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Electrospinning alginate-based nanofibers: From blends to crosslinked low molecular weight alginate-only systems

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ABSTRACT

We report here preparation of nanofibers containing alginate using two different molecular weights (MWs): 37 kDa and 196 kDa. Low MW alginates are attractive for *in vivo* tissue scaffolds where degradation and clearance from the body are desirable, whereas higher MW alginates are amenable for topical use as wound coverage because of its better mechanical properties. We use polyethylene oxide (PEO) as a carrier material to aid in electrospinning, and relate the solution properties, including entanglement concentration, relaxation time, conductivity, and surface tension, to their ability to be electrospun. In addition, we examine an FDA-approved, nonionic surfactant as a route to enhancing the alginate–PEO ratio (>80:20), and less toxic alternative to Triton X-100 surfactant. Finally, alginate-only nanofibers that are also water-insoluble are obtained by crosslinking the electrospun fibers with calcium and subsequently removing the PEO and surfactants by soaking the nanofibers in water.

1. Introduction

Electrospun nanofibers are promising materials for biomedical applications, such as drug delivery, wound dressings, and tissue scaffolds. Nanofibrous mats have high surface areas and tunable morphologies, which can influence cell proliferation and behavior (Chew, Wen, Dzenis, & Leong, 2006). Additionally, the abundance of polymers and polymer blends that can be electrospun provide researchers many options for tailoring the mechanical and biological properties for their desired application. Several synthetic polymers, such as poly(ε-caprolactone), polylactide, and polyglycolide, have been electrospun for use as tissue scaffolds (Liang, Hsiao, & Chu, 2007). However, one drawback of fibers made from these polymers is the use of cytotoxic organic solvents during fabrication, which would require thorough washing and/or solvent evaporation treatments on the mats prior to use with cells. Natural, water-soluble polymers are an attractive alternative, as they are readily soluble in aqueous media because of their hydrophilic nature, and have low immunogenicity (Lee, Jeong, Kang, Lee, & Park, 2009).

Sodium alginate is a water-soluble, biocompatible polysaccharide that is used in drug delivery (Augst, Kong, & Mooney, 2006), wound dressings (Hashimoto, Suzuki, Tanihara, Kakimaru, & Suzuki, 2004), and tissue engineering (Alsberg, Anderson, Albeiruti, Rowley, & Mooney, 2002). Ionically crosslinked alginate gels are biodegradable, a property which can be tuned by changing the composition and MW of the polymer chains. Alginate is composed of blocks of β-D-mannuronic acid (M) and α-L-guluronic acid (G). Only the G blocks in alginate can be crosslinked with divalent cations (e.g., Ca^{2+}) (Smidsrod & Skjakbraek, 1990). When used *in vivo*, ionically crosslinked alginate degrades when the calcium ions are exchanged with other ions in the body, such as Na^{+} (Shoichet, Li, White, & Winn, 1996). As such, the variation in the M to G ratio, based on the alginate source, can provide one avenue to control the degradation. However, compared to the composition of alginate, modification of the polymer MW is a more exploitable route to controlling *in vivo* degradation. Alginate chains can be shortened to lower MWs by exposure to gamma-irradiation. Low radiation doses (<8 Mrad) create chain scissions of the glycosidic bonds between the M and the G blocks, with minimal effects on the block content and length (Alsberg et al., 2003). When used as tissue scaffolds, ionically crosslinked low MW alginate (<50 kDa) degrades more quickly than higher MW. Shorter degradation times may be more optimal for some tissue regeneration applications that want to match tissue formation with polymer degradation rate (Alsberg et al., 2003). One

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significant advantage of low MW polymer chains is that polymers with MW less than 50 kDa can be passed by the kidneys when used *in vivo* (Al-Shamkhani & Duncan, 1995). Thus, nanofibers composed of low MW alginate are attractive for *in vivo* biomedical applications where degradation and clearance from the body are desirable.

The use of low MW polymers has important implications when developing material fabrication methods. In particular, electrospinning is influenced by polymer chain length, among many other variables (Shenoy, Bates, Frisch, & Wnek, 2005). Polymer chain entanglements in solution are essential to the formation of a continuous jet, leading to uniform, nonwoven fibers. Therefore, polymer solutions with inadequate entanglements, such as those in dilute concentrations or with low viscoelasticity or MW, may form only beaded fibers or droplets (McKee, Wilkes, Colby, & Long, 2004; Yu, Fridrikh, & Rutledge, 2006). These added challenges have likely prevented the widespread usage of low MW polymers in electrospinning. In addition, ionic conductivity and surface tension have significant effects on the electrospinning process (Fong, Chun, & Reneker, 1999; McKee et al., 2004; Yu et al., 2006). The ability of a polymer solution to form uniform nanofibers can be linked directly to its solution properties.

Nanofibrous mats of alginate can be fabricated by electrospinning. Even though attempts to electrospin pure alginate in water have been unsuccessful, alginate solutions have been blended with a second material (i.e. glycerol, polyvinyl alcohol (PVA), polyethylene oxide (PEO)) to successfully form nanofibers (Bhattarai, Li, Edmondson, & Zhang, 2006; Jeong et al., 2010; Lu, Zhu, Guo, Hu, & Yu, 2006; Nie et al., 2008, 2009; Safi, Morshed, Ravandi, & Ghiaci, 2007). The second co-solvent/polymer is believed to mitigate the charge repulsions between the alginate chains, improve chain flexibility, and create hydrogen bonds (Caykara, Demirci, Eroglu, & Guven, 2005; Nie et al., 2008). Blends of alginate with PEO have shown promise among the alginate-based solutions electrospun. The ratio of alginate to PEO in the electrospun fiber has been maximized to 80:20 with the addition of Triton X-100, a PEO-based non-ionic surfactant, and dimethyl sulfoxide (DMSO) (Bhattarai et al., 2006; Bhattarai & Zhang, 2007). The addition of the surfactant and organic solvent resulted in bead-free fibers, most likely from a reduction in surface tension. However, the study did not fully investigate the role of solution properties on electrospinnability. In fact, many studies that have electrospun alginate–PEO blends focused extensively on the morphology and properties of the nanofiber mats, whereas little work has been done to relate the pre-electrospinning alginate properties to the resultant nanofibers. The ability to relate solution properties to nanofiber formation will aid in the understanding of electrospinning alginate nanofibers, and can be extended to other polymer blends containing polyelectrolytes. One of the objectives of our study is to characterize the rheological and solution properties of blends containing high (196 kDa) or low (37 kDa) MW alginate and PEO, and relate these to the electrospun fiber morphology. While Bhattarai and Zhang reported to have electrospun different molecular weights of alginate that were obtained by sample degradation at room temperature, their study focused on solution viscosities and did not characterize MWs (Bhattarai & Zhang, 2007). Polymer chain lengths of the low MW alginate in our study were shortened by gamma-irradiation, which is a more controlled method than sample degradation. To the best of our knowledge, we are also the first to demonstrate the fabrication of nanofibers composed entirely of low MW alginate.

In addition to the MW of alginate, we also report methods to minimize the cytotoxicity involved in preparing the electrospun mats through the use solely of biocompatible materials. While some groups have previously shown the potential for alginate-based fibers as cell scaffolds, they used Triton X-100 and/or DMSO, which have possible cytotoxicity issues (Bhattarai et al., 2006; Dayeh, Chow, Schirmer, Lynn, & Bols, 2004; Rubin, 1975; Saquing, Manasco, Bonino, & Khan, 2008). In this study, we compare alginate–PEO blends containing Triton X-100 (without DMSO) to those containing Pluronic F127, an FDA-approved surfactant. Both Triton X-100 and F127 have hydrophilic PEO blocks. However, the hydrophobic polypropylene oxide (PPO) block is less toxic than the alkyl benzene block on Triton, one reason that Pluronic F127 is a viable material for biomedical applications (Khattak, Bhatia, & Roberts, 2005; Ren, Marquardt, & Lech, 1997; Sluzky, Klibanov, & Langer, 1992). After electrospinning, the alginate-based nanofibers are ionically crosslinked in a calcium solution, without the need for cytotoxic chemical crosslinkers (Birnbaum, Pendleton, Larsson, & Mosbach, 1981; Huanglee, Cheung, & Nimni, 1990). Subsequent removal of PEO via soaking in water leads to alginate-only nanofibers that retain their morphological integrity. An alginate variety with a high ratio (66%) of G blocks was selected to maximize crosslink density. The use of such biocompatible polymers, solvents, and crosslinkers therefore readily permits the fabrication of nanofibrous mats as scaffolds for potential use in regenerative medicine or drug delivery applications.

2. Materials and methods

2.1. Materials

Sodium alginate was obtained from FMC Biopolymers (Princeton, NJ) (M_W = 196 kDa, M_W/M_n = 1.6, 66:34 G:M blocks) and used as received. Low MW alginate (M_W = 37 kDa, M_W/M_n = 1.5) was prepared from the high MW by gamma irradiation. Dry alginate was irradiated with a 5 Mrad dose at 25 °C (Phoenix Laboratory, University of Michigan, Ann Arbor, MI), following a previously reported procedure (Alsberg et al., 2003). Additionally, polyethylene oxide (Polysciences, Warrington, PA, PEO, M_W = 600 kDa), polyethylene glycol (Polysciences, Warrington, PA, PEG, M_W = 35 kDa), Triton X-100 (Sigma Aldrich, St. Louis, MO), and Pluronic F127 (Sigma Aldrich, St. Louis, MO) were also used. Solutions were prepared by making separate solutions of PEO (4 wt%) or PEG (40 wt%) and 196 kDa (4 wt%) or 37 kDa (13.5 wt%) alginate in deionized water, and then combining with the surfactant. Solution ratios of up to 2.8:1.2:2.0 (196 kDa alginate:PEO:surfactant) and 8.0:1.6:2.0 (37 kDa alginate:PEO:surfactant) were investigated. Solutions were mixed overnight at room temperature with a magnetic stir bar. Crosslinking treatment of electrospun fibers used ethanol (reagent grade, Sigma Aldrich, St. Louis, MO) and calcium chloride (Sigma Aldrich, St. Louis, MO).

2.2. Solution characterization

Rheological experiments were performed with a TA Instruments (New Castle, DE) AR2000 stress-controlled rheometer with a 40 cm, 2° cone and plate geometry. All samples were measured at 25 °C. Dynamic and steady state shear experiments were conducted on each sample. The stresses applied in the frequency sweeps were selected from the linear viscoelastic (LVE) regime in the stress sweeps. All rheological measurements were repeated on at least two different samples to ensure repeatability within ±5%. Ionic conductivity measurements were made using a potentiostat from Gamry Instruments (Warminster, PA). Surface tensions were determined using a pendant drop analyzer from SEO Co. Ltd (model Phoenix 300, Lathes, South Korea).

2.3. Preparation of alginate nanofibers

The electrospinning setup consisted of a syringe pump (model NE-1010, New Era Pump Systems, Inc., Wantagh, NY), high voltage power supply (model AU-60P0.5, Matsusada Precision, Inc.

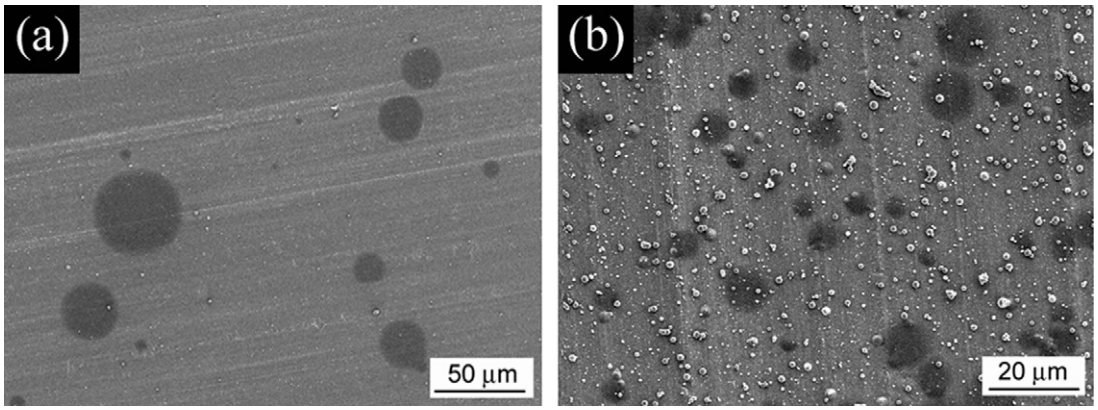

Fig. 1. Scanning electron micrographs of electrospun droplets of alginate (a) and alginate/Triton X-100 (b), both without PEO. Fibers were not seen with all aqueous alginate solutions investigated, regardless of the addition of a surfactant.

Kusatsu-City Japan), and collector plate covered in Reynolds Wrap non-stick aluminum foil for easy removal of mats. The polymer solution was pumped through a syringe with a 22 gauge needle at rates of 0.50–0.75 mL/h. The distance from the end of the needle to the collector plate was fixed at 15 cm. The voltage was varied from 10 to 15 kV until a stable Taylor cone was achieved, and then maintained at a constant level (Taylor, 1964). All nanofibers were made at room temperature (21–24 °C) and relative humidity 40–55%.

2.4. Crosslinking of alginate nanofibers

After electrospinning, nanofiber mats were removed from the collector plate and ionically crosslinked. Mats were soaked in ethanol (1 min), followed by a calcium chloride solution (2 wt%) in 1:5 ethanol:water (10 s). Finally, the mats were rinsed in water (1 min). The crosslinked fibers were submerged in water for up to four days, without agitation, at room temperature to confirm their stability. After soaking, mats were dried in a lyophilizer (VirTis 10-324 lyophilizer, Gardiner, NY) overnight.

2.5. Characterization of nanofibers

Nanofiber mats were analyzed with a scanning electron microscope (SEM). A FEI XL30 field emission SEM was used at the following settings: 6 mm working distance, 5 kV accelerating voltage, spot size 3, and ultrahigh resolution mode. Fiber diameters and standard deviations on 50 fibers per sample were measured using Adobe Photoshop C3. Spectra of stand alone fiber mats without salt windows were collected from 4000 to 400 cm^{-1} with 4 cm^{-1} resolution and 512 scans using a Fourier Transform Infrared (FTIR) Spectrometer (model Nicolet 6700, Thermo Electron Corp.) in the transmission mode.

3. Results and discussion

3.1. Solution rheology and electrospinning alginate

Our initial attempt at electrospinning pure alginate nanofibers focused on solutions containing alginate concentrations spanning two decades (0.04–5.0 wt% for 196 kDa alginate, and 0.15–15 wt% for 37 kDa alginate). Attempts to electrospin either MW resulted in beads or droplets but no nanofibers as shown in Fig. 1 for two representative samples. Other groups have also reported similar results when attempting to electrospin aqueous alginate solutions (Bhattarai et al., 2006; Nie et al., 2008, 2009; Safi et al., 2007). An explanation proposed by these groups has been the inability of alginate to form chain entanglements.

Solution rheology has been suggested to be a good indicator of the range of polymer concentration appropriate for electrospinning as well as determining the entanglement concentration. McKee et al. reported that neutral and charged polymer solutions could be electrospun into uniform fibers at concentrations 2–8 times

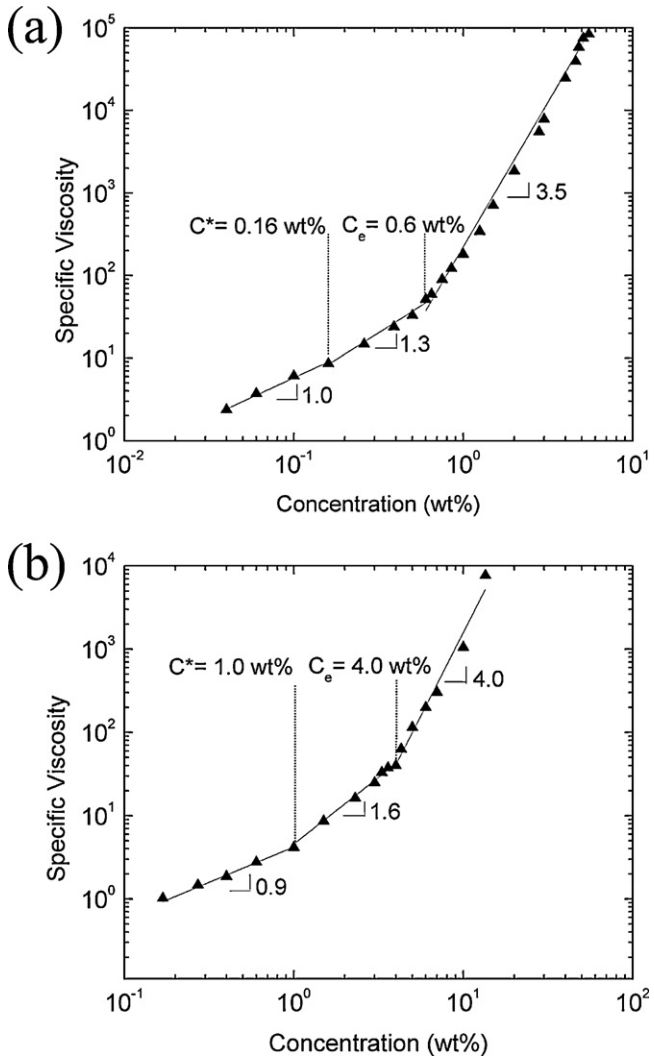

Fig. 2. Dependence of specific viscosity (η_{sp}) on concentration for (a) 196 kDa and (b) 37 kDa aqueous sodium alginate solutions. The entanglement concentrations (C_e) are 0.6 and 4 wt%, respectively.

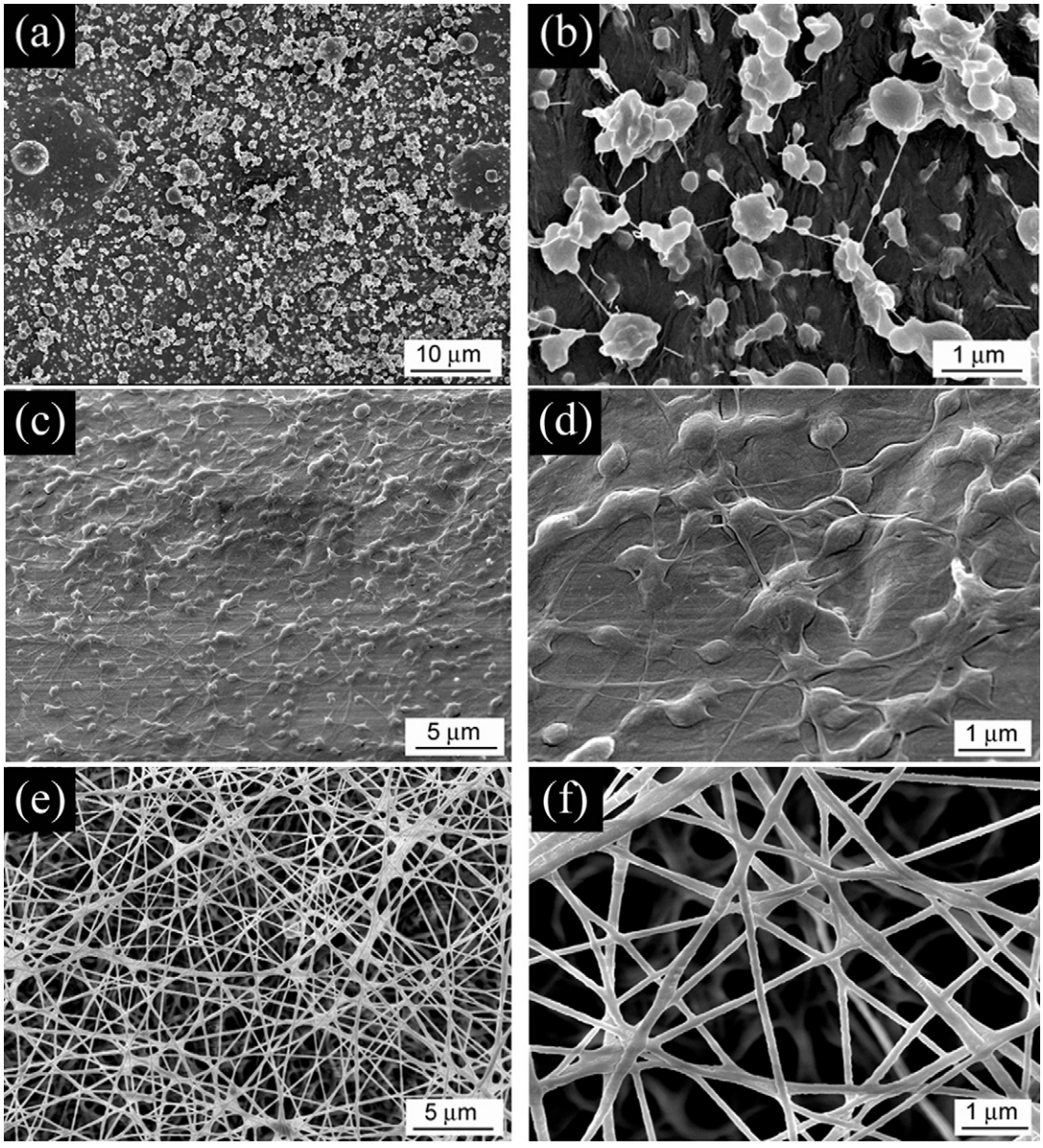

Fig. 3. Scanning electron micrographs of electrospun droplets of alginate–PEG blended (2.0:20.0 wt%) solution (a) and (b). PEG (35 kDa) is not an effective carrier polymer for alginate, due to its low MW and inability to be electrospun. Also shown are blended nanofibers prepared from alginate–PEO (600 kDa) (70:30 by wt) solutions. Beaded fibers (c) and (d) were made without surfactant, whereas uniform fibers (e) and (f) contained 1 wt% Triton X-100.

above the entanglement concentration (McKee et al., 2004; McKee, Hunley, Layman, & Long, 2006). To estimate the entanglement concentrations of our sample, the viscosity-shear stress behavior was measured for polymer concentrations that spanned two decades. Fig. 2 shows the specific viscosity ($\eta_{sp} = (\eta_o - \eta_s)/\eta_s$, where η_o is the solution zero shear viscosity and η_s the solvent viscosity) of these samples as a function of concentration (C). Several pieces of information can be obtained from these plots. First, there are two distinct changes in slope, corresponding to the overlap (C^*) and entanglement (C_e) concentrations. Alginates with MW 37 (Fig. 2a) and 196 (Fig. 2b) kDa were found to have entanglement concentrations (C_e) of approximately 4.0 and 0.6 weight percent in water solutions, respectively. Entanglement concentrations decrease with increasing polymer MW, as a result of the longer polymer chain lengths (Rubinstein & Colby, 2003). We also find that the entanglement concentrations of alginate solutions occur at zero shear viscosities ~0.05 Pa s, or $\sim 50 \times \eta_s$ which is consistent with theoretical predictions (Dobrynin, Colby, & Rubinstein, 1995). In addition, the slopes of the curves match well with scaling predictions of neutral polymers in good solvent: $\eta_{sp} \sim C^{1.0}$ for the dilute regime ($C < C^*$), $\eta_{sp} \sim C^{1.25}$ for the semidilute unentangled regime ($C^* < C < C_e$,), and, $\eta_{sp} \sim C^{3.75}$ for the semidilute entanglement regime ($C_e < C < C^{**}$) (Dobrynin et al., 1995). (C^{**} is the concentrated regime.) We speculate that the alginate solutions act as neutral polymers, instead of charged polyelectrolytes, due to counterions (e.g., Na^+) that effectively screen the carboxylic acid groups on alginate (Krause, Bellomo, & Colby, 2001). Purification of the alginate may be necessary to reduce counterions and cause alginate solutions to follow the scaling predictions of polyelectrolytes (Rubinstein, Colby, & Dobrynin, 1994). However, Nie et al. recently reported that concentration plots of unpurified alginate solutions matched charged polymer models (Nie et al., 2009). We hypothesize that the concentration of counterions varies with the source and distributor of alginate. Variation in the solution behavior of alginate based on its source is an important finding to consider for future materials research with the biopolymer.

Table 1a
Properties of electrospun solutions and observations of nanofiber morphology.

Sample[a]	Alginate	Alginate/Triton[b]	Alginate/PEO[c]	Alginate/PEO/Triton[b,c]
Zero shear visc (Pa s)	22.0	21.9	14.3	14.3
Relaxation time (s)	0.06	0.05	0.05	0.04
Conductivity (mS/cm)	7.15 ± 0.07	6.80 ± 0.07	4.79 ± 0.05	4.62 ± 0.05
Surface tension (mN/m)	63 ± 2	29 ± 1	55 ± 2	29 ± 2
Electrospun fibers	Droplets	Droplets	Beaded fibers	Uniform fibers

[a] Total polymer concentration was maintained at 4 wt%.
[b] Concentration of Triton X-100 was 1 wt%.
[c] Concentrations of alginate and PEO were 2.8 and 1.2 wt% (70:30 by wt), respectively (means ± standard deviations are reported).

In addition to solution behavior predictions from the concentration curves, we also found that alginate does in fact have an entanglement concentration well below the solution concentrations attempted for electrospinning. Our efforts to electrospin aqueous alginate solutions in concentrations up to 5.5% or $\sim 9 \times C_{ent}$ were unsuccessful, suggesting that chain entanglement is not the dictating factor for alginate solutions to electrospin (Fig. 1). It should be noted that strain rates at the transition between the Taylor cone and the jet during the electrospinning process are high (Han, Yarin, & Reneker, 2008); however, the zero shear viscosity provides information on chain entanglement of a system.

The inability of a polymer solution to be electrospun can often be overcome by blending it with another polymer. PEO has been blended with charged biopolymers, such as chitosan, or low MW polymers, such as polyethylene glycol (PEG), that are incapable of being electrospun alone to generate nanofibers (Klossner, Queen, Coughlin, & Krause, 2008; Yu et al., 2006). Recently, blends of sodium alginate and PEO have been electrospun (Bhattarai et al., 2006; Jeong et al., 2010; Lu et al., 2006; Safi et al., 2007). PEO and alginate chains can interact by hydrogen bonding, which contributes to their compatibility in solution (Caykara et al., 2005; He, Zhu, & Inoue, 2004). However, hydrogen bonding between alginate and its carrier polymer alone does not allow for a blend to be electrospun. To demonstrate this, attempts were made to electrospin blends of alginate and 35 kDa PEG (2.0:20.0). The solutions electrosprayed, but did not form continuous fibers (Fig. 3). We hypothesize that the chain length of the carrier polymer also plays a role in the electrospinning of alginate.

3.2. Effect of Triton X-100 surfactant on alginate/PEO blends

While blended nanofibers can be formed from electrospinning alginate and PEO, the amount of alginate in the blend can be increased with the addition of small amounts of surfactants (Bhattarai & Zhang, 2007). Initially, the effects of the nonionic surfactant Triton X-100 on the solution properties of alginate–PEO blends and on the morphology of the resultant nanofibers were investigated. Alginate–PEO solutions were maintained at a total concentration of 4 wt%, because PEO (600 kDa) can be electrospun at this concentration (data not shown). We find that alginate–PEO blends at a ratio of 70:30 by weight produce beaded nanofibers (Figs. 3c and d). However, the addition of small amounts (1 wt%) of Triton X-100 to the sample, generates bead-free fibers (Fig. 3). Surfactants lower the surface tension of the polymer solution, which suppresses bead defects (Fong et al., 1999). Table 1a shows the various properties of polymer solutions containing alginate and PEO. Alginate and PEO have limited surface activities and reduce the surface tension of water to 55 mN/m. The addition of Triton X-100 causes the surface tension of the polymer blend to decrease to 29 mN/m, which possibly contributes to the morphological transition from beaded to uniform fibers. However, it is important to note that reducing the surface tension of alginate solutions with surfactants did not lead to uniform fibers (Fig. 1b). Alginate solutions, regardless of added surfactants, that were not blended with PEO formed droplets (Saquing et al., 2008). Thus, reducing surface tension enhances the electrospinnability of alginate solutions for systems that have reached the onset of fiber formation.

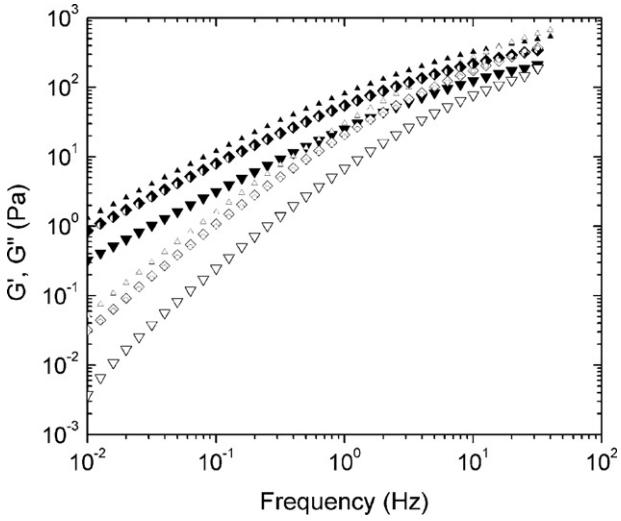

Fig. 4. Elastic (G') and viscous (G'') moduli as a function of frequency for 2.8 wt% (▼, ▽) and 4.0 wt% alginate (▲, △), and blends containing alginate/PEO/Triton X-100 (2.8:1.2:1.0 wt%) (◆, ◇) and alginate/PEO (2.8:1.2 wt%) (◑, ○).

The roles of other solution properties that could impact the electrospinnability of the alginate/PEO solutions were also examined. Rheological properties, such as zero shear viscosity and relaxation time have been known to affect fiber morphology (Talwar, Hinestroza, Pourdeyhimi, & Khan, 2008; Yu et al., 2006). However, we found that the addition of PEO did not have a significant effect on the solution relaxation time, as determined by dynamic oscillatory measurements. Solutions of alginate and alginate/PEO/Triton blends with similar zero shear viscosities also have similar moduli and relaxation times (Fig. 4). Additionally, the ionic conductivity of the alginate-only solutions was reduced after blending with PEO, thereby diluting its total solution concentration, showing that alginate is the greatest contributor of the solution conductivity due to its anionic nature in water. As expected, the addition of a nonionic surfactant did not affect the conductivity. These results (Figs. 3 and 4 and Table 1a) taken together indicate that the surface tension is the most important factor in electrospinning these polymers and that the additions of both PEO and Triton X-100 are necessary to generate nanofibers with up to 70% (relative to PEO) or 2.8 wt% alginate.

3.3. Role of biocompatible F127 nonionic surfactant

With the potential uses for alginate-based nanofibers in biomedical applications, there is strong interest in using only non-cytotoxic materials. Therefore, Triton X-100 was replaced with an

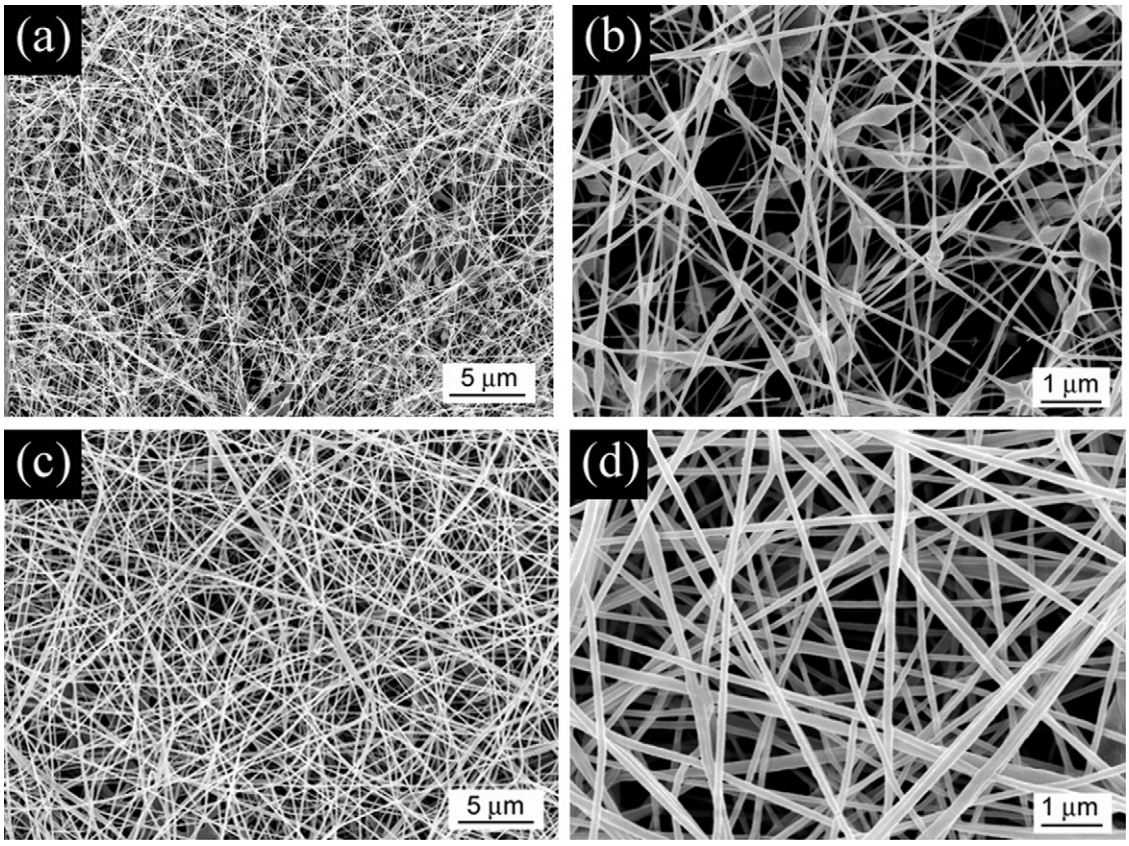

Fig. 5. Scanning electron micrographs of nanofibers composed of alginate and PEO (2.4:1.6 wt% or 60:40) without (a and b) and with Pluronic F127 (2 wt%) (c and d).

Table 1b
Properties of electrospun solutions containing Pluronic F127 and observations of nanofiber morphology.

Sample[a]	Alginate	Alginate/F127[b]	Alginate/PEO[c]	Alginate/PEO/F127[b,c]
Zero shear visc (Pa s)	22.0	21.5	14.0	15.5
Relaxation time (s)	0.06	0.06	0.05	0.06
Conductivity (mS/cm)	7.15 ± 0.07	7.04 ± 0.07	4.10 ± 0.04	3.53 ± 0.05
Surface tension (mN/m)	63 ± 2	35 ± 1	57 ± 1	36 ± 1
Electrospun fibers	Droplets	Droplets	Beaded fibers	Uniform fibers

[a] Total polymer concentration was maintained at 4 wt%.
[b] Concentration of Pluronic F127 was 2 wt%.
[c] Concentrations of alginate and PEO were 2.4 and 1.6 wt% (60:40 by wt), respectively (means ± standard deviations are reported).

FDA-approved surfactant, Pluronic F127. Using the same procedure as discussed in the previous section, alginate–PEO blended solutions were characterized and electrospun. As shown before with Triton X-100, the addition of Pluronic F127 effectively suppressed bead defects (Fig. 5). Blends of alginate and PEO were electrospun with minimal beads at weight ratios up to 60:40 (2.4:1.6 wt%). The formation of fibers with minimal bead defects were primarily due to the decrease in surface tension, since the rheology and ionic conductivity of blends with and without surfactant were similar. As shown in Table 1b, the addition of F127 could not match the same maximum amount of alginate for bead-free fibers as with Triton X-100 (70% by weight). This is because the surface tension of F127 solutions is 35–36 mN/m compared to 29 for Triton solutions. Pluronic is a triblock copolymer (PEO–PPO–PEO) and is expected to have lower packing density at the water–air interface compared to Triton X-100 (Hiemenz & Rajagopalan, 1997). However, the use of an FDA-approved surfactant in electrospun solutions as a method to enhance the alginate loading fiber morphology is an attractive option.

3.4. Electrospinning low MW alginate

From a materials perspective, low MW polymer chains are less desirable to use in electrospinning due to limited entanglements. However, from a tissue engineering perspective, small polymer chain lengths have a faster rate of *in vivo* degradation than high MW chains and can be cleared by the kidneys (Al-Shamkhani & Duncan, 1995; Alsberg et al., 2003). In this study, we attempted to electrospin irradiated alginate (M_W = 37 kDa). As observed previously with the high MW alginate, solutions containing only low MW alginate in concentrations >3 × C_e did not electrospin.

The inability to electrospin low MW alginate was overcome by blending it with PEO and Pluronic F127 surfactant, using the same approach as with the high MW alginate. However, compared to the high MW alginate case, the alginate content was raised from 2.8:1.2 to 8.0:1.6 (by polymer wt% relative to PEO) using the low MW polymer. A shorter chain length polymer requires a greater concentration to achieve comparable solution viscosities to a high MW polymer. Despite a greater concentration of alginate (8 wt%)

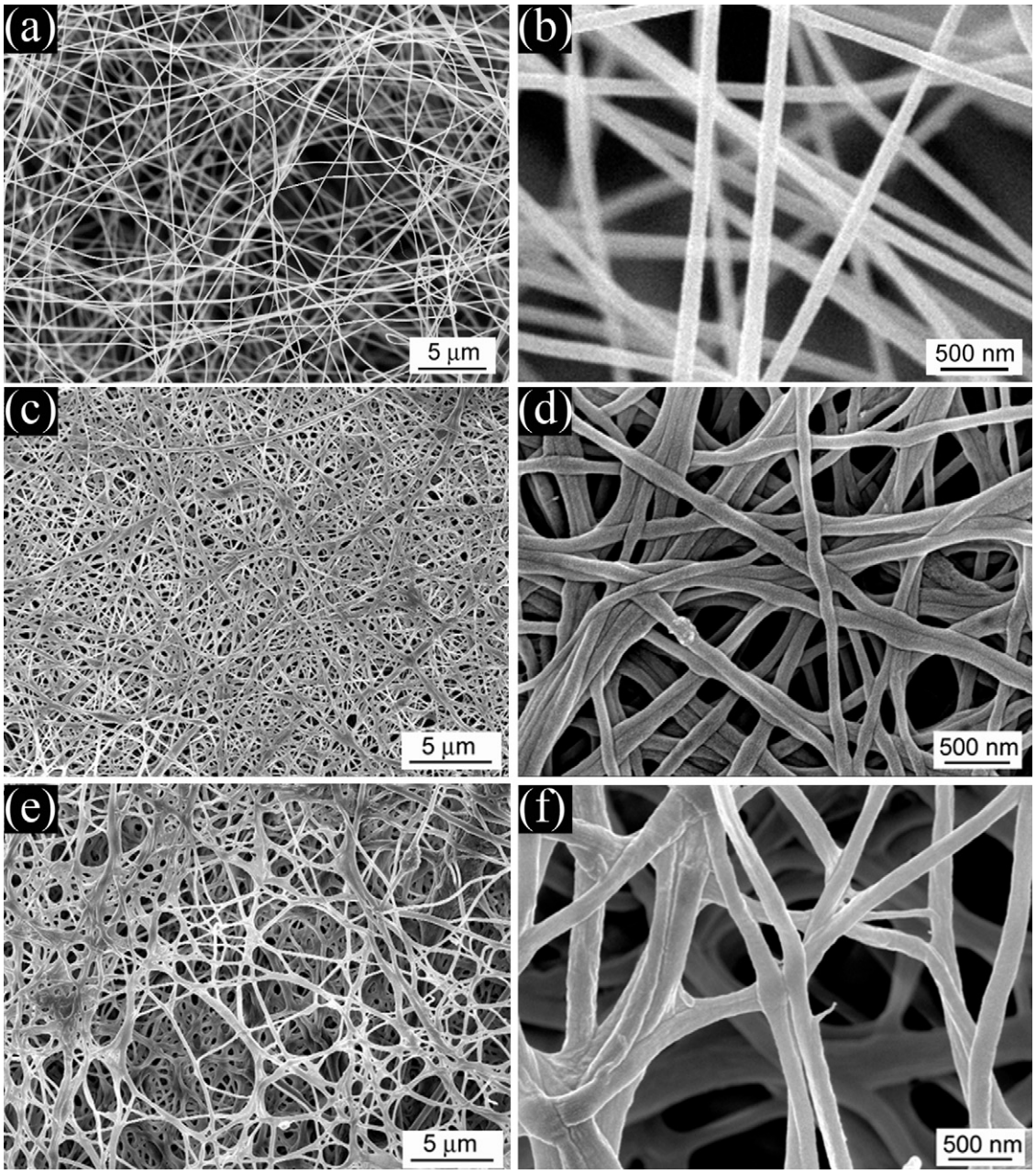

Fig. 6. Scanning electron micrographs of nanofibers containing 37 kDa alginate/PEO/Pluronic F127 (8.0:1.6:2.0 wt%) (a and b) prior to crosslinking. Nanofibers composed of 196 kDa (c and d) and 37 kDa (e and f) alginate after crosslinking with calcium and soaking in water for 4 days to remove PEO.

for the low MW solution, the zero shear viscosity was less than the high MW alginate solution (2.8 wt%) (2.3 vs. 14.5 Pa s, Supplemental Fig. 1). In addition, the longest relaxation time of the low MW solution was an order of magnitude less than the high MW solution. Polymers with short chain lengths have fewer entanglements than high MW chains, and relax sooner (Rubinstein & Colby, 2003).

Uniform nanofibers containing low MW alginate, PEO, and F127 surfactant were obtained (Fig. 6a and b). To our knowledge, we are the first to prepare alginate-based nanofibers from a low MW alginate. The average fiber diameters were ~150 nm, the same size as fibers with high MW alginate. Thus, the final fiber diameter was not dependent on solution composition of the concentrations investigated, despite using a solution with a low MW polymer as the majority species in the blend. We speculate the consistency in the solution and processing conditions (solution viscosity, temperature, humidity) contributed to similarities in the fiber sizes between the two different MW solutions (Tripatanasuwan, Zhong, & Reneker, 2007; Yu et al., 2006). Electrospinning blends containing low MW alginate reduced the amount of PEO as the inert, carrier polymer, thereby allowing for a greater polysaccharide component, which is of greater interest when these nanofibers are used as tissue scaffolds. In our case, we achieved nanofibers with alginate/PEO ratio of 8.0/16, i.e., with over 83% alginate in the blend. In addition, the complete removal of PEO (M_W 600 kDa) from the nanofibers would leave only the low MW material, which would be capable of renal clearance if used *in vivo*.

3.5. Crosslinked alginate-only nanofibers

Prior to use as tissue engineering scaffolds, alginate-based materials need to be crosslinked to preserve their structure in an aqueous environment. Ionic crosslinking with divalent ions (e.g. Ca^{2+}) avoids

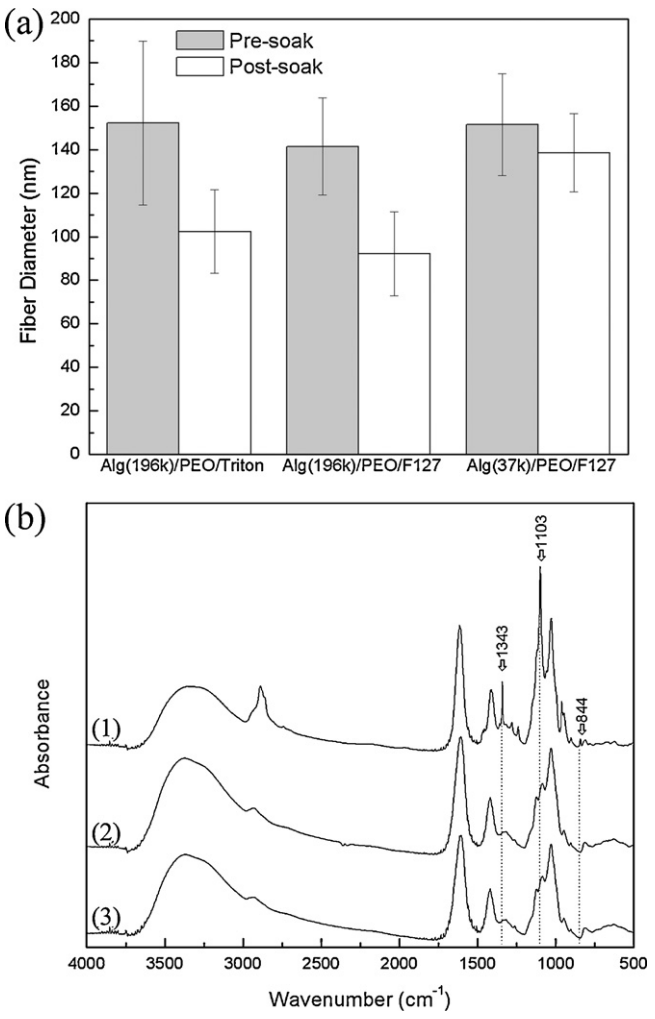

Fig. 7. Fiber diameters (a) of alginate (196 kDa)/PEO/Triton X-100 (2.8:1.2:1.0), alginate (196 kDa)/PEO/F127 (2.4:1.6:2.0), and alginate (37 kDa)/PEO/F127 (8.0:1.6:2.0) before and after crosslinking and soaking in water. (Error bars = 1 standard deviation.) FTIR spectrographs (b) of nanofibers containing 37 or 196 kDa MW alginate and PEO before (37 kDa (1)) and after (37 kDa (2), 196 kDa (3)) soaking. PEO peaks at 844, 1102, and 1342 cm^{-1} disappear after soaking.

potentially toxic materials used in chemical crosslinking reactions (Birnbaum et al., 1981; Huanglee et al., 1990; Smidsrod & Skjakbraek, 1990). The alginate used throughout these studies has a high G–M block ratio (66–34) to increase ionic crosslinking sites. We determined that the fiber morphology could be best maintained using ethanol in a pre-crosslinking treatment and as part of the crosslinking solution. Mats were soaked in ethanol first, followed by a calcium chloride solution (2 wt%) in 1:5 ethanol:water, as delineated in the methods section. PEO is soluble in ethanol, but alginate is not, which prevents the alginate from being dissolved into solution before it is effectively crosslinked. After crosslinking, the nanofiber mats were soaked in water to test their structural and durability. The nanofibers composed of both high and low MW alginates were shown to remain intact after exposure to water (Fig. 6c–f).

Soaking fibers in water to remove PEO reduced fiber diameters, which was dependent on fiber composition. Diameters of fibers containing blends of high MW alginate (2.4–2.8 wt%) and PEO (1.2–1.6 wt%) shrunk by ~34% (Fig. 7a). In comparison, fiber blends containing the low MW alginate (8.0 wt%) and PEO (1.6 wt%) decreased by only ~9%, due to the smaller concentration of PEO. Minimal changes in fiber diameter after PEO removal may help maintain the fiber mat integrity, which is another advantage of using the low MW blend. Future work with mechanical testing will evaluate fiber strength after crosslinking and PEO removal.

FTIR analysis of the fibers was conducted in order to determine if PEO was completely removed in the soaking step. Fig. 7b shows the spectrum for fibers prior to and following soaking. Peaks that correspond to C–O–C bond stretching in PEO located at 844, 1103 cm^{-1}, and $-CH_2$ bond stretching at 1343 cm^{-1}, disappear after soaking (Ji et al., 2006). Our results reveal that PEO is removed during the soak treatment, which is important because the remaining mat is pure alginate; as our group has previously shown, pure alginate can be modified with cell adhesion ligands to be interactive with cells, whereas PEO is inert and does not contribute to cellular interactions (Jeong et al., 2010). In addition, nanofibrous mats containing low MW alginate are of particular interest for *in vivo* studies after the high MW PEO is removed. It is worth noting that, despite the effective removal of PEO, the outer surfaces do not appear to be highly porous (Zhang, Feng, Huang, Ramakrishna, & Lim, 2006). We speculate that the PEO was present in small domains within the fiber, which could not be detected with SEM analysis. Blends of PEO and alginate are miscible, which explains the smooth surface appearance of the soaked fibers (Caykara et al., 2005).

4. Conclusions

The addition of small amounts of surfactants can enhance the concentration of alginate and morphology of electrospun fibers from alginate/PEO blends. We found this concept to hold true for low molecular alginates and an FDA approved Pluronic surfactant, both of which holds great promise for *in vivo* applications. In particular, nanofibers of alginate blends with compositions greater than 80/20 for alginate-to-PEO ratio were obtained in such systems. Alginate-only fibers, evidenced from FTIR, were also achieved by crosslinking the nanofibers and removing the PEO and surfactant. Finally, compared to the rheological properties and ionic conductivity, we found surface tension of the solutions had the greatest effect on whether the solution blends could be electrospun. Reducing the surface tension could thus be an effective method to tune the fiber morphology while maximizing the amount of alginate.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carbpol.2011.02.002.

References

Al-Shamkhani, A., & Duncan, R. (1995). Radioiodination of alginate via covalently-bound tyrosinamide allows monitoring of its fate in-vivo. *Journal of Bioactive and Compatible Polymers*, *10*, 4–13.

Alsberg, E., Anderson, K. W., Albeiruti, A., Rowley, J. A., & Mooney, D. J. (2002). Engineering growing tissues. *Proceedings of the National Academy of Sciences of the United States of America*, *99*, 12025–12030.

Alsberg, E., Kong, H. J., Hirano, Y., Smith, M. K., Albeiruti, A., & Mooney, D. J. (2003). Regulating bone formation via controlled scaffold degradation. *Journal of Dental Research*, *82*, 903–908.

Augst, A. D., Kong, H. J., & Mooney, D. J. (2006). Alginate hydrogels as biomaterials. *Macromolecular Bioscience*, *6*, 623–633.

Bhattarai, N., Li, Z. S., Edmondson, D., & Zhang, M. Q. (2006). Alginate-based nanofibrous scaffolds: Structural, mechanical, and biological properties. *Advanced Materials*, *18*, 1463–1467.

Bhattarai, N., & Zhang, M. (2007). Controlled synthesis and structural stability of alginate-based nanofibers. *Nanotechnology*, *18*, 10.

Birnbaum, S., Pendleton, R., Larsson, P. O., & Mosbach, K. (1981). Covalent stabilization of alginate gel for the entrapment of living whole cells. *Biotechnology Letters*, *3*, 393–400.

Caykara, T., Demirci, S., Eroglu, M. S., & Guven, O. (2005). Poly(ethylene oxide) and its blends with sodium alginate. *Polymer*, *46*, 10750–10757.

Chew, S. Y., Wen, Y., Dzenis, Y., & Leong, K. W. (2006). The role of electrospinning in the emerging field of nanomedicine. *Current Pharmaceutical Design*, *12*, 4751–4770.

Dayeh, V. R., Chow, S. L., Schirmer, K., Lynn, D. H., & Bols, N. C. (2004). Evaluating the toxicity of Triton X-100 to protozoan, fish, and mammalian cells using fluorescent dyes as indicators of cell viability. *Ecotoxicology and Environmental Safety*, *57*, 375–382.

Dobrynin, A. V., Colby, R. H., & Rubinstein, M. (1995). Scaling theory of polyelectrolyte solutions. *Macromolecules*, *28*, 1859–1871.

Fong, H., Chun, I., & Reneker, D. H. (1999). Beaded nanofibers formed during electrospinning. *Polymer*, *40*, 4585–4592.

Han, T., Yarin, A. L., & Reneker, D. H. (2008). Viscoelastic electrospun jets: Initial stresses and elongational rheometry. *Polymer*, *49*, 1651–1658.

Hashimoto, T., Suzuki, Y., Tanihara, M., Kakimaru, Y., & Suzuki, K. (2004). Development of alginate wound dressings linked with hybrid peptides derived from laminin and elastin. *Biomaterials*, *25*, 1407–1414.

He, Y., Zhu, B., & Inoue, Y. (2004). Hydrogen bonds in polymer blends. *Progress in Polymer Science*, *29*, 1021–1051.

Hiemenz, P. C., & Rajagopalan, R. (1997). *Principles of colloid and surface chemistry*. NY: Marcel Dekker.

Huanglee, L. L. H., Cheung, D. T., & Nimni, M. E. (1990). Biochemical-changes and cytotoxicity associated with the degradation of polymeric glutaraldehyde derived cross-links. *Journal of Biomedical Materials Research*, *24*, 1185–1201.

Jeong, S. I., Krebs, M. D., Bonino, C. A., Khan, S. A., & Alsberg, E. (2010). Electrospun alginate nanofibers with controlled cell adhesion for tissue engineering. *Macromolecular Bioscience*, doi:10.1002/mabi.201000046

Ji, Y., Ghosh, K., Shu, X. Z., Li, B. Q., Sokolov, J. C., Prestwich, G. D., et al. (2006). Electrospun three-dimensional hyaluronic acid nanofibrous scaffolds. *Biomaterials*, *27*, 3782–3792.

Khattak, S. F., Bhatia, S. R., & Roberts, S. C. (2005). Pluronic F127 as a cell encapsulation material: Utilization of membrane-stabilizing agents. *Tissue Engineering*, *11*, 974–983.

Klossner, R. R., Queen, H. A., Coughlin, A. J., & Krause, W. E. (2008). Correlation of chitosan's rheological properties and its ability to electrospin. *Biomacromolecules*, *9*, 2947–2953.

Krause, W. E., Bellomo, E. G., & Colby, R. H. (2001). Rheology of sodium hyaluronate under physiological conditions. *Biomacromolecules*, *2*, 65–69.

Lee, K. Y., Jeong, L., Kang, Y. O., Lee, S. J., & Park, W. H. (2009). Electrospinning of polysaccharides for regenerative medicine. *Advanced Drug Delivery Reviews*, *61*, 1020–1032.

Liang, D., Hsiao, B. S., & Chu, B. (2007). Functional electrospun nanofibrous scaffolds for biomedical applications. *Advanced Drug Delivery Reviews*, *59*, 1392–1412.

Lu, J.-W., Zhu, Y.-L., Guo, Z.-X., Hu, P., & Yu, J. (2006). Electrospinning of sodium alginate with poly(ethylene oxide). *Polymer*, *47*, 8026–8031.

McKee, M. G., Hunley, M. T., Layman, J. M., & Long, T. E. (2006). Solution rheological behavior and electrospinning of cationic polyelectrolytes. *Macromolecules*, *39*, 575–583.

McKee, M. G., Wilkes, G. L., Colby, R. H., & Long, T. E. (2004). Correlations of solution rheology with electrospun fiber formation of linear and branched polyesters. *Macromolecules*, *37*, 1760–1767.

Nie, H., He, A., Zheng, J., Xu, S., Li, J., & Han, C. C. (2008). Effects of chain conformation and entanglement on the electrospinning of pure alginate. *Biomacromolecules*, *9*, 1362–1365.

Nie, H. R., He, A. H., Wu, W. L., Zheng, J. F., Xu, S. S., Li, J. X., et al. (2009). Effect of poly(ethylene oxide) with different molecular weights on the electrospinnability of sodium alginate. *Polymer*, *50*, 4926–4934.

Ren, L. F., Marquardt, M. A., & Lech, J. J. (1997). Estrogenic effects of nonylphenol on pS2, ER and MUC1 gene expression in human breast cancer cells-MCF-7. *Chemico-Biological Interactions*, *104*, 55–64.

Rubin, L. F. (1975). Toxicity of dimethyl-sulfoxide, alone and in combination. *Annals of the New York Academy of Sciences*, *243*, 98–103.

Rubinstein, M., & Colby, R. H. (2003). *Polymer physics*. New York: Oxford University Press.

Rubinstein, M., Colby, R. H., & Dobrynin, A. V. (1994). Dynamics of semidilute polyelectrolyte solutions. *Physical Review Letters*, *73*, 2776–2779.

Safi, S., Morshed, M., Ravandi, S. A. H., & Ghiaci, M. (2007). Study of electrospinning of sodium alginate, blended solutions of sodium alginate/poly(vinyl alcohol) and sodium alginate/poly(ethylene oxide). *Journal of Applied Polymer Science*, *104*, 3245–3255.

Saquing, C. D., Manasco, J. L., Bonino, C. A., & Khan, S. A. (2008). Electrospun metal nanoparticle-alginate based polymer blend nanofiber composites for biomedical applications. In *American Chemical Society National Meeting* New Orleans, LA,

Shenoy, S. L., Bates, W. D., Frisch, H. L., & Wnek, G. E. (2005). Role of chain entanglements on fiber formation during electrospinning of polymer solutions: Good solvent, non-specific polymer–polymer interaction limit. *Polymer*, *46*, 3372–3384.

Shoichet, M. S., Li, R. H., White, M. L., & Winn, S. R. (1996). Stability of hydrogels used in cell encapsulation: An in vitro comparison of alginate and agarose. *Biotechnology and Bioengineering*, *50*, 374–381.

Sluzky, V., Klibanov, A. M., & Langer, R. (1992). Mechanism of insulin aggregation and stabilization in agitated aqueous-solutions. *Biotechnology and Bioengineering*, *40*, 895–903.

Smidsrod, O., & Skjakbraek, G. (1990). Alginate as immobilization matrix for cells. *Trends in Biotechnology*, *8*, 71–78.

Talwar, S., Hinestroza, J., Pourdeyhimi, B., & Khan, S. A. (2008). Associative polymer facilitated electrospinning of nanofibers. *Macromolecules*, *41*, 4275–4283.

Taylor, G. (1964). Disintegration of water drops in electric field. *Proceedings of the Royal Society of London Series A-Mathematical and Physical Sciences*, *280*, 383.

Tripatanasuwan, S., Zhong, Z. X., & Reneker, D. H. (2007). Effect of evaporation and solidification of the charged jet in electrospinning of poly(ethylene oxide) aqueous solution. *Polymer*, *48*, 5742–5746.

Yu, J. H., Fridrikh, S. V., & Rutledge, G. C. (2006). The role of elasticity in the formation of electrospun fibers. *Polymer*, *47*, 4789–4797.

Zhang, Y. Z., Feng, Y., Huang, Z. M., Ramakrishna, S., & Lim, C. T. (2006). Fabrication of porous electrospun nanofibres. *Nanotechnology*, *17*, 901–908.