Carbohydrate Polymers 87 (2012) 2357–2361

Contents lists available at SciVerse ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Short communication

Preparation of electrospun alginate fibers with chitosan sheath

Jung-Jhih Chang[a], Yen-Hsien Lee[b], Meng-Hsiu Wu[a], Ming-Chien Yang[a,*], Chiang-Ting Chien[c,**]

[a] Department of Materials Science and Engineering, National Taiwan University of Science and Technology, 43, Sec. 4, Keelung Rd, Taipei 106, Taiwan, ROC
[b] Graduate Institute of Engineering, National Taiwan University of Science and Technology, 43, Sec. 4, Keelung Rd, Taipei 106, Taiwan, ROC
[c] Department of Medical Research, National Taiwan University Hospital and National Taiwan University College of Medicine, No. 7, Chung Shan S. Rd, Taipei 100, Taiwan, ROC

ARTICLE INFO

Article history:
Received 30 September 2011
Received in revised form 18 October 2011
Accepted 19 October 2011
Available online 28 October 2011

Keywords:
Sodium alginate
Chitosan
Electrospinning
Core–sheath structure
Fluorescent labeling

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

* Corresponding author. Tel.: +886 2 2737 6528; fax: +886 2 2737 6544.
** Corresponding author. Tel: +886 2 2312 3456x65720; fax: +886 2 2394 7927.
E-mail addresses: myang@mail.ntust.edu.tw (M.-C. Yang), ctchien@ntuh.gov.tw (C.-T. Chien).

0144-8617/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2011.10.054

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.