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Electrospinning of polysaccharides for regenerative medicine ☆

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ABSTRACT

Electrospinning techniques enable the production of continuous fibers with dimensions on the scale of nanometers from a wide range of natural and synthetic polymers. The number of recent studies regarding electrospun polysaccharides and their derivatives, which are potentially useful for regenerative medicine, is increasing dramatically. However, difficulties regarding the processibility of the polysaccharides (e.g., poor solubility and high surface tension) have limited their application. In this review, we summarize the characteristics of various polysaccharides such as alginate, cellulose, chitin, chitosan, hyaluronic acid, starch, dextran, and heparin, which are either currently being used or have potential to be used for electrospinning. The recent progress of nanofiber matrices electrospun from polysaccharides and their biomedical applications in tissue engineering, wound dressings, drug delivery, and enzyme immobilization are discussed.

Contents

1. Introduction

Recent advances in electrospinning techniques have enabled the production of continuous fibers with dimensions on the scale of nanometers. A wide range of natural and synthetic polymers can be electrospun into nanofiber matrices with structural integrity and specific fiber arrangements. Nanofiber matrices have been widely used in industrial applications (e.g., multifunctional membranes, filters, textiles, and templates for hollow fibers) and biomedical applications

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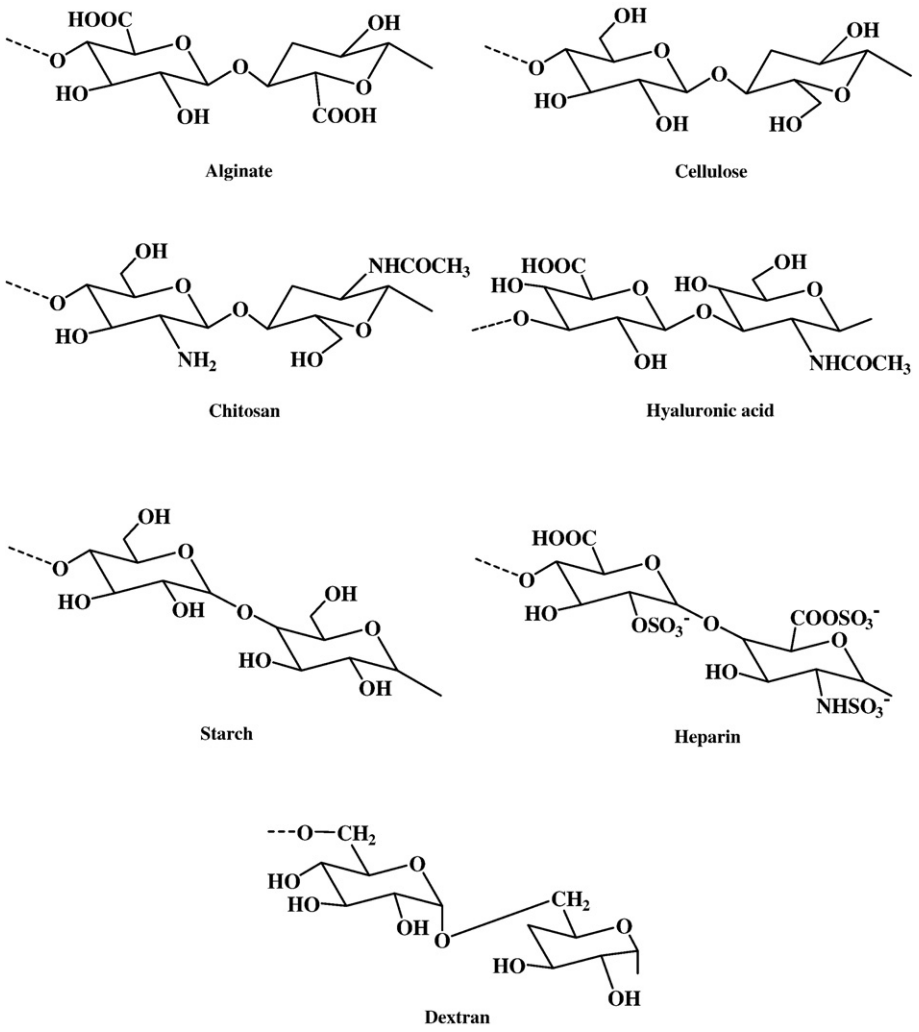

Fig. 1. Chemical structures of polysaccharides currently used or that have a potential for use in electrospinning.

(e.g., tissue engineering scaffolds, wound dressing, vascular grafts, and drug delivery systems) [1,2].

Polysaccharides are the homopolymers or copolymers of monosaccharides. In nature, polysaccharides can be found in many organisms, including polysaccharides of algal origin (e.g. alginate), plant origin (e.g. cellulose and starch), microbial origin (e.g. dextran), and animal origin (e.g. chitosan and hyaluronic acid) [3]. Polysaccharides are also diverse in their chemical structure (Fig. 1), chemical composition, molecular weight, and ionic character, all of which contribute to their functionality and biological activity. Several fabrication methods for nanofibers, including drawing [4], template synthesis [5], phase separation [6], self-assembly [7], and electrospinning [8], have been developed. Among these, the electrospinning process has become the most attractive because it is cost-effective, highly productive, and applicable to a variety of polysaccharides. Many studies have been conducted to date using polysaccharides and their derivatives for the fabrication of electrospun nanofibers that could be potentially useful in regenerative medicine. Fig. 2 shows the significant increase in the number of scientific publications since 2002 regarding electrospun polysaccharides.

Electrospun nanofibers from natural and synthetic polymers have been widely used in regenerative medicine, including tissue engineering applications. Tissue engineering aims to provide man-made tissues or organs to patients who suffer the loss or failure of a tissue or organ. Tissues and organs are typically engineered using a combination of a patient's own cells and polymer scaffolds. The polymer scaffold used in this approach is designed to mimic many of the roles of extracellular matrices (ECMs) of tissues in the body. These roles include properly arranging cells, controlling the tissue structure, regulating the function of cells, and allowing the diffusion of nutrients, metabolites, and soluble factors [9].

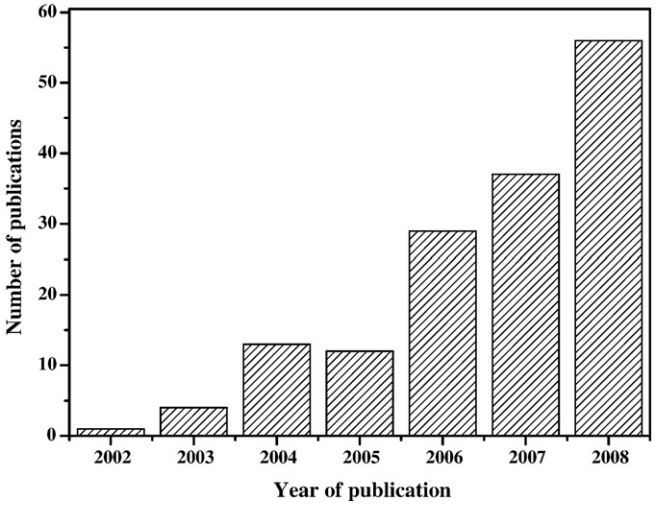

Fig. 2. Scientific publications on electrospun polysaccharide nanofibers found in the SciFinder Scholar search system.

Nanofiber matrices have recently become attractive in tissue engineering due to their large surface area, high porosity, controlled mechanical properties, and their ability to interact with cells in a manner which mimics the natural ECMs. This review will discuss the recent progress of several nanofiber matrices electrospun from polysaccharides and their biomedical applications, including tissue engineering scaffolds, wound dressings, controlled drug release systems, and immobilization substrates of biocatalysts and enzymes.

2. Potential polysaccharides for electrospinning

2.1. Alginate

2.1.1. Introduction

Alginate is an anionic polysaccharide derived from brown seaweed. Alginate is a linear copolymer that contains blockwise structures of (1,4)-linked β-D-mannuronic acid (M) and α-L-guluronic acid (G) residues [10]. Alginate can form hydrogels, porous sponges, beads, and microfibers, all of which have been used for many biomedical applications. These include tissue engineering applications, such as skin [11], cartilage [12], bone [13,14], liver [15,16], and cardiac tissue [17] regeneration. Alginate has excellent biocompatibility, low toxicity, non-immunogenicity, relatively low cost, and simple gelation behavior with divalent cations such as Ca^{2+}, Mg^{2+}, Ba^{2+}, and Sr^{2+} [18].

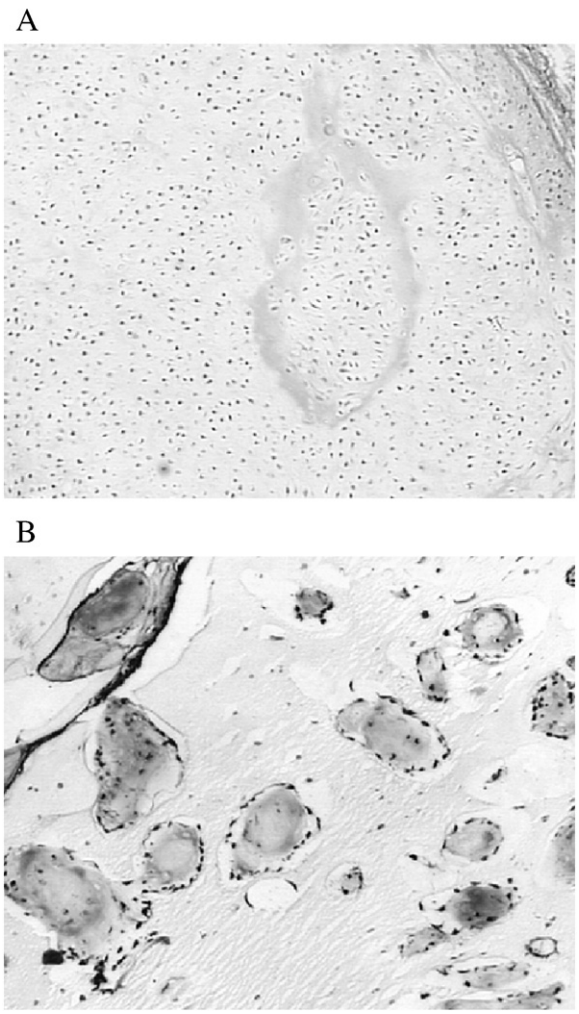

Fig. 3. Photomicrographs of (A) degradable alginate/chondrocyte and (B) non-degradable alginate/chondrocyte constructs after 7 weeks of subcutaneous implantation in the backs of mice. Tissue sections were stained with trichrome blue to visualize cartilage (from ref. [21] with permission).

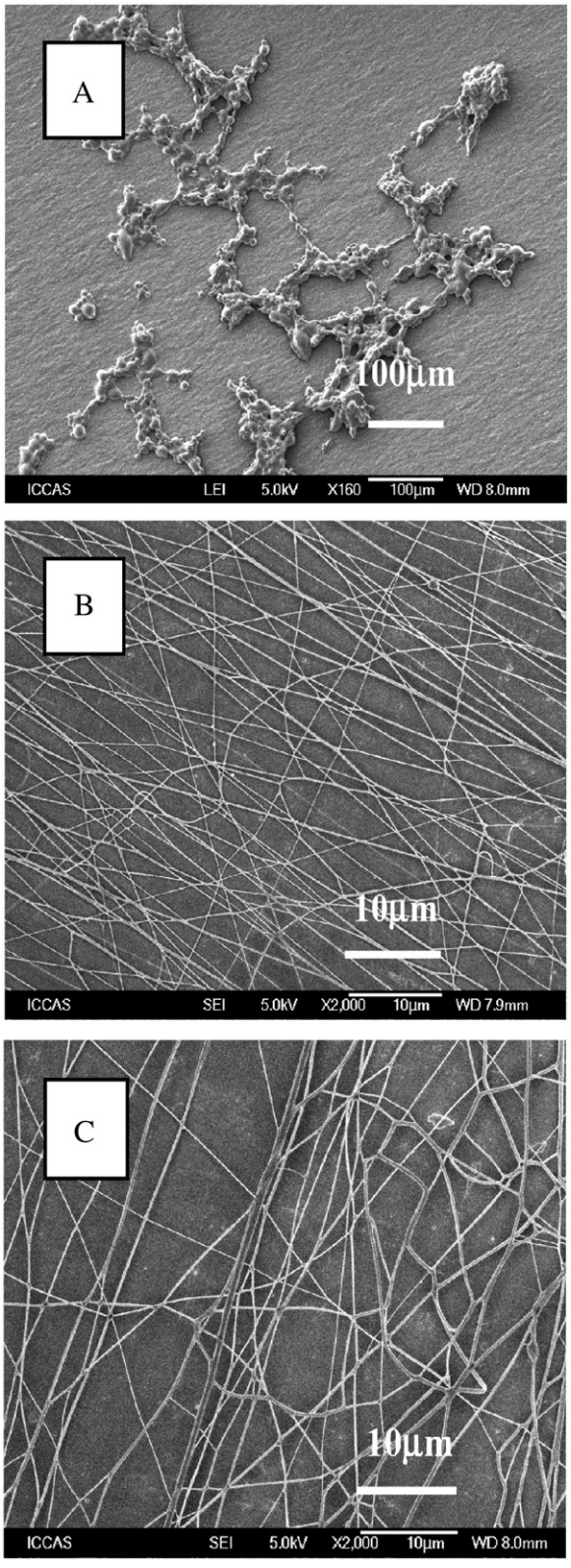

Fig. 4. SEM images of alginate nanofibers electrospun from aqueous alginate solutions with different volume ratios of glycerol to water (v/v) of (A) 0.5, (B) 1, and (C) 2 ([alginate] = 2%) (from ref. [22] with permission).

The composition, sequence, and molecular weight generally determine the physical properties of alginate [19].

Alginate is inherently non-degradable in physiological conditions, but ionically cross-linked alginate gels can be dissolved via a process involving a loss of divalent ions into the surrounding media rather

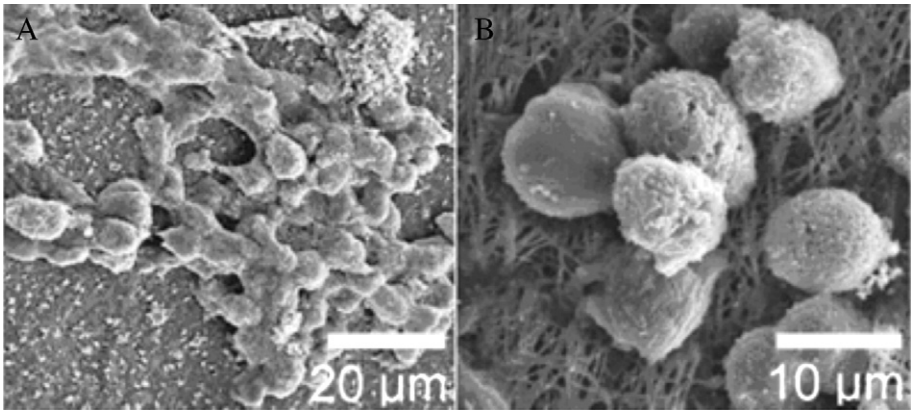

Fig. 5. SEM images of chondrocytes grown on alginate/PEO nanofibers (alginate/PEO = 80/20, w/w). (A) Low and (B) high magnification images (from ref. [23] with permission).

than actual degradation. However, the molecular weights of many commercially available alginates are typically above the renal clearance threshold of the kidney [20]. Alginate slightly oxidized by sodium periodate was degradable in aqueous media and has demonstrated a potential in the delivery of chondrocytes for cartilage regeneration [21]. A significant reduction in the molecular weight of the oxidized alginate was observed during incubation, depending on the pH and temperature of the medium. Degradable alginate gels containing chondrocytes were subcutaneously injected into mice and resulted in the formation of cartilage tissues after 7 weeks of implantation that were consistent with the appearance of native cartilage (Fig. 3).

2.1.2. Electrospinning and its applications

Although alginate can be easily dissolved in water, it is difficult to obtain continuous and uniform nanofibrous structures by electrospinning due to the lack of chain entanglements caused by the rigid and extended chain conformation in aqueous solution. Therefore, the electrospinning of aqueous alginate solution is still challenging. Nie et al. [22] reported that alginate can be successfully electrospun by using glycerol as a co-solvent. Smooth and uniform alginate nanofibers were fabricated by simply adjusting the volume ratio between glycerol and water (Fig. 4). Glycerol enhanced the entanglement of the alginate chains by forming new hydrogen bonds. Two different schematic models were proposed to describe the conformation of alginate chains in aqueous solution with and without glycerol.

To overcome the poor electrospinnability of aqueous alginate solution, water-soluble synthetic polymers such as poly(ethylene oxide) (PEO) and poly(vinyl alcohol) (PVA) were added to the alginate solution [10,23–27]. Generally, the electrospinnability increased as the concentration of synthetic polymers increased, and continuous nanofibers were obtained at synthetic polymer-rich compositions, that is, alginate/synthetic polymer ratio of 50/50 or below. Alginate/PEO nanofibers exhibited good uniformity, structural integrity, and cellular compatibility with cartilage chondrocyte-like cells (HTB-94) [23]. The cells adhered well and formed cell clusters on the alginate/PEO nanofibrous matrices and maintained their characteristic phenotypes (Fig. 5). Cell viability was determined to be approximately 95%.

2.2. Cellulose

2.2.1. Introduction

Cellulose consists of (1,4)-linked β-D-glucose units and has been of particular interest due to its abundance as a renewable resource, biodegradability, and compatibility with biological systems. Cellulose-based materials have been extensively used in the pharmaceutical

Table 1
Electrospun cellulose nanofibers.

Polymer	Molecular weight	Solvent	Reference
Cellulose	210–1600[a]	LiCl/DMAc, NMMO/water	[29,30,44,49]
Cellulose	1600[a]	AMIMCl/DMSO	[34]
Cellulose acetate	30 kDa[b]	Acetone/water	[37,47,50,51]
Cellulose acetate	30 kDa[b]	Acetone/DMAc	[28,36,54–58]
Cellulose acetate	29 kDa[c]	Acetone/DMF/trifluro-ethanol	[49]
Cellulose acetate	30 kDa[d]	Acetic acid/water	[39]
Cellulose acetate	30 kDa[d]	Acetone, chloroform, DMAc, DMF, DCM, formic acid, methanol, pyridine, water	[38]
Cellulose triacetate	–	Methylene chloride/ethanol	[40]
Ethyl cellulose	72 kDa[d], 160 kDa[d]	THF/DMAc	[42,43]
Ethyl–cyanoethyl cellulose	97 kDa[d]	THF	[45,46]
Hydroxypropyl methylcellulose	350 kDa[d]	Water/ethanol	[44]
Hydroxypropyl methyl cellulose phthalate	74 kDa[b]	Acetone/ethanol	[41]
Methyl cellulose	350 kDa[d]	Water/ethanol	[44]
Cellulose acetate/hydroxyapatite	29 kDa[c] (CA)	Acetic acid/acetone	[62]
Cellulose acetate/PEG	29 kDa[b] (CA), 10 kDa[b] (PEG)	DMAc/acetone	[61]
Cellulose acetate/PEO	30–60 kDa[b] (CA), 10–600 kDa[b] (PEO)	DMF, DMF/dioxane	[52,60]
Carboxymethyl cellulose/PEO	120–500 kDa[d] (CMC),		
400 kDa[d] (PEO)	Water	44	

Abbreviation: LiCl, lithium chloride; DMAc, dimethylacetamide; NMMO, N-methyl-morpholine N-oxide; AMIMCl, 1-allyl-3-methylimidazolium chloride ; DMF, dimethylformamide; DCM, dichloromethane ; THF, tetrahydrofuran.

[a] Degree of polymerization.
[b] Number-average molecular weight.
[c] Relative molecular weight.
[d] Weight-average molecular weight.

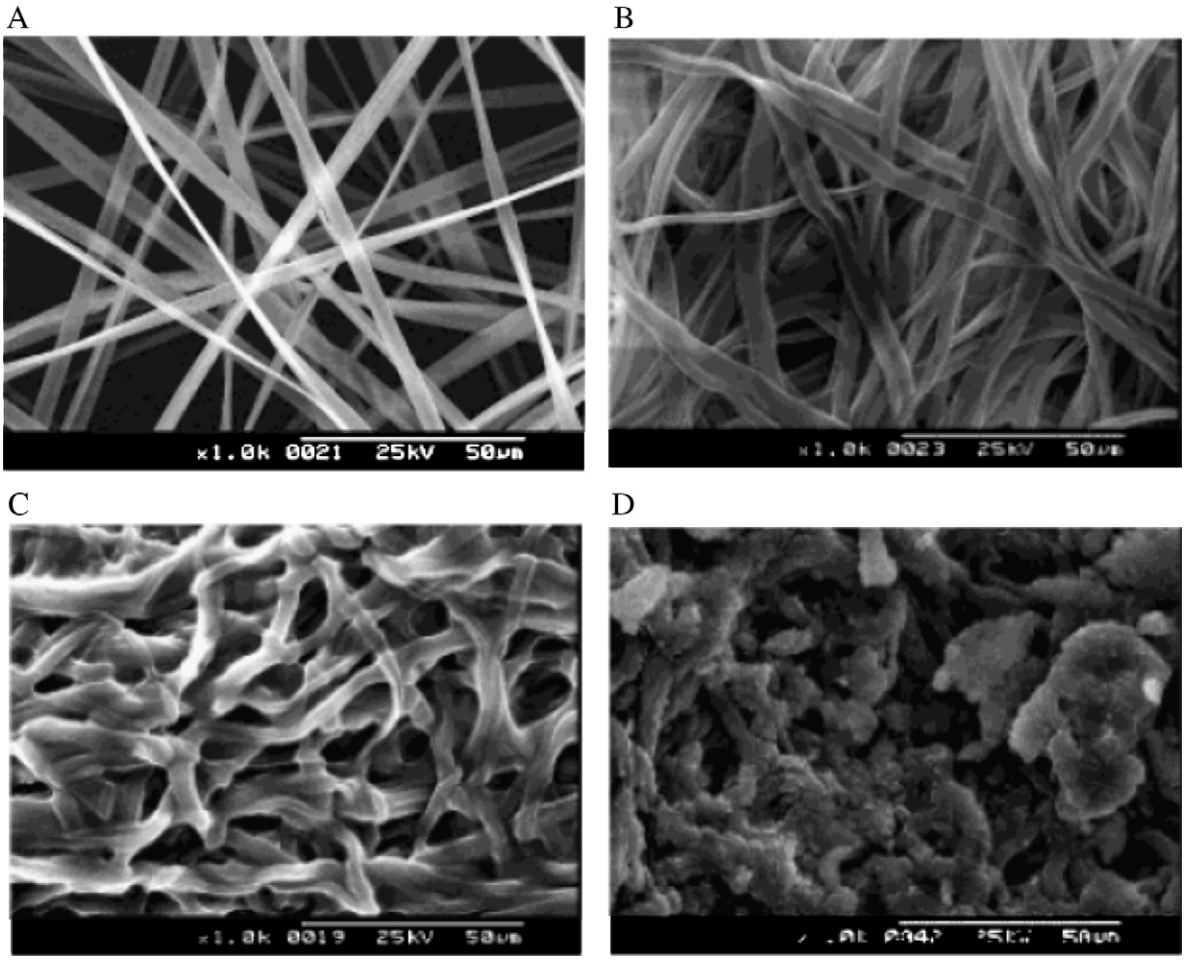

Fig. 6. SEM images of electrospun nanofiber matrices of (A) cellulose acetate, (B) deacetylated cellulose acetate, (C) oxidized cellulose prepared with a mixture of HNO_3/H_3PO_4–$NaNO_2$, and (D) oxidized cellulose prepared with a mixture of HNO_3/H_2SO_4–$NaNO_2$ (from ref. [47] with permission).

and biomedical fields, including applications as adsorbent beads, filters, artificial tissue/skin, and protective clothing. Unfortunately, the processibility of cellulose is extremely restricted by its limited solubility in common organic solvents and its inability to melt due to strong inter- and intra-molecular hydrogen bonds [28]. Cellulose is much more crystalline than starch, and cellulose requires a temperature of 320 °C and a pressure of 25 MPa to become amorphous in water.

2.2.2. Electrospinning and its applications

Since cellulose does not melt, it must be processed from solution. Several solvents that directly dissolve cellulose have been investigated and used for electrospinning, including N-methyl-morpholine N-oxide/water (NMMO/water) and lithium chloride/dimethylacetamide (LiCl/DMAc) [29–33]. In addition, ionic liquids have been recently used to fabricate electrospun cellulose nanofibers [34]. However, these solvents have low volatility, and thus cannot completely evaporate during the electrospinning process. In addition, the electrospinning temperature must be raised above the melting temperature of the solvent (e.g. approximately 85 °C for NMMO/water). For a LiCl/DMAc solvent system, it is difficult to completely remove lithium or chlorine ions by coagulation after the electrospinning process [35].

Cellulose derivatives have been extensively exploited to enhance the solubility of cellulose, and thus improve its electrospinnability. Cellulose derivatives can be easily electrospun into fibers and then converted to cellulose by aqueous or ethanolic hydrolysis. Cellulose derivatives used for electrospinning include cellulose acetate (CA) [36–39], cellulose triacetate (CTA) [40], hydroxypropyl cellulose (HPC) [41], ethyl cellulose (EC) [42,43], methyl cellulose (MC) [44],

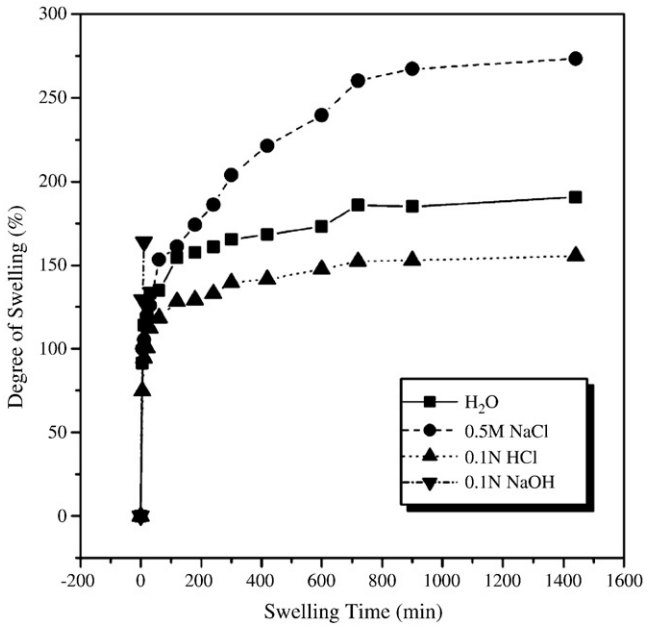

Fig. 7. Changes in the degree of swelling of ultrafine oxidized cellulose nanofiber matrices with time in various aqueous solutions at 25 °C (from ref. [47] with permission).

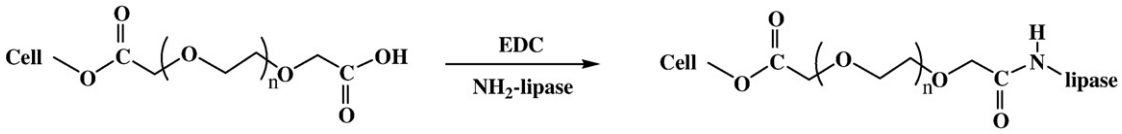

Fig. 8. Schematic description for immobilization of enzymes by electrospun cellulose nanofibers using poly(ethylene glycol) spacers (from ref. [54] with permission).

and ethyl–cyanoethyl cellulose (E-CE C) [45,46]. Table 1 summarizes various experimental conditions that have been used to fabricate electrospun cellulose-based nanofibers.

Electrospun cellulose-based nanofiber matrices have been used as affinity or barrier membranes [47–49], antimicrobial membranes [50–52], three-dimensional structures resembling the urinary bladder matrix [53], membranes for enzyme immobilization [54], and membranes for drug delivery [55–58]. Ultrafine oxidized cellulose (OC) matrices were prepared by the oxidation of ultrafine cellulose matrices produced by electrospinning CA and subsequent deacetylation [47]. Nonwoven OC matrices have potential applications, such as nonwoven adhesion barriers, as they degrade under physiological conditions. When ultrafine cellulose matrices were oxidized with a mixture of HNO_3/H_3PO_4–$NaNO_2$, the matrix structure was maintained without any severe change. However, when they were oxidized with a mixture of HNO_3/H_2SO_4–$NaNO_2$, small fibers and aggregated particles were obtained because of severe degradation (Fig. 6). In a physiological salt solution, the degree of swelling was approximately 280% (Fig. 7). Khil et al. [48] prepared OC matrices with different carboxyl contents by using NO_2 as an oxidant. The weight loss of the OC matrices was more than 90% within 4 days of incubation in PBS.

An electrospun CA nanofiber membrane was oxidized with sodium periodate to generate aldehyde groups, upon which protein A/G ligand containing IgG binding domains was covalently attached [49]. This membrane provided a useful tool for fast purification of antibody at a small scale. Zhang et al. [28] also fabricated regenerated cellulose nanofibrous membranes functionalized with diethylaminoethyl (DEAE) groups as a weak anion-exchange group, and evaluated their potential for bioseparation applications. The DEAE-functionalized cellulose nanofiber membrane had enhanced binding capacity for bovine serum albumin (BSA). Ultrafine cellulose fibers with diameters of 100 nm were produced by electrospinning and alkaline hydrolysis of CA [54]. The surfaces of the electrospun nanofibers were activated by reacting with PEG diacylchloride, followed by covalently binding with lipase using simple carbodiimide chemistry (Fig. 8). The bound lipase exhibited significantly higher catalytic activity at elevated temperatures than the free lipase, up to 8–10 times at 60 and 70 °C.

Electrospun nanofibers from cellulose and its derivatives were functionalized by incorporating functional compounds (e.g. drug) into a spinning solution. The antimicrobial CA nanofibrous membranes were prepared by adding silver nitrate to a CA solution in acetone/water (80/20, w/w) [51,52]. Silver ions were subsequently photoreduced into silver nanoparticles by irradiating the electrospun fibers with UV light. The particles were uniformly dispersed on the fiber surface with the particle size ranging from 10 to 20 nm (Fig. 9). The CA fibers containing silver nanoparticles showed very strong antimicrobial activity against *S. aureus*, *K. pneumoniae*, *E. coli*, and *P. aeruginosa*. The nanofibers with bactericidal properties were also prepared from electrospinning a CA solution containing chlorhexidine (CHX) as a bactericidal agent and organic titanate Tyzor® TE (TTE) as a cross-linker [52]. The resultant fiber matrices exhibited bactericidal properties against *S. epidermidis* and *E. coli* on contact due to the CHX immobilized on the fibers and within a zone of inhibition due to the release of unbound CHX.

Vitamin-loaded CA matrices of electrospun nanofibers were successfully fabricated by electrospinning a 17 wt% CA solution in an acetone/DMAc (2/2, v/v) solvent mixture [55]. Vitamins A and E were loaded as model drugs. Vitamin-loaded as-spun fiber matrices exhibited a gradual and monotonous increase in the cumulative release of the vitamins over time, while a burst release of the vitamins from as-cast films was also observed. CA nanofibrous matrices containing four types of model drugs including naproxen (NAP), indomethacin (IND), ibuprofen (IBU), and sulidac (SUL) were prepared by an electrospinning method. The amount of the drugs released from the drug-loaded CA nanofiber matrices in acetate buffer solution at 37 °C was greater than that from as-cast films. The cumulative release of the drugs from CA nanofiber matrices was found in the following order: NAP > IBU > IND > SUL (Fig. 10) [56]. Wang et al. [41] fabricated erythromycin-containing electrospun

Fig. 9. TEM image of electrospun cellulose acetate nanofibers containing silver nitrate after irradiation with UV light at 365 nm for 240 min (scale bar, 60 nm) (from ref. [50] with permission).

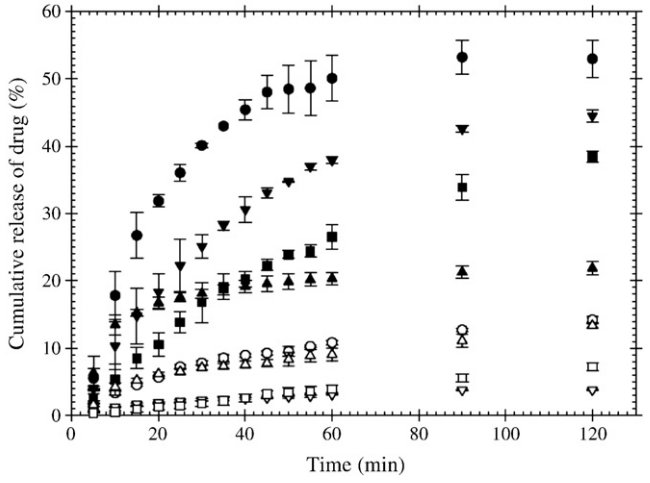

Fig. 10. Cumulative release of model drugs from (●) NAP-, (■) IND-, (▲) IBU-, and (▼) SUL-loaded electrospun cellulose acetate fiber matrices and (○) NAP-, (□), IND-, (△) IBU-, and (▽) SUL-loaded solvent-cast films (from ref. [56] with permission).

hydroxypropyl methyl cellulose phthalate fiber matrices with various fiber diameters using an electrospinning technique. The fiber diameter plays an important role in controlling the release rate and total amount of the drug released both in the stomach and in the intestine. Ultrafine CA fibrous matrices containing herb compounds (e.g. curcumin and asaiaticoside) were fabricated by electrospinning [57,58] and were proven to be non-toxic to normal human dermal fibroblasts, indicating a potential for use as transdermal patches or wound dressings.

Recent progress in the electrospinning of dual- or multi-component systems allows the production of fibers with novel properties and structures. Cellulose-based bicomponent fibrous matrices have been fabricated by electrospinning a binary mixture of CA with synthetic polymers, such as PVA [59] and PEO [44,60,61]. Formation of CA/PEO bicomponent fibers was strongly influenced by their chain length, concentrations and mixed ratios as well as the solvents employed. The bicomponent fibers also exhibited phase-separated structure with CA core and PEO sheath [60]. Organic/inorganic composites were also prepared by electrospinning a CA solution mixed with hydroxyapatite (HAp) for the development of structurally stable prosthetic devices [62].

2.3. Chitin and chitosan

2.3.1. Introduction

Chitin, composed of (1,4)-linked N-acetyl-β-D-glucosamine, is the second most abundant natural polymer in the world, and is primarily obtained from shrimp and crab shells. When the degree of deacetylation (DD) of chitin reaches approximately 50%, it becomes soluble in aqueous acidic solution and is called chitosan. Chitosan has a repeated structure of (1,4)-linked β-D-glucosamine and an apparent pK of 6.5. Commercial products are traditionally composed of 80% β-D-glucosamine and 20% N-acetyl-β-D-glucosamine [63]. Chitosan is generally soluble at pHs below 6, and its solution properties depend on its molecular weight, degree of deacetylation, and distribution of acetyl groups in the backbone [64,65].

Chitosan is a cationic polymer and has been widely used in food, cosmetic, biomedical, and pharmaceutical applications [66–69]. In particular, chitin and chitosan have been extensively used in many biomedical applications due to their (1) biocompatibility and biodegradability, (2) cellular binding capability, (3) acceleration of wound healing, (4) hemostatic properties, and (5) anti-bacterial and anti-fungal properties. A large variety of useful forms, including beads, films, sponges, tubes, powders, and fibers, can be obtained from chitosan [70]. Chitosan is degraded by enzymes, such as lysozyme and chitosanase, and the degradation rate is dependent on the temperature, ionic strength, and pH of the medium *in vitro* [71]. In general, the lower the degree of deacetylation of chitosan, the faster the degradation rate. Chitosan has also been proven to be biodegradable when implanted into animals [72].

2.3.2. Electrospinning and its applications

Chitosan is difficult to electrospin into a fibrous structure because it has a polycationic character in an acidic aqueous solution due to the many amino groups in its backbone. Its polycationic nature excessively increases the surface tension of the solution. High electrical force is thus required to produce electrospun chitosan nanofibers, and particles are often formed during the electrospinning process, likely due to the repulsive forces between ionic groups in the chitosan backbone in an acidic solution [73].

Fibrous structures were successfully formed by electrospinning chitosan solutions in 90 wt% aqueous acetic acid solution [74] or by using an environmentally harmful and toxic solvent like trifluoroacetic acid (TFA) or TFA/dichloromethane (DCM) [75]. Chitosan initially forms stable salts in TFA, which may destroy strong interactions

Table 2
Electrospun chitosan nanofibers.

Polymer	Molecular weight	Degree of deacetylation (%)	Solvent	Reference
Single component				
Hexanoyl chitosan	576 kDa[a]	88	Chloroform	[102,104]
PEGylated chitosan	405 kDa[a]	84.7	DMF/THF	[112]
Chitosan	–	–	GA/TFA	[77]
Chitosan	106–310 kDa	54–85	aq AA	[74,76]
Chitosan	106 kDa[a]	54	TFA-MC	[75]
Chitosan-*g*-L-lactide	–	80	2-butanone	[111]
Blend				
Chitosan/PVA	120–1600 kDa	82.5–90	aq AA	[84,87,90,91,93,94]
Chitosan/PVA	120 kDa	82.5	aq AcrA	[92]
Chitosan/PLA			TFA-TCM	[96]
Chitosan/PEO	148, 276 kDa	82	aq acid	[80,83]
Chitosan/PEO	190–1400[a] kDa	85	aq AA	[78,79,82]
Chitosan/UHMWPEO	–	>85	aq AA/DMSO	[81]
Chitosan/PET	–	85	TFA	[122]
Chitosan/silk fibroin	220 kDa	86	FA	[98]
Chitosan/HAp	–	–	AA/DMSO	[120]
Chitosan/P(LLA-CL)	600 kDa	80	aq AA	[97]
Chitosan/collagen	1000 kDa	85	HFIP/TFA	[99]
CMCS/PVA	405 kDa[a]	84.7	Water	[110]
CECS/PVA	390 kDa[a]	–	aq AcrA	[108]
CECS/PVA	120 kDa	82.5	Water	[109]
Q-Chitosan/PVP	380 kDa	80	Water	[106]
Q-Chitosan/PVA	400 kDa	80	aq AA	[105]
Chitosan-*g*-PEG/PLGA	–	85	DMF/THF	[121]
Hybrid				
Chitosan-PLGA	600 kDa	85	TFA-MC	[95]
Chitosan/PVA-PLGA	165 kDa[a]	90	aq AA	[89]

Abbreviation: UHMWPEO, ultrahigh-molecular-weight poly(ethylene oxide); PET, poly(ethylene terephthalate) ; HAp, hydroxyapatite ; P(LLA-CL), poly(L-lactic acid-*co*-ε-caprolactone); CMCS, carboxymethyl chitosan; CECS, carboxyethyl chitosan; PVA, poly(vinyl alcohol); Q-Chitosan, quaternized chitosan; PVP, poly(vinyl pyrrolidone); DMF, dimethylformamide; THF, tetrahydrofuran; GA, glutaraldehyde ; TFA, trifluoroacetic acid; aq AA, aqueous acetic acid solution; MC, methylene chloride; aq AcrA, aqueous acrylic acid solution; TCM, trichloromethane; DMSO, dimethyl sulfoxide; HFIP, 1,1,1,3,3,3,-hexafluoro-2-propanol.

[a] Viscosity average molecular weight.

between the chitosan chains, making them more adequate for electrospinning. Additionally, its high volatility is advantageous for the rapid solidification of the electrified jet of the chitosan/TFA solution [75]. Chitosan nanofibers can also be made from deacetylation of electrospun chitin fibers using 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) as a solvent [73]. However, electrospinning conditions are relatively limited in terms of concentration, molecular weight, and degree of deacetylation [76]. The resultant chitosan fibers need to be cross-linked to maintain their structural integrity, as they can readily swell in aqueous solution [77].

Since the electrospinning of chitosan itself proved to be difficult, chitosan was mixed with other synthetic or natural polymers, such as PEO [78–83], PVA, [84–94], poly(lactic acid) (PLA) or its copolymers [95–97], silk fibroin (SF) [98], and collagen [99–101]. As an alternative approach to improve the solubility and electrospinnability of chitosan, several chitosan derivatives, including hexanoyl chitosan [102–104], quaternized chitosan [105,106], N-carboxyethylchitosan [107–110], and chitosan grafts with L-lactide or PEG oligomer [111,112], were synthesized and electrospun with or without polymer additives. Table 2 summarizes the experimental conditions adapted for the fabrication of electrospun chitosan nanofibers.

Chitin- and chitosan-based electrospun nanofibers have shown potential for many biomedical applications as they are structurally similar to glycosaminoglycans in the ECM and because of their morphological proximity to fibrous collagen structures in the ECM on the scale of nanometers (50–500 nm in diameter). They also have good biocompatibility and biodegradability as well as various biofunctionalities including antithrombogenic, hemostatic, and wound healing properties [113]. The use of nanofibrous chitosan matrices is thus expected to mimic the natural ECM, in which cells attach, proliferate, and differentiate.

Chitin nanofibrous matrices (Chi-N) were fabricated and their biodegradability and cellular behavior were compared with a commercially available chitin microfiber (Beschitin W®, Chi-M). Chi-N (mean diameter = 163 nm) was approximately two orders of magnitude smaller than Chi-M (mean diameter = 8.77 μm), and thus Chi-N degraded faster than Chi-M *in vitro* and *in vivo*. Chi-N was found to promote the attachment and spreading of normal human epidermal keratinocytes (NHEKs) and normal human epidermal fibroblasts (NHEFs) compared to Chi-M (Fig. 11) [114]. Chi-N fibrous matrices were considered useful for wound healing and regeneration of oral mucosa and skin.

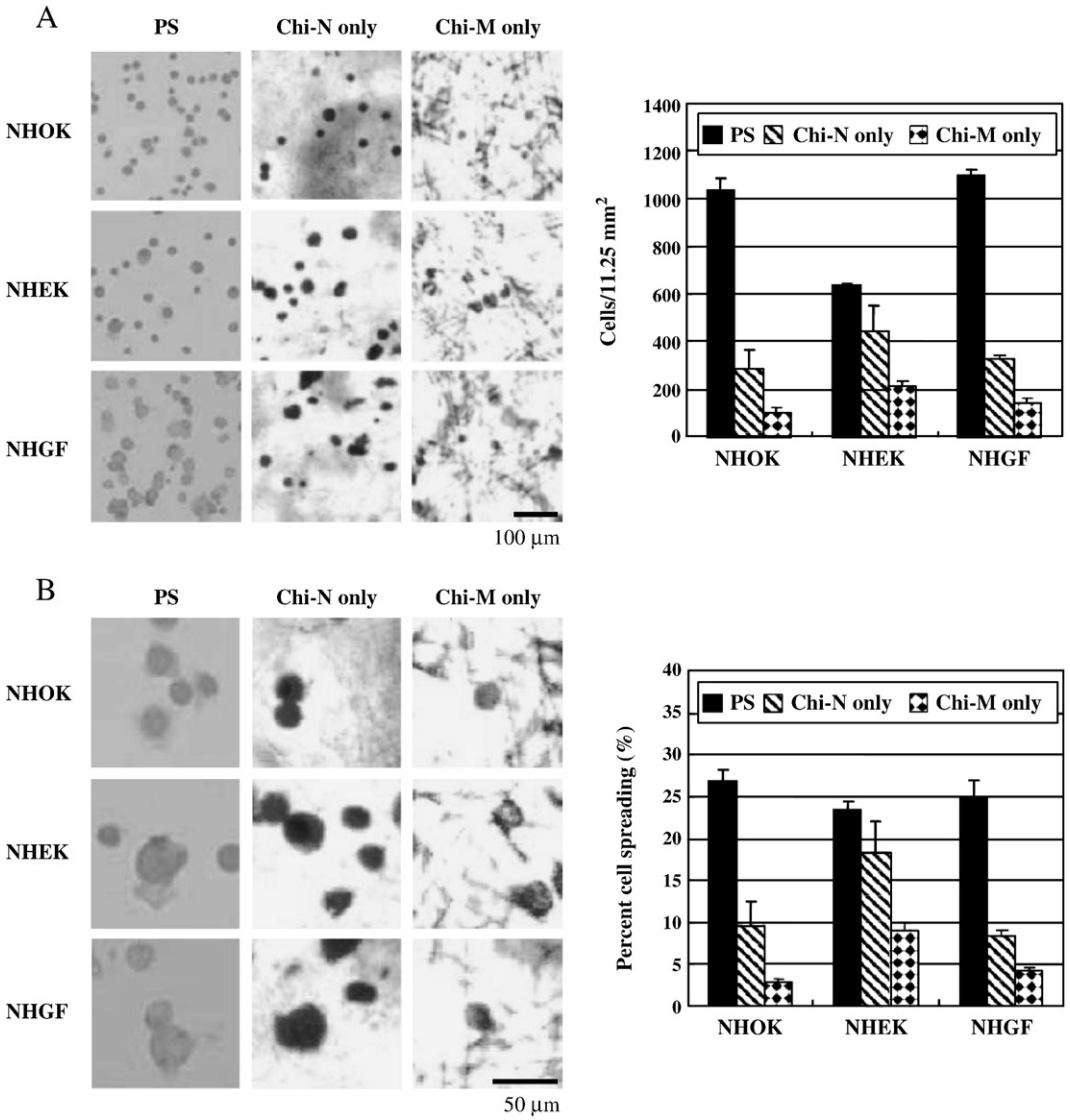

Fig. 11. (A) Attachment and (B) spreading of normal human epidermal keratinocytes (NHEKs), fibroblasts (NHEFs), and gingival fibroblasts (NHGFs) plated onto either chitin nanofibrous (Chi-N) or microfibrous (Chi-M) matrices without ECM protein coating (PS indicates polystyrene tissue culture plates) (from ref. [114] with permission).

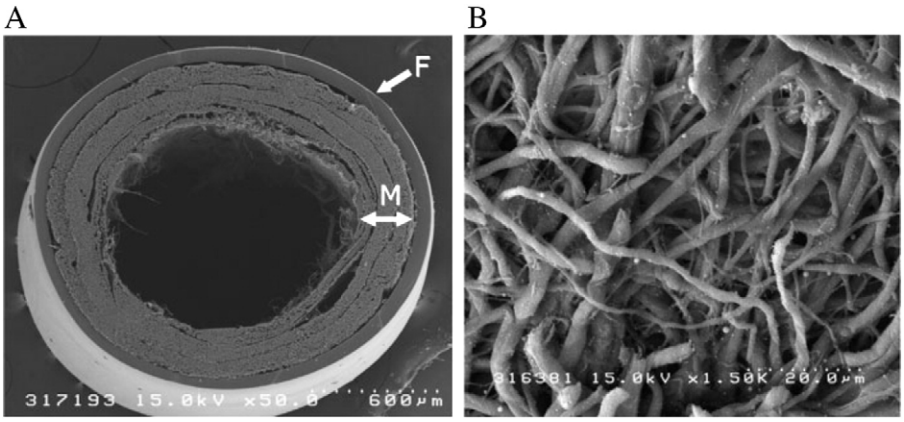

Fig. 12. SEM images of (A) a bilayered chitosan tube (F, chitosan film; M, electrospun nano/microfiber mesh) and (B) enlargement of the electrospun nano/microfiber mesh (from ref. [119] with permission).

A nanostructured composite matrix was produced from PLGA/chitin at the ratio of 80/20 (w/w) by simultaneous electrospinning [115]. PLGA nanofibers with an average diameter of 310 nm were obtained by electrospinning a 15 wt% PLGA solution in HFIP. Chitin was simultaneously electrospun in the form of nanosized particles because chitin itself was unable to produce continuous fibers, even at high concentrations. When normal human epidermal keratinocytes (NHEKs) were seeded on the nanofibrous matrix, cell adhesion and spreading were substantially improved compared with the PLGA matrices. The cellular responses of NHEKs and NHEFs to the chitin/silk fibroin blend and bicomponent nanofibrous matrices were also significantly improved [116,117]. PLGA-chitosan/PVA hybrid matrices were obtained by simultaneous electrospinning, and were considered promising for culturing human embryo skin fibroblasts [88,89]. Bhattarai et al. [79] fabricated chitosan-based nanofibers by electrospinning mixed solutions of chitosan, PEO, and Triton X-100. The fibrous matrix with a ratio of chitosan/PEO (90/10, w/w) retained its excellent structural integrity in water and promoted the attachment of chondrocytes. Wang et al. [118,119] prepared a bilayered chitosan tube composed of an outer layer of chitosan film and an inner layer of chitosan nonwoven nano/micro mesh by electrospinning (Fig. 12). The functional domain of laminin-1 (e.g., CYIGSR) was introduced to the nano/microfiber mesh surface of chitosan by covalent bonding. Nerve regeneration in animals using the chitosan tubes was efficient, similar to that of the isograft, indicating a promising scaffold for peripheral nerve repair.

An organic/inorganic composite scaffold of hydroxyapatite (HAp) and electrospun nanofibrous matrix was prepared by using chitosan/poly(vinyl alcohol) (CS/PVA) and N-carboxyethyl chitosan/PVA (CECS/PVA) electrospun membranes, and HAp was formed in supersaturated $CaCl_2$ and KH_2PO_4 solution [120]. The addition of poly (acrylic acid) (PAA) to the incubation solution appeared to improve HAp formation with uniform distribution on the membrane. Mouse fibroblasts (L929) grew on the surface of the HAp-CECS/PVA nanofibrous membrane and the cell morphology and viability were well maintained, indicating potential applications in bone tissue engineering.

Recently, electrospun nanofibrous matrices have found potential as a novel drug delivery system. Jiang et al. [121] prepared ibuprofen-loaded composite membranes composed of PLGA and PEG-*g*-chitosan by electrospinning. The presence of PEG-*g*-chitosan significantly reduced the initial burst of ibuprofen from the electrospun PLGA membranes. Moreover, ibuprofen could be conjugated to the side chains of PEG-*g*-chitosan to sustain its release for more than two weeks (Fig. 13). However, few studies have been reported until now regarding applications of chitosan nanofiber matrices in regenerative medicine. In contrast, a large number of studies have been conducted on drug delivery applications of chitosan micro or nanoparticles, likely due to its ease of fabrication.

Electrospun nanofiber membranes are considered promising for the immobilization of enzymes because of their high specific surface area and porous structure. Huang et al. [91] immobilized lipase in a nanofibrous chitosan/PVA membrane using glutaraldehyde as a coupling agent. The lipase loading on this nanofibrous membrane was up to 63.6 mg/g, and the residual activities of the immobilized lipase were more than 50% after 30 days, which indicates excellent reusability and storage stability.

Chitosan is well known to have antimicrobial activity due to its cationic nature. Poly(ethylene terephthalate) (PET) has been used in cardiovascular implants, including artificial blood vessels and artificial heart sewing rings. Jung et al. [122] fabricated electrospun chitosan/PET matrices using TFA/HFIP as a solvent, which inhibited the growth of *S. aureus* and *K. pneumoniae* much more effectively than pure PET matrices. Spasova et al. [123] investigated the effect of potassium 5-nitro-8-quinolinolate (K5N8Q) incorporated into chitosan/PEO nanofiber matrices on antimicrobial and antimycotic activity against gram negative and gram positive bacteria (*E. coli* and *S. aureus*) and fungi (*C. albicans*). Sterile zones were observed only for the electrospun matrices containing K5N8Q. Useful functionality of fibrous matrices was achieved by simple coating of the electrospun microfibrous or nanofibrous matrices with a thin chitosan film. Fibrous PLA and bicomponent PLA/PEG matrices were prepared by electrospinning and then coated with chitosan [124]. The hemostatic activity of the matrices increased with increasing amounts of chitosan. It was also

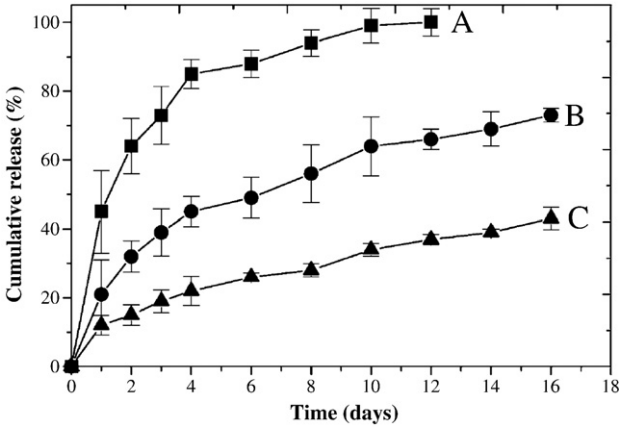

Fig. 13. Release profiles of ibuprofen from an electrospun (A) PLGA membrane (5% ibuprofen), (B) a PLGA/PEG-*g*-chitosan membrane (5% ibuprofen), and (C) a PLGA/PEG-*g*-chitosan membrane conjugated with ibuprofen (4.4% ibuprofen). Electrospun membranes were incubated in 0.1 M PBS (pH 7.4) at 37 °C (from ref. [121] with permission).

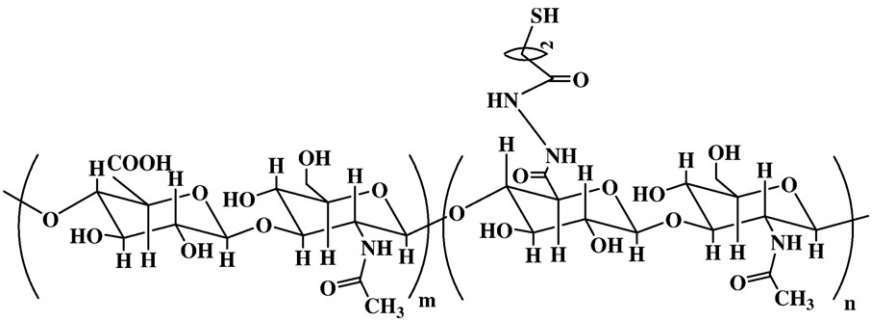

Fig. 14. Chemical structure of 3,3′-dithiobis(propanoic dihydrazide)-modified hyaluronic acid (HA-DTPH) (from ref. [129] with permission).

demonstrated that chitosan-coated hybrid matrices had anti-bacterial activity against *S. aureus*.

2.4. Hyaluronic acid

2.4.1. Introduction

Hyaluronic acid (HA) is a linear polysaccharide consisting of alternating disaccharide units of (1,4)-linked α-D-gluconic acid and (1,3)-linked β-N-acetyl-D-glucosamine. HA is a main component of the ECM of connective tissues and has various important biological functions [125]. Because of excellent biocompatibility and biodegradability, HA and its derivatives have been extensively used in biomedical areas including tissue engineering scaffolds, wound dressings, drug delivery systems, and implant materials.

2.4.2. Electrospinning and its applications

As a major component of the natural ECM, HA has also attracted a considerable amount of attention in electrospinning. Similar to alginate, it is very difficult to electrospin an aqueous HA solution because the unusually high viscosity and surface tension of an aqueous HA solution hinder the electrospinning process. In addition, the strong water retention ability of HA leads to the fusion of electrospun nanofibers on the collector due to the insufficient evaporation of the solvents during electrospinning [125]. The fabrication of HA into nanofibrous membranes from aqueous solution was successfully carried out only after the development of blowing-assisted electrospinning (electro-blowing system) [126]. HA nanofibers were fabricated using a DMF/water mixture (mean diameter = 200 nm). The use of DMF significantly decreased the surface tension without changing the viscosity of the HA solution. HA/gelatin nanofibrous matrices were also able to be produced by this method (mean diameter = 190–500 nm).

HA has been electrospun by blending it with gelatin [125,127], PEO [128,129], and zein, a major protein in corn [130]. The addition of HA improved the electrospinnability of the aqueous gelatin solution to form a gelatin/HA nanofibrous membrane [127]. A series of zein/HA blend fibrous membranes cross-linked with methylene diphenyl diisocyanate (MDI) was prepared, and the average diameter of the blend fibers increased with an increase of zein contents [130].

HA-based nanofibrous membranes have been extremely attractive as biomimetic tissue engineering scaffolds, wound healing materials, and drug delivery systems. In order to mimic the architecture of the natural ECM using electrospinning, a thiolated-HA derivative (e.g., 3,3′-dithiobis(propanoic dihydrazide)-modified hyaluronic acid; HA-DTPH) was synthesized and electrospun to form nanofibrous matrices (Fig. 14) [129]. NIH 3 T3 fibroblasts were attached to the matrix and spread with an extended dendritic morphology within the matrix, which suggests potential applications of HA-DTPH nanofibrous matrices in cell encapsulation and tissue regeneration (Fig. 15).

2.5. Others

Other polysaccharides, such as starch [131], dextran [132,133], and heparin [134–136], have also shown potential in the electrospinning process, with or without polymer additives. Starch is the major carbohydrate reserve in plants and consists of two types of molecules, amylose (normally 20–30%) and amylopectin (normally 70–80%). Amylose consists of a single linear chain of (1,4)-linked α-D-glucose units and amylopectin is formed by (1,6)-linked branching of the

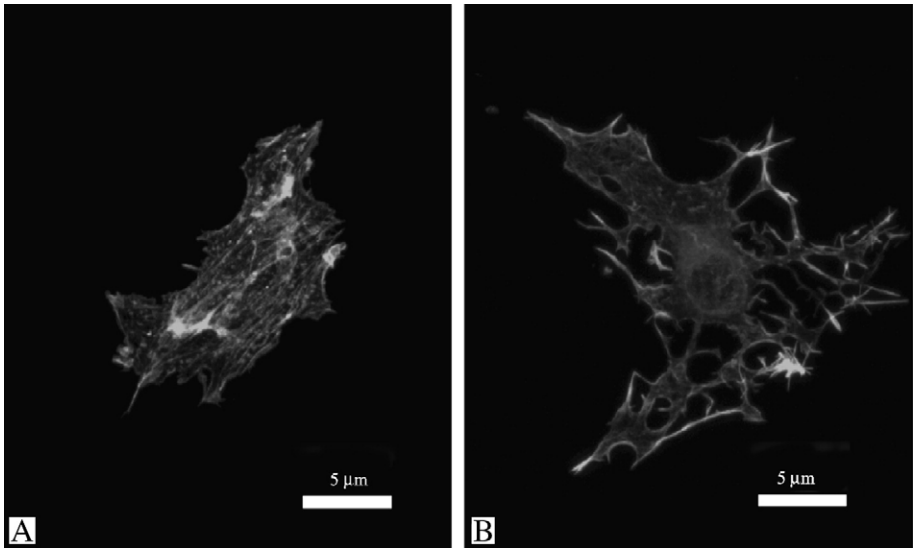

Fig. 15. Confocal microscopic images of NIH 3T3 fibroblasts on (A) a fibronectin-adsorbed glass cover slip and (B) a fibronectin-adsorbed HA-DTPH scaffold (scale bar, 5 μm) (from ref. [129] with permission).

(1,4)-linked α-D-glucose structure. Starch-based nano- and microfiber combined scaffolds (SPCL) were prepared from a starch/poly(ε-caprolactone) blend (30/70, w/w) by a fiber bonding process [131]. Human osteoblast-like cells (SaOs-2) were organized to bridge between microfibers and the presence of nanofiber enhanced spreading of the cells. Furthermore, the level of alkaline phosphatase activity of the cells cultured on the combined scaffolds increased, indicating that a combination of nano- and microfibers can provide a useful structure for cell adhesion, organization, and differentiation.

Dextran, a bacterial polysaccharide, consists of (1,6)-linked α-D-glucopyranose residues with some α-1,4 linked branches. Dextran is synthesized from sucrose by *L. mesenteroides* and *S. mutans*. Due to its biocompatible and biodegradable characteristics, dextran and its derivatives have been frequently used as blood substitutes and drug delivery carriers. Jiang et al. [133] prepared biodegradable core-shell structured fibers with poly(ε-caprolactone) as the shell and bovine serum albumin (BSA)-containing dextran as the core by coaxial electrospinning. The loading efficiency of BSA in the fibers and its release rate were increased as the feed rate of the inner dope increased.

Heparin is a highly-sulfated linear glycosaminoglycan that plays a critical role in regulating various biological activities [134] and has been widely used as an anticoagulant. The most common disaccharide unit of heparin is composed of a 2-O-sulfated iduronic acid and 6-O-sulfated, N-sulfated glucosamine residues. Degradable heparin-loaded PCL or cellulose fiber matrices were successfully fabricated by electrospinning [134,136]. Heparin was evenly distributed throughout the electrospun nanofibers. A sustained release of heparin was achieved from the nanofiber over 14 days, indicating a potential delivery system for the localized administration of heparin to the site of vascular grafts [136].

3. Conclusions

Electrospun polysaccharide nanofibers have shown great potential in many biomedical applications, including regenerative medicine. A critical future challenge of electrospun polysaccharide nanofibers may include proper selection of polysaccharides, use of mixed solvents, synthesis of various derivatives, hybrid of natural and/or synthetic polymers, fabrication of core-shell structures, blowing-assisted electrospinning, and fabrication of micro/nanofiber composites. In this review, we summarized the general characteristics and biomedical applications of polysaccharides that are currently being used or have the potential for use in electrospinning. Typically used polysaccharides are alginate, cellulose, chitin, chitosan, hyaluronic acid, starch, dextran, and heparin. Although most of these polysaccharides are of fundamental interest for electrospinning and have been found to be useful in many biomedical applications, there are still limitations to be overcome for electrospinning. In particular, one of the most difficult barriers to overcome may be the limited solubility of several polysaccharides, such as cellulose and chitin. A variety of approaches have been reported to improve the solubility, including the synthesis of derivatives and the use of mixed solvent systems. A high viscosity caused by inherently high molecular weights and electrical charges also produces poor electrospinnability of some polysaccharides, such as alginate, chitosan, and hyaluronic acid. These issues can be overcome by varying the blend ratio with other polymers and by varying the solvent composition.

Naturally occurring polysaccharides have been known to be biocompatible and safe for many biomedical applications. However, improving the surface functionality of electrospun nanofibers with bioactive molecules could be very important for specific biomedical applications. The surface chemistry, microstructure, and architecture of nanofibrous matrices significantly influence cellular adhesion, proliferation, and differentiation. Aligned nanofibers significantly induced neurite outgrowth and enhanced skin cell migration during wound healing compared to randomly oriented nanofibers. Furthermore, immobilized biochemical factors (e.g. soluble factors) significantly promoted neurite outgrowth [137]. Electrospun nanofiber matrices were chemically modified by oxygen or ammonia plasma treatment, and the adhesion and proliferation of fibroblasts seeded onto the plasma-treated matrices were significantly improved compared with non-treated ones [138]. It would also be challenging to fabricate polysaccharide-based electrospun micro/nanofiber composites for various applications in regenerative medicine, as nanofiber matrices neither provide sufficient space for cell migration within the matrices nor effective points of cell attachment [131]. Thus, composites composed of microfibers and nanofibers electrospun from synthetic polymers in the same construct have been studied [139]. In addition, the critical issue of electrospun polysaccharide nanofibers in regenerative medicine is that more animal studies may be required, and rapid progress is certainly expected by collaboration between material scientists and clinicians.

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