

Composite polysaccharide fibers prepared by electrospinning and coating

N. Maeda^{a,b}, J. Miao^{a,b,c}, T.J. Simmons^{a,b,d}, J.S. Dordick^{a,b,c}, R.J. Linhardt^{a,b,c,e,*}

^a Rensselaer Nanotechnology Center, Rensselaer Polytechnic Institute, Troy, NY 12180, USA

^b Center for Biotechnology and Interdisciplinary Studies, Rensselaer Polytechnic Institute, Troy, NY 12180, USA

^c Chemical and Biological Engineering, Rensselaer Polytechnic Institute, Troy, NY 12180, USA

^d Center for Future Energy Systems, Rensselaer Polytechnic Institute, Troy, NY 12180, USA

^e Chemistry and Chemical Biology, Rensselaer Polytechnic Institute, Troy, NY 12180, USA

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ABSTRACT

Composite polysaccharide fibers composed two oppositely charged natural polysaccharides, chitosan and hyaluronic acid, were prepared by electrospinning and subsequent coating. The fiber size distribution was characterized by scanning electron microscopy. Chitosan/hyaluronic acid composite fibers were stable in water but showed controlled release of hyaluronic acid into phosphate buffered saline, and the presence of 3-wt% hyaluronic acid coating improved the swelling ratio to 30%. The resulting composite polysaccharide fibers have a number of potential biomedical applications in wound healing applications and in drug delivery systems.

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1. Introduction

Electrospinning is a simple and widely used technique to prepare fibers of various polymers with diameters on the nanometer to micron scale. In recent years electrospinning has become a rapidly growing field of research in nanotechnology. The electrospinning of biopolymers has generated particular interest for biomedical application (Huang, Zhang, Kotaki, & Ramakrishna, 2003; Meli, Miao, Dordick, & Linhardt, 2010; Ramakrishna et al., 2006; Viswanathan et al., 2006). Biopolymers have clearly demonstrated lower toxicity, immunogenicity, and improved biocompatibility compared to synthetic polymers. However, electrospinning biopolymers remain challenging because of a lack of understanding of the fundamental reasons for electrospinnability (Bhardwaj & Kundu 2010).

Chitosan is a cationic biopolymer obtained by partial de-N-acetylation of chitin, a major component of the shells of crustaceans including crab, crawfish, and shrimp. Chitosan is biocompatible, biodegradable, non-toxic and exhibits antimicrobial activity, wound healing properties, and anti-tumor activity (Croisier &

Jérôme, 2013). Indeed, chitosan has been evaluated in many clinical studies as an accelerating agent for wound healing (Xu, Ma, Shi, Gao, & Han, 2007). Unfortunately, chitosan has inferior mechanical properties and very high swelling ratio, causing it to be easily deformed. These undesirable properties can be generally improved by blending chitosan with other polymers, including both non-ionic polymers and negatively charged anionic polymers.

Chitosan can be processed into various forms, including films, hydrogels, nanoparticles, microparticles, scaffolds, beads, and sponges (Muzzarelli, 2009), leading to a wide variety of proposed applications. Chitosan can also be formed into fibers, including nanofibers, which hold promise as materials for novel biomedical applications due to their large surface area-to-volume ratio, high porosity, and small diameter of nanofibers. In wound healing applications, high porosity allows rapid exchange of gases, wound moisturizing, and the drainage of wound fluid. Smaller diameter fibers allow for a better tissue interface that can promote healing, and since chitosan-based biomaterials accelerate wound healing, chitosan nanofibers appear particularly promising for such applications.

Electrospinning of chitosan poses many challenges because of the low solubility and high viscosity of chitosan. Previous reports have shown that chitosan nanofibers can be obtained directly from a solution of pure chitosan dissolved in concentrated acetic acid or trifluoroacetic acid (Sencadas et al., 2012). However, such solvents

* Corresponding author at: Center for Biotechnology and Interdisciplinary Studies, Biotech 4005, Rensselaer Polytechnic Institute, Troy, NY 12180, USA. Tel.: +1 518 276 3404; fax: +1 518 276 3405.

E-mail address: linhar@rpi.edu (R.J. Linhardt).

Table 1
Electrospinning conditions of chitosan (Ch)/poly(ethylene) oxide (PEO) fibers.

No.	Ch composition (w/v%)	PEO composition (w/v%)	Solvent composition	Spinnability
1	Chitosan low M_w 2.5	–	Acetic acid/H ₂ O = 40/60	–
2		PEO 900 kDa		–
3		1.5		–
4		PEO 4000 kDa		–
5		0.75		–
6		PEO 4000 kDa		Fiber
7		0.6		Fiber
8		PEO 4000 kDa		Fiber
9		0.5		Fiber
10		PEO 4000 kDa		Fiber
11		0.45		Fiber
12		PEO 4000 kDa		Fiber
13		0.4		Fiber
14		PEO 4000 kDa		Fiber
15		0.375		Fiber

are not suitable for biomedical applications because they are difficult to remove and are often toxic. In an attempt to overcome this obstacle, soluble derivatives of chitosan, such as hexanoyl chitosan, PEGylated chitosan, carboxymethyl chitosan, and quaternized chitosan have been used for electrospinning (Elsabee, Naguib, & Morsi, 2012; Jayakumar, Prabakaran, Nair, & Tamura, 2010).

Chitosan nanofibers have also been spun by blending with polymers that are known to be easily electrospun, such as poly(ethylene oxide) (PEO), poly[(L-lactide)-co-(D,L-lactide)] (PLA), poly(vinyl alcohol) (PVA), and poly(vinyl pyrrolidone) (PVP) (Ignatova, Manolova, Markova, & Rashkov, 2009; Zhang, Su, Ramakrishna, & Lim, 2008). This method can improve electrospinnability while improving the physical and mechanical properties of the resultant chitosan-containing fiber. PEO is particularly useful for blending with chitosan because of its low toxicity, excellent electrospinnability, hydrophilicity, and biocompatibility. Moreover PEO can be easily removed from electrospun chitosan fibers by washing with water.

Hyaluronic acid is a naturally occurring linear polysaccharide consisting of alternating disaccharide units of α -1,4-D-glucuronic acid and β -1,3-N-acetyl-D-glucosamine. Hyaluronic acid is the main component of the extracellular matrix surrounding all human tissues. Due to the excellent biocompatibility and biodegradability, hyaluronic acid has been widely used in biomedical applications (Liu et al., 2011).

Like chitosan, hyaluronic acid is also difficult to electrospin into nanofibers having poor processability due to its high viscosity at relatively low concentrations (Young, 2006). Due to these processing issues, there are few reports describing the electrospinning of hyaluronic acid (Brenner, Schiffman, Thompson, Toth, & Schauer, 2012; Um, Fang, Hsiao, Okamoto, & Chu, 2004). As with chitosan, hyaluronic acid is well known for accelerating wound healing in humans (Thakur, Florek, Kohn, & Michniak, 2008). This suggests that electrospinning chitosan/hyaluronic acid composite fibers may result in an improved wound-healing matrix. However, because hyaluronic acid is highly soluble in aqueous solvents, only a small surface coating of hyaluronic acid can be formed on a chitosan core when using co-axial electrospinning. Furthermore, the non-complexed hyaluronic acid is quickly dissolved by water (Ma et al., 2012). In the current study, we have developed a simple method to obtain chitosan/hyaluronic acid polyelectrolyte complexed (PEC) nanofibers using the combination of electrospinning and coating methods.

2. Experimental

2.1. Materials and methods

Low molecular weight chitosan (M_w ~50–190 kDa) and poly(ethylene oxide) (M_w = 4 MDa) were purchased from

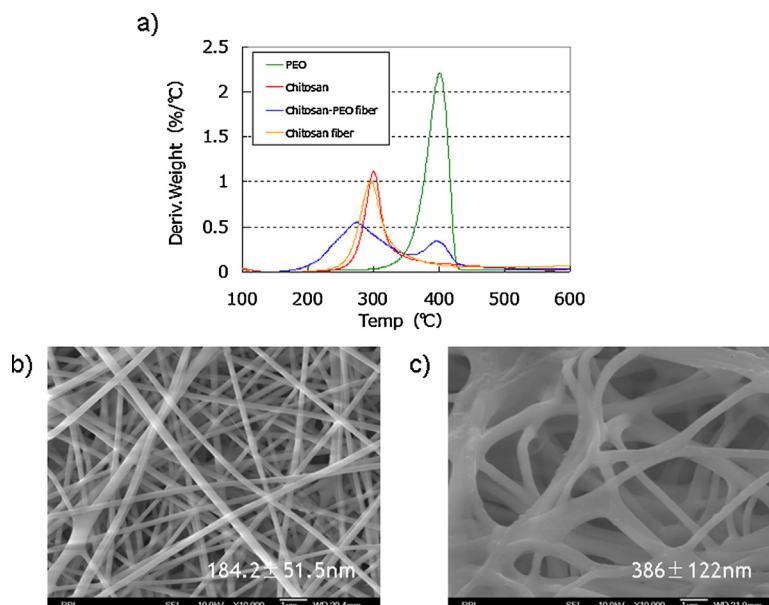


Fig. 1. (a) Thermogravimetric analysis and (b and c) SEM images showing morphologies of spun chitosan/PEO fibers and chitosan fibers after PEO extraction, respectively.

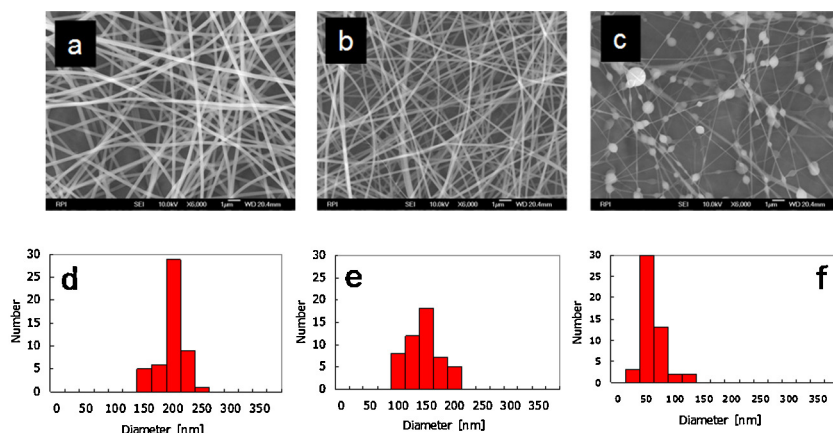


Fig. 2. The effect of humidity on spinnability and morphology of chitosan/PEO fiber. (a) 30%, (b) 50%, (c) 70%, and fiber size distribution at (d) 30% humidity, (e) 50% humidity, and (f) 70% humidity (chitosan 2.5 wt%, PEO 4000 kDa 0.4 wt%).

Sigma–Aldrich. Hyaluronic acid (sodium salt, $M_w \sim 1$ MDa) was purchased from Dali Co., China. Glacial acetic acid and ammonium hydroxide aqueous solution (28.0–30.0, NH_3 basis) were purchased from Sigma–Aldrich. Sodium chloride was purchased from Acros Organics. Dulbecco's phosphate buffered saline was purchased from Sigma–Aldrich. All chemicals and solvents were used without further purification.

2.2. Electrospinning and coating to prepare composite chitosan–hyaluronic acid PEC nanofibers

The electrospinning apparatus consisted of syringe pumps (New Era Pump Systems Inc., Hauppauge, NY), a high voltage supply (Spellman, CZE 1000R, High Voltage Electronics Corporation, Hauppauge, NY) and a spinneret made of stainless steel with an outer diameter of 1.2 mm and an inner diameter of 0.94 mm (Terumo Co., Japan) and fitted with a single 18-gauge needle to which a single needle was attached. Chitosan/PEO fibers were electrospun from acetic acid solutions, collected on grounded aluminum foil, and dried in a vacuum oven. Briefly, chitosan (low molecular weight, 2.5 wt%), PEO (4 M, 0–0.6 wt%), were individually dissolved in acetic acid/water (40/60 vol), and allowed to mix at a pre-determined ratio for 4–6 h (Table 1). This homogenous mixture was electrospun into nanofibers and then treated with 1% ammonium hydroxide aqueous solution for three hours to remove residual acetic acid.

The neutralized chitosan/PEO fibers were immersed in deionized water for 24 h to remove PEO from the fibers. The fiber mats produced were then freeze-dried overnight to remove the absorbed water. Thermogravimetric analysis was carried out to confirm the complete extraction of PEO (Fig. 1).

Various concentration of hyaluronic acid solutions (0.1, 0.3, 0.5, 1.0, and 1.5 wt%) were used for coating the chitosan fibers. Chitosan fiber mats were immersed into the hyaluronic acid solution for 24 h after which residual hyaluronic acid was washed from the fibers with deionized water. The coated fiber mats of chitosan/PEO PEC nanofibers were then freeze-dried overnight.

2.3. Scanning electron microscopy

Samples were imaged using a field emission scanning electron microscope (FE-SEM) JSM-6335 (Tachikawa, Tokyo, Japan). All samples were sputter-coated with a layer (approximately 10 Å thick) of palladium (Denton Desk II, Moorestown, NJ) prior to imaging. Images were obtained at a working distance of 15 mm using an accelerating voltage of 5–10 kV. The size distribution of chitosan

and composite fibers was analyzed by ImageJ software from the NIH.

2.4. Controlled release experiments

Each fiber mat was weighed (~ 50 mg) and immersed in 10 mL release media at 37 °C and 225 rpm. The media used was phosphate buffered saline (10 mM sodium phosphate and 137 mM sodium chloride at pH 7.4), deionized water, and 1 M sodium chloride. Supernatants of each sample were collected at different time intervals, and the amount of released hyaluronic acid was determined by a carbazole assay.

2.5. Fiber swelling

Dry fiber mats were accurately weighed and then soaked in deionized water. The wet samples were withdrawn from the water after 1 h and the excess surface water was removed by blotting gently with filter paper. The sample mats were weighed again. The following formula was used to estimate the swelling ratio at given time.

$$\text{Swelling Ratio (\%)} = ((W_s - W_d)/W_d) \times 100$$

where W_s and W_d represent the weight of swollen and dry states samples, respectively.

3. Results and discussion

3.1. Electrospinning of chitosan–PEO fiber

3.1.1. The effect of humidity

The relative humidity of the spinning environment affects both the morphology of the electrospun fibers and bead formation. The effects of the humidity on spinnability and morphology of the electrospun fibers of chitosan/PEO were studied by FE-SEM (Fig. 2). When the humidity was kept at or below 50%, stable spinnability was maintained and the obtained fibers showed a random orientation and were free of beads. The surface of the fiber was also smooth and the average diameter was <200 nm. However, when the humidity was above 50% the spinnability was unstable and the fibers showed the presence of many beads and had significantly smaller diameter.

3.1.2. The effect of PEO molecular weight and PEO concentration

The molecular weight and concentration of PEO had a significant affect on spinning conditions and spinnability. In general the

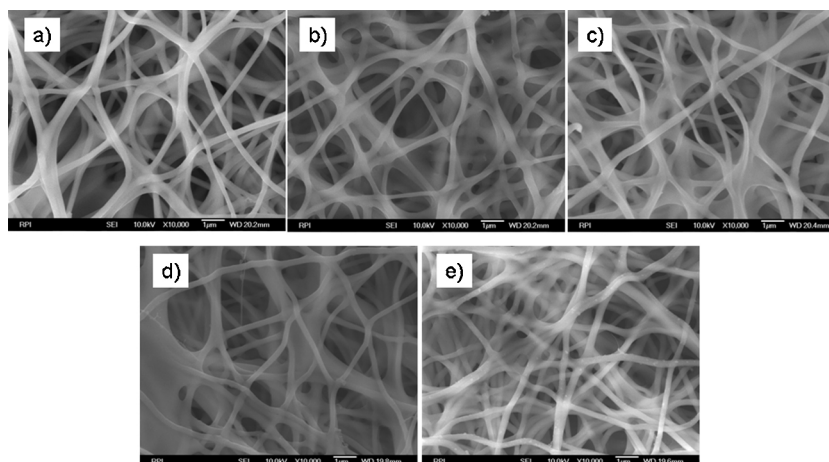


Fig. 3. The effect of hyaluronic acid concentration on morphology of chitosan/hyaluronic acid composite fibers, hyaluronic acid concentration at (a) 0.1%, (b) 0.3%, (c) 0.5%, (d) 1.0%, and (e) 1.5%.

addition of PEO improved spinnability. Above a certain molecular weight and concentration of PEO, chitosan/PEO fibers were produced. Several molecular weights and concentrations of PEO were used to examine the affect on spinnability. When the molecular weight of the PEO was 4 MDa, the minimum PEO concentration needed was 0.45 wt%. When the molecular weight of the PEO was 2 MDa, the minimum concentration for good spinnability and fiber formation was 0.75 wt%. When the molecular weight of the PEO was 900 kDa, the PEO concentration needed 1.5 wt%. These results clearly demonstrate that with higher molecular weight PEO, the minimum concentration of PEO required for spinnability is lower.

3.2. Fiber morphology

Pure chitosan did not form fibers when electrospun from acetic acid. This was due to the limited solubility of chitosan in common organic solvents, resulting in concentrations of chitosan too low to allow for the high degree of entanglement required for effective electrospinning. However, by adding PEO to chitosan solution the entanglement becomes sufficiently high to allow for effective electrospinning. Fig. 1(b) and (c) shows the morphology of chitosan/PEO fiber and chitosan fiber. The ratio of the chitosan/PEO solution was 86/14 by weight and the molecular weight of PEO was 4 MDa. The surface of the chitosan/PEO fiber was smooth and uniform with an average fiber diameter of 184 nm.

After complete extraction of PEO from chitosan/PEO fibers, which was confirmed by the absence of a derivative weight peak for PEO in thermogravimetric analysis (Fig. 1a), some portions of the fibers were stuck together due to swelling. The average diameter of the chitosan fibers increased to 386 nm, more than twice that of the chitosan/PEO fibers.

3.3. Hyaluronic acid coated chitosan fibers

Aqueous hyaluronic acid solutions (0.1–1.5 wt %) were prepared for coating of the chitosan/PEO electrospun fibers. Fig. 3 shows the SEM images of various hyaluronic acid concentrations and their effect on fiber morphologies. When electrospun chitosan fibers were coated with hyaluronic acid at concentrations higher than 0.5 wt%, some fibers join together and swell. The hyaluronic acid coating on the chitosan fibers was nearly identical for all of the hyaluronic acid concentrations tested. The increase in mass in combination with thermogravimetric analysis confirmed that hyaluronic acid had indeed been coated onto the chitosan fibers.

3.4. Controlled release from hyaluronic acid-coated chitosan fibers

The sustained release of hyaluronic acid from hyaluronic acid-coated electrospun chitosan fibers was determined in three representative solvent systems: phosphate buffered saline, sodium chloride solution, and double distilled deionized water (Fig. 4a). In sodium chloride solution the chitosan/hyaluronic acid fibers showed a burst release of hyaluronic acid. In contrast, no hyaluronic acid was released from the hyaluronic acid-coated chitosan fibers in pure water. In the phosphate buffered saline solution hyaluronic acid showed a controlled rate of release with no initial burst. These results suggest that the chitosan and hyaluronic acid form an ionic complex in the fibers. Uncomplexed hyaluronic acid would simply dissolve away in water as it is highly soluble in aqueous solutions. After 2 h in 250- μ L of phosphate buffered saline, almost 40- μ g of hyaluronic acid was released. After the release of hyaluronic acid over 2 h, some of the fibers were observed adhering to one another, but overall fiber morphology was maintained and the fiber surface remained smooth. Furthermore, both chitosan fibers and chitosan/hyaluronic acid fibers showed a reduced average fiber diameter. The chitosan/hyaluronic acid fibers exhibited less of a diameter reduction compared to the chitosan fibers. This is likely the result of the complexed hyaluronic acid had forming a gel on the surface of the fibers (Fig. 4b–e).

3.5. Chitosan/hyaluronic acid fiber swelling

The polysaccharide ionic complex of the chitosan/hyaluronic acid fiber is insoluble in water. Table 2 shows the value of the swelling ratio of chitosan/hyaluronic acid fibers in deionized water at room temperature after 1 h. Chitosan fibers showed 19.0% swelling, while chitosan/hyaluronic acid fibers showed 25% swelling (hyaluronic acid represents just 3 wt% of chitosan). The increased swelling of the chitosan/hyaluronic acid fibers demonstrates that they are more hydrophilic than the chitosan fibers. Despite this high swelling ratio, the controlled release of hyaluronic

Table 2
Swelling test on chitosan/hyaluronic acid composite fibers.

	HA coating ratio (wt%)	Swelling ratio (%)
Chitosan/HA fiber	3.8	25.2 $\pm$ 3.6
Chitosan fiber	–	18.9 $\pm$ 2.2

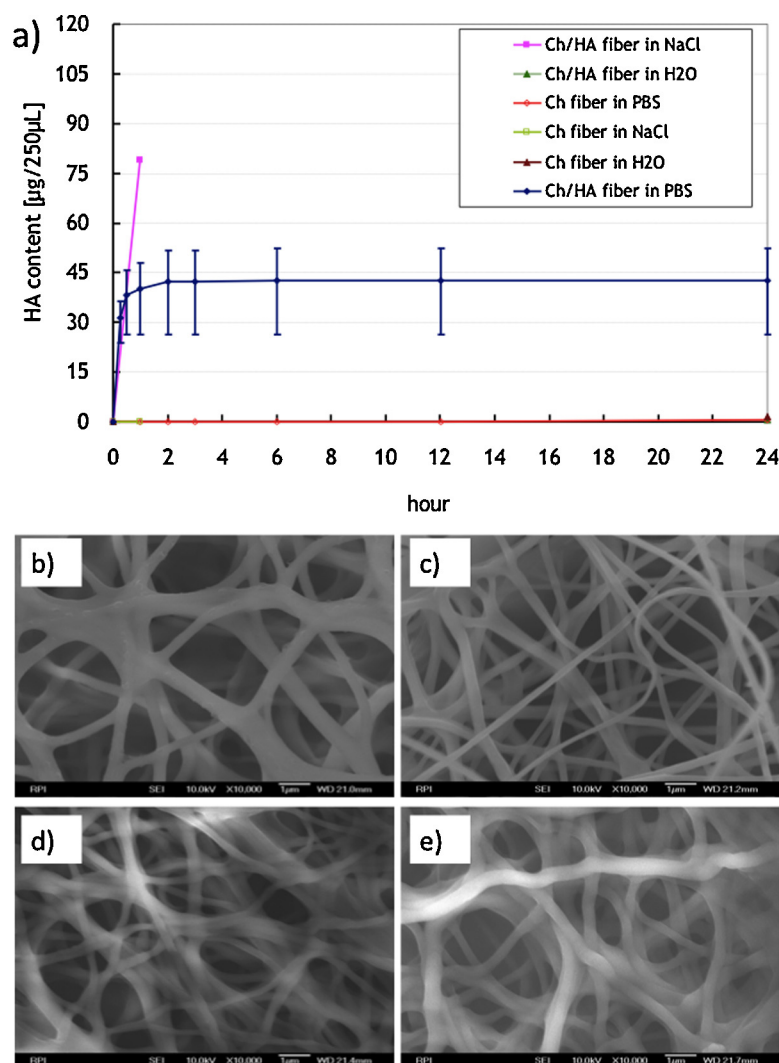


Fig. 4. Controlled release of hyaluronic acid within 24 h for chitosan/hyaluronic acid composite fiber in PBS, sodium chloride, and double distilled water. (a) SEM images for fiber morphologies, (b) chitosan (Ch) fiber, (c) chitosan fiber in phosphate buffered saline (PBS), (d) chitosan (Ch)/hyaluronic acid (HA) fiber, and (e) chitosan (Ch)/hyaluronic acid (HA) fiber in phosphate buffered saline (PBS).

acid in water revealed that chitosan/hyaluronic acid fibers are quite stable.

4. Conclusions

In this work we have demonstrated that chitosan/hyaluronic acid composite fibers can be fabricated through a simple electrospinning method followed by a coating procedure. Furthermore, less than 5 wt% hyaluronic acid coating significantly improved the swelling ratio of these fibers in water. The results presented in the current work suggest that this simple and effective method may be used to prepare many types of polyanion/polycation complex fibers. Chitosan/hyaluronic acid composite fibers show a controlled release of hyaluronic acid in phosphate buffered saline, suggesting that these chitosan/hyaluronic acid composite fibers have potential biomedical applications in wound healing and drug delivery systems.

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