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Short communication

Preparation of electrospun alginate fibers with chitosan sheath

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ABSTRACT

In this study, alginate (AL) fibers were electrospun and coagulated with chitosan (ChS) and ethanol using a single spinneret. These fibers exhibited a core–sheath structure that was revealed using a confocal laser scanning microscope (CLSM) and fluorescence-labeled polymers. The resulting fibers were examined using a field emission scanning electron microscope (FESEM) for the fiber size and morphology. The average diameter of the fibers ranged from 600 to 900 nm depending on the electrospinning parameters. To mimic the stability of alginate fibers in physiological fluids, the release of alginate from these fibers in normal saline was also tested. The results demonstrated that the core–sheath structure of alginate fiber can greatly reduce the degradation by 40% for 3 d in physiological environment.

1. Introduction

Electrospinning is frequently employed for making micrometer or nanometer-sized fibers for biomedical or tissue engineering applications (Agarwal, Wendorff, & Greiner, 2008; Fang, Liu, Jiang, Nie, & Ma, 2011; Muzzarelli, 2004). The fiber diameter and porosity of the fibrous mats can be controlled by process parameters including voltage, flow rate, distance between needle tip and collector, and solution parameters, such as polymer concentration, solvent conductivity, and surface tension (Sill & von Recum, 2008).

Several reports about electrospinning of alginate (AL) nanofibers can be found in the literature. Alginate-based nanofibers were electrospun successfully by blending with polyethylene oxide (PEO) (Bhattarai, Li, Edmondson, & Zhang, 2006). Pure alginate nanofibers were electrospun by using glycerol as a cosolvent (Nie et al., 2008). Alginate/chitosan (ChS) complexed nanofibers were coagulated *in situ* during the electrospinning process (Jeong et al., 2011). Nevertheless, the alginate matrices in these works were all coagulated with Ca^{2+} after electrospinning. When in physiological environment or in buffer solutions with high concentration of phosphate or citrate ions, Ca^{2+} would be extracted from the alginate leading to structure damage (Taqieddin & Amiji, 2004).

Alginate and chitosan can form polyanion–polycation complexes (Muzzarelli, Stanic, Gobbi, Tosi, & Muzzarelli, 2004; Muzzarelli, Tosi, Francescangeli, & Muzzarelli, 2003; Sæther, Holme, Maurstad, Smidsrød, & Stokke, 2008). The AL–ChS hybrid polymer fibers promoted biological responses of seeded chondrocytes including enhancing cell attachment and proliferation (Iwasaki et al., 2004). A novel method of enzyme immobilization using AL–ChS core–shell microcapsules technology was developed (Taqieddin & Amiji, 2004).

The purpose of this work is to electrospin a core–sheath fibrous mat composing of alginate (the core) and chitosan (the sheath). Such a fibrous mat will avoid the structure damage due to the extraction of calcium in physiological environment. The viscosity, surface tension, and specific gravity of the spinning solution were measured.

2. Experimental

2.1. Materials

Sodium alginate (Mw 22 kDa), and chitosan (Mw 20 kDa) were purchased from Acros (USA). 1-Ethyl-3-(-3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), fluorescein isothiocynate (FITC), rhodamine B isothiocynate (RITC), glycerol, ethanol, and acetic acid were obtained from Sigma, USA.

2.2. Preparation of polymer solutions

Alginate was dissolved in 50 wt% glycerol under stirring for 1 d at 25 °C. Chitosan was dissolved in 5 wt% acetic acid at 25 °C under stirring for 1 d to form a homogeneous solution of 1 wt%. Then

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Table 1
The compositions and physical properties of alginate solution.

Code	Solution composition[a]		Solution properties		
	Alginate (wt%)	Glycerol (wt%)	Viscosity (mPa s)	Surface tension (dyne/cm)	Specific gravity
A1	1.25	49.375	2532 ± 146	51.4 ± 0	1.147
A2	1.50	49.250	5988 ± 197	52.8 ± 0	1.145
A3	1.75	49.125	9967 ± 29	57.6 ± 0	1.143

[a] The balance was water.

the chitosan solution was mixed with ethanol. The compositions and physical properties of the electrospinning solutions and the coagulant were presented in Tables 1 and 2.

2.3. Electrospinning

The electrospinning system was consisted of a power supply (ES30P, Gamma High Voltage Research, USA), a syringe pump (LSP04-1A, Baoding Longer Precision Pump Co., Ltd, China), and a coagulating bath. The alginate solution was spun through a needle spinneret (OD 1.07 mm, ID 0.77 mm). The flow rate ranged from 0.1 to 0.5 ml/h. The applied voltage ranged from 13 to 15 kV, and the distance between the needle and the coagulating bath was 70 mm. The resulting samples were labeled as AL–ChS. For comparison, alginate solution was electrospun into 80 wt% ethanol aqueous solution containing 10 wt% $CaCl_2$. The resulting sample was labeled as calcium alginate. All these resulting samples were immersed in ethanol for 1 h and then rinsed with ethanol to remove residual glycerol. Afterwards, the samples were dried at 60 °C for 1 d.

2.4. Characterization

Polymer solution properties including viscosity, surface tension, and specific gravity were measured with a viscometer (LVTDV-II, Brookfield Engineering Laboratories, Inc., Middleboro, MA, USA), surface tension meter (CBVP-A3, Kyowa Interface Science Co., Tokyo, Japan), and a pycnometer, respectively. The morphology of the fibrous mats was examined using an FE-SEM (JSM-6500F, JEOL, Japan). Diameters of the fibers were analyzed from the SEM images using image analysis software (Image J, National Institutes of Health, USA).

2.5. Confocal laser scanning microscope (CLSM) observation of fibrous mats

To examine the core–sheath structure of the fibers, alginate was labeled with FITC (alginate-[FITC]) and chitosan was labeled with RITC (chitosan-[RITC]) (Hsieh, Tsai, Wang, Chang, & Hsieh, 2005; Hsu, Hung, Liou, & Shen, 2010). Briefly, alginate-[FITC] was prepared by mixing 100 ml of 1.76% alginate with 10 mg FITC and 20 mg EDC at 4 °C for 1 d. Chitosan-[RITC] was prepared by mixing 100 ml of 1% chitosan with 50 mg RITC and 20 mg EDC at 4 °C for 1 d. The residual free dyes were then dialyzed off with doubly distilled water for 4 wk. These labeled polymers were mixed with neat polymers to prepare fibrous mats. Cryomicrotome sections of the fibrous mat were then examined using a confocal microscope (LSM 510 META, Carl Zeiss Inc. USA).

2.6. Stability test

Dry fibrous mats were weighted and placed in normal saline (0.9% NaCl, pH 7.4) at 25 °C. At specified time intervals, 0.1 ml of the saline were withdrawn and the concentration of alginate-[FITC] and chitosan-[RITC] released were determined using an ELISA reader (Wallac 1420 Victor^2 multilabel counter, EG&G Wallac, USA).

3. Results and discussion

3.1. Effect of alginate concentration on the solution properties

Table 1 lists the viscosity, surface tension, and specific gravity of the alginate solution in the present study. In general, the viscosity and surface tension of the alginate solutions increased with increasing alginate concentration, whereas the specific gravity changed insignificantly.

3.2. Effect of coagulating solution on fiber morphology

Instead of Ca^{2+}, we employed chitosan to coagulate alginate fibers. To facilitate the coagulation of electrospun alginate fibers, ethanol was added into the coagulation solution.

Fibrous structure was not resulted until the coagulant contained more than 30 wt% or ethanol. Fig. 1A shows that a flat sheet was resulted when the coagulant did not contained ethanol. Fig. 1B shows that the alginate solution piled up, forming a rugged surface. Fig. 1C shows that fibrous structure was observable. As the coagulant containing 50 wt% of ethanol (Fig. 1D), continuous fibers could be produced. This result suggests that the addition of ethanol into the coagulant can facilitate the formation of alginate fibers.

Table 2 shows that the addition of ethanol can reduce both the surface tension and specific gravity of the coagulant. These two factors would affect the submerging speed of the nascent alginate fiber during electrospinning. We observed that the nascent fiber would not submerge into C9 and C10, leading to the formation of a flat sheet on top of the coagulant. Therefore, the addition of ethanol can reduce the surface tension of coagulant, allowing the fiber to submerge and leading to the coagulation of nascent fibers. Furthermore, ethanol can facilitate the extraction of solvent from

Table 2
The compositions and physical properties of coagulant.

Code	Solution composition[a]		Solution properties		
	Chitosan (wt%)	Ethanol (wt%)	Viscosity (mPa s)	Surface tension (dyne/cm)	Specific gravity
C10	1.0	0	31.6 ± 0.5	39.4 ± 0.1	1.01
C9	0.9	10	31.8 ± 0.3	39.2 ± 0.1	0.995
C7	0.7	30	25.8 ± 0.5	38.2 ± 0	0.974
C5	0.5	50	22.4 ± 0.3	28.5 ± 0.1	0.931
C0	0	100	11.5 ± 0.5	21.8 ± 0	0.792

[a] The balance was 5 wt% acetic acid.

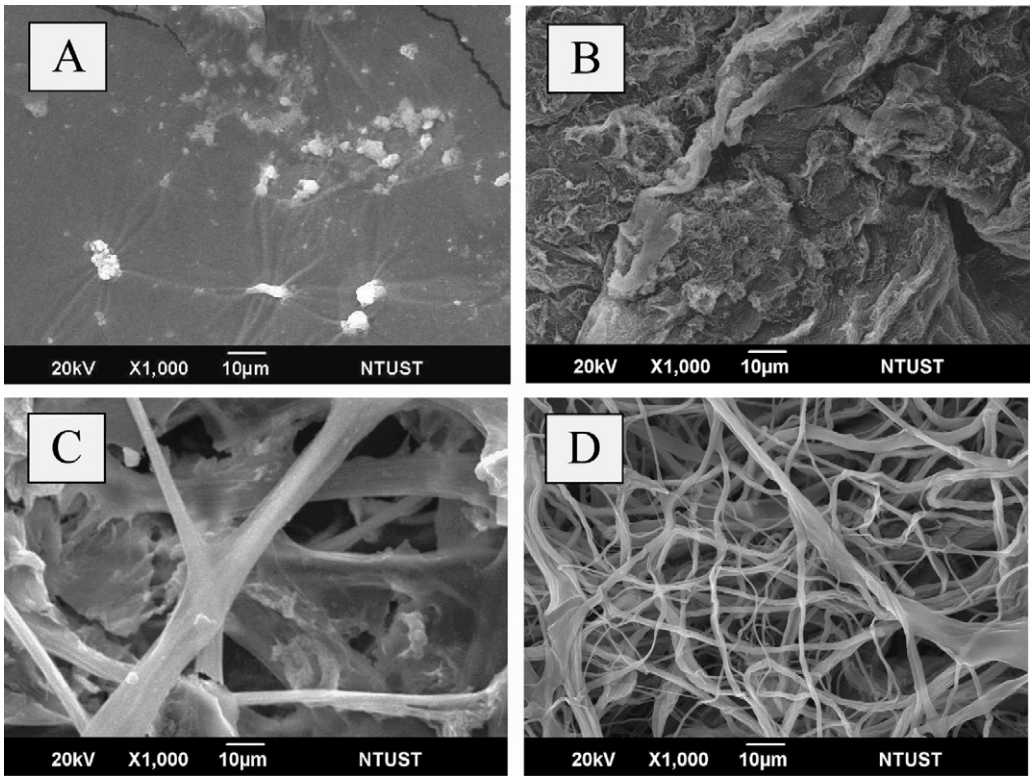

Fig. 1. Effect of adding ethanol in chitosan solution on fibrous mats morphology. (A) A2/C10; (B) A2/C9; (C) A2/C7; and (D) A2/C5.

the nascent fibers, hence accelerating the coagulation. Thus ethanol was indispensible for the formation of this core–sheath fiber.

3.3. Core–sheath structure of electrospun fibers

Fig. 2 reveals that the electrospun AL–ChS fiber exhibits a core–sheath structure. In this image, the mixing ratio of alginate to alginate-[FITC] was 9/1, while that of chitosan to chitosan-[RITC] was 1/1. After electrospinning into coagulant, alginate fiber was coated with a thin layer of chitosan.

In the literature, core–sheath fibers, including poly(ε-caprolactone) (PCL)/poly(ethylene glycol) (PEG) (Saraf et al., 2009) and PCL/gelatin fibers (Drexler & Powell, 2011) were electrospun using coaxial spinnerets. Unlike these previous works, the core–sheath fiber in this work consisting of alginate (the core) and chitosan (the sheath) was prepared using a single spinneret. Although alginate is soluble in water, the chitosan in the collecting bath coagulated alginate and generated the core–sheath morphology via polyelectrolyte formation.

Fig. 3 shows that the fiber diameter distribution of electrospun fibers varies with the alginate concentration. In general, the

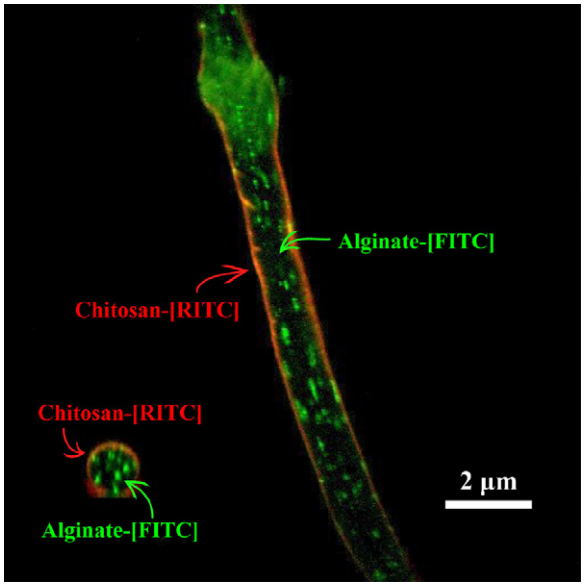

Fig. 2. CLSM image of the core-sheath structure of AL–ChS.

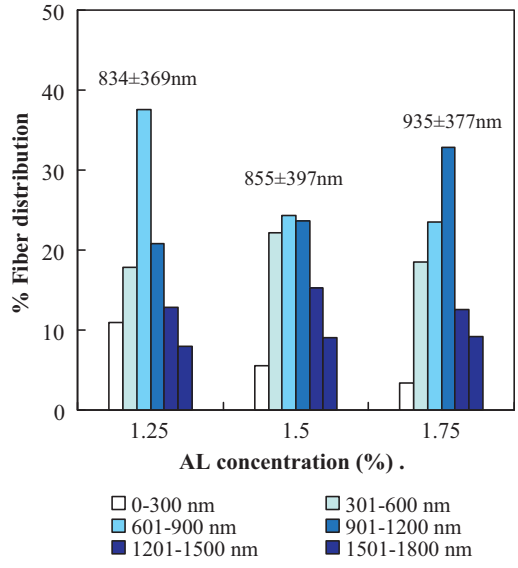

Fig. 3. The effect of alginate concentration on fiber diameter distribution. Data are expressed as mean ± standard deviation with $n = 100$ (1.25% vs 1.75%, $^{*}p < 0.05$).

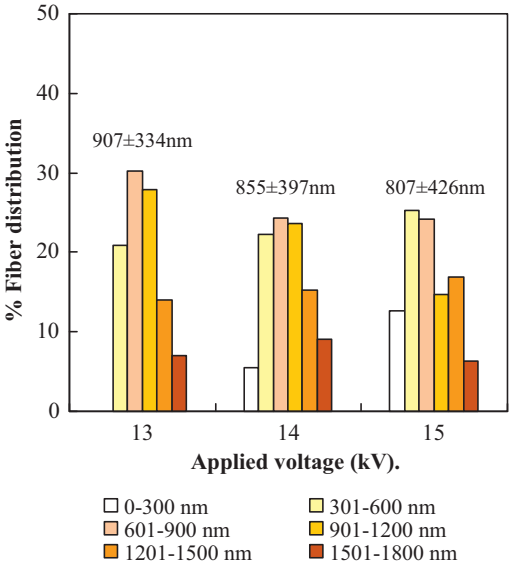

Fig. 4. The effect of applied voltage on fiber diameter distribution. Data are expressed as mean ± standard deviation with $n = 100$ (13 kV vs 14 kV; 13 kV vs 15 kV, $^{*}p < 0.05$).

diameters of the fibers increased with the viscosity of the alginate solution (Ki et al., 2005). In addition, the average fiber diameter increased with the increase in the alginate concentration (Nie et al., 2008). Because higher viscosity makes the fiber stream more difficult to be elongated, hence the diameter was larger.

Fig. 4 shows the effect of applied voltage on the distribution of the fiber diameters. The applied voltage did not significantly affect the average diameter and the distribution of electrospun fiber, because the range of variation in the applied voltage was only 2 kV.

Fig. 5 shows the effect of flow rate on the diameter distribution. For fibers spun at 0.1 ml/h, about 43% of the fibers exhibited diameter fell in the range 300–600 nm. Whereas for fibers spun at 0.3 ml/h, only about 23% of the fibers exhibited diameter between 300 and 600 nm, and about 40% of the fibers exhibited diameter between 600 and 900 nm. This agrees with the literature that lower

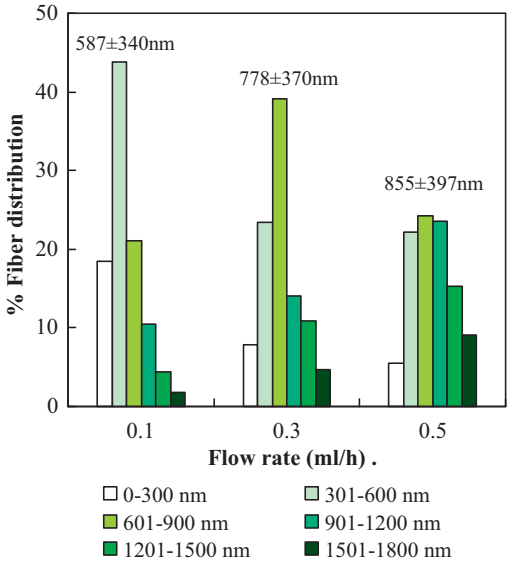

Fig. 5. The effect of flow rate on fiber diameter distribution. Data are expressed as mean ± standard deviation with $n = 100$ (0.1 ml/h vs 0.3 ml/h; 0.1 ml/h vs 0.5 ml/h, $^{*}p < 0.05$).

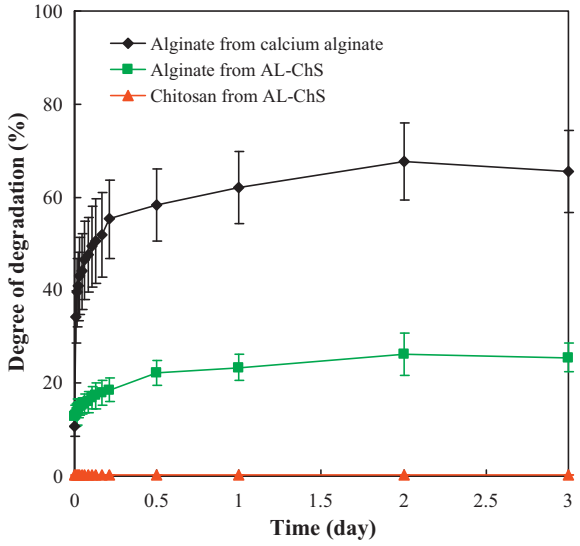

Fig. 6. The stability test of fibrous mats of AL–ChS and calcium alginate.

flow rates yielded fibers with smaller diameters (Pham, Sharma, & Mikos, 2006).

3.4. Stability test

Fig. 6 shows the time-course of the degradation of alginate fiber in normal saline. The release of alginate from calcium alginate was much faster than that from AL–ChS. After 3 d, calcium alginate lost about 65% of alginate, while AL–ChS lost only 25%. This can be attributed to the exchange of Ca^{2+} with Na^{+} from normal saline, leading to the disintegration of calcium alginate (Bajpai & Sharma, 2004). In the case of AL–ChS, the thin chitosan sheath was insoluble in saline, hence reduced the release of alginate from the core of the fiber. Therefore, AL–ChS would be more stable than calcium alginate in physiological environment.

4. Conclusion

In this work, we demonstrated that by electrospinning alginate fiber into a coagulant containing chitosan and alcohol can result in a core–sheath structure. The addition of ethanol can facilitate the extraction of glycerol from the nascent fibers, hence accelerating the coagulation. The core–sheath structure was revealed using confocal microscopy. When the flow rate increased from 0.1 to 0.5 ml/h, the fiber diameter increased from 587 to 855 nm. In normal saline, the degree of degradation of AL–ChS was about 40% of that of calcium alginate. This suggests that alginate fibers with chitosan sheath would be more stable in physiological environment. Thus, this core–sheath electrospun fiber has a potential for biomedical applications.

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