluronic acid

Hyaluronic acid has multiple structural and physiological functions in the soft periodontal tissue, ligaments, gingiva, alveolar bone, and cementum. Some of the key roles are as follows:[7]

• Inflammatory response

The high-molecular–weight HA degrades to lower-molecular–weight molecules in chronically inflamed tissues, such as gingival tissue or in the postoperative period after implant or sinus lift surgery. The fragmentation is influenced by reactive oxygen species. This low-molecular–weight HA damages the tissues and mobilizes the immune cells. The high-molecular–weight HA plays a role in immune response suppression, thus preventing exacerbation of inflammation.[7]

• Role in infection control

The use of HA-based membranes, sponges, or gel in surgeries may reduce bacterial contamination, thus alleviating the risk of infection after surgery, and promoting regeneration.[2]

• Role in wound healing

Hyaluronic acid functions as a regulator of migration and cellular defense mechanism when bound to receptors on active defense cells. It plays a vital role in the healing of chronic injuries, of both mineralized and non-mineralized periodontal tissues, during the inflammatory phase, the granulation tissue formation, and epithelial remodeling.[6]

Efficacy of HA in dental treatments: Clinical evidence

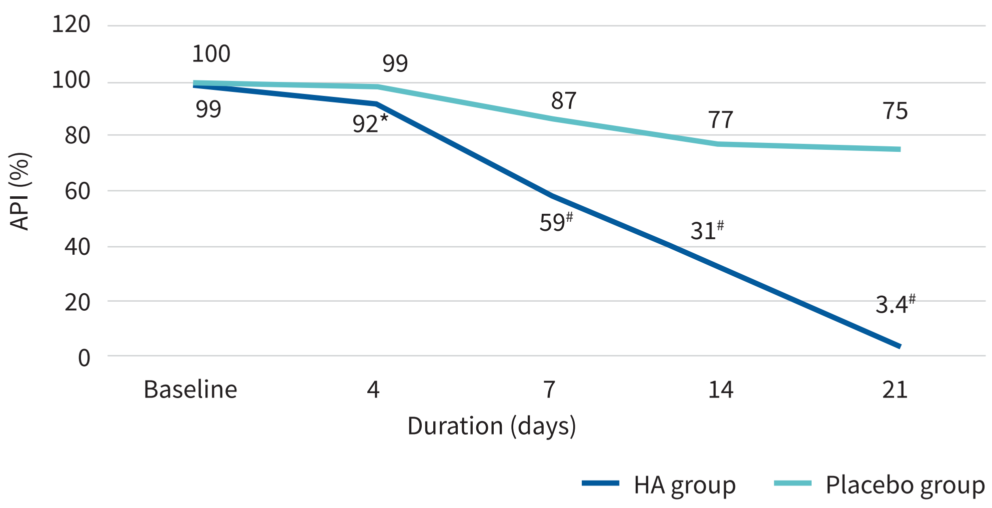
• Hyaluronic acid in gingivitis[8]

Aim: To evaluate the effect of hyaluronic acid in the treatment of plaque-induced gingivitis.

Patient groups: HA group (n=25), Placebo group (n=25)

Results:

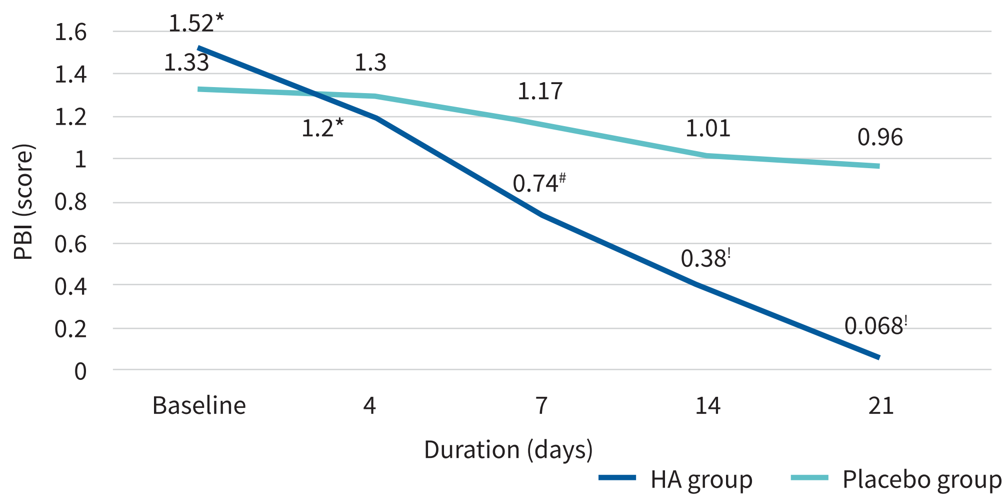
Graph 1: Evaluation of API in the treatment groups.



API: Approximal plaque indices; *: P=0.011; #: P<0.001.

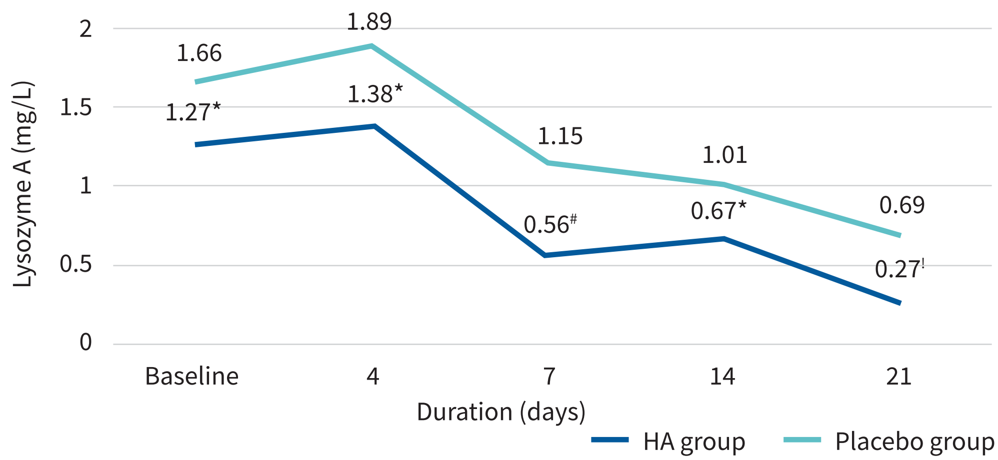
- A significant improvement was observed in approximal plaque indices (p=0.011) (Graph 1) and the papilla bleeding index (p=0.001) (Graph 2) from the fourth and the seventh day of treatment initiation, respectively, in the HA patients. A significant decrease was also reported in lysozyme A, from 1.27 mg/L to 0.27 mg/L, in the HA group (Graph 3).

Graph 2: Evaluation of PBI score in the treatment groups.



PBI: Papilla bleeding index; *: p=non-significant; #: p=0.001; !: p<0.001.

Graph 3: Evaluation of lysozyme A in the treatment groups.



*: p=non-significant; #: p=0.015; !:p=0.039.

Hyaluronic acid in surgical correction of the infra-bone defects[9]

Aim: To study the osteoinductive effect of the hyaluronic acid (HA) with an esterified low-molecular preparation.

Results based on radiographic re-evaluation:

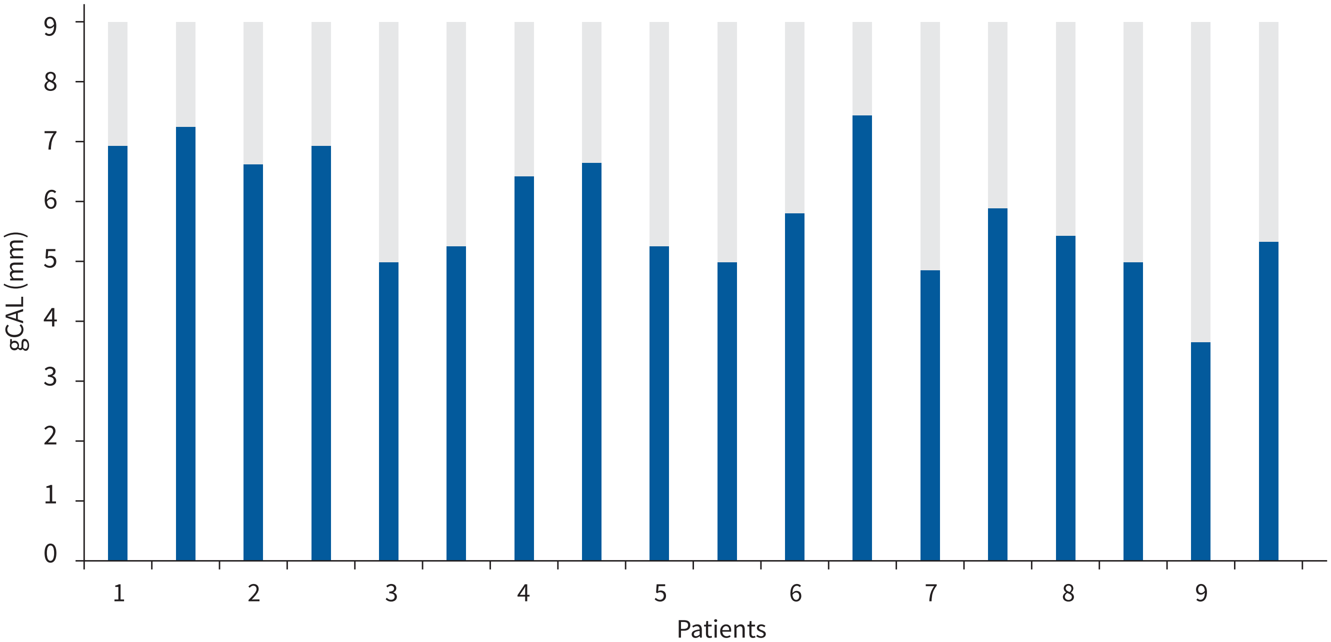
Hyaluronic acid demonstrated potent efficacy in accelerating the formation of new bone in patients (n=9) with infra-bone defects.

- Post 6 months of surgery: Mild bone remodeling present and excellent infra-bone filling (Figure 8b).
- Post 9 months of surgery: A mean gain in clinical attachment (gCAL) of 2.6 mm was achieved; filling defect and good prognosis (Figure 8c, Figure 9).
- Post 24 months of surgery: Satisfactory filling of the infra bony defect (Figure 8d).

Figure 8: Pre-operative and post-operative radiographs.

(a) Pre-operative radiograph (b) Radiographic at 6 months post-surgery (c) Radiograph at 9 months post-surgery (d) Radiograph at 24 months post-surgery.

Figure 9: Graphical representation of gain hi clinical attachment in each patient.



gCAL: gain hi clinical attachment.

The mean gCAL indicates new bone formation in patients with the infra-bone defects. The data shows that autologous bone graft, when combined with esterified low-molecular HA, demonstrates potential to accelerate formation of new bone.[9]

Hyaluronic acid in oral ulcers[10]

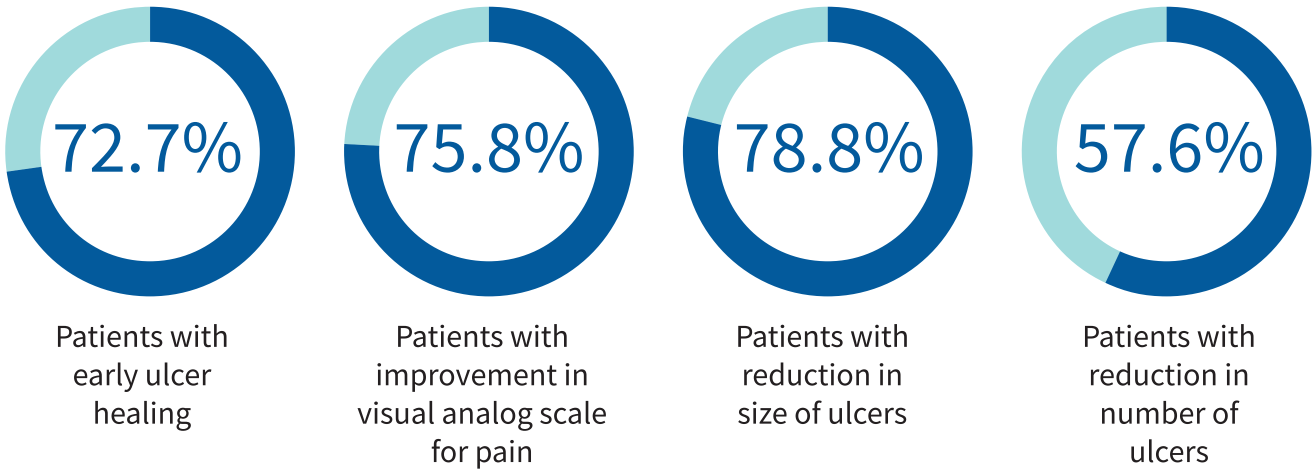
Aim: To evaluate the efficacy and safety of the topical application of 0.2% HA gel in patients with recurrent aphthous ulcers (RAU) and the ulcers of Behçet's disease (BD).

Number of patients: 17 patients: Behçet's disease, 16 patients: Recurrent aphthous ulcers

Results:

0.2% HA gel demonstrated potent efficacy in patients with RAU and the ulcers of BD over a period of 2 weeks. The results are summarized as below (Figure 10).

Figure 10: Clinical efficacy of HA.



Conclusion

Based on promising results in clinical trials, acceptability by patients, it is evident that hyaluronic acid has multiple applications in the field of dentistry. It is a safe option for children during the second dentition, the elderly, and pregnant females.[6]

Key messages

- Hyaluronic acid is a naturally occurring, non-sulfated glycosaminoglycan. It is an unbranched biodegradable polymer.[1]
- Some of the key properties of HA are that it is biocompatible, hygroscopic, anti-inflammatory, non-antigenic, bacteriostatic, and viscoelastic.[2]
- Hyaluronic acid functions as a key player in cell signaling, regeneration of tissues, healing of wounds, morphogenesis, matrix organization, and pathobiology.[3]
- Hyaluronic acid is used as a biomaterial in dentistry to regenerate soft tissues, build a barrier between the soft and hard tissues (bone), promote healing of wounds, and facilitate regeneration of hard tissues.[4]
- Hyaluronic acid acts as both a passive structural molecule and as a signaling molecule.[5]
- Hyaluronic acid has multiple structural and physiological functions in the soft periodontal tissue, ligament, gingiva, alveolar bone, and cementum. It plays a significant role in infection control, wound healing, and inflammatory response.[2,6,7]
- Data from clinical studies show that HA demonstrates a potent benefit in the treatment of plaque-induced gingivitis, accelerating new bone formation in infra bony defects, and treating recurrent aphthous ulcers and oral ulcers of Behçet's disease.[8–10]

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