ChemPlusChem

Review
doi.org/10.1002/cplu.202300599

Chemistry Europe
European Chemical Societies Publishing

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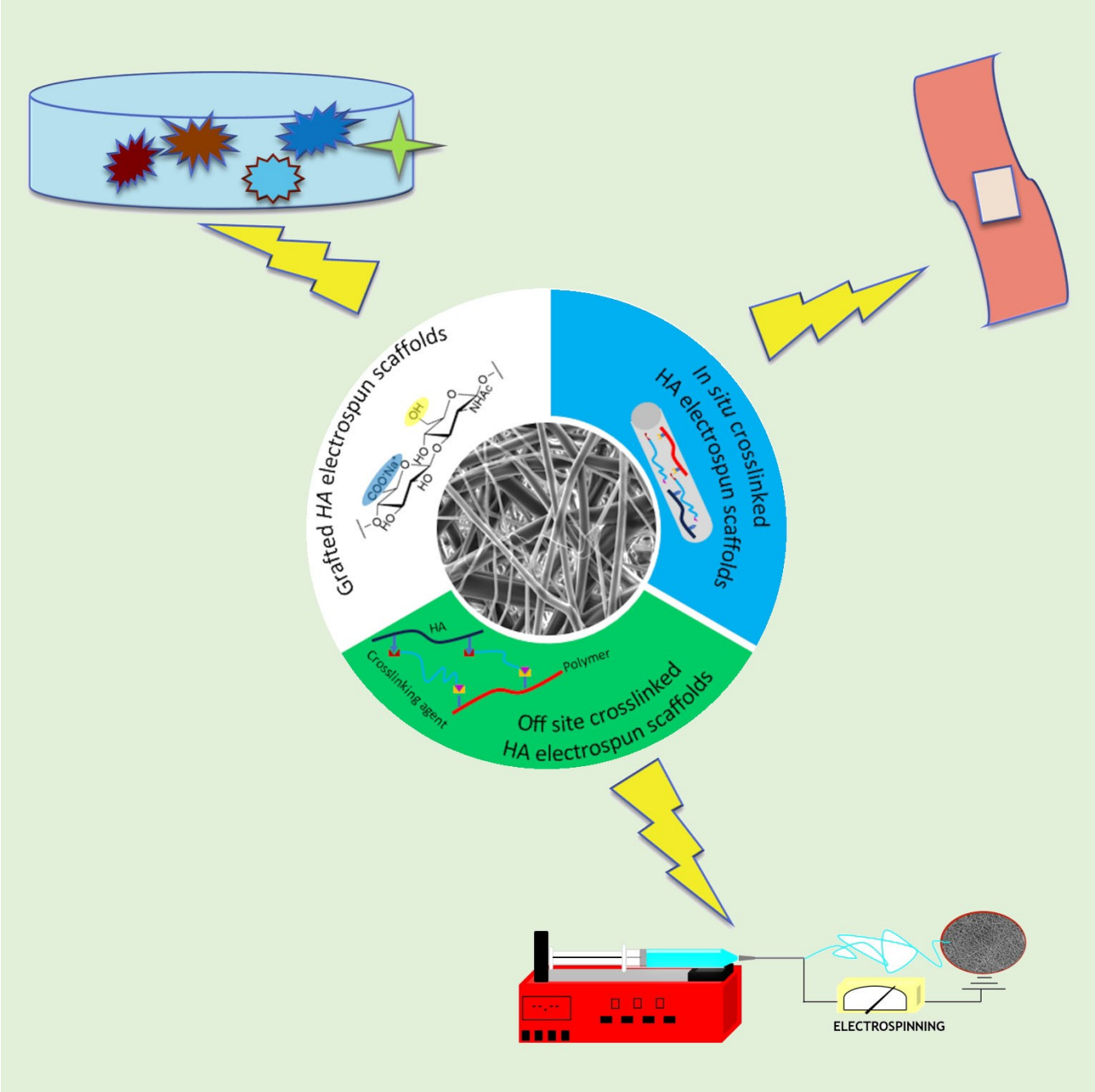
Chemical Modifications in Hyaluronic Acid-Based Electrospun Scaffolds

Antonietta Pepe,[a] Antonio Laezza,[a] Francesca Armiento,[a] and Brigida Bochicchio*[a]



Hyaluronic acid (HA) is a natural, non-sulfated glycosaminoglycan (GAG) present in ECM. It is involved in different biological functions with appealing properties in cosmetics and pharmaceutical preparations as well as in tissue engineering. Generally, HA has been electrospun in blends with natural or synthetic polymers to produce fibers having diameters in the order of nano and micro-scale whose pores can host cells able to regenerate damaged tissues. In the last decade, a rich literature on electrospun HA-based materials arose. Chemical modifications were generally introduced in HA scaffolds to favour crosslinking or conjugation with bioactive molecules. Considering the high solubility of HA in water, HA-based electrospun scaffolds are cross-linked to increase the stability in biological fluids. Crosslinking is necessary also to avoid the release of HA from the hybrid scaffold when implanted *in-vivo*. Furthermore, to endow the HA based scaffolds with new chemical or biological properties, conjugation of bioactive molecules to HA was widely reported. Herein, we review the existing research classifying chemical modifications on HA and HA-based electrospun fibers into three categories: i) *in-situ* crosslinking of electrospun HA-based scaffolds ii) off-site crosslinking of electrospun HA-based scaffolds; iii) conjugation of biofunctional molecules to HA with focus on peptides.

1. Introduction

Hyaluronic acid (HA) is a glycosaminoglycan which is a linear polysaccharide found in skin, cartilage, vitreous humor, and synovial liquid.[1] This natural polymer consists of alternating disaccharide units of β-1,4-D-glucuronic acid and β-1,3-*N*-acetyl-D-glucosamine *n*-fold repeated (Figure 1). One of the main drawbacks in HA use are source limitations as well as high production costs. Generally, the production of HA has been based in the past on its extraction from bovine vitreous humor, umbilical cord, or rooster comb, while microbial HA from *Streptococcus* strains is currently the main valid alternative to traditional methods. HA, it is one of the most appealing macromolecules not only for cosmetics and pharmaceutical formulations but also for regenerative medicine with applications ranging from myocardial tissue engineering to neural growth to wound healing. HA is a natural hydrogel owning a physiological water holding capacity and raising deep interest also in cancer therapy considered its ability to target receptors overexpressed in cancer cells, i.e. CD44.[2] However, the debate concerning HA inflammatory reactions is still challenging. Recent studies have demonstrated pro-inflammatory functions of low molecular weight (LMW) HA and its capability to promote the activation of macrophage.[3] That finding undoubtedly deserves to be kept under consideration in the formulations of HA-based nanodevices. Natural hydrogels have physicochemical and biological properties superior to synthetic ones. The most important advantages in the use of natural hydrogels are low immunogenicity, excellent biocompatibility and cytocompatibility, biodegradability, specific cellular/tissue response, adequate stability, superior structural design, controllable solubility, and 3D geometry.[4] On the other hand, weak mechanical properties, rapid *in vivo* degradation, and clearance[5] must be taken into account too. An answer is offered by the versatility of HA structure containing different functional groups such as acetamide, carboxyl, hydroxyl, and terminus aldehyde affording a multitude of potential functionalization strategies. In Figure 1, the most common functionalization sites are highlighted.[6] Commonly, HA esterification or amidation were employed to introduce functional groups able to form covalent bonds with different biomolecules through Michael-type addition or Schiff-base synthetic methodologies. HA functionalization is also useful to the formation of cross-links conferring stability to HA in aqueous solution. The most diffuse crosslinkers are dihydrazide, dialdehyde, butanediol-diglycidyl ether (BDDE), divinyl sulfone (DVS), and genipin.[7] Furthermore, HA has been widely photo-crosslinked by preliminary introduction of methacrylic groups.[8] However, great attention deserves the cross-linker choice which must be operated in the perspective of ensuring HA stability and preserving the cytocompatibility of the final scaffolds. Recent studies have demonstrated that the esterification of HA through carbodiimide induced self-esterification equivalent to a self-crosslinking.[9] The main advantage inside is represented by the exclusive presence of HA as a bridged molecule. Self-crosslinked HA has great potential in applications such as drug release where the simultaneous release of drug and side-reaction products derived from commercial cross-linkers is avoided.

In addition, cross-linking confers to HA a 3D structure able to mimic the extracellular matrix and hosting cells for regenerative purposes. Higher is the relationship between surface area and volume of pores in 3D structure of the hydrogel, better would be the performance as biomaterial. Electrospinning is a valuable technique aimed at the production of micro- and nano-fibers hierarchically organized in interconnected porous structures assuring nutrients and waste exchanges essential for the survival of cells. Electrospinning is an

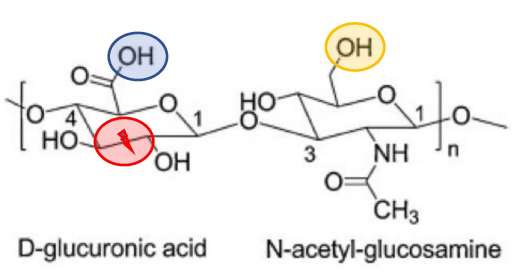
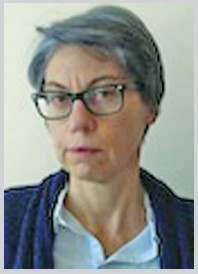


Figure 1. Chemical structure of hyaluronic acid and target sites for chemical modification.

[a] *Prof. A. Pepe, Dr. A. Laezza, Mrs. F. Armiento, Prof. B. Bochicchio*
Department of Science
University of Basilicata
Via Ateneo Lucano, 10
85100 Potenza, Italy
E-mail: brigida.bochicchio@unibas.it

electrohydrodynamic process where a liquid drop from a polymer solution is electrified to produce a jet elongating in a straight-line direction and undergoing thinning and solidification onto a grounded collector. The schematic set-up of electrospinning consists of a high-voltage supply, a syringe-pump, a spinneret consisting of a needle and a ground collector. The solvent evaporates during the path from spinneret to the collector and then the solid appears as a web of fibres. Therefore, volatile organic solvents are preferred in electrospinning because solvent evaporation induces fiber solidification. However HA is not soluble in organic solvents while it is soluble in aqueous solution characterized by high surface tension, low evaporability and high viscosity level which significantly hinders the process of electrospinning. Also HA concentration is a critical issue in spinnability, given that hyaluronan concentration can be used only in a narrow range in order to successfully realise fibrous structures. At high polymer concentration, the electric field may not be able to overcome the high viscosity of the solution, and thus a continuous jet stream from the polymer solution is not obtained and fiber production fails. On the other hand, at low polymer concentrations an excess amount of solvent must be removed between the jet fluid stream leaving the spinneret and the fiber reaching the ground with the consequence that the process turns into the formation of particles instead of fibers. The contradiction could be overcome by accurate tuning of concentration and electrospinning parameters such as voltage, tip to collector distance, needle gauche diameter, flow rate. Additionally, another parameter worthy of attention is HA molecular weight. Low molecular weight HA is less viscous than that of high molecular weight HA at the same concentration. However, the electrospinning results from the lower molecular weight HA do not produce nanofibers as the polymer chains are not sufficiently entangled to form fibrous morphology].[10] Overcoming the high viscosity to successfully find the threshold between molecular weight, viscosity, and spinning parameters, are the challenges faced in fabricating nanofibers of HA. A successful attempt was the introduction of electro-blowing assisted electrospinning where solvent evaporation was accelerated by blowing air at high temperature.[10] Alternatively, to improve the electrospinnability, HA has been widely electrospun with other synthetic or natural polymers for the production of hybrid scaffolds as recently highlighted in several reviews.[11] Finally, chemical modifications of HA functional groups are extensively carried out to improve electrospinnability by changing HA viscosity. In this way, intermolecular hydrogen bond network found in the hyaluronan solution is interrupted with beneficial effects on the viscosity. This review focuses on the electrospinning of properly designed chemical modifications on hyaluronans. Indeed, even if a rich literature on chemical modifications of hyaluronic acid exists, few are the examples of electrospun scaffolds obtained from HA-derivatives containing covalent bonds between hyaluronic acid and bioactive molecules. In this review, we survey the recent research carried out on the chemical modifications made on hyaluronan-based electrospun scaffolds through the classification into three main categories: i) *in-situ* crosslinked HA electro-



Brigida Bochicchio is Associate Professor in Organic Chemistry at University of Basilicata, Potenza (Italy). She is graduated in Chemistry and received the PhD in Chemistry on 2003 from University of Basilicata and from University of Reims Champagne-Ardenne (France) where she was Invited Professor in 2007 and 2009. Her research interests are focused on the synthesis of bioactive molecules such as polypeptides and glycopeptides, production of bioinspired electrospun scaffolds as smart biomaterials with applications ranging from waste recovery to drug delivery.

Antonietta Pepe received her PhD degree in Chemistry of Natural Products from University of Naples in 1998. In the same year she joined as Postdoc the Laboratories of Protein Chemistry at the University of Basilicata. In 2000 she was visiting fellow at the Biophysics Laboratories of University of Portsmouth, UK. At present she is Associate Professor in Organic Chemistry at University of Basilicata. Her current research activities are mainly focused on electrospinning of biodegradable and bioactive biopolymer to obtain scaffolds for skin, vascular or bone tissue engineering; design production and characterization of electrospun multicomponent scaffolds as drug delivery systems and as eco-friendly membranes for separators in lithium ion batteries

Antonio Laezza was born in 1987 in Naples (Italy). He received the Ph.D. in Chemical Sciences in 2017 under the guidance of Professor Emiliano Bedini at the University of Naples Federico II (Italy), working on the regioselective modifications of polysaccharides. In 2018, he joined the group of Professor Matthew Gibson at the University of Warwick (UK) as Postdoc working on chemoenzymatic approaches to new glycomaterials. In 2019, he was hired as a researcher at the University of Basilicata in the group led by Professor Brigida Bochicchio, where his research is focused on the chemical modification of polysaccharides and biopolymers, to produce multicomponent electrospun scaffolds for tissue engineering and drug delivery

Francesca Armiento was born in 1996 in Potenza, Italy. She held a Master's Degree in Biotechnologies for Medical, Pharmaceutical and Veterinary Diagnostics in 2022 at University of Basilicata, where she is currently a PhD student in Chemical Sciences in the Laboratory of Bioinspired Materials. Her present research is focused on development of hyaluronic acid based biomaterial technology.

Table 1. Summary of main characteristics of *in situ* crosslinked HA-based electrospinning methodologies.

Chemical modification	Crosslinker/ Modifier[a]	Polymer Blend[b]/ Drug	Methodology	Proposed Application	Ref.
In situ crosslinking	Divinylsulfone (DVS)	HA + PLLA	Bilayered Scaffold	Patch for preventing post-surgical adhesions	[12]
	PEGDA	HA-DTPH/ PEO	PEO removing after electrospinning	soft-tissue scaffold in 3D cell cultures for tissue engineering.	[14]
	Citric acid (CA)	HA/PVA/L-Arg HA/PVA/ CNCs/L-Arg	Additional thermal treatment (100 °C, 6 h)	Multifunctional wound dressings	[15]
	EDC/NHS	HA/PVA/ HPβCD	multi-needles emitter; additional annealing (60 °C, 24 h)	Drug delivering wound dressings	[18]
	Maleic Anhydride (MA)	HA/PVA	Additional Photo-crosslinking (UV)	Controlled release modulated devices	[19]

spun scaffolds; ii) off-site crosslinked HA electrospun scaffolds; iii) (peptide)-decorated HA electrospun scaffolds.

2. In Situ Crosslinking

In-situ crosslinking of hyaluronan-based electrospun fibers is defined as a process consisting of a single crosslinking step carried out during electrospinning with instruments equipped with multi-syringe apparatus. The syringes contain either crosslinker or electrospinnable polymer(s) solutions which are mixed and ejected simultaneously. Herein, some examples are presented (Table 1). Arnald-Pastòr *et al.* reported an bilayered, *in situ* crosslinked electrospun HA/PLLA scaffolds. Poly-L-lactic acid (PLLA) is FDA approved polymer widely used for applications in biomedicine. It is easily electrospun alone or blended with other polymers as HA. In this example, bilayered HA/PLLA crosslinked electrospun scaffolds were produced (Figure 2a). Crosslinked electrospun HA scaffolds were obtained in a single step by combining HA with a divinylsulfone (DVS) crosslinker solution in sodium hydroxide in a three-way valve (Figure 2b). They were electrospun and deposited onto previously electrospun PLLA materials in order to obtain bilayered HA/PLLA membranes. These membranes showed two distinct faces with complementary properties: the PLLA side was cell friendly promoting cell attachment and spreading, while the HA face hindered cell adhesion and thus may prevent post-surgical adherences. Bilayered membranes made up of polymers and HA are widely described in literature with potential applications in wound dressing and tissue engineering.[12] Kim *et al.* described the dual syringe reactive electrospinning.

The *in situ* or reactive electrospinning allows the crosslinking reaction to occur simultaneously during the electrospinning process.[13] In the example herein reported, HA was previously chemically modified with dithiobis(propanoic dihydrazide) (DTP) by carbidiimide chemistry.

Next, disulfide bonds were reduced using dithiothreitol (DTT) to give, after exhaustive dialysis, the corresponding thiol-modified macromolecular derivatives HA-DTPH (Figure 3). The HA-derivative containing thiol groups (HA-DTPH) has been electrospun (Figure 3a) and simultaneously crosslinked with a homobifunctional poly(ethylene glycol)diacrylate (PEGDA), shown in Figure 3b, through a dual-syringe apparatus. The crosslinking reaction occurred at room temperature in approximately ten minutes and in aqueous solution at pH 7.4 giving

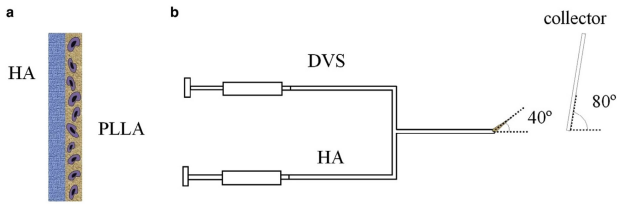


Figure 2. Schematic representation of: a) bilayer membrane b) electrospinning apparatus setup (Adapted from [12]. Copyright (2013) with permission from Elsevier).

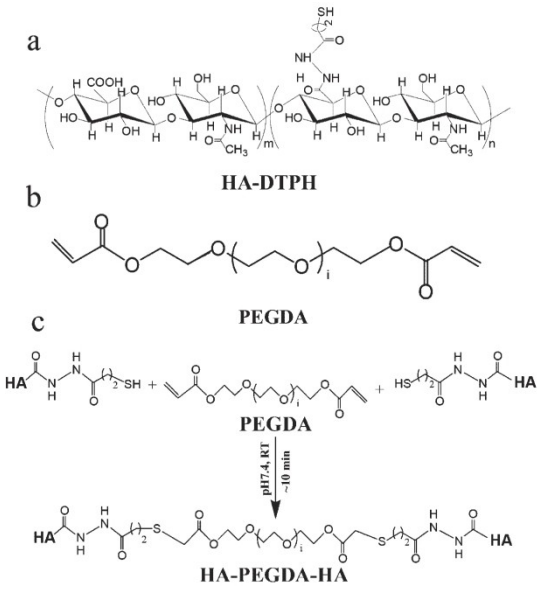


Figure 3. a) Structure of HA-DTPH. b) Structure of PEGDA. c) Crosslinking reaction to form HA-PEGDA-HA linkages. Reprinted from [14]. Copyright (2006) with permission from Wiley.

rise to HA-PEGDA-HA electrospun scaffold (Figure 3c). The electrospinning apparatus is schematized in Figure 4.[14] Electrospinning in water is highly recommended in terms of the adoption of harmless practices for the environment and sustainability, even if the electrospinning of HA in water is a huge task given the high viscosity of the biopolymer. This limit was here successfully addressed by adding a viscosity modifier as PEG which facilitated fiber formation. HA-PEGDA-HA electrospun scaffold was proposed as a potential soft-tissue scaffold material for tissue engineering. Another example is represented by electrospun polyvinylalcohol(PVA)/Hyaluronic acid/L-arginine nanofibers *in-situ* crosslinked by citric acid. L-arginine-loaded citric acid (CA) crosslinked Poly-Vinyl-Alcohol/Hyaluronic Acid (PVA-HA) nanofibers were fabricated by electrospinning and proposed for potential wound healing purposes. These nanofibrous scaffolds were prepared by dissolving first L-arginine and CA in PVA/HA solution and then by electrospinning them. CA is a tri-dentated crosslinker showing the advantages of being water soluble and harmless. CA started the crosslinking reaction during electrodeposition, whereas a final heating treatment of electrospun nanofibers at 100 °C for 6 h was applied to ensure complete crosslinking. L-arginine loading was expected to accelerate wound healing process through nitric oxide (NO) release stimulating angiogenesis, collagen synthesis, and cell proliferation.[15] Tomihata *et al.* reported the *in situ* crosslinking of hyaluronic acid/cyclodextrin nanofibers electrospun in aqueous solution. In order to broaden the scope of potential applications, the incorporation of functional compounds such as cyclodextrins in the electrospun fibers is one of the novel strategies envisaged. Herein, HA was electrospun in water with a natural polysaccharide as hydroxypropyl-β-cyclodextrin (HPβCD) in presence of poly-vinyl alcohol (PVA) as carrier polymer. *In-situ* crosslinking was carried out simultaneously to electrospinning by adding *N*-(3-Dimethylaminopropyl), *N'*-ethylcarbodiimide hydrochloride and *N*-hydroxysuccinimide (EDC/NHS) to the polymeric solution (Figure 5a). In general terms, carbodiimide derivatives are widely used because they are water soluble and atoxic.[16] The coupling of EDC with NHS allows to form ester bonds between carboxylic acid groups and hydroxyl groups of HA itself or of other molecules present at its side giving rise to an extensive self-crosslinking which stabilizes the molecule (Figure 5b).[17] In order to preserve the bioactivity of such compounds that could be lost during harvest heating, the mildest annealing conditions allowing an efficient crosslinking were sought. An annealing step at 60 °C during 24 h was necessary to ensure the efficient crosslinking for which the reaction scheme and the proposed resulting structure of the nanofibers are shown in Fig. 5c. It has been demonstrated that the nanostructure of the final membrane as revealed by SEM was not altered by annealing, highlighting the role of HPβCD in ensuring an efficient crosslinking reaction mainly due to the combination of the electrospinning and freeze-drying steps.[18] An envisaged application for these scaffolds is the drug delivery system exploiting cyclodextrin structure.

Finally, the production of crosslinked HA/PVA electrospun membranes free from organic solvent was reported as another example of environmentally-friendly procedure. The membranes were crosslinked during electrospinning through maleic anhydride (MA) addition improving also the electrospinning performances. To ensure the stability of the membranes, since PVA and HA functional groups compete for the possible active reaction sites as shown in Figure 6, a final crosslinking step was performed through photo-crosslinking by exposure to a UV light reactor for 40 min[19] Previously, Yang *et al.* showed that PVA hydroxyl and MA acyl groups efficiently reacted giving rise to a crosslinking during the electrospinning process, without

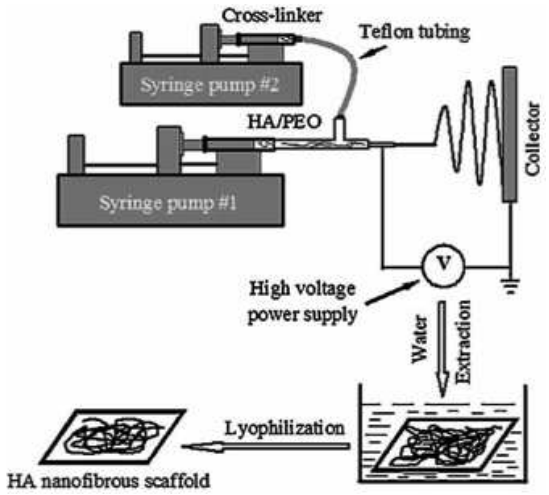


Figure 4. Schematic illustration of the electrospinning setup and the procedure for the fabrication of HA-DTPH nanofibrous scaffolds. HA-DTPH/PEO blend solution was loaded into the primary syringe (#1). A PEGDA solution was loaded into the secondary syringe (#2) and delivered to the primary syringe (#1) using Teflon tubing. A 'T'-shaped three-way steel adapter was designed to mix the solution and transport them to the spinneret. The electrospun HA-DTPH/PEO blend scaffold was soaked in deionized water for 24 h to extract PEO, followed by lyophilization to obtain an electrospun HA-DTPH nanofibrous scaffold. Reprinted from [14]. Copyright (2006) Wiley.

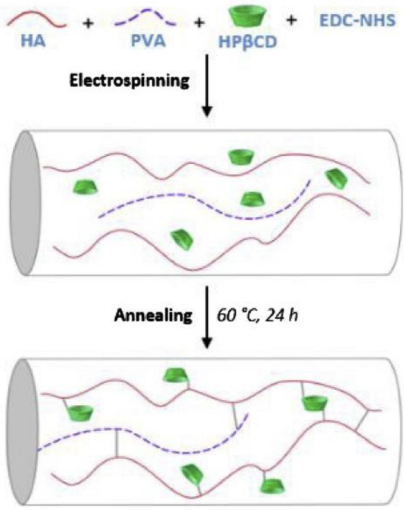


Figure 5. Schematic illustration of the processing to obtain crosslinked HA-PVA-HPβCD fibers. Adapted from [18]. Copyright (2018) with permission from Elsevier.

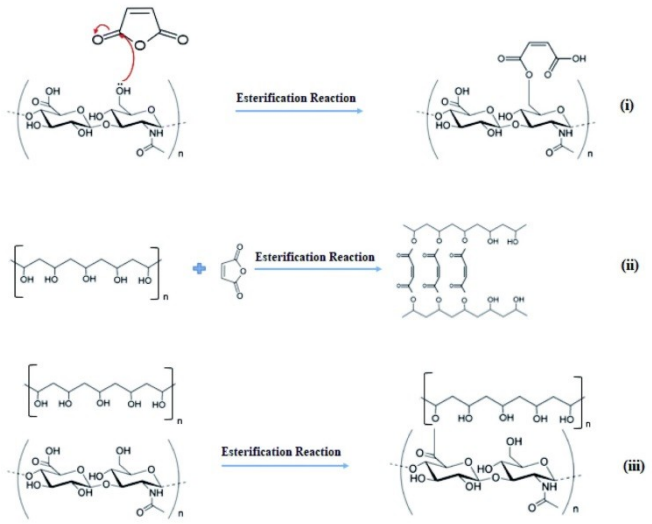


Figure 6. Schematic representation of possible esterification reactions between (i) HA and MA, (ii) PVA and MA, (iii) PVA and HA. Reprinted from [19], copyright (2020) with permission from RSC.

the need of heat treatment or UV radiation.[20] PVA and MA reacted to form mono esters or bis-esters during electrospinning thanks to the high electric field that can stimulate the chemical activity of the molecules and causing the rapid evaporation of water can accelerate the esterification reaction.

3. Off-Site Crosslinked HA Electrospun Scaffolds

In this section some examples of crosslinking reactions carried out on blended HA scaffolds after electrospinning are reviewed (Table 2). In many cases a modification step of HA before its processing by electrospinning has been reported to promote the crosslinking of HA electrospun fibers.[21] In some cases, HA was used as a crosslinker by forming amide or ester bonds with other electrospun polymers. For example, hybrid Gelatin/Poly-L–Lactide Scaffolds (GE/PLA) were crosslinked by tethering HA to electrospun nanofibers through carbodiimide and *N*-hydroxysuccinimide (NHS). The high-water solubility of GE in the electrospun scaffolds made the crosslinking mandatory before any use in aqueous solutions. Hyaluronate crosslinking by EDC/NHS reaction to GE/PLA electrospun scaffolds was performed also to control hydrophilicity and cell adhesion. The scaffolds after crosslinking and sterilization in 70% ethanol solution were allowed to stay overnight at r.t. to complete evaporation of any residual solvent. Fluorescence lifetime imaging microscopy (FLIM) was used to image human dermal fibroblasts (HDFs) seeded on the hybrid scaffolds. Besides cell imaging, FLIM enabled the measurement of the HDF glycolytic activity by assessing the ratios between free and protein-bound NAD(P)H.[22]

Chen *et al.* crosslinked a dispersion of homogenized GE/PLA electrospun nanofibers by addition of HA and EDC/NHS activating agents. After freeze-drying, a superabsorbent 3D

Table 2. Summary of main characteristics of off-site crosslinking HA-based electrospinning methodologies.

Chemical modification	Crosslinker/Modifier[a]	Polymer Blend[b]/ Drug	Methodology	Proposed Application	Ref.
Off-site cross-linking	HA + EDC/NHS	GE/PLLA	Pouring and crosslinking	in vitro drug testing system	[11]
			Nanofiber dispersion crosslinking	Superadsorbent scaffolds for Cartilage Tissue Engineering	[23]
	Methacrylated HA	Aminolyzed PCL	Michael reaction and photocrosslinking	Cell culture matrix	[24]
	EDC	HA/Gelatin	Pouring in crosslinking solution	Biomedical materials	[25]
		HA/collagen	Salt leaching of NaCl particulates	Scaffolds for tissue engineering	[26]
	UV-irradiation	Maleilated-HA/Methacrylated PVA	Photo-crosslinking	wound dressings	[27]
	UV-irradiation	Methacrylated HA/PEO	Photopatterning	scaffolds with channels for tissue engineering	[28]
	UV-irradiation	Methacrylated HA/PCL/IGF-1	Blended or shell(PCL)/core(HAMA + IGF-1)	artificial dura mater for application in neurosurgery.	[29]
	UV-irradiation	PCL/poly(glycerol sebacate) (PCL-PGS) + HAMA/PEO/Pluronic F127	Bilayered scaffold	artificial nerve grafts as tubular nerve guide conduit	[30]
	UV-irradiation	NO-releasing methacrylated HA/PEO	Photo-crosslinking	Wound healing applications	[31]
	Genipin, Glutaraldehyde, EDC/NHS	HA/CTL/PEO	Different chemical crosslinking methodologies	wound dressings	[33]
	$FeCl_3$ BDDE	HA/Ibuprofen	ionic crosslinking + covalent crosslinking	Prevention of post surgery tendon adhesion	[34]
	Methylene diisocyanate (MDI)	HA/zein//PVP		Scaffolds for tissue engineering and wound healing	[35]

scaffold based on electrospun nanofibers for cartilage tissue engineering was obtained.[23] In another report Cho *et al.* decorated PCL nanofibers on the surface with methacrylated HA via Michael type addition and by photo-crosslinking with the aim to reduce hydrophobicity and improve cell viability and proliferation of the scaffold.[24]

3.1. Carbodiimide Promoted Crosslinking of Electrospun HA-Based Scaffolds

Water soluble carbodiimide EDC was frequently employed as activating agent of carboxyl groups, both in protein-based or uronic acid -based biopolymers. Further reaction with amine or hydroxyl groups introduces amide or ester linkages, respectively. HA solubility in water permits to avoid organic solvents in electrospun scaffolds. Gelatin (GE) outstanding biocompatibility and water solubility characteristics induced the fabrication of GE/HA nanofibrous membranes. The addition of HA improved the electrospinnability of GE solutions. Electrospun scaffolds showing aligned nanofibers were fabricated. The crosslinking of GE/HA electrospun scaffolds was carried out by immersion of electrospun scaffolds in EDC solution.[25]

The same crosslinking methodology was employed by Kim et al. for the production of HA/collagen electrospun scaffolds for cartilage tissue engineering. In order to avoid collapse of HA/collagen electrospun scaffolds at the end of electrospinning and to create macropores facilitating cell infiltration NaCl salt particulates were deposited on electrospun scaffolds. After EDC crosslinking the scaffolds were exposed to a salt leaching process with deionized water. Collagen introduction triggered a slow degradation of scaffolds as demonstrated by *in vitro* studies. Furthermore, chondrocyte adhesion was greatly enhanced in presence of collagen showing 4.5-fold higher cell number. This work demonstrated that HA/collagen scaffolds after crosslinking process and salt-leaching treatment gave rise to biomaterials with strong potentialities in tissue engineering.[26]

3.2. Photo-Crosslinking of Electrospun HA-Based Scaffolds

Photo-crosslinking is largely employed as a crosslinking strategy both *in-situ* and off-site as well. Below, some examples of off-site photo-crosslinking procedures are reported.

Maleilated hyaluronate (MHA) and methacrylated poly-vinyl-alcohol (MaPVA) were synthesized. Afterwards, MHA/MaPVA bicomponent nanofibrous scaffolds were prepared using water as solvent for electrospinning and subsequently photo-polymerized. The nanofibers were irradiated directly under the Hg lamp. The application proposed is a hydrogel useful in wound dressing.[27] Another example of off-site crosslinking is represented by the photo-patterning strategy to fabricate scaffolds with pore size useful to enhance cell infiltration. Patterns are obtained through the introduction of masks between the light source and the scaffold (Figure 7).

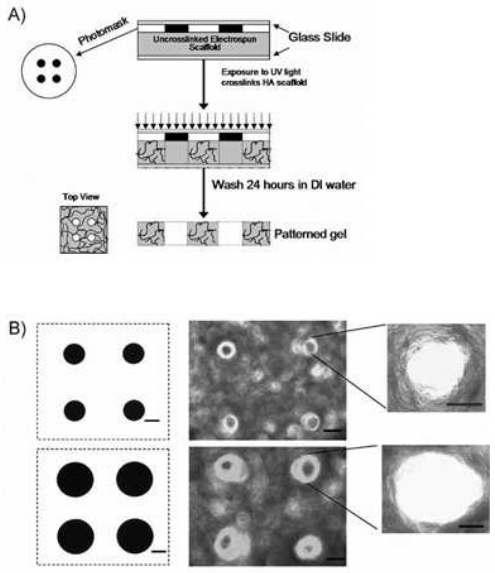


Figure 7. Schematic representation of photopatterned macro-channels into electrospun scaffolds using light transmittance through masks. (B) Light micrographs of photomasks and top-view of patterned HA scaffolds (scale bars = 100 μm) Adapted from [27], copyright 2010 with permission from Wiley.

Herein, methacrylated HA (MAHA), PEO, and the photo-initiator electrospun scaffolds were photo-crosslinked by 365 nm light in nitrogen chamber because the oxygen can inhibit crosslinking.[28]

An additional example of off-site photo-crosslinking was carried out on PCL and methacrylated HA electrospun scaffolds proposed as scaffolds for patients suffering from traumatic brain injury. Crosslinking was accomplished after electrospinning by UV irradiation for 20 s time. The encapsulation of insulin-like growth factor IGF-1, essential for improving the survival and neurite outgrowth of neural cells after traumatic brain injury, ensured a controlled release and a higher survival of neurons.[29] Other reports describe off-site photo-crosslinking of methacrylated HA/PEO/Pluronic F127 blend as layer of a scaffold deposited on polycaprolactone-poly(glycerol sebacate) (PCL-PGS) layer. The produced bilayered scaffolds self-folds into tubular structures in aqueous solutions forming nerve guide conduit as artificial nerve grafts.[30]

In the work of Gwon *et al.* hyaluronic acid was functionalized with methacrylic groups for UV photo-crosslinking and with N-diazeniumdiolate (NONOate)-NO donor groups as bioactive molecule developing NO-releasing HA-based nanofibers with the potential application in wound healing.[31] Multifunctional HA derivatives were also prepared by Miller *et al.* starting from methacrylated HA (HAMA) by conjugating separately adamantane (AD) and β-cyclodextrin (CD) for the development of a host-guest system. In electrospun scaffolds containing both AD-HAMA and CD-HAMA a strong association of hydrophobic AD (guest) groups with the hydrophobic core of CD (host) molecules is observed, resulting in a stable, yet reversible, supramolecular guest–host interaction that could mechanically stabilize the UV photo-crosslinked nanofibers.[32]

3.3. Alternative Crosslinking Methodologies for HA-Based Electrospun Scaffolds

Electrospun membranes prepared using hyaluronic acid, a bioactive lactose-modified chitosan (CTL), and polyethylene oxide (HA/CTL/PEO) have been produced. The obtained scaffolds showed weak mechanical properties, thus they have been crosslinked following different protocols using glutaraldehyde (GTA), genipin, EDC/NHS or thermal treatments and the results have been discussed in terms of scaffold stability and fiber loss.[33]

Chen *et al.* prepared a multi-functional nanofibrous membrane (NFM) for prevention of postoperative tendon adhesion by electrospinning hyaluronic acid (HA)/ibuprofen (IBU) (HAI), followed by dual ionic crosslinking with $FeCl_3$ and covalent crosslinking with 1,4-butanediol diglycidyl ether (BDDE) to produce nanofibers endowed with abilities to prevent fibroblast attachment and penetration and contextually exert anti-inflammation effects. Results of the studies showed that the presence of HA in the multifunctional scaffold was able to prevent fibroblast penetration, while at appropriate concentration IBU imparted anti-inflammation response.[34]

Other biopolymers were blended with HA and electrospun. For example, zein is the major corn protein and gained some interest in tissue engineering because it is electrospinnable from ethanol aqueous solutions. In this study, blended zein/HA solutions were electrospun after the introduction of polyvinylpyrrolidone (PVP) in aqueous ethanol solution and zein/HA/PVP scaffolds were fabricated. The scaffolds were crosslinked by methylene diphenyl diisocyanate (MDI) in THF solution. The crosslinked scaffold morphology was characterized by SEM and the mechanical properties were assessed by stress-strain measurements. The results have suggested that the combination of three different polymers such as zein, HA and PVP was successful for the fabrication of electrospun scaffolds with good mechanical properties with great potential in tissue engineering and wound healing.[35]

4. Electrospun Scaffolds Based on Hyaluronic Acid Derivatives

In general terms, the modification of a polymer backbone to impart a variety of functional groups through chemical methodologies is referred to as decoration.[36] The final aim of polymer decoration is to improve physico-chemical properties such as conductivity, solubility, mechanical characteristics, as well as bioactivity.[37] HA decoration is widely carried out mainly to improve electrospinnability by changing HA viscosity. However, other reasons for grafting have to be also considered. In the following, some examples of electrospun grafted HA will be discussed (Table 3).

Table 3. Summary of main characteristics of grafted HA-based electrospinning methodologies.

Chemical modification	grafted molecules	Polymer Blend[b]/ Drug	Methodology	Proposed Application	Ref.
Grafting	2-(methylthio)ethylamine	thioether grafted HA/	EDC/HOBT ionic crosslinking (Fe^{3+})	wound-dressing for chronic diabetic wounds	[38]
	benzyl bromide	HA-benzyl ester/ Doxorubicin	Doxorubicin encapsulation	anti-melanoma drug delivery system	[39]
	$C_8/C_{12}/C_{18}$ Alkyl Amine + EDA	alkylated-HA/ PVA/HPβCD/dexamethasone	EDC/NHS crosslinking	scaffold for periodontal regeneration	[40]
	Temporin Ra	HA/Chitosan/PVA	Peptide Loading	Antimicrobial wound healing dressing	[52]
	REDV peptide (P1)	PCL	Layer-by-layer deposition of PDDA[a], SePEI[b] and P1-HA	Small diameter vascular graft with catalytic NO generation and promoted EC adhesion	[61]
	GCGYGRGDSPG peptide (P2)	P2-HA/PEO Or HAMA/PEO	P2 conjugation by Thio-ene to methacrylated HA + UV crosslinking	electrospun scaffolds with mechanical and adhesive gradients	[72]
	CAAAAAAAAAAKAAKYGAAAGL (P3)	PDLLA/P3-HA	P3 conjugation by Thio-ene to methacrylated HA	wound dressings	[76]
	RGD-TCO[c] (P4)	PCL-Tz[d]	LbL deposition of HA-TCO and HA-Tz + final P4-TCO deposition	therapeutic implants for the treatment of vocal fold scarring	[80]
	Ac-KGGPQVTRGDVFTMP (P5)	PVA/HA	P5 conjugation with EDC/NHS on glutaraldehyde crosslinked nanofibers	Scaffold for iPSCs growth and proliferation	[81]

[a]PDDA: Polydiallyldimethylammonium chloride; [b]SePEI: organoselenium immobilized polyethyleneimine; [c]TCO : trans-cyclooctene; [d]Tz: *s*-tetrazine.

4.1. Small Molecule Decorated HA Derivatives

With the aim to produce an absorbable bioactive wound dressing device, thioether grafted HA (HHA-S) was synthesized from high molecular weight HA (HHA) and 2-(methylthio)ethylamine using EDC and 1-hydroxybenzotriazole (HOBT) as condensation reagents. Afterwards, decorated HHA-S was electrospun into nanofibers and further crosslinked by ferric ions (Fe^{3+}). The thioether moiety was shown to scavenge the ROS quickly in the early inflammation phase, conferring to the matrice significant antioxidant properties, while the Fe^{3+} showed broad-spectrum antibacterial action.[38] Moreover, high molecular weight HA (HHA) was able to promote the transformation of macrophages from a pro-inflammatory M1 to a reparative M2 phenotype, thus reducing inflammation and promoting proliferation by releasing anti-inflammatory cytokines and growth factors.[3] The electrospun thioether decorated HHA-S nanofibrous hydrogel was shown to accelerate the healing phase transition from inflammation to proliferation and remodeling and could be proposed as improved dressing for chronic diabetic wounds.

Nowadays, the employment of HA electrospun scaffolds in tissue regeneration and regenerative medicine is largely assessed. Additionally, they play an additional role as therapeutic agents acting as vehicles in drug delivery. Doxorubicin (DOX) is a widely employed anticancer drug also used in the treatment of melanoma. One of the main limits in its use is the rapid release affecting the efficacy of the treatment. Han et al. esterified HA with benzyl-bromide in presence of tetrabutylammonium (TBA) and electrospun the resulting product in order to obtain HA-benzyl ester (HA-Bn) nanofibers. The anticancer drug doxorubicin (DOX) was encapsulated into nanofibers via electrospinning. Taking advantage of the π–π^* stacking interactions between the benzene ring of DOX and the benzyl group of HA-Bn, the scaffolds were designed in order to favor a sustained release of DOX resulting in an efficiency of about 90%, achieved within 7 days. *In vitro* cell experiments demonstrated that HA-DOX nanofiber had a considerable inhibitory effect on murine melanoma cell-lines.[39]

The introduction of alkylamine pendant moieties at different lengths (C_x) to HA (HA-EDA-C_x) was carried out by Federico *et al.* and the osteoinductive potential of HA-based electrospun scaffolds was evaluated).[40] HA derivatives were blended with PVA and 2-hydroxypropylcyclodextrin to improve electrospinning process. The polymeric solutions were also loaded with dexamethasone (DEX) before electrospinning in order to stimulate an osteoinductive effect.[41] Finally, the electrospun scaffolds were crosslinked with EDC/NHS. The main purpose of this study was to assess if the increasing hydrophobicity of the growing alkyl chains provided superior resistance to enzymatic hydrolysis without affecting surface properties of the scaffolds, important for cell adhesion and proliferation. The results of cell cultures on DEX-loaded alkylated HA scaffolds showed high cytocompatibility and induced osteogenic response with improved mineralization, thus holding great potential for periodontal regeneration.

4.2. C_6-Decorated Hyaluronic Acid for Enhanced Resistance to Hyaluronidase-Mediated Digestion

The hyaluronidase enzyme (Hyal-2) and the CD44 (Cluster of Differentiation-44) cell surface receptors are involved in HA degradation.[42] Molecular modeling based on crystallographic studies indicated that the recognition sites of the Hyal-2 enzymes and CD44 receptors are the carboxylate group of HA .[43] As a consequence, the functionalization of HA carboxylic groups into ester or amide, could affect enzymatic recognition and stability of HA towards hyaluronidase-mediated hydrolysis. Vandamme and co-workers evaluated the effect of conjugating alanine onto HA through the amidation of HA carboxyl group.[44] They demonstrated that for higher degree of modification slower HA enzymatic degradation was observed. One of the most popular methods to link amines to HA carboxylic acid is the activation with EDC, because it can be carried out in aqueous solution, which is the native solvent for HA, and at slightly acidic pH.[45] EDC first reacts with the carboxylic acid to form a highly reactive O-acyl isourea intermediate (Figure 8a) which can react with the N-terminal amino group of an aminoacid residue or of a peptide. However, the stable *N*-acyl urea product could be formed by reaction of O-acyl isourea intermediate with competitive water nucleophile thus precluding the amide formation (Figure 8).[17,46] One of the strategies to avoid this collateral reaction, is the use of *N*-hydroxysuccinimide (NHS) at pH = 7.5. Also in that case, as reported by Vandamme and co-workers, the degree of substitution (DS), defined as a percentage value expressing the number of HA molecules esterified at carboxyl groups, was relatively low being in the

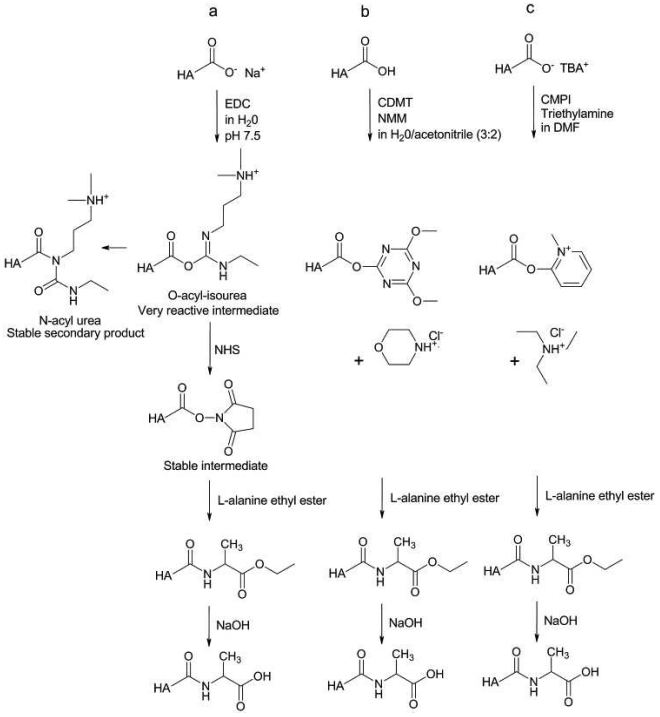


Figure 8. Synthetic routes to N-alanyl hyaluronamide. Reprinted from [43], Copyright (2011), with permission from Elsevier.

range of 10 to 13%, according to literature data.[47] An alternative strategy which avoided the use of NHS and pH monitoring was performed in water/acetonitrile mixture using 2-chlorodimethoxy-1,3,5-triazine (CDMT) with a final increased DS value of 50% (Figure 8b).[47d,48] To obtain a complete substitution at the HA carboxylic acid, the reaction was carried out in anhydrous conditions using 2-chloro-1-methylpyridinium iodide (CMPI) in *N,N*-dimethylformamide (DMF) after conversion of HA into a tetrabutylammonium (TBA) salt (Figure 8c).[49] The results indicated that amidation of hyaluronic acid in an anhydrous solvent was carried out with a degree of substitution of the carboxylic groups up to 100%. They also demonstrated the hyaluronamides obtained through amino acid conjugation to HA enhanced resistance towards enzymatic digestion while forming solutions with viscosities similar to the solutions of hyaluronic acids of similar lengths.[47d] However, to the best of our knowledge amino acid-decorated hyaluronamides were not electrospun.

4.3. Peptide-Decorated HA Electrospun Scaffold

Peptides were widely employed as bioactive components in tissue engineering and regenerative medicine. Indeed, peptides able to self-assemble into nanofibers are generally used as biomaterials with a wide range of applications from tissue engineering, biosensors to drug delivery systems.[50] Peptides are produced on medium scale by Solid Phase Peptide Synthesis (SPPS) with affordable costs and the possibility to introduce bioactive sequences and non-proteinogenic or modified amino acid residues such as L–DOPA, ornithine, hydroxyproline, and methoxyproline inside the primary structure improved their versatility.[51] On the other hand, synthetic polymers are easily available at low cost while natural polymers like gelatin promise better biocompatibility. A successful strategy representing a good compromise is the electrospinning of peptides in combination with polymers. Some examples are described in literature where the polymeric solutions were loaded with bioactive peptides. Koohzad and Asoodeh produced by electrospinning a nanofibrous scaffold composed of HA/Chitosan/PVA adding temporin-Ra peptide as antimicrobial agent for improved wound healing dressing.[52] The main drawback of blended mixtures consisting of peptides and polymers is represented by the fast release of the peptides when the scaffolds are implanted *in-vivo*. To avoid this problem, the covalent link between the peptide and the polymers represents a valid alternative. A general overview of peptide conjugation strategies to diverse polymers before and after electrospinning was reviewed by Bucci et al.[53] A less explored, however full potential route, is peptide conjugation to HA through its functional groups for endowing novel biological/pharmacological properties to HA electrospun scaffolds. Generally, the main strategy currently used to covalently bind peptides, consists of the modification of the polymer prior to electrospinning.[54] Different methodologies were used to link peptides. In the following we summarize the main synthetic strategies aimed to peptide conjugation on chemically modified HA.

As previously shown for the conjugation of amino acids to HA, a high number of reports described peptide grafting to the C_6 carboxylic group of glucuronic acid of HA by amidation. The most explored route envisaged the activation of the COOH with water-soluble carbodiimide (EDC) in presence of NHS or HOBT for the synthesis of the intermediate activated esters. The following reaction with the *N*-terminal amino group, or eventual ε-amino group of lysine residues caused the formation of the amide linkage. In this way antimicrobial peptides (AMPs) like nisin,[55] or colistin,[56] cell penetrating peptides G_4R_8,[57] fibronectin-derived cell adhesive peptides, like $G_{1/4}$RGDS peptides,[58] BMP-2 peptide,[59] acne alleviating peptide,[60] as well as endothelial cell specific peptide REDV[61] were successfully conjugated to HA. In some cases EDC alone was employed as activating agent for conjugating peptides like the cell penetrating peptide SS-31 the cell binding peptide,[62] GRGDSY,[63] or the collagen binding peptide LSELRLHNN.[64] In these conditions the degree of substitution (DS) was very low, due to the competitive side reaction that took to the stable *N*-acyl urea by-product.

With the aim to avoid reaction of amino acid side chain functional groups with the activated COOH, alternative chemoselective methodologies were developed. Some of these reactions are based on the coupling of aldehyde moieties with amine- or aminoxy- groups forming Schiff's base or oxime, respectively. Usually, aldehydes were introduced on the HA by coupling of small molecule containing masked-aldehyde moieties using carbodiimide-mediated coupling. For example, aminoacetaldehyde dimethyl acetal was easily conjugated to HA and then converted to aldehyde by mild acidic treatment.[45a] Alternatively, periodate oxidation of the C_2, C_3 diols function of the glucuronic acid was performed. The oxidation of HA by periodate treatment on the proximal hydroxyl groups at C_2 and C_3 carbons of glucuronic acid moiety generates two aldehyde groups, thereby opening the sugar ring to form a linear chain. An antimicrobial peptide (KR12) was successfully conjugated onto periodate oxidized HA by reductive amination,[65] while Insulin growth factor-1 derived peptide (IGF-1 C) with potential effect on neovascularization was conjugated by Schiff's base linkage.[66] While periodate oxidation allows for the formation of a large number of functional groups, the disadvantage is the loss of the native backbone structure that could be responsible for limited biological activity of HA. Consequently, the generated derivative presumably could not be recognized as HA by cells.[45a]

4.4. Peptide Bioconjugation by Bioorthogonal Chemistry

Biologists were historically involved in the study of biomolecules in their native state. Nowadays, the interest to study biological processes is growing also among chemists and it is finalized to the chemical modification of proteins, carbohydrates, nucleic acids. The reaction of compounds with bioorthogonal functional groups is required to proceed in the

presence of all functional groups present in living systems such as carboxylic, thiol, aldehyde, hydroxyl groups. In other terms,"bioorthogonal reactions should be mutually and selectively reactive, and neither interact nor interfere with the biological system".[54] Such reactions must be inert toward the multitude of functional groups of biomolecules. Bioorthogonal chemistry well fits peptide conjugation to HA because the two components to be linked-peptide and carbohydrate- share most of the functional groups that could interfere in common reactions. Accordingly, to promote bioorthogonal chemistry, specific functional groups have to be introduced on both molecules. Commonly, HA was decorated with electron-poor activated unsaturated groups by adding (meth)acrylate or maleimide moieties in order to exploit the thiol-ene reaction, in both its radical and base/nucleophilic forms. The thiol-ene click reaction has already been demonstrated to be a powerful and versatile method for site specific functionalization also for biopolymers, and as a convenient conjugation tool.[67] Methacrylic anhydride was preferentially reacted with C_6 primary hydroxyl group of *N*-acetyl glucosamine sugar ring of HA to obtain methacrylated HA (HAMA). Peptides, containing a cysteine residue in the sequence, preferentially at the *N*- or C-terminus, were then grafted by thiol-ene click reaction, through a Michael-type addition. The insertion of cysteine in the peptide sequence by SPPS is straightforward, rendering this chemical strategy widely explored. He and Li conjugated a tumor recurrence inhibiting peptide, juxtamembrane 2 peptide (JM2), to HAMA by triethanolamine nucleophile catalyzed thiol-ene reaction[68] while Teng et al. conjugated a RGD peptide in a similar manner.[69] *N*-cadherin mimetic peptides were also grafted to HAMA to promote the osteogenesis of human mesenchymal stem cells.[70] In another report, Deng et al. employed tris(2-carboxyethyl) phosphine (TCEP) as nucleophilic catalyst to bioconjugate cysteine-containing Wnt5a mimetic ligand (Foxy5 hexapeptide) to HAMA with the aim to promote the chondrogenesis of hMSCs.[71] Furthermore, HAMA was decorated by thiol-ene Michael addition with an adhesive peptide showing G**C**GYG**RGD**SPG sequence and subsequently co- electrospun with PEO to fabricate HA scaffolds with adhesive gradient. Adhesive gradients in the HA-based electrospun scaffold were created by programming the flow rate of two solutions where one with high concentration of Peptide-conjugated HAMA/PEO increases in concentration, whereas the other solution composed by HAMA /PEO decreases in concentration over the scaffold collection period. In this way the incorporation of an adhesive peptide was modulated.[72] In all these reports the methacrylic group was exploited for peptide grafting as well as for hydrogel production by UV photocrosslinking. Accordingly, the reagents ratios (HAMA/peptide) were carefully determined to have a DS of peptide conjugation in the order of 20% with respect to methacrylic groups. To improve enzymatic degradation resistance the functionalization of HA with activated ene groups was also performed at the glucuronic C_6 COOH site using two-step synthetic methods.

In the first step a molecule containing the activated -ene group was anchored to HA usually by amidation.As an example, 2-aminoethyl maleimide was conjugated with carboxylic function of HA by EDC/NHS activation. The subsequent Michael addition by thiol containing peptides determined peptide anchoring. In this way a collagen mimetic peptide,[73] and GRGDSPC cell adhesive peptide[74] were added. In a recent report, an elastin-derived peptide was grafted to glucuronic C_6 methacrylated HA by thiol-ene chemistry. The peptide sequence- (C)AAAAAAAAAAKAAKYGAAAGL- was modified by the insertion of a cysteine at C-terminal end (EL) and corresponds to the region 302–322 of human tropoelastin (HTE) promoting cell attachment and spreading.[75] In the first step, the carboxyl groups of HA activated by EDC/NHS reacted in water with 2-aminoethyl methacrylate hydrochloride (2-AEMA·HCl), affording the C_6-methacrylated hyaluronic acid (MAHA). In the second step, the electron-poor double bond of MAHA was linked to the thiolated elastin-peptide via Michael-type addition (Scheme 1b) to produce an elastin-hyaluronan bioconjugate (ELHA) as a bioactive component of electrospun scaffolds.[76] The authors demonstrated by circular dichroism conformational studies that the linkage to HA preserved the native α-helix conformation of the peptide, considered important for bioactivity. The ELHA bioconjugate was successfully electrospun with poly-D,L-lactide (PDLLA) giving rise to a nanofibrous structure. Interestingly, SEM micrographs of ELHA/PDLLA electrospun scaffolds notably showed improved morphology with absence of defects in comparison to electrospun scaffolds composed of HA/PDLLA. In summary, the bioconjugation facilitated the electrospinning process of HA in aqueous solution thus rendering more appealing the obtained scaffolds in terms of biocompatibility through the elimination of organic solvents. Other examples of bioorthogonal click chemistry used for peptide conjugation to HA are presented below. HA polymers were first modified at glucuronic C_6 carboxyl groups with masked aldehyde groups and then with methylfuran moieties. HA-methylfuran groups were conjugated with maleimide-functionalized bioactive peptides via Diels–Alder reaction to introduce biochemical properties, whereas the HA-aldehyde groups were crosslinked with bis(oxyamine)-poly(ethylene glycol) (PEG) via oxime ligation to control hydrogel mechanical properties.[77] Maleimide-functionalized peptides were also conjugated by Fowler et al. to thiol-functionalized HA derivatives by thiol-ene chemistry.[78] By this route, biomimetic, hyaluronic acid (HA)-based hydrogel platform containing covalently immobilized bioactive peptides derived from perlecan domain IV (TWSKV), laminin-111 (YIGSR, IKVAV), and fibronectin (RGDSP) were produced.

Recently, peptide decoration to HA was at the basis of the fabrication of a tunable hydrogel platform having protease susceptibility and cell adhesive properties employing bioorthogonal inverse electron tetrazine ligation with strained alkenes like *trans*-cyclooctene (TCO).[79] In this study, tetrazine-functionalized HA (HA-Tz) was first crosslinked by a bis-norbornene-functionalized protease susceptible peptide used as crosslinker to obtain the hydrogel. Then the hydrogel was modified via a diffusion-controlled method using a *trans*-cyclooctene-functionalized cell adhesive peptide reacting to HA-Tz by cycloaddition (Figure 9). The reaction was extremely fast and highly selective, and succeeded in producing an

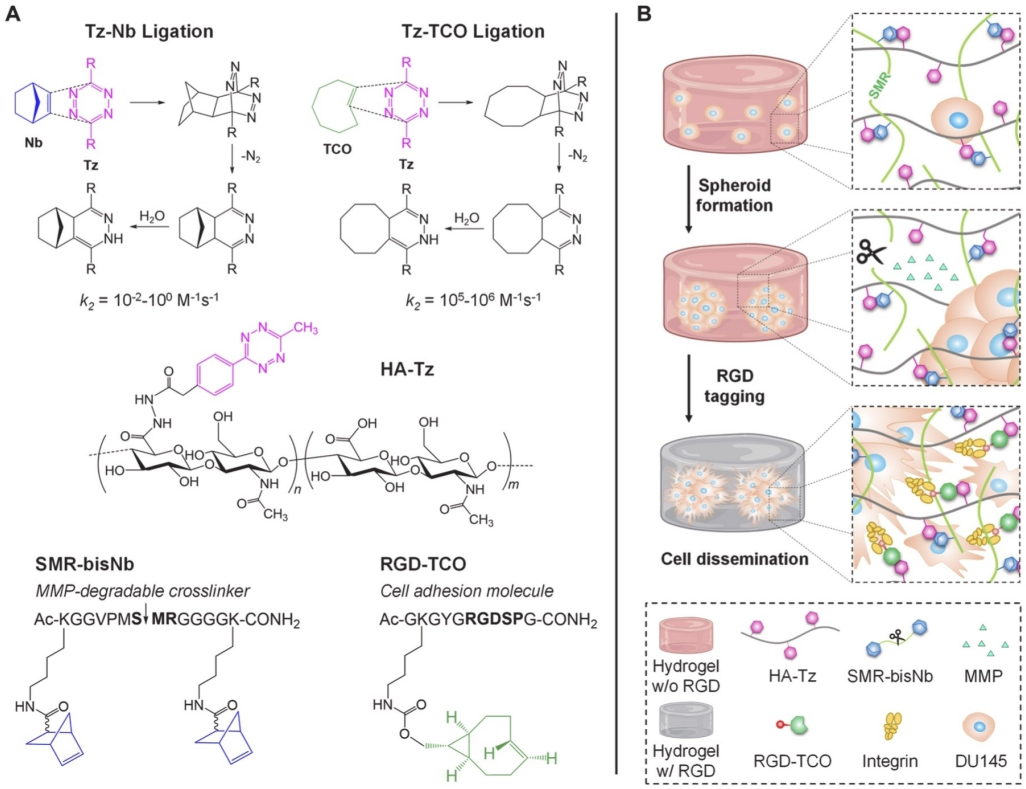


Figure 9. Bioorthogonal tuning of hydrogel properties to induce EMT. (A) Hydrogels were fabricated via slow Tz-Nb cycloaddition reaction using tetrazine modified HA (HA-Tz) and an MMP-degradable crosslinker (SMR-bisNb) at a Tz/Nb molar ratio of 2/1. RGD ligand was covalently conjugated to the synthetic ECM via fast Tz-TCO ligation through a diffusion-controlled mechanism. RGD density was tuned by varying the molar ratio of RGD-TCO and RGE-TCO. (B) DU145 cells in MMP-degradable HA gels spontaneously assembled into multicellular spheroids in 3D. Seven days post encapsulation, the RGD signal was introduced. RGD tagging promoted decompaction of spheroids and invasive processes were developed around the spheroids. Reprinted from [78], copyright (2023) with permission from Elsevier.

innovative hydrogel platform that supports the growth, assembly, and migration of cancer cells in 3D.

The biorthogonal inverse electron tetrazine ligation with strained alkenes was also employed to form core-shell microfibers by layer-by-layer assembly of decorated HA-Tz and HA-TCO as outer shells of electrospun PCL microfibers. The outermost layer constituted of HA -Tz was reacted with TCO-conjugated RGD peptide, to promote cell adhesion. The scaffolds fostered the attachment and growth of primary porcine vocal fold fibroblasts limiting the induction of the myofibroblast phenotype. The electrospun microfibers, consisting of a stiff PCL core and a soft HA based shell, were considered promising as therapeutic implants for the treatment of vocal fold scarring.[80]

Finally, peptide conjugation performed on scaffolds after electrospinning was reported by Deng et al. .[81] PVA/HA electrospun nanofibers were crosslinked by glutaraldehyde vapors and thereafter Vitronectin peptide (VP) was immobilized onto the electrospun mats through EDC/NHS chemistry. Decoration of scaffolds with VP peptide was able to promote proliferation of human induced pluripotent stem cells (hiPSCs).

5. Summary

This review focuses on electrospun hyaluronic acid-based materials commonly used in various applications, from cosmetics to tissue engineering. Nowadays, electrospinning techniques have raised great interest in the field of biomedical devices. On that basis, hyaluronic acid has been generally electrospun in blend with other polymers for optimizing the performance of the final scaffold, sharing complementary properties such as the biocompatibility deriving from the use of polysaccharide and the mechanical strength of nanofibers. Electrospun polymers are characterized by porous structures that facilitate the exchange of nutrients and the elimination of waste products by cells populating the scaffolds.

Hyaluronic acid is highly versatile thanks to the presence of different functional groups, which give rise to the possibility of achieving chemical modifications on the glycosaminoglycan backbone to anchor bioactive molecules and functionalized polymers as well. Furthermore, the glycosaminoglycan structure is also functional for the crosslinking of hyaluronic acid-inspired scaffolds, which is mandatory to prolong the half-life when implanted in-vivo.

Analogously, decorated HA biomolecules such as peptides are also worthy of note because the covalent bond slows HA's

degradation rate, as the crosslinking does. Recent advances in the field have been herein reviewed and discussed.

6. Outlook

Over the last years, great interest has been devoted to the production of hyaluronic acid derived products because they are appealing as biomedical devices and cosmetics as well. Besides, the electrospinning technique has attracted a great attention to produce nonwoven scaffolds made of nanofibers. However, electrospinning of hyaluronan-based biomaterials is still a challenging process that requires from the research community peculiar attention and further efforts forwarded in the direction of implementing sustainable and eco-friendly processes especially in the perspective of extending electrospinning adoption to large-scale production. Therefore, the material and production cost, and societal and environmental concerns need to be considered too. Bioorganic chemistry could be the answer to this difficult task because it enables the tuning of the properties of hyaluronan through the creation of covalent bonds with proper bioactive molecules using mild conditions. This review is an anthology of the recent findings in the field and opens new perspectives in terms of innovative and smart chemical strategies on hyaluronan.

With the outlook of smart biomaterials production, the future perspective is represented by the set-up of crosslinking methodologies to be carried out during electrospinning both on plain HA and HA-based scaffolds. While several literature data exist, in fact, on HA fibers crosslinked after electrospinning, few data are present on crosslinking carried out simultaneously with electrospinning.

Acknowledgements

F. A. acknowledges support from INPS within PhD Programme Industry 4.0.

Conflict of Interests

The authors declare no conflict of interest.

Keywords: Hyaluronic acid · electrospinning · cross-linking · bioconjugation · peptide

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Manuscript received: October 20, 2023
Revised manuscript received: March 19, 2024
Accepted manuscript online: March 20, 2024
Version of record online: April 12, 2024