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Review

Hyaluronic acid—Based wound dressings: A review

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ABSTRACT

Hyaluronic acid (HA), a non-sulfated glycosaminoglycan (GAG), is a major component of skin extracellular matrix (ECM) and it is involved in the inflammatory response, angiogenesis, and tissue regeneration process. Due to the intrinsic properties of HA (such as biocompatibility, biodegradability and hydrophilic character), it has been used to produce different wound dressings, namely sponges, films, hydrogels, and electrospun membranes. Herein, an overview of the different HA-based wound dressings that have been produced so far is provided as well as the future directions regarding the strategies aimed to improve the mechanical stability of HA-based wound dressings, along with the incorporation of biomolecules intended to ameliorate their biological performance during the healing process.

1. Introduction

Skin, as a primary immunological barrier of our body, provides protection against microorganisms infiltration and dehydration (Byrd, Belkaid, & Segre, 2018). After a skin injury occurs, induced by physical or thermal agents, the wound healing process is initiated. However, infection, fluid loss, exuberant inflammation and other complications may delay or not allow such process, leading to the emergence of chronic wounds (Byrd et al., 2018; Simões et al., 2018).

To circumvent these restrictions of the healing process, different types of biomaterials like sponges, films, hydrogels, electrospun membranes have been developed by researchers from Tissue Engineering area, aiming to produce an ideal wound dressing that is capable of fulfil specific requirements such as: i) provide/assure a moist environment at wound site; ii) improve the epidermal migration, sponsoring the angiogenesis and connective tissue synthesis; iii) allow gas and nutrient exchanges; iv) provide protection against bacterial infection and v) must be sterilizable, non-toxic, biodegradable and non-allergic (Dhivya,

Abbreviations: α-SMA, alpha-smooth muscle actin; ADH, adipic dihydrazide; AFM, atomic force microscopy; AgNPs, silver nanoparticles; AHA, aldehyde hyaluronic acid; ALG, alginate; AND, andrographolide; Arg, arginine; bFGF, basic fibroblast growth factor; BP, pendant bisphosphonate; CMC-Na, carboxymethylcellulose sodium; CNC, cellulose nanocrystals; COL, collagen; COL-P, collagen I-hydroxybenzoic acid; CS, chitosan; CSH, cornstarch; DEX-PDM, poly((2-dimethyl amino)-ethyl methacrylate)-grafted dextran; DN, dopamine; *E. coli*, *Escherichia coli*; ECM, extracellular matrix; EDC, 1-ethyl-3-[3-(dimethylaminopropyl)] carbodiimide; EEP, ethanolic extract of propolis; EGCG, epigallocatechin-3-O-gallate; EGF, endothelial growth factor; GAG, glycosaminoglycan; GEL, gelatin; GS, gentamicin; GTA, glutaraldehyde; HA, hyaluronic acid; HA-ADH, hyaluronic acid modified with adipic acid dihydrazide; HA-BP, pendant bisphosphonate-modified hyaluronic acid; HA-EDA, hyaluronic-(2-aminoethyl)-carbamate acid; HA-*g*-Pu, hyaluronic acid grafted pullulan; HA-Tyr, hyaluronic acid-tyramine; HMEC, human microvascular endothelial cells; hMSCs, human mesenchymal stem cells; HMW, high molecular weight; HPβCD, hydroxypropyl-βcyclodextrin; HRP, horseradish peroxidase; HUVECs, human umbilical vein endothelial cells; HYAL, hyaluronidases; IL, interleukin; LbL, layer-by-layer; *L. monocytogenes*, *Listeria monocytogenes*; LMW, low molecular weight; MMP-2, matrix metalloproteinase-2; MMT, montmorillonite; MMW, medium molecular weight; MPO, myeloperoxidase; *MRSA*, *Methicillin-resistant Staphylococcus aureus*; MW, molecular weight; NHS, *N*-hydroxysuccinimide; NOCC, N,O- carboxymethyl chitosan; O-HA, hyaluronic acid oligosaccharides; OHEC, oxidized hydroxyethyl cellulose; *P. aeruginosa*, *Pseudomonas aeruginosa*; PCL, poly(caprolactone); PDGF, platelet-derived growth factor; PEO, poly(ethylene oxide); PLA, poly(lactic acid); PLC, poly(l-lactide-*co*-ε-caprolactone); PLGA, poly(lactic-*co*-glycolic acid; PLL, poly(L-lysine); Pu, pullulan; PVA, poly(vinyl alcohol); RHAMM, hyaluronan-mediated motility receptor; ROS, reactive oxygen species; *S. aureus*, *Staphylococcus aureus*; *S. epidermidis*, *Staphylococcus epidermidis*; SA, sodium alginate; SD, sulfadiazine; SEM, scanning electron microscopy; Ser, sericin; SF, silk fibroin; STMP, sodium trimetaphosphate; TA, tranexamic acid; THY, thymol; TGF-β, transforming growth factor-beta; TLR, toll-like receptors; TNF-α, tumor necrosis factor-alfa; usSN, ultrasmall silver nanoparticles; VEGF, vascular endothelial growth factor; vHMW, very high-molecular weight; Vit.C, vitamin C; WCA, water contact angle; ZIF-8, zeolite imidazolate frameworks; ZN, zein

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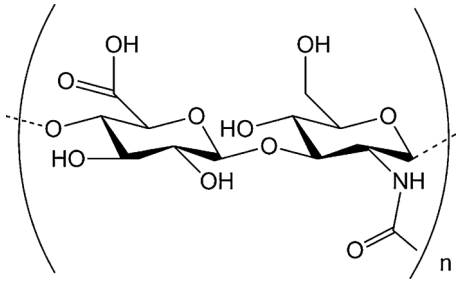

Fig. 1. Molecular structure of Hyaluronic acid.

Padma, & Santhini, 2015).

To accomplish such objective, different polymers (poly(caprolactone) (PCL), poly(vinyl alcohol) (PVA), collagen (COL), chitosan (CS), alginate (ALG), hyaluronic acid (HA)) have been used for the production of different wound dressings. Among them, HA or hyaluronan, which is composed of repeating disaccharide units of β-D-glucuronic acid and N-acetyl-D-glucosamine, alternately linked by β-1,3 and β-1,4 glycosidic bonds (as represented in Fig. 1) has capture the attention of researchers from the Tissue Engineering field (Knopf-Marques et al., 2016).

The large number of carboxyl and hydroxyl groups, available within the HA structure, are responsible for conferring it a highly hydrophilic character. Such feature allows HA to perform exudate absorption as well as enhance cell adhesion. Further, HA biocompatibility, biodegradability (through the enzymatic action of hyaluronidases (HYAL)) and easy chemical modification propelled its application in the wound healing, intending to promote the hemostasis phase, regulate the inflammation and encourage the re-epithelialization process (Mahedia, Shah, & Amirlak, 2016; Mohandas, Anisha, Chennazhi, & Jayakumar, 2015; Zhu, Wang, Yang, & Luo, 2017).

In this review, different HA-based wound dressings are described, highlighting the different strategies used to enhance their water stability/mechanical properties as well as biological performance.

2. The physicochemical and biological properties of HA

HA is a natural polymer that belongs to a group of heteropolysaccharides known as glycosaminoglycans (GAGs), which are found in the human vitreous humour, joints, rooster comb, umbilical cord, skin and connective tissue (Fallacara, Baldini, Manfredini, & Vertuani, 2018). In addition, HA can also be obtained through microbial fermentation (Gupta, Lall, Srivastava, & Sinha, 2019; Liu, Liu, Li, Du, & Chen, 2011). HA may present different molecular weights (MW), namely HA oligosaccharides (O-HA, $< 1 \times 10^4$ Da), low molecular weight HA (LMW-HA, $1–25 \times 10^4$ Da), medium molecular weight HA (MMW-HA, $25–10 \times 10^4$ Da), high molecular weight HA (HMW-HA, $> 1 \times 10^6$ Da), and very high-molecular weight HA (vHMW-HA, $> 6 \times 10^6$ Da) (Tavianatou et al., 2019).

At physiological pH, each carboxylic group found within HA structure displays an anionic charge. Hence, HA is able to establish H-bonds with the water molecules through the carboxyl and acetamido groups available on HA structure, leading to an stabilization of the secondary structure of this biopolymer (Fallacara et al., 2018). Kobayashi and collaborators reported that the establishment of these H-bonds is dependent on the HA MW, *i.e.*, HA networks displaying an increased stability, viscosity and viscoelasticity are produced when HA with high MW is used (Kobayashi, Okamoto, & Nishinari, 1994). On the other side, the rheological properties of HA are quite dependent on the solution ionic strength, pH and temperature: when the solution pH < 4 or pH > 11, the HA is degraded by hydrolysis, prompting a decline on HA viscosity and polymeric network integrity (Maleki, Kjøniksen, & Nyström, 2008; Miguel, Simões, Moreira, Sequeira, & Correia, 2019).

Such pH influence on HA properties/effectiveness must be considered when HA is aimed to be used for wound healing management, since the wound bed pH suffers a variation along the healing process. After an injury occurs, the pH at the wound site is approximately 8, decreasing to pH ≈ 5 when the healing process ceases (Maleki et al., 2008; Miguel, Simões et al., 2019; Morgado et al., 2014).

In addition, the MW of HA can also influence its biological performance during the healing process, which will be described in the following sections.

2.1. HA roles in the wound healing process

Immediately after a skin injury occurs, the healing process begins to re-establish, as soon as possible, the skin tissue architecture as well as halt the bleeding (Tavianatou et al., 2019). To accomplish that, platelets release large amounts of HMW-HA, that prompt the deposition of fibrinogen and formation of an initial clot. Further, HA, as a major component of the edema fluid, also promotes the recruitment of neutrophils cells, involved in the phagocytosis of the debris and removal of dead tissue, and the subsequent release of tumor necrosis factor-alpha (TNF-α), IL-1β, IL-8 (Tavianatou et al., 2019). Further, the secretion of inflammatory cytokines will also contribute to HMW-HA fragmentation into LMW-HA, which is involved in the recruitment of leucocytes and monocytes, a process that is triggered by the binding of HA to the CD44 receptors available on monocytes and granulocytes' surface (Wolny et al., 2010).

In the last stage of the inflammatory phase, the lymphocytes and macrophages migrate into the wound site, where their toll-like receptors (TLR2 and TLR4) interact with HA fragments (LMW-HA), and prompt the expression of TNF-α and interleukins such as IL-6, IL-8 and IL-1β (Aya & Stern, 2014; Chen & Abatangelo, 1999; Zamboni, Vieira, Reis, Oliveira, & Collins, 2018). In addition, LMW-HA together with fibronectin guide the fibroblasts invasion and proliferation, which is mandatory for collagen deposition within the wound, as well as promotes the differentiation of fibroblasts into myofibroblasts (cells expressing smooth-muscle actin and myosin), that play a pivotal role in the wound contraction (Webber, Jenkins, Meran, Phillips, & Steadman, 2009). Moreover, Stern and collaborators demonstrated that HA fragments composed of 6–20 disaccharides can stimulate dermal fibroblast migration and proliferation, with the subsequent deposition of type III collagen, leading to the formation of a new ECM (Stern, Asari, & Sugahara, 2006).

Moreover, in the re-epithelialization phase, CD44 receptors available in keratinocytes cells interact with the LMW-HA present at the wound margins, regulating the re-epithelialization process (Aya & Stern, 2014; Chen & Abatangelo, 1999). An illustration of the HA main roles in the wound healing process is depicted in Fig. 2.

2.2. HA molecular weight impacts on its performance during the wound healing process

As previously mentioned, HA with different molecular weights (resulting from the cleavage of the polymeric chain), have a distinct effect on the wound. HA with HMW presents anti-inflammatory effect, through the control of the inflammatory cells recruitment, cytokines production and the migration of stem cells (Fallacara et al., 2018). HMW-HA can also inhibit endothelial cell growth as well as limit the supply of nutrients, impairing the skin regeneration process (Dreifke, Jayasuriya, & Jayasuriya, 2015; Gallo et al., 2019). In addition, HMW-HA can interact with the CD44 receptors available on the monocytes and granulocytes surfaces (Fallacara et al., 2018). The HMW-HA-CD44 interaction can affect a variety of intracellular signalling pathways that control biological processes such as: angiogenesis, cell migration, proliferation, and adhesion to ECM components, the elimination of intracellular reactive oxygen species (ROS) as well as the reduction of the DNA damage (Litwiniuk, Krejner, Speyrer, Gauto, & Grzela, 2016). Such interaction is more relevant with HMW-HA because it possesses

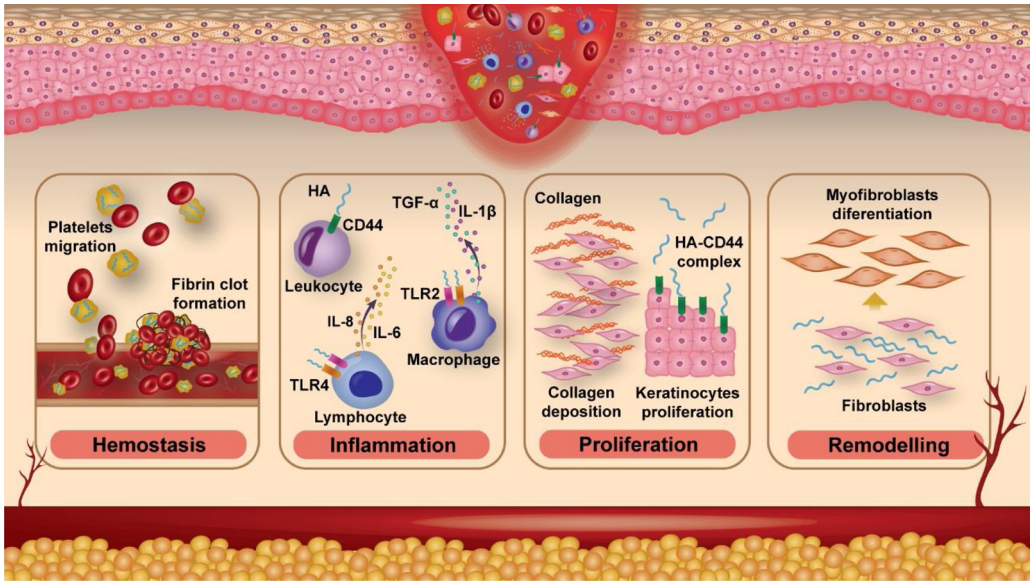

Fig. 2. Illustration of HA main roles in the wound healing process.

multivalent sites available to bind to CD44, while oligomers only display one or two binding sites (Wolny et al., 2010; Yang et al., 2012).

On the other hand, LMW-HA is pro-angiogenic, stimulates the production of pro-inflammatory cytokines as well as growth factors enrolled in the remodelling of skin ECM (Fallacara et al., 2018). Kouvidi et al. (2011) reported that LMW-HA (15–40 × 10^3 Da) specifically binds to Hyaluronan-mediated motility receptor (RHAMM) in fibrosarcoma cells, triggering the cell adhesion onto fibronectin, while HMW-HA inhibits cell adhesion and the RHAMM expression (Kouvidi et al., 2011). Moreover, O-HA (sized 2–10 disaccharides units) stimulates angiogenesis *via* RHAMM-mediated signalling pathways in epithelial cells during wound healing. Such observations suggested that RHAMM is expressed during the inflammatory phase of the healing process (Gao, Yang, Mo, Liu, & He, 2008).

Matsumoto, Arai, Momose, and Kuroyanagi (2009) produced different sponges using HA with diverse MWs (HMW and LMW), through freeze-drying technique. To obtain crosslinked HMW-HA (cHMW-HA)/LMW-HA sponges, the HMW-HA sponges were immersed in an LMW-HA solution. The *in vivo* data obtained revealed that after 1 week, the group treated with cHMW-HA/LMW-HA displayed blood vessels with a higher area (≈ 0.05 mm^2), contrasting with ≈ 0.03 mm^2 presented by animals treated with cHMW-HA sponges. Such results demonstrated that the incorporation of LMW-HA on the sponges had a positive effect on the angiogenesis process. Additionally, LMW-HA also evade an exuberant inflammatory response, which was confirmed through the determination of amount of myeloperoxidase (MPO) present in neutrophils (the presence of this inflammatory enzyme is proportional to the extension of the inflammatory process) (Matsumoto et al., 2009).

On the other side, the O-HA (MW < 10^4 Da) resulting from the degradation of HA, through the action of HYAL or ROS, also play important roles in the healing process (Fallacara et al., 2018; Knopf-Marques et al., 2016). O-HA displays pro-angiogenic activity, *i.e.* it stimulates the migration and differentiation of endothelial cells as well as enhance the migration and proliferation of dermal fibroblasts and keratinocytes (Fallacara et al., 2018). In a recent study, Wang, Han, Guo, and Huang (2016) noticed that O-HA prompted the *in vitro* migration of human umbilical vein endothelial cells (HUVECs) into the wounded area, *i.e.* ≈ 30 % the wound was covered with HUVECs incubated with O-HA cream, while in control group only ≈ 15 % of wound area was covered by these cells. On the other hand, the animals (diabetic rats) treated with O-HA cream also presented a lower expression of CD31, increased capillary density and blood supply, as well as improved granulation and re-epithelization of the wound.

3. HA-chemical derivatives

HA is also known by its high versality. Researchers have functionalized the polymeric backbone of HA with different functional groups in order to improve its mechanical, rheological and swelling properties as well as to modulate its degradation rate (Fallacara et al., 2018). The hydroxyl and the carboxyl groups present in HA structure are usually selected to perform its chemical modification (Schanté, Zuber, Herlin, & Vandamme, 2011). The hydroxyl groups of HA have been chemically modified with mono- or bi-functional agents, resulting in different HA derivatives like ethers, hemiacetals, esters and carbamates (Fallacara et al., 2018). In a work performed by Laurent, Gelotte, and Hellsing (1964), HA was crosslinked for the first time with 1,2,3,4-diepoxybutane to improve its stability in aqueous solutions. In a similar way, Piron and Tholin, 2005 mixed butanediol-diglycidylether in sodium hydroxide solution and added to the HA powder, leading to the formation of a homogeneous and stable hydrogel (Piron & Tholin, 2005).

HA hydroxyl groups have been also functionalized with alkyl succinic anhydrides, such as octenyl succinic anhydride, under alkaline conditions. In this reaction, the hydroxyl groups of HA react with the anhydride to form ester bonds aimed to improve the mechanical properties as well as decrease the degradation rate of HA (Tommeraas & Eenschooten, 2009). Moreover, the HA esterification with methacrylic anhydride has been also performed to obtain methacrylated HA. The presence of methacrylate groups enabled the photo-crosslinking of the HA derivatives (Burdick, Chung, Jia, Randolph, & Langer, 2005; Seidlits et al., 2010).

The activation of polymeric carboxyl groups of HA involves esterification and amidation processes (Schanté et al., 2011). The esterification can be performed by alkylation of HA carboxyl groups using alkyl halides or tosylate activation (Huin-Amargier, Marchal, Payan, Netter, & Dellacherie, 2006). Moreover, HA esters can be synthesized using diazomethane as the activator of the carboxyl groups. In turn, the HA amidation with 1,1´-carbonyldiimidazole or 2-chloro-1-methylpyridinium iodide, as activating agents, is also performed (Hirano et al., 2005; Huin-Amargier et al., 2006). However, the most efficient mechanism to perform the activation of HA carboxyl groups is by using carbodiimide (*i.e.* N-(3-dimethylaminopropyl)-N´-ethylcarbodiimide hydrochloride) and co-activators like N-hydroxysuccinimide (NHS) or 1-hydroxybenzotriazole in water. The activation with carbodiimide

Table 1
List of some examples of commercially available HA-based wound dressings.

Commercially available product	Characteristics	References
Bionect®	Topical solution composed of LMW-HA sodium salt (0.2 %), that is used to prevent abrasion, removal of the harmful agents and skin integrity restoration.	Vasir, Tambwekar, and Garg (2003)
Connettivina®	Cream containing 10 mg/ml of hyaluronate sodium used for the treatment of skin irritations. It assures a hydrated environment that promotes cell migration and skin regeneration.	Paghetti, Bellingeri, Pomponio, Sansoni, and Paladino (2009)
Hyalofill®	Cream-coloured non-adherent produced of a derived-HA (HYAFF). It is applied for the management of chronic wounds, including diabetic foot ulcers. It is available either as a flat sheet (Hyalofill-F) or as a rope (Hyalofill-R). When applied at wound site, it interacts with wound exudate and a hydrophilic gel is produced. The gel creates an HA-rich tissue interface, providing a moist wound environment that promotes cell activity and the healing process.	Colletta, Dioguardi, Di Lonardo, Maggio, and Torasso (2003)
Hyalomatrix®	It is a transparent membrane composed of HYAFF that allows cellular invasion and capillary growth, processes that are fundamental for encouraging skin re-epithelialization. It is indicated for the management of partial- and full-thickness wounds, second-degree burns, pressure ulcers, venous ulcers, chronic vascular ulcers, as well as surgical and trauma wounds.	Longinotti, 2014)
Hyalosafe®	This transparent film is designed to cover superficial wounds and create a moist healing environment. It is composed of HYAFF (a total benzyl ester of HA) and is applied directly at wound site, since its transparency allows the continuous monitoring of healing process. It is indicated for the management of superficial moderately exuding wounds, such as post-laser surgery wounds, superficial surgical wounds, and first and second-degree burns.	Longinotti (2014)
Hylase Wound Gel®	It is a gel composed of a combination of emollients and sodium hyaluronate (2.5 %), that avoids tissue dehydration and support the healing process. It is suitable to treat different types of wounds namely: leg, pressure and diabetic ulcers as well as for the management of wounds that are prone to bleeding.	Khelfi (2018)
HylaSponge®	It is a sponge that possesses a network of large hyaluronan molecular chain assemblies. It can absorb and release large volumes of water, assuring the skin hydration along the healing process. It acts as an equilibrium barrier or elastic “second skin”.	Mahedia et al. (2016)
Laserskin®	It is a HYAFF-biopolymer-based scaffold, which possesses a microperforated HA membrane allowing the growth and migration of autologous keratinocytes and fibroblasts to the wound bed. It is used in the management of acute and chronic wounds.	Price, Berry, and Navsaria (2007)

allows the formation of ester bonds between the hydroxyl and carboxyl groups, leading to the production of biomaterials with improved stiffness and resistance to degradation (Bulpitt & Aeschlimann, 1999; Kaczmarek, Sionkowska, Kozlowska, & Osyczka, 2018; Kirk et al., 2013). In the following sections, different HA derivatives-based wound dressings will be described in further detail.

4. HA-based wound dressings

Nowadays, different HA-based wound dressings, such as HylaSponge® System, Hyalomatrix® and Hyalosafe®, are available to be used in the clinic (Longinotti, 2014; Mahedia et al., 2016) (as described in Table 1). The HylaSponge® System is produced through a free-radical polymerization process, leading to the formation of a complex HA network that is capable of providing protection and grant a high hydration to the wound site (Mahedia et al., 2016). Hyalomatrix® is a flexible, conformable and bilayered dermal substitute conceived to promote wound closure as well as dermis regeneration. The bottom layer (layer in contact with the wound) is a 3D fibrous matrix composed of HYAFF® 11, whereas the top layer is formed by a thin sheet of transparent silicone. HYAFF® 11 is a HA-derived, which is obtained through the esterification of the free carboxylic group of HA with benzyl alcohol (Longinotti, 2014). This esterification process prevents the water infiltration into the macromolecule, *i.e.* increases the hydrophobic character of HA. Further, such process also increases the degradation time of the polymer: 75 % of esterified HA degrades over the course of 7–14 days, while the 95 % esterified HA may require up to 2 months to degrade (Benedetti et al., 1993; Fallacara et al., 2018).

In addition, the transparency of the top layer is fundamental to perform a continuous monitoring of the healing process (Longinotti, 2014). Gravante et al. (2010) and Osti (2008) evaluated the therapeutic efficacy of Hyalomatrix® in clinical assays and the data obtained revealed that after 29 days of treatment, a complete wound closure was achieved in 85.7 % of the patients (28/57), while 14.3 % of them only displayed a partial re-epithelization.

In turn, Hyalosafe® is a transparent film used for the treatment of second-degree superficial burns. The degradation of this film leads to the release of HA, that encourages the proliferation of epithelial cells (Longinotti, 2014).

Apart from the physicochemical and biological properties of HA-based wound dressings, the sterilization of these dressings is also crucial for their application in tissue engineering (Galante, Pinto, Colaço, & Serro, 2018). The sterilization method used may impact on the mechanical, chemical and biological properties of HA-based wound dressings (Galante et al., 2018; Huerta-Angeles, Nesporova, Ambrozova, Kubala, & Velebny, 2018; O'Connell et al., 2019). In the literature, natural polysaccharide-based biomaterials are usually sterilized through filtration, high-pressure-high-temperature (autoclave), ethylene oxide gas, UV-radiation, gamma-radiation and electron beam (Huerta-Angeles et al., 2018).

Indeed, despite all the efforts that have been performed so far, the commercially available HA-based wound dressings, still present some shortcomings, namely high production costs, possible presence of contaminants (due to the extraction process used to obtain HA), limited cell adhesion/proliferation and low mechanical stability (Gallo et al., 2019). To overcome these drawbacks, researchers from the Tissue Engineering area have been developing alternative solutions. In the following sections, different examples HA-based wound dressings (namely sponges, films, hydrogels, and electrospun membranes) are highlighted.

4.1. Sponges

Sponges, due to their biodegradability, porosity, and swelling profile, are capable of absorbing large amounts of wound exudate, as well as maintain a moist environment at the wound site (Simões et al., 2018; Villamizar-Sarmiento et al., 2019). Further, the sponges are generally non-adhesive and require secondary dressings or tapes/bandages that grant their maintenance at the wound site (Simões et al., 2018). To surpass the weak mechanical properties exhibited by sponges, researchers have been combining HA with other polymers or producing HA derivatives through chemical synthesis (as listed in Table 2).

Orellana et al. (2016) produced a blend of HA (MW = 417 Da) with

Table 2
Examples of HA-based sponges developed so far to be used as wound dressings, aiming to improve the water stability/mechanical properties and biological properties of sponges.

Main goal	Sponges composition	HA molecular weight	Production technique	Main findings	Refs.
Improve the water stability and mechanical properties	CS/ALG/HA	MW = 417 Da	Freeze-drying	The presence of HA allowed to obtain a microporous structure favourable for cell adhesion and proliferation.	Orellana et al. (2016)
	HA/CMC-Na	MW = 1.3×10^6 Da	Freeze-drying	The ADH and EDC were used as the crosslinker and carboxyl-activating agent, respectively; The DSC analysis verified that more energy was required for HA-CMCNa sponges' degradation; The increase on ADH and EDC prolong the degradation time of HA-CMCNa sponges.	Liu et al. (2007)
	HA/DEX-PDM	MW = $2-4 \times 10^6$ Da	Self-foaming	STMP was used as chemical crosslinking agent of hydroxyl groups of HA and dextran; The crosslinked sponges presented a higher porosity (> 70 % vs 48.9 %) and swelling ratios (> 1000 % vs 520 %) in comparison to uncrosslinked sponges; The crosslinked sponges were hemocompatible, presenting a low haemolysis ratio (below 0.5 %).	Liu et al. (2018)
Improve biological properties	ALG/HA/TA	MW = $1.5-1.8 \times 10^6$ Da	Freeze-drying	95 % of TA was released from ALG/HA sponges after 6 h of incubation; The ALG/HA sponges loaded with TA reduced 40 % of blood clotting index; ALG/HA/TA sponges presented promising properties for controlling the hemostasis phase of healing process.	Catanzano et al. (2018)
	CS/HA/AgNPs	Not available	Freeze-drying	The sponges displayed antibacterial activity against *S. aureus*, *E. coli* and *MRSA* due to the AgNPs incorporation; The CS/ALG/AgNPs sponges inhibited the microorganism growth, without impairing the cell viability.	Anisha et al. (2013)
	HA/Arg/EGF	MW = 2×10^6 Da	Freeze-drying and coating	The animals treated with sponges coated with Arg and EGF (group II) presented a wound size area of ≈ 3.3 cm^2, whereas the control group had ≈ 6.8 cm^2; The functionalization of sponges with Arg allowed to avoid an exuberant inflammatory response; The synergic effect of HA, Arg and EGF promoted an enhanced wound closure and epithelialization process.	Matsumoto and Kuroyanagi(2010)
	CS/HA_VEGF loaded nanofibrin	Not available	Freeze-drying	More than 60 % of VEGF was release from CS/HA sponges after 3 days of incubation; The capillary like tube formation was evidenced by the HUVECs cells seeded on VEGF containing sponges; The CS/HA_VEGF loaded nanofibrin sponges demonstrated potential to induce angiogenesis process in wound healing.	Mohandas et al. (2015)
	HA/COL/EGF/Vit.C	MW = 2×10^6 Da	Freeze-drying	*In vitro* assays showed that sponges incorporating EGF and Vit.C stimulated the fibroblasts to release 2 times more HGF in comparison to HA/COL/EGF sponges; The HA/COL/EGF/Vit.C promoted a more effective collagen deposition, granulation tissue formation, and angiogenesis process in animal experiments.	Niiyama and Kuroyanagi (2014)
	CS/HA_AND loaded lipid nanocarriers	MW = $1.5-1.8 \times 10^6$ Da	Freeze-drying	The CS/HA sponges incorporating AND presented a total porosity of ≈ 70% with enhanced swelling; The combined anti-inflammatory and antioxidant effects of CS, HA and AND improved the healing process and reduce the scar formation.	Sanad and Abdel-Bar (2017)

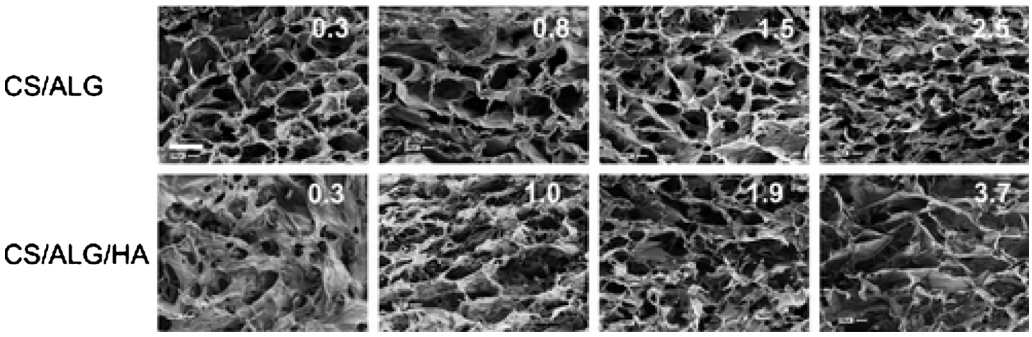

Fig. 3. SEM images of CS/ALG and CS/ALG/HA sponges produced using a freeze-drying technique. Reprinted from Journal of Biomedical Materials Research Part A, vol. 104, Orellana et al., Relevance of charge balance and hyaluronic acid on alginate-chitosan sponge microstructure and its influence on fibroblast growth, 2537– 2543. Orellana et al., 2016, with permission from Wiley.

CS and ALG to obtain porous sponges by using the freeze-drying technique. Scanning electron microscopy (SEM) images of the produced CS/ALG/HA sponges revealed that these sponges exhibited a higher porosity than those without containing HA, *i.e.* CS/ALG (Fig. 3). Such structural variation is responsible for enhancing the O_2 supply, nutritional flow and cell proliferation at the wound site (Bružauskaitė, Bironaitė, Bagdonas, & Bernotienė, 2016). Moreover, the cell proliferation rate has increased when they were incubated with sponges containing HA (Orellana et al., 2016).

In another study, Liu, Liu, Wang, Du, and Chen (2007) mixed HA (MW = 1.3×10^6 Da) with carboxymethylcellulose sodium (CMC-Na), that was then crosslinked with adipic dihydrazide (ADH) to produce a HA based sponge. The HA-CMCNa sponges displayed a lower degradation rate when higher concentrations of ADH and 1-ethyl-3-[3-(dimethylaminopropyl)] carbodiimide (EDC) were used. Overall, the extra stability displayed by HA-CMCNa sponges is fundamental for their successful application in skin regeneration.

Apart from the enhancement of the physicochemical properties presented by HA-based sponges, other researchers have been focused on the improvement of their biological features (hemostatic, antibacterial and healing). To accomplish that, bioactive molecules have been incorporated into sponges' structure. Catanzano, D'Esposito, Formisano, Boateng, and Quaglia (2018) incorporated tranexamic acid (TA) into ALG/HA (MW = $1.5-1.8 \times 10^6$ Da) sponges to avoid excessive blood loss, which is fundamental to promote the hemostasis as well as the subsequent phases of healing process.

Anisha, Biswas, Chennazhi, and Jayakumar (2013) incorporated silver nanoparticles (AgNPs) into CS/HA sponges to improve their bactericidal activity and propel their use in the treatment of diabetic foot ulcers. The antimicrobial activity of the produced sponges was assessed using *Staphylococcus aureus* (*S. aureus), Escherichia coli* (*E. coli)* and *Methicillin-resistant Staphylococcus aureus* (*MRSA*) as model bacteria. The obtained results show that CS–HA loaded with different concentrations of AgNPs (0.001 %, 0.005 %, and 0.01 %) induced the formation of inhibitory halos with diameter values of 7 ± 1 mm, 11 ± 2 mm and 14 ± 2 mm for *S. aureus*; 8 ± 1 mm, 11 ± 2 mm and 13 ± 2 mm for *E. coli*, and 9 ± 1 mm, 10 ± 2 mm and 10 ± 2 mm were noticed for *MRSA*. Such values demonstrate that the incorporation of AgNPs (even at low concentrations) into CS-HA sponges avoided bacterial growth, without compromising the eukaryotic cell viability, evidencing the potential of this composite sponge for being applied in the treatment of diabetic foot ulcers infected with multidrug-resistant bacteria (Anisha et al., 2013).

Additionally, other biological molecules (amino acids and/or growth factors) have also been incorporated into HA-based sponges to enhance their biological performance (Hussain, Thu, Katas, & Bukhari, 2017; Kondo & Kuroyanagi, 2012).

Matsumoto and Kuroyanagi (2010) functionalized HA (MW = 2×10^6 Da) -based sponges with arginine (Arg) and epidermal growth factor (EGF) and then they evaluated sponges' performance in the healing process. Full-thickness wounds (15 mm of diameter) were induced on rats' skin and, after one week, they noticed that animals treated with sponges containing Arg and EGF presented a significant decrease in the wound area. Further, the ability of sponges to control the inflammatory response was also assessed through the determination of amount of MPO produced by neutrophils. The obtained results show that the Arg induces a moderate inflammatory response (≈ 0.13 units/mg of MPO) in the group treated with c-HMW-HA/LMW-HA/Arg sponge, contrasting with ≈0.21 units/mg of MPO in the group treated with c-HMW-HA/LMW-HA/Arg/EGF. Overall, the gattered results suggest that a synergic effect occurs between HA, Arg and EGF, leading to an enhancement of the wound closure and epithelization process (Matsumoto & Kuroyanagi, 2010).

4.2. Films

Films are highly elastic and flexible structures, composed of adherent and transparent polymers that allow O_2 and CO_2 exchange, as well as water vapor transmission from the wound site and avoid bacteria penetration. Furthermore, their transparency enables the continuous wound monitoring, without demanding the removal of the wound dressing (Felgueiras & Amorim, 2017). Films also promote the autolytic debridement of eschar (Dhivya et al., 2015). However, this type of wound dressings present a reduced capacity to absorb the exudate and may induce trauma if not appropriately removed (Simões et al., 2018).

Up to now, the development of HA-based films has been focused on the enhancement of their biological performance, through the incorporation of bioactive molecules (like growth factors, natural product extracts and sulfadiazine (SD)), and inorganic compounds. Furthermore, these films have been also functionalized with other polymers or HA-derivatives. Examples of the strategies used to improve the biological properties of the HA-based films aimed to be used for the treatment of wounds are listed in Table 3.

Li et al. (2018) produced for the first time HA-based films using HA (MW = 5.4×10^3 Da) grafted pullulan (HA-*g*-Pu) in order to enhance HA stability and biological performance during the healing process. The acquired data showed that the HA-*g*-Pu films presented a higher swelling ratio (40 %) in comparison to the pullulan (Pu) and HA films (≈ 30 % and ≈ 34 %, respectively). Further, HA films presented a 100 % of weight loss after ± 3 days of incubation, whereas the HA-*g*-Pu films only become completely degraded after 12–14 days. Thus, confirming that the grafting of Pu into HA chain improved the films' water stability. In addition, the SEM analysis demonstrated that the HA-*g*-Pu films presented a porous structure with an average pore size of 73.13 ± 29.36 μm, which is compatible with cell migration. Finally, the *in vitro* and *in vivo* results showed that HA-*g*-Pu films are biocompatible, and a faster wound healing process occurred for those animals covered with the produced film (Li et al., 2018).

In another study, Zhou et al. (2016) produced HA (MW = 6.8×10^3 Da) /silk fibroin (SF) films loaded with vascular endothelial growth factor (VEGF). Different ratios of SF and HA were used, and the water absorption, degradation and mechanical assays revealed that those films produced with 5% HA/SF presented the most promising results.

Table 3
Examples of strategies used to improve the biological properties of HA-based films aimed to be used as wound dressing.

Strategy to improve the biological properties	Films composition	HA molecular weight	Production technique	Main findings	Refs.
Incorporation of bioactive molecules	HA/SF/VEGF	MW = 6.8 × 10^3 Da	Casting	The HA/SF films presented an increased water absorption 51.45 ± 0.53 %) in comparison to the pure SF films (45.40 ± 1.82%); The HA/SF films produced at 60 °C presented an improved water stability and mechanical properties; The VEGF released profile was more controlled from HA/SF films formed at 60 °C, than those produced at 37 °C; The addition of HA and increase the film temperature production allowed to control the films structural integrity and VEGF release profile.	Zhou et al. (2016)
	CSH/HA/EEP	MW = 1.73−1.54 × 10^5 Da	Casting	The concentration of released EEP from CSH/HA/0.25 %EEP, CSH/HA/0.5 %EEP and CSH/HA/1%EEP films was 60 %, 69 % and 74 % after 48 h, respectively; The CSH/HA/EEP films presented antimicrobial activity against *S. aureus*, *E. coli*, *S. epidermidis* and *P. aeruginosa*; *In vivo* assays showed that CSH/HA/EEP films promoted the healing process after 14 days on skin incision induced on rats.	Eskandarinia et al. (2019)
	HA/SA/SD/AgNPs	Not available	Casting	The crosslinking of films was performed with divalent metal cations (Ca^{2+}, Zn^{2+}, Cu^{2+}); The characterization of physicochemical properties of films revealed that HA/SA films crosslinked with Ca^{2+} showed the most promising properties; The HA/SA/Ca^{2+}/SD/AgNPs films presented a higher reduction percentage of *S.aureus* and *E.coli* growth; *In vivo* results evidenced that the wounds treated with HA/SA/Ca^{2+} films presented a higher reduction percentage of wound area in comparison to the SA/Ca^{2+}; *In vivo* assays, the anti-inflammatory and antioxidant properties of HA were also demonstrated, which allows the rapid restoration of skin structure; The incorporation of both SD and AgNPs into films augment the antibacterial properties.	Abou-Okeil et al. (2018)
	HA/ZIF-8	MW = 1 × 10^6 Da	Casting	The films were crosslinked with EDC/NHS chemistry; The ZIF-8 incorporation into HA films increased the Young modulus and tensile stress values; The HA films incorporating ZIF-8 also presented an enhanced antibacterial activity against *E. coli* and *S. aureus*.	Abednejad et al. (2019)
	MMT/HA/GS	MW = 2.5 × 10^4 Da	Layer-by-layer assembly	The loading dosage of GS was 0.85 mg/cm^2; The multilayer films presented high roughness and are biodegradable, when incubated in contact with hyaluronidase solution; The GS release from multilayer films contributed for efficient antibacterial properties and long-term biofilm inhibition functions for *E. coli* and *S. aureus*.	Wang et al. (2018)
Synthesis of HA-derivatives or combination with other polymers	HA-*g*-Pu	MW = 5.4 × 10^3 Da	Freeze-drying	The HA-*g*-Pu films presented a higher swelling ratio in comparison to Pu and HA films; The *in vitro* enzymatic degradation assays also demonstrated that the grafting of Pu into HA chain improves the water stability of films; The chemical modification of HA with Pu allowed to obtain a film that may be used in the treatment of skin injuries.	Li et al. (2018)
	COL/HA/CS	MW = 1.8 × 10^6 Da	Casting	COL/HA and COL/HA/CS films presented rough surfaces, demonstrated by SEM and AFM analysis; The thermal stability was better on COL/HA/CS films; The crosslinking reactions between polymer chains improved the physical properties of films.	Lewandowska, Sionkowska, Grabska, and Kaczmarek (2016)
	HA/PLL	MW = 6.4 × 10^4 Da; MW = 3.51−6 × 10^5 Da;	Spray layer-by-layer	HA and PLL were used to produce a stable membrane, acting as epidermal component, which was sprayed on top of porous HA scaffold (dermal component); The opposite charges of HA and PLL allowed nanometer-scale control over the film thickness; The rough surfaces of both components promoted the cell adhesion;	Monteiro, Shukla, Marques, Reis, and Hammond (2015)

(*continued on next page*)

Table 3 (*continued*)

Strategy to improve the biological properties	Films composition	HA molecular weight	Production technique	Main findings	Refs.
		MW = 1.2−1.8 × 10^6 Da		The LbL thin film allowed the formation of a monolayer of keratinocytes; The combination of top LbL thin film with bottom porous scaffold enabled the formation of the normal skin architecture.	
	CS/ALG/HA-DN	MW = 5.95 × 10^5 Da	Layer-by-layer assembly	The free-template multilayer patch to treat skin wounds were fabricated, without the use of any organic solvent; The modification of HA with dopamine (catechol groups) improved the cell adhesion and spreading; The application of the DN-containing multilayer membranes in treatment of dermal wounds resulted in decrease on inflammation process; The multilayer films demonstrated great potential for supporting the skin wound healing.	Sousa et al. (2018)
	HA/PLL_HA/CS	Not available	Layer-by-layer assembly	The multilayer films are composed of a top layer (HA/PLL) and bottom layer (HA/CS); The increased secretion of hyaluronidase and chymotrypsin in the bacterial infection microenvironment led to the fast degradation of the outer (HA/PLL) multilayer film; The composite multilayer films presented effective anti-infection properties, avoiding the bacterial adhesion *in vitro* and *in vivo* assays.	Yao et al. (2017)

Furthermore, other researchers have also been working to ameliorate the antibacterial properties of HA-based films for improving the healing process. Eskandarinia et al. (2019) produced a cornstarch (CSH)/HA (MW = 1.73−1.54 × 10^5 Da) dressing through the incorporation of an ethanolic extract of propolis (EEP), using a casting technique. The EEP release profile was studied, and the results show that 60 %, 69 % and 74 % of EEP were released from CSH/HA/0.25 % EEP, CSH/HA/0.5 %EEP and CSH/HA/1%EEP films after 48 h, respectively. Such EEP release profile can grant an aseptic environment at wound site, during at least 48 h. In fact, the CSH/HA/EEP 0.25 %, CSH/HA/EEP 0.5 % and CSH/HA/EEP 1% films induced the formation of inhibitory halos with diameter values of 0.93 ± 0.25 mm, 2.08 ± 0.14 mm and 4.68 ± 0.12 mm for *S. aureus*, 1.21 ± 0.39 mm, 2.64 ± 0.18 mm and 4.33 ± 0.27 mm for *E. coli*, and 0 mm, 1.02 ± 0.15 mm and 2.92 ± 0.26 mm for *Staphylococcus epidermidis*. In addition, these films were also applied on wounds induced in animals. The attained results show that the wounds treated with films (as can be observed in Fig. 4), were almost healed after 14 days, whereas those only covered with gauze did not. Such results clearly demonstrated that the CSH/HA films loaded with EEP have potential to enhance the healing process as well as inhibit microorganisms' growth at the surface of the wound (Eskandarinia et al., 2019).

In a similar way, Abou-Okeil, Fahmy, El-Bisi, and Ahmed-Farid (2018) produced HA/sodium alginate (SA) films crosslinked with Ca^{2+}, Zn^{2+}, and Cu^{2+} metal cations. Then, the physicochemical properties determined for these films revealed that HA/SA films crosslinked with Ca^{2+} showed the most promising properties. To improve the antimicrobial activity of HA/SA films, AgNPs and SD were incorporated into these films. The *in vivo* data obtained show that the wounds treated with these films display a higher reduction of the wound area. In addition, HA also prompted a decreased production of inflammatory mediators (nitric oxide), as well as oxidative stress markers (malondialdehyde).

Abednejad, Ghaee, Nourmohammadi, and Mehrizi (2019) incorporated zeolite imidazolate frameworks (ZIF-8) nanoparticles into HA (MW = 1 × 10^6 Da) films for improving its mechanical and antibacterial properties. Their results demonstrated that those films incorporating FZIF-8 nanoparticles, with concentrations ranging from 0.5 % to 2%, display an increase in the Young modulus (from 145 ± 3 K Pa to 176 ± 2 K Pa) and tensile stress values (from 105 ± 3 K Pa to 128 ± 3 K Pa). A similar trend was also observed for the antimicrobial activity, *i.e.* a higher concentration of the FZIF-8 nanoparticles lead to an improved bactericidal effect. In summary, the results obtained in this study show that the HA films loaded with FZIF-8 nanoparticles display enhanced mechanical and antibacterial properties, without affecting fibroblasts adhesion and proliferation.

4.3. Hydrogels

Hydrogels are known as 3D polymeric networks able to absorb massive quantities of water. Its highly hydrated networks provide a moist and biomimetic environment for cellular outgrowth (Hoare & Kohane, 2008; Hoffman, 2002). Additionally, hydrogels also display a highly porous structure that allows the accommodation of living cells as well as gases, nutrients, and waste products diffusion (Nguyen et al., 2019). Such features capture researchers attention and trigger their application in the treatment of wounds (Hoffman, 2002). Indeed, different hydrogels composed of synthetic and/or natural polymers (CS, ALG, HA, COL, PVA, PCL, poly(ethylene glycol)) have been produced and some of them displayed the required properties to be used in the area of tissue regeneration (Fan, Yang, Yang, Peng, & Hu, 2016; Jeong, Park, & Lee, 2017; Kamoun, Kenawy, & Chen, 2017; Khorasani, Joorabloo, Moghaddam, Shamsi, & MansooriMoghadam, 2018).

Among them, HA based hydrogels have been widely studied for wound dressing applications, due to their intrinsic properties, namely biocompatibility, ability to provide a moist environment as well as

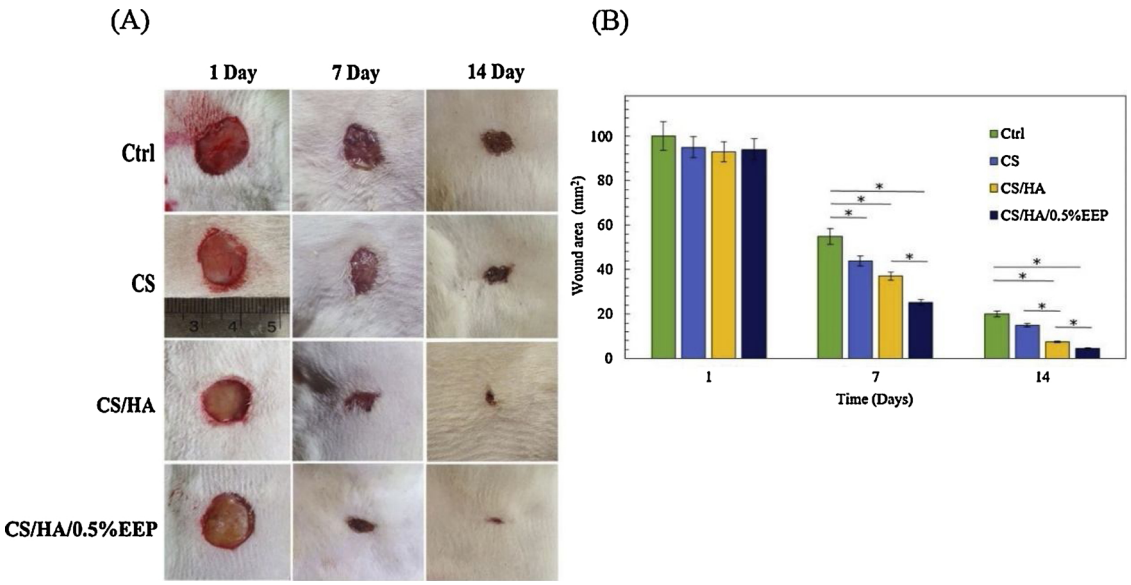

Fig. 4. Monitorization of the wound healing process along 14 days. Photos of the wounds after 1, 7 and 14 days can be observed in (A); The wound area along time (1-14 days) are presented in (B). Reprinted from Carbohydrate Polymers, vol. 216, Eskandarinia et al., Cornstarch-based wound dressing incorporated with hyaluronic acid and propolis: *in vitro* and *in vivo* studies, 25–35. Eskandarinia et al., 2019, with permission from Elsevier.

promote the cell infiltration and proliferation (Lam, Truong, & Segura, 2014).

However, HA based hydrogels present some drawbacks, like weak mechanical properties and fast degradation (Simões et al., 2018). To widening their applicability, researchers have been pursuing different strategies to improve hydrogels features, some of them listed in Table 4.

Wu et al. (2017) used EDC to promote the crosslinking of HA with gelatin (GEL) aiming to improve hydrogels' water stability. Initially, these authors prepared different ratios of GEL and HA (8:2, 5:5 and 2:8), that were subsequently crosslinked with 0.1 % EDC, in order to promote the chemical interaction between compounds. Through the morphological characterization, it was possible to verify that the use of a crosslinking agent did not impair the porous structure of GEL-HA hydrogels (which presented a total porosity of 40 %–70 %, with an average pore size of 100 − 400 mm), a feature that is essential for cell infiltration and gaseous/nutrients exchange. Furthermore, the *in vivo* assays demonstrated that the wounds treated with GEL/HA 8:2 hydrogel had a decrease in the area of ≈ 95 %, thus demonstrating that the combination between GEL and HA provide a suitable moist environment for fibroblasts proliferation and migration.

In another study, Hong et al. (2018) compared the potential of uncrosslinked/crosslinked HA hydrogels (MW = 2×10^6 Da) (HA1 and HA2, respectively) to be used in the treatment of full-thickness skin injuries induced in rabbits. The results obtained show that the wound area of animals treated with HA2 was smaller than that displayed by the other groups (control, HA and HA1) after 14 days. In addition, the expression of alpha-smooth muscle actin (α-SMA), VEGF and transforming growth factor-beta (TGF-β) were also quantified. A higher expression of α-SMA and VEGF was accomplished for the HA2 treated group, which is indicative of high proliferation of myofibroblasts as well as the occurrence of the angiogenesis process, respectively. On the other hand, the TGF-β expression was reduced for HA2 group. Such decrease is important to attain a smaller inflammatory response and a reduced scar formation. Overall, the results obtained reveal that HA2 (crosslinked HA hydrogel) presented the most auspicious properties for future application on wound therapy (Hong et al., 2018).

Ying et al. (2019) produced a hydrogel with improved mechanical properties by mixing HA (MW = 2×10^5 Da)-tyramine (HA-Tyr) with collagen I-hydroxybenzoic acid derivative (COL-P), and then performed the crosslink of the blend with horseradish peroxidase (HRP) and H_2O_2. In addition, these authors also performed the encapsulation of fibroblasts and human microvascular endothelial cells (HMEC) within the hydrogels to induce the angiogenesis process. Based on the collected data, animals treated with COL-HA hydrogels exhibited a decrease of 96.44 ± 0.47 % of the wound area, whereas those treated with COL-P and HA-Tyr hydrogels presented a reduction of 93.87 ± 1.12 % and 93.83 ± 2.81 % of the wound area after 2 weeks, respectively.

Fiorica et al. (2018) produced a hydrogel capable of containing and delivering VEGF, by performing the crosslinking of a copolymer of HA (MW = 1.5×10^6 Da) (hyaluronic-(2-aminoethyl)-carbamate acid (HA-EDA)) with α-elastin. The release assays demonstrated that the produced hydrogels retained the VEGF, and approximately 50 % of the incorporated growth factor remains within polymeric network, after 5 days of incubation. An appropriate VEGF release profile is fundamental to stimulate the proliferation of HUVECs enrolled in the formation of the new blood vessels during the wound healing process.

Shi et al. (2018) developed a modified HA (MW = 1.5×10^5 Da) polymer functionalized with pendant bisphosphonate (BP) groups through EDC coupling. Then, Ag^+ ions were also added to a solution of BP-modified HA (HA-BP), producing the HA-BP·Ag^+ hydrogel. The *in vitro* assays showed that the produced hydrogels were able to inhibit the growth *S. aureus* and *E. coli*. Furthermore, the *in vivo* assays demonstrated that the animals treated with hydrogel presented a lower wound area in comparison to the non-treated group, 6 days after the wound be induced (as can be observed in Fig. 5). Overall, self-healing hydrogels were able to fill the wound defects and exhibited antimicrobial activity against both Gram-positive and Gram-negative bacterial strains. Such features are a *sine qua non* condition for the improvement of the healing process.

4.4. Electrospun membranes

In recent years, the simplicity and versatility of the electrospinning technique allowed the production of different types of electrospun membranes. The fibrous network presented by this type of membranes mimics the native structure of the ECM of the skin and encourages cell adhesion, growth, migration, and differentiation (Miguel, Sequeira et al., 2019). The therapeutic potential of these membranes on the wound healing process has been assessed in different works, where natural/synthetic polymers and bioactive molecules have been used to

Table 4
Examples of strategies used to improve the water stability of HA-based hydrogels for wound dressing applications.

Strategy to improve the water stability	Hydrogels composition	HA molecular weight	Production technique	Main findings	Refs.
Use of crosslinked agents	GEL/HA	Not available	Polymeric mixture	EDC was used as crosslinked agent between HA and GEL; The GEL/HA hydrogels presented porosity and pore size suitable for cell infiltration; *In vitro* migration assay showed that GEL/HA hydrogels promoted a faster cell migration in comparison to control groups; The wound healing ratio on GEL/HA 8:2 group (≈ 95%) was higher than control group; EDC granted the interaction between GEL and HA, without impairing the cell proliferation and healing process.	Wu et al. (2017)
	HA	MW = 2×10^6 Da	Polymeric mixture	A polysaccharide extracted from kelp was used to crosslink HA hydrogel; The wound area of animals treated with crosslinked HA hydrogel was smaller than in other groups; The expression of VEGF and α-SMA was higher in groups treated with crosslinked HA hydrogels; The crosslinked HA hydrogel is also able to control the inflammation process (decrease the TGF-β expression).	Hong et al. (2018)
	PVA/SA/HA	MW = 8×10^5 Da	Freeze/thaw cycle	The composite hydrogel was produced using $CaCl_2$ as a crosslinker agent; An increase on amount of crosslinker promoted a decrease on pore size and an increase on density of hydrogels; The crosslinker content also influenced the swelling ratio and hydrophilicity of hydrogels.	Jiang et al. (2019)
	ALG/HA/usSN	MW = $1.5-1.8 \times 10^6$ Da	Internal gelation	The hydrogels were produced through internal gelation method using $CaCO_3$ and glucono-δ-lactone as gelation agents promoters; The usSN was incorporated into ALG/HA hydrogels, before gelation process, to confer antimicrobial properties; The combination between HA bioactivity with antimicrobial properties of usSN demonstrated great potential to produce biofunctional wound dressings.	Catanzano et al. (2017)
	GEL/HA/CNC	MW = 2×10^5 Da	Freeze-drying	GEL/HA hydrogels were produced through freeze-drying technique and using EDC/NHS as crosslinking agents; GEL/HA/CNC hydrogels presented pores with diameter values of about 80 − 120 μm; CNC improved the rheological properties and swelling ability; The fibroblast cells attached and proliferated on hydrogels' surface.	Yin, Lin, and Zhan (2019)
HA- chemical derivatives	HA-Tyr/COL-P	MW = 2×10^5 Da	Polymeric mixture	The hydrogel synthesis was accomplished through the covalently crosslinked between HA-Tyr and COL-P, using HRP and H_2O_2 as crosslinking agents; COL-HA composite hydrogels presented higher glass transition temperature and thermal transition temperature; COL-HA hydrogels exhibited a best wound healing ratio in relation to other groups.	Ying et al. (2019)
	HA-EDA/α-elastin	MW = 1.5×10^6 Da HA-LMW = 2.5×10^5 Da	Polymeric mixture	The α-elastin (extracted from elastin of bovine neck) was grafted to copolymer of HA (HA-EDA), yielding to HA-EDA-g-α-elastin hydrogel; The biodegradation assays confirmed that the HA-EDA-g-α-elastin hydrogel was susceptible to enzymatic degradation; The composite hydrogels were able to retain 50 % of incorporated VEGF; The release VEGF stimulated the HUVECs proliferation, which is crucial for formation of the new blood vessels.	Fiorica et al. (2018)
	HA-BP	MW = 1.5×10^5 Da	Self-healing	The HA polymer was modified with BP groups by EDC coupling and chemoselective "click" reactions; The self-healing hydrogel formation occurred due to crosslinking between Ag^+ ions and BP groups linked to HA backbone; The HA-BP·Ag^+ hydrogel presented antibacterial activity against *S.aureus* and *E. coli*; The animals treated with HA-BP·Ag^+ hydrogel presented a higher wound closure percentage (48.2 ± 3.7%), after 6 days post-wound induction.	Shi et al. (2018)
	NOCC-AHA	Not available	Schiff base linkage	The gelation process was induced by forming Schiff base linkage between aldehyde groups of AHA and amino groups of NOCC, without adding any additional crosslinker; The oxidation degree of AHA had a direct impact on biocompatibility and rheological properties of hydrogels;	Nguyen et al. (2019)

(*continued on next page*)

Table 4 (*continued*)

Strategy to improve the water stability	Hydrogels composition	HA molecular weight	Production technique	Main findings	Refs.
				NOCC-AHA40 hydrogel is able to promote cell proliferation, cell attachment and wound healing.	
	HA-ADH/OHEC	MW = 1 × 10^6 Da	Schiff base linkage	HA-ADH was synthesized by coupling primary carboxyl group of HA with amino group of ADH; OHEC was used as biological crosslinking agent to promote the gelation process; The composite hydrogel resulted from interaction between the aldehyde of OHEC and the amino of HA-ADH; The gelation time was about 106 s, while the swelling rate was 2888%; The hydrogels demonstrated to be hemocompatible (haemolysis rates < 5%) and biocompatible (cell viability of 85 %).	Luo, Liu, Xu, Fan, and Nie (2018)

accomplish the production of such wound dressings (Augustine, Kalarikkal, & Thomas, 2016, 2018; Miguel et al., 2018; Miguel, Sequeira et al., 2019).

HA has been selected by researchers for the production of electrospun membranes, since it is an ECM component, presents high-water retention capacity, biodegradability and beneficial effects on wound healing process (Aya & Stern, 2014; Chanda et al., 2018; Shin et al., 2016). However, the electrospinning of pure solutions of HA is extremely difficult. To overcome this shortcoming, researchers have been blending HA with synthetic polymers (to enhance its mechanical properties as well as electrospinnability) and natural polymers or bioactive agents (to augment the HA' biological performance). In Table 5, are summarized the different strategies followed so far to produce HA-based electrospun membranes aimed for wound healing applications.

Kenar et al. (2019) prepared a blend of HA with COL and poly(L-lactide-co-ε-caprolactone) (PLC), that was then used to produce a nanofibrous membrane able to support cell proliferation and promote the vascularization process. The characterization of morphological properties and swelling profile of the produced membrane demonstrated that the PLC/COL/HA fibers presented a lower diameter values (569 ± 188 nm) and a higher water uptake capacity (103 ± 13 %), in comparison to the PLC membrane (which presented mean diameter values of 641 ± 104 nm and 66 ± 4% of swelling ability). Further, these authors also noticed that the HA membranes promoted the vascularization process (Kenar et al., 2019).

Shin et al. (2016) produced co-axial nanofibers of HA (MW = $0.8-1.8 \times 10^6$ Da) and poly(lactic-co-glycolic acid) (PLGA) loaded with epigallocatechin-3-O-gallate (EGCG) (HA/PLGA-E), through coaxial electrospinning, aiming to use them for the treatment of full thickness wounds. The data obtained in this study revealed that the animals treated with HA/PLGA-E membranes presented a lower wound size after 14 days of treatment, in comparison with the other groups (as presented in Fig. 6). Such results suggest that a synergistic effect occurs between HA and EGCG and it can improve the healing process by scavenging ROS, mitigating inflammation, enhancing the re--epithelialization, promoting angiogenesis and ECM re-organization. Overall, these results reveal the potential of HA/PLGA-E core/shell fiber matrices to be used in the treatment of diabetic wounds.

More recently, the electrospinning technique has also been explored for the production of bilayered membranes, which are aimed to reproduce both layers of the skin, *i.e.* the epidermis and dermis (Miguel, Sequeira et al., 2019). In these membranes, the top layer is conceived to avoid bacterial invasion and wound dehydration, whereas the bottom layer is aimed to remove the wound exudate and promote cell infiltration and proliferation (Miguel, Ribeiro, Coutinho, & Correia, 2017; Miguel, Sequeira et al., 2019).

Chanda et al. (2018) produced an electrospun bilayered membrane composed of a CS/PCL and HA (MW = $1-2 \times 10^6$ Da) /Poly(ethylene oxide) (PEO) layer. The bilayered membranes were produced through the deposition of nanofibrous HA/PEO layer over pre-formed layers of CS/PCL, in order to obtain membranes with the required mechanical properties. Overall, the bilayered CS/PCL-HA-PEO dressing showed improved physicochemical and biological properties (biocompatibility, promoting cell adhesion and proliferation), that are essential for its application as a wound dressing.

Figueira, Miguel, de Sá, and Correia (2016) produced bilayer membranes composed of a dense top layer (formed by HA (MW = $1.5-2.2 \times 10^6$ Da) and PCL), and a bottom layer (produced with CS and zein (ZN)) loaded with salicylic acid. These authors used HA to produce the top layer aiming to mimic the epidermis' layer of the skin. The HA_PCL nanofibers produced through electrospinning displayed a mean diameter value of 472 ± 192 nm, which is within the range displayed by collagen fibers (50–500 nm) found in skin. Such feature promoted cell proliferation, differentiation and adhesion. Furthermore, the HA_PCL membranes' porosity below 90 % and a water contact angle

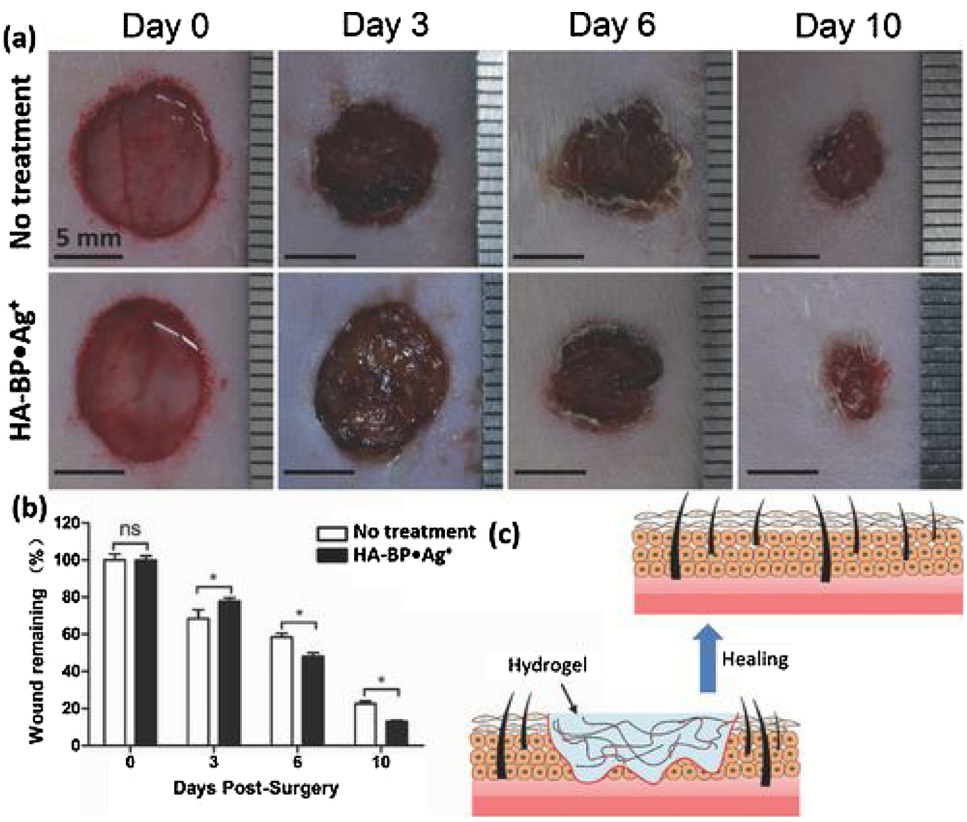

Fig. 5. Evaluation of HA-BP·Ag^+ hydrogel potential for improving the skin regeneration.: a) Macroscopic images of wounds at different time points (0, 3, 6, and 10 days). Scale bar: 5 mm; b) Variation of the size of the wound along time; c) Schematic representation of the application of the HA-BP·Ag^+ hydrogel at the wound surface. Reprinted from Advanced Healthcare Materials, vol. 7, Shi et al., Moldable hyaluronan hydrogel enabled by dynamic metal–bisphosphonate coordination chemistry for wound healing, 1700973. Shi et al., 2018, with permission from Wiley.

(WCA) value of 120.20 ± 0.85° (hydrophobic character) avoided the bacterial colonization of the wound.

Miguel, Simões et al. (2019) produced an asymmetric electrospun membrane composed of PCL and SF in the top layer and HA (MW = $8-15 \times 10^3$ Da) plus SF in the bottom one. The authors combined HA with SF to produce a layer that mimics the dermis' properties. Additionally, they also incorporated thymol (THY) into this layer, in order to confer it antioxidant and antibacterial properties. The SF_HA_THY nanofibrous bottom layer presented a total porosity of 85.24 ± 2.47 %, which is compatible with cell adhesion, migration, and proliferation. Moreover, the authors also analysed the swelling capacity of the SF_HA_THY membrane and the data obtained revealed that this membrane had a higher swelling ratio values (≈ 45 and ≈ 39, at pH 8 and at pH 5, respectively). Thus, demonstrating that the bottom layer is able to provide a moist environment, avoid wound dehydration as well as remove the wound exudate. Moreover, the SF_HA_THY layer possessed a hydrophilic character (WCA value of 38.77 ± 5.32°), which is considered an ideal wettability for cell adhesion and proliferation. The *in vitro* assays showed that the presence of HA in the membranes improved the fibroblasts' attachment and proliferation on membrane surface (confirmed by SEM and confocal scanning laser microscope images). The combination of HA with SF incorporating an essential oil (THY) resulted in the production of a bilayered membrane that is capable of absorb the wound exudate as well as promote the cell adhesion and proliferation.

5. Conclusions and future perspectives

HA is a major component of the ECM of the skin, that plays crucial roles in the wound healing process, like promoting the formation of a fibrin clot, production and release of interleukins and proinflammatory cytokines. In addition, it also encourages the fibroblasts/keratinocytes proliferation as well as propels the fibroblast differentiation into myofibroblasts. Apart from these biological effects, HA is also characterized by its hydrophilicity, biocompatibility, and ability to be chemically modified, widening its applicability to different areas. In this way, different HA-based wound dressings have been produced so far, namely sponges, hydrogels, films, and electrospun membranes, where different strategies have been followed to overcome the low water stability and weak mechanical properties of HA, as well as increment its biological performance. Overall, the presence of the HA in these dressings improves their porosity and swelling (features that are essential to enhance the O_2 and nutrients exchanges), promotes the exudate absorption besides potentiates the cell migration and proliferation. Moreover, HA also decreases the inflammatory cells infiltration, improves the re-epithelization and granulation as well as increases the formation of blood vessels, that are of utmost importance for improving skin regeneration. However, despite all the promising properties exhibited by HA-based wound dressings, they still present some limitations, such as low mechanical stability and inappropriate biodegradation profile. Up to now, the chemical modifications performed, aiming to increase the stability of HA-based wound dressings, using toxic agents and involving complex reactions, which can compromise the wound dressings' biocompatibility. Further, the rapid and high *in vitro/in vivo* degradation of HA demands a periodic replacement of wound dressing, which may lead to the formation of new lesions, tissue exacerbation, increased risk of infection as well as pain to the patient.

In a near future, these aspects need to be further addressed by using alternative approaches (*e.g.* solvent-free methods and "click chemistry") to produce HA derivatives that fulfil the required properties. Such approaches will overcome the use of toxic agents and contribute for enhancing the mechanical properties of HA-based dressings. Furthermore, a reproductible process to accomplish the production of HA derivatives is required and their pharmacokinetic/pharmacodynamic properties must be optimized to allow their successful commercialization. In addition, the biological activity of HA-based wound dressings could be improved through the incorporation of other biomolecules such as adhesive proteins (*e.g.* fibronectin, laminin, fibrinogen), stem cells and/or antimicrobial agents (*e.g.* antibiotics, silver nanoparticles or natural products) for accomplishing an improved healing process.

Table 5
Description of works reporting the production of HA-based electrospun membranes aimed to be used as wound dressing, highlighting the improvement of HA mechanical properties, electrospinnability and its biological properties.

Strategies used on HA-based electrospun membranes	Membranes composition	HA molecular weight	Type of electrospinning	Main findings	Refs.
Improve the mechanical properties and electrospinnability	HA/COL/PLC	Not available	Blend electrospinning	PLC was used to grant the electrospinning process of membranes; The presence of HA on membranes' composition improved the water uptake ability; The membranes composed of ECM components (COL and HA) supported cell adhesion and proliferation.	Kenar et al. (2019)
	HA/PVA/HPβCD	MW = 57 × 10^3 Da	Blend electrospinning	-The addition of HPβCD stabilized the electrospinning process, resulting in production of uniform nanofibrous membranes; An *in situ* crosslinking process (based on EDC/NHS reaction) was proposed; The naproxen was impregnated into electrospun membranes, showing its maximum release, from HA/PVA/HPβCD membranes, during first 24 h.	Séon-Lutz, Couffin, Vignoud, Schlatter, and Hébraud (2019)
	PEO/HA	MW ≈ 0.6–1.1 × 10^6 Da	Blend electrospinning	PEO was used since the electrospinning of HA is difficult due to its high viscosity at very low concentrations; Kanamycin was incorporated into PEO/HA nanofibers to provide antibacterial properties; The nanofibers presented excellent antimicrobial activity against *L. monocytogenes,* which can be used as prosthetic implants coating.	Ahire, Robertson, van Reenen, and Dicks (2017)
	PCL/SF/HA	MW = 2.5 × 10^6 Da	Emulsion electrospinning	PCL and SF acted as main structural and cell adhesion components; HA provided a hydrated microenvironment and promoted the cell infiltration; HA improved the resistance non-specific protein adsorption, which may lead to reduce the macrophages adhesion and fibrosis formation.	Li et al. (2012)
	PLA/HA	Not available	Blend electrospinning	A layered membrane was obtained through the production of a PLA membrane covered with HA layer; The parameters like amount of solvents and polymer concentration influenced the diameter and properties of polymer fibers; All fibers displayed a biocompatible profile.	Stodolak-Zych et al. (2018)
	GEL/HA	MW = 1.2 × 10^6 Da	Blend electrospinning	GTA vapor was used to improve the stability of the electrospun membranes in a moist environment; GEL/HA membranes promoted a formation of more epidermis in comparison to the control group; The inflammation process was also controlled by the presence of GEL/HA membranes.	Ebrahimi-Hosseinzadeh et al. (2016)
	PCL/HA	MW = 2.5 × 10^6 Da	Blend electrospinning	PCL acted as mains structural component and HA provided an ECM biomimetic surface to promote cell migration; PCL/HA nanofibers exhibited good mechanical properties and up-regulated collagen III expression and down-regulated collagen I expression; HA/CD44 interactions activated the TGF-β/MMP-2 signalling pathway, which promotes cell motility.	Qian et al. (2015)
Augment the biological properties	HA/PLGA/EGCG	MW = 0.8 − 1.8 × 10^6 Da	Coaxial electrospinning	The HA/PLGA-E fibers presented excellent results on study of *in vivo* wound healing effect; The synergistic effect of HA and EGCG accelerate the healing process, by controlling the inflammation phase and stimulating the epithelialization and angiogenesis processes.	Shin et al. (2016)
	GEL/HA/chondroitin sulfate/Ser	Not available	Blend electrospinning	Ser, HA and chondroitin sulfate were used as bioactive compounds, while GEL was selected as a base polymer to prepare electrospun membranes; The presence of Ser clearly augmented the adhesion and proliferation of fibroblasts, keratinocytes and hMSCs; The Ser loaded electrospun membranes stimulated the differentiation of hMSCs.	Bhowmick, Scharnweber, and Koul (2016)

(*continued on next page*)

Table 5 (*continued*)

Strategies used on HA-based electrospun membranes	Membranes composition	HA molecular weight	Type of electrospinning	Main findings	Refs.
	COL/HA	MW = 2.05×10^6 Da	Blend electrospinning	COL/HA membranes were produced with the programmable release of multiple angiogenic growth factors; The EGF and bFGF were directly incorporated into COL/HA nanofibers to accelerate the epithelialization and vasculature sprouting at early stage; The PDGF and VEGF were pre-loaded into gelatin nanoparticles to induce the blood vessels maturation at late stage; This composite nanofibrous membrane presented promising properties to be used for treatment of chronic wound healing.	Lai et al. (2014)
	PCL/HA/EGF	MW = 2.5×10^9 Da	Emulsion electrospinning	The synergistic effects of HA and EGF promoted cell proliferation and infiltration; PCL/HA/EGF membranes accelerated the epidermis regeneration; PCL/HA/EGF nanofibrous membranes were able to encapsulate and control the release of growth factors.	Wang et al. (2015)
	CS/PCL_HA/PEO	MW = $1-2 \times 10^6$ Da	Blend electrospinning-asymmetric membrane	The different polymeric combinations resulted on layer with distinct properties; The bilayered membrane presented similar mechanical properties to those exhibited by native skin; The CS/PCL_HA/PEO membrane supported the cell adhesion, proliferation and migration.	Chanda et al. (2018)
	HA_PCL/CS_ZN	MW = $1.5-2.2 \times 10^6$ Da	Blend electrospinning-asymmetric membrane	The HA was combined with PCL to produce a top layer able to mimic the epidermis' layer of the skin; The top layer (HA_PCL) exhibited a total porosity below to 90 %, which is crucial to avoid the bacterial invasion; The hydrophobic character presented by top layer is essential to avoid the bacterial colonization at wound site.	Figueira et al. (2016)
	SF_PCL/HA_SF_THY	MW = $8-15 \times 10^3$ Da	Blend electrospinning-asymmetric membrane	The combination between HA and SF promoted to produce a bottom layer with similar features to the dermis layer of native skin; The HA_SF_THY nanofibrous layer presented a higher porosity (85.24 ± 2.47%), which is suitable for cell adhesion, proliferation and migration; THY was incorporated into HA_SF nanofibers to confer antioxidant and antibacterial properties to the bottom layer; The HA_SF bottom layer promoted the fibroblast attachment and proliferation, as well as, avoided the bacterial growth at its surface.	Miguel, Simões et al. (2019)

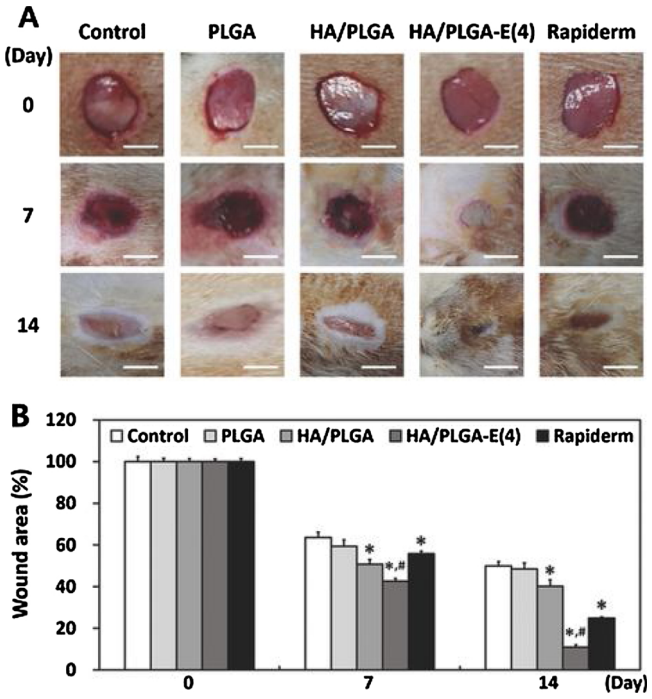

Fig. 6. Evaluation of the performance of HA/PLGA-E core/shell fiber matrices in the treatment of wounded diabetic rats: A) macroscopic images of the wound area in the control, PLGA, HA/PLGA, HA/PLGA-E(4), and Rapiderm groups on 0, 7, and 14 days; B) Percentage of remaining wound area of the control, PLGA, HA/PLGA, HA/PLGA-E(4), and Rapiderm groups at 0, 7 and 14 days. Reprinted from Advanced Healthcare Materials, vol. 5, Shin et al., Hyaluronic acid/PLGA core/shell fiber matrices loaded with EGCG beneficial to diabetic wound healing, 3035-3045. Copyright (2016), with permission from Wiley.

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