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Review

Hyaluronic acid in wound dressings

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Abstract: Human skin possesses an essential function in the maintenance of individuals' health. However, it may undergo a variety of lesions that produce wounds of distinct severity. In this respect, instantly after any skin wound, the process of tissue regeneration and repair initiates. Nevertheless, diverse factors can delay this process, including bacterial infections, nutritional status, age, hypoxia, chronic diseases, necrosis, and vascular and arterial diseases. Thus, wound dressings are frequently used to improve wound healing. Those wound dressings are fabricated with diverse materials, which confer them different properties. In this regard, hyaluronic acid is a natural polysaccharide widely distributed in extracellular matrices of mammal tissues, which possesses remarkable attributes in terms of biocompatibility, biodegradability, and low cost. Moreover, hyaluronic acid exhibits several beneficial effects on wound healing, such as the decrease of inflammatory processes, regulation of tissue remodeling, and enhancement of angiogenesis. Therefore, in recent years, there is growing attention in this polysaccharide for the design and manufacture of novel wound dressings, which have shown encouraging properties. Here, we describe the different approaches of hyaluronic acid for the production of wound dressings, encompassing hydrogels, films, scaffolds, foams, topical formulations, and nanoformulations, as well as its beneficial effects on wound healing. Finally, we discuss perspectives about the use of hyaluronic acid in wound dressings.

Key words: Hyaluronic acid; Wound; Wound dressing; Hydrogel; Scaffold.

Introduction

Human skin serves as the first line of defense against harmful agents; thus, its function is crucial in the maintenance of individuals' health (1,2). However, the skin may undergo a variety of lesions that disturb its correct function and produce wounds.

In this regard, after any skin wound, the organism activates a mechanism for wound healing, which is a complex process that involves three main stages: hemostasis/inflammation, cell migration and proliferation, and remodeling and reepithelialization (3). Wound healing is a crucial process to repair tissue structure and recover the organism's homeostasis. However, this process frequently delays because this may be affected by factors such as bacterial infections, nutritional status, age, hypoxia, chronic diseases, necrosis, and vascular and arterial diseases (4). Thus, distinct kinds of wound dressings have been proposed to improve wound healing (5). These may differ in their materials and properties, depending on the type of injury (e.g., burns, cuts, pressure ulcers) and the kind of wound (e.g., acute or chronic). Some authors have proposed biomaterials as a good choice because these possess excellent properties in terms of biocompatibility, biodegradability, and low cost. Furthermore, these materials generally produce less activation of inflammatory and immune responses (3,5,6).

In this respect, hyaluronic acid (HA) is a natural polysaccharide that abundantly exists in extracellular

matrices (ECM) of mammal tissues, such as skin, synovial liquid, umbilical cord, vitreous body, and epithelial and connective tissues (7). HA is a non-toxic and non-allergic polymer that has demonstrated exciting effects on wound healing, including the promotion of epithelial cell migration, the decrease of inflammatory processes, enhancement of angiogenesis, and stimulation of endothelial cell proliferation and migration (8–12). Thus, in recent years, HA is increasingly employed for the design and manufacture of wound dressings with highly promising results (13–20).

Therefore, in this article, we describe the biological properties of HA and its beneficial effects on wound healing. Moreover, we mention the different approaches of HA for the production of different types of wound dressings, including hydrogels, films, scaffolds, foams, topical formulations, and nanoformulations. Finally, we discuss perspectives about the use of HA in future proposals of wound dressings.

Wound healing process

When the skin suffers an injury, it comprises the breaking of the continuity of some functional tissue due to trauma o resulting from a pathological disorder (21,22). In this regard, after the lesion formation, the tissue regeneration and repair process for the wound healing begins. This sophisticated mechanism involves the interaction of different systems in a continuous process, and it is divided into three stages: hemostasis/inflammatory phase, proliferative phase, and remodeling phase, which are represented in Figure 1.

Hemostasis/Inflammatory Phase

This phase begins immediately after that the lesion is formed and ends four or five days after the injury production. The hemostasis is the coagulation process of the wound to prevent bleeding or hemorrhage, whereas tissue regeneration occurs (Figure 1A). After the injury, vasoconstrictors such as thromboxane A2 are released; at the same time, a network (clot) of platelets, fibrin, and thrombin is formed. This clot reestablishes the homeostasis and acts as a scaffold for the arriving cells. Following the clot formation, a cellular distress signal is sent out, being the neutrophils the first cells to appear in the injured area (23,24). Then, the vasodilation of the closer blood vessels occurs, increasing cellular circulation. The neutrophils are attracted by different inflammatory mediators such as interleukin (IL)-1, tumor necrosis factor alfa (TNF-α), and transforming growth factor-beta (TGF-β). After that, (48 to 96 h after injury), macrophages (which were monocytes transformed) arrive at the lesion site (Figure 1B). Besides participating in phagocytosis, these macrophages release different pro-angiogenic, inflammatory, and fibrogenic factors, such as Platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), TGF-β, and fibroblast growth factor (FGF). These factors attract other inflammatory cells, which also results in the stimulation of the granulation tissue formation (proliferative phase) (25).

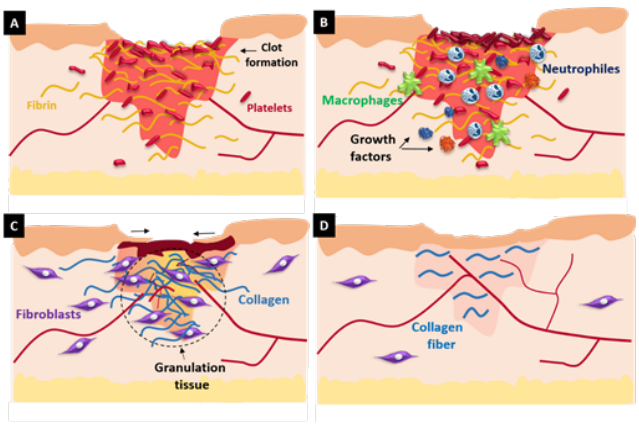

Figure 1. The wound healing process. A) Hemostasis: the coagulation begins. B) Inflammation: white blood cells arrive in the injury area; inflammatory growth factors are released. C) Proliferation: fibroblasts synthesize abundant collagen, and the angiogenesis is stimulated. D) Remodeling: collagen matrix remodeling.

Poliferative phase

The proliferative phase begins around the third day after wounding and lasts approximately two weeks. During this phase occurs the re-epithelization, angiogenesis, granulation tissue formation, and collagen deposition. The fibroblasts, which increased during the first three days in the surrounding tissues, were stimulated to migrate, and they are attracted to the damaged area by factors like PDGF and TGF-β (Figure 1C) (26–29). Once in the wound, the massive proliferation of fibroblasts continues, as well as the synthesis of ECM components (e.g., hyaluronan, fibronectin, procollagen, proteoglycans, and elastin), forming the granulation tissue. Furthermore, the neovascularization is carried out through angiogenesis and vasculogenesis. The adjacent epithelial cells migrate to achieve the re-epithelization, forming a thin layer that subsequently shall be thicker and more resistant (30–32).

Remodeling phase

The remodeling phase begins before the proliferative phase ends, and it could last from six months to two years. In this stage, a continuous synthesis and breakdown of collagen occur to achieve the remodeling. Furthermore, the degradation of HA and fibronectin takes place (25,33). At the same time, the slow process of the wound contraction begins, showing the maximal tensile strength of the incision wound after 11-14 weeks.

In summary, there are many molecules and cells involved in the wound healing process. Particularly, HA, in combination with other molecules and cells, modulates several process in wound healing such as angiogenic respose, in cell adhesion within the ECM as well as collagen deposition in the remodeling phase.

Hyaluronic acid properties

HA is a natural biopolymer belonging to the group of glycosaminoglycans, which is one of the main components of the ECM. HA molecular structure is a linear repeating disaccharide unit β-(1→4) linked D-glucopyranuronic acid, and β-(1→3) linked 2-acetamido-2-deoxy-D-glucopyranose, with anionic charge (34,35) (Figure 2). The orientation of HA films in the solid-state was revealed by X-ray diffraction assay, describing two forms: single two-handed helices with 2-, 3-, and 4-fold

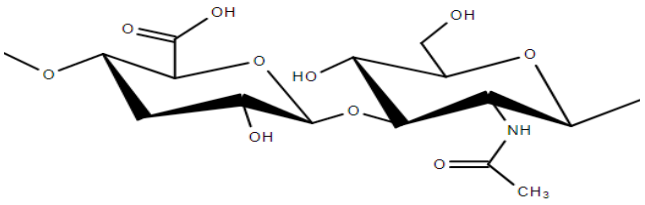

Figure 2. Chemical structure of Hyaluronic Acid. Empirical formula $(C_{14}H_{21}NO_{11})_n$.

symmetries, and a double-helical structure stabilized by intra-chain hydrogen bonds linking the two adjacent sugar residues, with inter-chain hydrogen bonds and cation/H_2O bridges (35–37). Likewise, the molecular weight of the HA is between 3 and 7 x 10^6 g/mol, and up 25,000 disaccharides units can form the HA chains. Furthermore, HA structure is highly dependent on pH; in physiological conditions, it possesses a negative charge and forms salts generally called hyaluronan or hyaluronate (34,35,38).

HA has a solubility of 0.5 g/L in aqueous solution (reported in commercial products), and high moisture retention capacity. In this regard, carboxylic and acetamide groups from HA are responsible for enhancing the water-retention ability. Through molecular dynamic simulation, the number of bonds among water with the carboxylic and acetamide groups has been estimated in 10-15 per disaccharide unit (38). Likewise, the presence of N-Acetyl groups in HA is also related to its moisture retention capacity. Zhang et al. demonstrated that both moisture-absorption and moisture-retention of HA significantly decrease when there is a reduction in the number of N-Acetyl groups (38,39). Interestingly, HA retention-moisture capacity is not altered when the chemical structure is transformed by irradiation with γ-rays to obtain a low molecular weight HA (LMWHA) (17).

On the other hand, at physiological conditions, the carboxyl groups of HA with anionic charge can be balanced with cations such as Na^+, K^+, Ca^{2+}, or Mg^2 (40). This behavior could enhance the biocompatibility, hygroscopicity, and the viscoelasticity, imparting flexibility to the tissues (41,42). In contrast to other glycosaminoglycans types, HA is not attached to a core protein, and not present sulfate groups. Thus, the HA solutions properties mainly depend on the HA molecular weight and its concentration (34,42). Concerning this, HA with high molecular weight (HMWHA) can produce strength networks, and high concentrations of HA lead to a progressive increase in the viscosity and viscoelasticity of solutions (40,43). HA chains can provide a significant viscosity, even at concentrations of 0.1%. Thus, a marked decline in solution viscosity suggests a weakening of the interactions among the polymer chains and a strong decrease in HA concentration. (40,44).

As mentioned before, the HA structure is susceptible to the pH changes; in high alkaline or acidic conditions, the balance between the repulsive and attractive forces can be altered, whereas in pH <4 or >11, HA is susceptible to hydrolysis. Hence, the breakdown of the polymeric network is transient and reversible (38,40,45). This unusual and unique behavior is determinant on the physiological interactions of HA, and make it able to be applied as a lubricant, joint stabilizer, water balance, and flow resistance regulator in pharmaceutical, medical, and cosmetic applications (40,41).

In the body, the HA is synthesized by hyaluronan synthase (HAS). Vertebrates present three types of HAS, the HAS-1, HAS-2, and HAS-3. These isozymes can produce different HA sizes, which are modulated by alternative splicing, cellular localization, and epigenetic processes (41). In this respect, the HMWHA can be related to inflammation and immune response, whereas the LMWHA can be involved with enzymatic activity (42).

The improvement of the HA properties is crucial in the development of new health technologies, and the increase of solubility of HA in aprotic solvents is the first step to facilitate the obtaining of HA derivatives. The carboxyl groups in the HA are the target for esterification reactions to obtain HA esters. Briefly, HA is converted in HA tetrabutylammonium salt by the reaction with DMSO and benzyl iodide at 30 °C/12 h, with the addition of saline water in small amounts. Finally, precipitation with acetone produces a benzyl HA ester. This kind of HA esters as well the ethyl-, propyl-, and benzyl- esters are employed in different products, including sutures, films, microspheres, membranes, and implants in corneal shields (35).

As observed, HA has several characteristics that make it an excellent option to be applied as a wound dressing. Additionally, HA participates in the wound healing process, as is deeply described in the next section.

Hyaluronic acid in wound healing

In the different stages of the wound healing process, the molecular components of the ECM are of great importance since they generate a suitable microenvironment for tissue remodeling. Furthermore, some of these components play a role key in diverse events of cell communication, regulating many signaling pathways.

HA has an active role through all steps of wound healing, modulating several processes of tissue remodeling and the innate immune response to tissue injury. The reactions elicited by the interaction of HA molecules in several regulation processes are strongly correlated to their size or molecular weight (46). The HMWHA molecules are the natural form in which this molecule is translocated to the extracellular space, and it is known that they present anti-inflammatory and anti-angiogenic activities. During the wound healing process in adults, HMWHA concentration reaches a maximum peak, followed by a catalytic degradation, which is mediated by hyaluronidases and reactive oxygen species (ROS). Inversely, LMWHA fractions stimulate the expression of pro-inflammatory cytokines and are angiogenic, which allows an adequate blood supply to the damaged zone (47).

On the other hand, the participation of HA in wound healing is mediated by its interaction with diverse cell surface receptors. In this respect, the molecular weight of HA is of high relevance to determine its biological activity because it alters its ability to bind to specific receptors to activate signaling pathways. Once activated, those receptors trigger other responses related to inflam-

matory response, cell migration, antioxidant effects, tissue repair, which could have either a positive or negative impact in the wound healing process. However, not all mechanisms are fully understood.

Some of the main molecules involved in the regulation of different stages of HA-mediated wound healing are cluster determinant 44 (CD44), the receptor for hyaluronate-mediated motility (RHAMM or CD168), toll-like receptors 2 and 4 (TLR2, TLR4) and hyaluronan receptor for endocytosis (HARE) (48). CD44 is a transmembrane glycoprotein, and it is a receptor for HA, collagen, and fibronectin, among others (49). Once HA binds to CD44, it induces fibroblast migration to the damaged area.

The binding of CD44 to HA produces its internalization, which allows its interaction with actin filaments and microtubules. This interaction is strongly correlated with the stimulation of cell motility (50) (fibroblast migration to injury sites), the induction of angiogenesis (51), as well as the reduction of cell death apoptosis due to the formation of a pericellular coating, which masks cell death receptors. Moreover, that suppression of CD44 results in decreased keratinocyte proliferation and impaired ECM remodeling and re-epithelialization process (52).

Additionally, the presence of HA in its free form modulates the host reaction to bacterial endotoxins, forming a jelly barrier that limits the accessibility of endotoxins to receptors like TLR4 (53). The major component of the external membrane of Gram-Negative bacteria is an endotoxin named lipopolysaccharide (LPS) that is involved in the activation of TLR4, which triggers a signaling pathway that promotes the production of pro-inflammatory cytokines mediated by factor nuclear kappa B (NF-ϰB).

Therefore, HA has been widely studied for applications related to the fabrication of wound dressings that allow an improvement in the healing process of diverse types of injuries.

Hyaluronic acid in wound dressings

Hydrogels

Hydrogels are polymeric hydrated networks, due to its high-water content, these possess a high potential of use in tissue engineering. Hydrogels provide favorable surrounding environmental conditions to promote wound closure, are cheap and easily manufactured, and offer controlled release of drugs, making them superior to other dressing forms. In this regard, HA-based hydrogels provide desirable properties such as biocompatibility, biodegradability, high exudates absorption capacity (only in the dry state), transparency, and pleasant sensation (non-grease/cool).

The polymeric network in HA-based hydrogels could be formed by two approaches: covalent crosslinking and non-covalent assembly. In the first one, chemical entities are intentionally bond to the HA chain to provide direct functionality to the hydrogel and to keep the mesh structure to retain abundant absorbed water, fluids, or drugs. Nowadays, one of the most promising strategies for chemical covalent crosslinking is "click chemistry", where the use of a toxic crosslinking agent, solvents, and chemical reagents is avoided. The click chemistry can be easily adapted to the fabrication of wound dressing hydrogels. For example, the carbodiimide-coupling reaction was used by Zhou et al. for the manufacture of gelatin/sodium alginate/HA composite (54). Similarly, enzymatic crosslinking is another way to form hydrogels in mild conditions. In this manner, Ying et al. prepared a wound dressing hydrogel based on collagen type I and HA crosslinked through horseradish peroxidase (55). Otherwise, only a few methods have been developed to achieve covalent crosslinking by physical methods. In this regard, the Freeze-thawing (F-T) technique has been applied by some authors due to is the safest physical crosslinking method for hydrogel formation (56). For instance, some authors reported the synthesis of PVA-HA hydrogels membranes for wound dressing applications through this method, highlighting the mechanical stability (57,58). In the same way, Rossi et al. described the synthesis of wound dressings by freeze-drying, based on chitosan hydrochloride, 5-methyl-pyrrolidinone chitosan, and HA, besides chlorhexidine diacetate to be used in the treatment of skin ulcers (59).

Commercial HA-based hydrogels dressings are available in the form of a gel, frequently in combination with other actives and inactive compounds. In 2016, Gentex Pharma (USA) launched HyGel™, a 2.5% HA-based gel utilizing Ionic Polymer Matrix technology, which provides a sustained delivery of HA. It is indicated for the management of exudative wounds, such as leg ulcers, pressure ulcers, diabetic ulcers, surgical wounds, mechanically or surgically debrided wounds, and for second-degree burns (60,61). Other examples of hydrogels products that included HA in the formulation are Regenecare™ HA (MPM Medical, USA) and DermaPlex™ Gel (MPM Medical, USA). Therefore, hydrogels represent a suitable option due to their excellent physicochemical characteristics; however, in cases of deep wounds, the use of scaffolds could constitute a better strategy of treatment.

Scaffolds/Films

A scaffold is a temporary supporting structure for growing cells and *novo* tissue formation (62). The scaffolds can be natural, synthetic, or composite, and the choice of the material depends on the type of tissue to regenerate (63,64). Due to its properties, HA has been widely employed in the development of scaffolds.

For example, in 2019, HA-based scaffolds were elaborated by jet-spinning, and their effectiveness in wound healing was analyzed *in vivo*. The results showed that the scaffold stimulated the granulation process, and promoted the angiogenesis and the reepithelization (65).

There are several manufacturing techniques to produce scaffolds; however, to obtain wound dressings, one of the most applied is the spinning (electrospinning technique and solution blow spinning technique). This method allows mainly to obtain nanofibrous structures with high mechanical resistance, and it is employed for drug release without losing the structure. Through this technique, naproxen loaded HA-based scaffold (66), gelatin/ HA nanofibrous dressing crosslinked with glutaraldehyde (67), and bilayered polymeric scaffold consisting of chitosan/polycaprolactone and HA, were developed for wound healing applications. Furthermore,

methods based on the phase separation of a polymer solution are employed in scaffolds elaboration, obtaining porous structures that could promote the healing. HA also can coacervate in the presence of oppositely charged polyelectrolytes. This characteristic was exploited by Nath et al. to develop chitosan–HA polyelectrolyte complex scaffold for protein delivery and bone tissue regeneration (68).

HA-based scaffolds enriched with nanoparticles and formulated with other polymers have been prepared by freeze-drying (69-71). Examples of freeze-drying HA-based scaffolds are chitosan–HA composite sponge scaffold enriched with andrographolide-loaded lipid (72), HA/poly-L-Lysine multilayer films attached to a porous HA scaffold (73), a silk fibroin/HA/sodium alginate composite scaffold for wound healing effects (19), and a blend membrane obtained with carboxymethyl chitosan/gelatin/HA as epithelia transplanting scaffold for corneal wound healing (74).

Finally, methods such as micropatterning could provide a versatile and straightforward technique to produce HA-scaffolds. However, natural polymers have not offered yet the resolution and mechanical integrity for the fabrication of fully 3D structures, almost by direct laser writing technology or two-photon polymerization. This limitation requires the modification of HA and other natural polymers. Nevertheless, photopolymerizable HA has been successfully generated using glycidyl methacrylate-based (75) and poly(ethylene glycol) diacrylate (76), producing highly hopeful porous scaffolds for wound dressing. Finally, these scaffolds could be enriched with delivery systems to enhance wound healing effectiveness. In this regard, HA also has been applied as based material for the development of drug release systems.

Controlled drug release

HA is employed for the manufacture of controlled-release carrier systems to obtain additional biological effects on wounds. First, the HA effect on the injury, second, the result derived from the action of the charged molecule on the carrier. In this sense, the preparation of microparticles for the encapsulation of growth factors is an example of new trends in the search for strategies to improve the management of wound healing. For these purposes, one study described HA microparticles with Epidermal growth factor (EGF) for wound healing (77). The particles were 21 ± 5.3 μm in size and applied on an 8 mm in depth excisional wound in mice. The authors mentioned that both microparticles with and without EGF favored the acceleration of the inflammatory process to facilitate the proliferation and remodeling phase. Histological analysis also confirmed that the microparticles remained at the injury site until day 7, and on day 14, there were no residues of the carrier. Moreover, the authors confirmed the deposition of a thinner layer of collagen in the dermis with the EGF.

Similarly, on a smaller size scale but about 1 micron, another investigation described the obtaining of porous nanoparticles of HA by the gas anti-solvent precipitation method for the incorporation of growth factors and their application in the healing of circular excisional wounds in Wistar rats (78). The authors mentioned the optimization of some operating variables with the most considerable influence, such as injection pressure and injection needle diameter. The big particle manufacturing strategy for incorporating growth factors is employed frequently with other natural and synthetic polymers as well. In particular, pore formation allows for higher loading capacity, unlike traditional encapsulation, where the growth factor is loaded inside at the same time of obtaining the microparticles. Even some strategies also involve additional pore coating to offer superior protection from growth factors and greater release control. In the study described, a bimodal distribution was observed with a population in the range of 400 nm and another of 900 nm with irregular shape and highly porous surface (78). With this system, PDGF achieved a release of approximately 100% in 72 hours, a crucial time in the wound healing process. HA nanoparticles with PDGF affected the stimulation of the ECM and collagen deposition, increasing the breaking strength. Interestingly, there was an effect of HA nanoparticles without growth factors in time-dependent (Fibroblasts, endothelial cells, and type I collagen), and close to the effect of the proposed formulation containing the factors (78).

On the other hand, nanofibers are controlled release systems that are increasingly employing HA for wound healing (7). In this sense, one study reported the obtaining of HA nanofibers containing type I collagen with a diameter of 200 nm, the authors had the objective of simulating the architectural dimensions of the ECM (79). Remarkably, the new wound dressing decreased the presence of tissue inhibitor of metalloproteins, which could suggest that the formulation could act as a true tissue regenerator without scarring in wounds. Another more sophisticated strategy consisted of the combination of HA and type I collagen for the manufacture of nanofibers embedded with bFGF and EFG. The fibers had encapsulated gelatin nanoparticles loaded with PDGF and VEGF. This innovative formulation stimulated the adequate epithelialization and maturation of blood vessels in diabetic rats. Other HA combinations in the manufacture of wound dressing nanofibers with encouraging results consist of the use of poly-ε-caprolactone, chitosan, and poly(lactic-co-glycolic acid) (80,81). In general, the presentation in nanofibers allows for higher exudate absorption capacity, greater regulation of transepidermal water loss, and the guide action of nanofibers in cell migration and proliferation (7).

Conclusion and perspectives

The primary purpose of wound dressings is to help in wound healing through the decrease of inflammation, the protection against harmful microorganisms, and the improvement of cell proliferation. In this respect, HA is a natural polymer with a wide range of beneficial properties in wound healing, including intrinsic antimicrobial and healing activities. Thus, in recent years, HA is increasingly utilized for the development of new proposals of wound dressings with encouraging effects.

However, the design of wound dressings for some hurts can be challenging because these may exhibit specific needs, particularly chronic wounds. In this respect, the addition of several substances with biological activity could improve current dressings through potentiation

of the healing effect. Moreover, complex nanoformulations composed by nanoparticles and nanofibers may provide an extended-release of the active compounds, producing multifunctional wound dressings. A potential advantage of these multifunctional systems would be the enhancement of healing through different biological mechanisms.

In this regard, the incorporation of growth factors constitutes an exciting proposal to enhance wound healing. Similarly, innovative wound dressings could include siRNAs and stem cells. For example, siRNAs could silence genes that encode inflammatory proteins, improving the healing of chronic cutaneous lesions. Likewise, stem cells may regulate angiogenesis and the immune system, which they would offer the possibility of restoring injured tissues to their original condition.

Finally, the manufacture of HA-based wound dressings by recent technologies such as 3D printing opens a plethora of possibilities to design wound-specific dressings through computerized tools. However, an extensive investigation will be needed to develop and optimize these novel wound dressings.

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Interest conflict
The authors declare no conflict of interest.

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