Preparation of chitosan/ poly (lactic-co glycolic acid)(PLGA) nanocoposite for tissue engineering scaffold

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In this study, highly tubular porous chitosan/poly (DL lactic-co-glycolic acid) (PLGA) nanocomposite structures were produced via electrospinning and unidirectional freeze drying techniques. The 3D porous structure of chitosan/PLGA was characterized by scanning electron microscopy (SEM). The properties of the chitosan/PLGA nanocomposite, including porosity and mechanical properties, were investigated.

(Received February 17, 2012; accepted April 11, 2012)

Keywords: Freeze-drying, Electrospinning, Highly tubular, Porous nanocomposites, Tissue engineering scaffolds

1. Introduction

In the past few years, considerable attention has been paid to the effects of nano-topography on cell behaviors, such as morphology, adhesion, and orientation, because the cells surrounding the natural extracellular matrix (ECM) are often comprised of nanoscale components. Therefore, fabricating 3-D, highly porous scaffolds with nano-topography is an important task for tissue engineering that could enable cell growth in a 3-D environment by enhancing cell–cell contact, promoting the spatial arrangement of cells, and supporting a high cell density due to the high surface area-to-volume ratio.

Among a variety of polymers proposed as scaffold materials, chitosan has been found to be an excellent candidate for many tissue engineering applications, either in isolation and/or in combination with other materials [6]chitosan**110** | View Record in Scopus | | Full Text via CrossRef [1-5]. Chitosan is the second most abundant natural polymer in the world. It is biodegradable, non-toxic and hydrophilic, has a remarkable affinity for proteins, and exhibits antibacterial, haemostatic, and fungistatic properties. However, the use of chitosan has been limited by its poor mechanical properties.

In this paper, three different chitosan concentrations (1.0, 1.5, 2.0%) were chosen to study the effect of chitosan concentration on the mechanical properties of chitosan scaffolds. Moreover, the effect of PLGA nanofiber content on the mechanical properties of chitosan/ PLGA nanocomposite scaffolds was investigated. These scaffolds were fabricated at three different electrospinning times—10, 20, and 30 mins—while the chitosan concentration remained the same at 1.5 %.

2. Experimental

2.1. Materials

Chitosan (75 to 85% deacetylated, medium molecular weight) in powder form was purchased from Sigma-Aldrich. Poly (DL lactic-co-glycolic acid) (PLGA 50/50, M_w = 100,000 kDa) was supplied by JiNan Daiyue Corp. Ltd. Acetic acid (AcOH), *N*, *N*-dimethylformamide (DMA), and tetrahydrofuran (THF) were purchased from Sigma-Aldrich. All chemicals were used as received.

2.2. Preparation of Chitosan/PLGA Nanocomposites

The acetic acid was diluted to 0.18 mol/l by the addition of deionized water. Then a proper amount of chitosan was put into the 0.18 mol/l diluted acetic acid and a homogenizer was used to mechanically agitate the solvent for 8 hours to form a homogeneous chitosan solution at different concentrations of 1.0%, 1.5% and 2.0% (w/v, g/ml).

In this study, 30 wt% PLGA was dissolved in a 1:1 DMF:THF mixture and mixed well by vortexing overnight until the solution became homogeneous. The polymer solution was placed in a 10 mL glass syringe fitted with an 8 cm, 20-G blunt tipped needle. The parameters of the electrospinning process included a solution flow rate of 0.4 ml/h and a high voltage of 22 kV supplied directly from a high DC voltage power supply. The copper plate was covered by aluminum foil. Then, a borosilicate glass petri dish (100 by 15 mm), which was pre-coated with a thin layer of chitosan solution, was put onto the aluminum foil. The PLGA nanofiber was collected through the glass petri dish 15 cm away from the tip of the syringe needle. The collected PLGA nanofibers floated on the surface of

the chitosan solution due to the high interfacial tension between the chitosan solution and the PLGA nanofibers. In order to overcome the interfacial tension and let the PLGA nanofibers enter into the chitosan solution, additional chitosan solution was sprayed into the glass petri dish at 2 minute intervals by a plastic mist sprayer.

The prepared chitosan and chitosan/PLGA mixed solution were poured into a plastic (polystyrene) six-well multidish plate. The plastic plate was placed into an insulating Styrofoam container, with only the bottom surface of the plastic plate exposed. The Styrofoam container was then placed onto the surface of a block of metal that was 13 cm in length and 10 cm in width, which in turn rested in a 6 cm deep pool of liquid nitrogen to create a uniaxial thermal gradient. Linear ice crystals on the surface of the samples, which extended through the polymer, could be seen after the samples were completely frozen for 15 mins. Next, the pre-frozen samples were freeze-dried for 72 h to form chitosan and chitosan/PLGA nanocomposite scaffolds.

2.3 Characterization

The cross section and vertical section morphologies of the chitosan and chitosan/PLGA scaffolds were evaluated using an SEM LEO 1530 with an accelerating voltage of 5 kV.

The various PLGA contents of the chitosan/PLGA scaffolds fabricated at the different electrospinning process times (0, 10, 20, and 30 mins) were analyzed with a TA Instruments Q500 TGA. All of the samples were heated in a nitrogen atmosphere from 25 to 600 °C at a heating rate of 20 °C/min.

The porosities of the chitosan and chitosan/PLGA scaffolds were determined by weighing them and measuring the dimensions of the weighed samples to obtain the volume. Equation (1) was then applied to determine the porosity,

$$P = \left(\frac{V_t - V_a}{V_t} \right) \times 100 = \left(\frac{V_t - (W_a / \rho)}{V_t} \right) \times 100 \quad (1)$$

where P is the porosity of the samples, V_t is the total volume of the chitosan or chitosan/PLGA scaffolds (cm^3), V_a is the actual volume taken by the chitosan or chitosan/PLGA (cm^3), W_a is the actual mass of the scaffold (g), and ρ is the density of the chitosan or chitosan/PLGA. For chitosan scaffolds, ρ is the density of chitosan (1.275 g/cm^3); for chitosan/PLGA scaffolds, ρ varies with the PLGA content.

A dynamic mechanical analysis (DMA) experiment was carried out using a dynamic mechanical analyzer (TA Instruments Q800). Round specimens with diameters of 15 mm were cut from freeze-dried parts and then tested using the compression method at 37 °C. The compression force ramped from 3 N/min to 15 N. All the samples were tested along the vertical (longitudinal) direction.

3. Results and discussion

3.1 Morphology of chitosan and chitosan/PLGA samples

Fig. 1 shows representative images of the vertical section of the chitosan/PLGA composite scaffolds at different concentrations. It can be seen that the microtubule-like orientation structure was formed in the chitosan matrix, and the PLGA nanofiber insert or spin on the microtubule-like structure was formed layer by layer. This indicates that 3-D highly tubular, porous scaffolds with nanoscale architectures were fabricated. These scaffolds could present more binding sites to cell membrane receptors and a larger surface area for absorbing proteins when the cultured cells contact with PLGA nanofiber. In addition, the mechanical properties were fortified by the addition of PLGA nanofibers in the chitosan/PLGA scaffolds, which is discussed in the following section. The SEM images of the electrospinning PLGA nanofibers collected on the aluminum foil is provided in Fig. 1 (d).

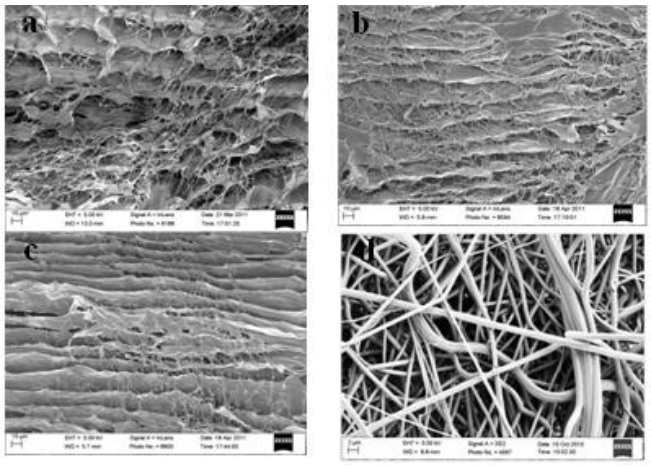

Fig. 1. SEM pictures of chitosan/PLGA scaffolds with different concentrations of PLGA nanofibers at (a) 1%, (b) 1.5% , (c) 2% chitosan, and (d) pure PLGA nanofibers.

Fig. 2 (a) shows the TGA curves of pure PLGA and chitosan/PLGA composites at different electrospinning times. As shown in Fig. 4 (a), for pure PLGA, when the temperature is higher than 600 °C, the weight ratio of PLGA is nearly 0. This indicates that the PLGA is completely degraded after 600 °C. For chitosan and chitosan/PLGA composites, two weight loss stages were observed. The weight loss in the range of 25 to 200 °C is due to the loss of water associated with the hydrophilic groups of chitosan [6, 7]. The weight loss in the range of 250 to 350°C is attributed to the degradation of chitosan molecules and the PLGA nanofibers. Sakurai et al. [8], Wang et al [9], and Zeng et al. [10] also reported that the thermal degradation of chitosan begins at about 250 °C. If the following assumptions are considered—(1) the weight ratio of the remaining chitosan is constant when it is

heated up to 600 °C, and (2) the PLGA is degraded completely when it is heated up to 600 °C—then the content of PLGA can be calculated by the following equation,

$$PLGA\,\% = (1 - \frac{R_{ch/PLGA}}{R_{ch}}) \times 100 \qquad (3)$$

where $R_{ch/PLGA}$ is the weight ratio of the residual product of heated chitosan/PLGA composite after 600 °C, R_{ch} is the weight ratio of the residual product of heated chitosan after 600 °C. Both weight ratios can be determined from the TGA curves (cf. Fig. 2 (a)).

The PLGA content (wt%) of the chitosan/PLGA composites at different electrospinning times were presented in Fig. 2 (b). It can be seen that the content of PLGA reached 2.7, 8.7, and 13.5% at the different electrospinning times of 10, 20, and 30 min, respectively.

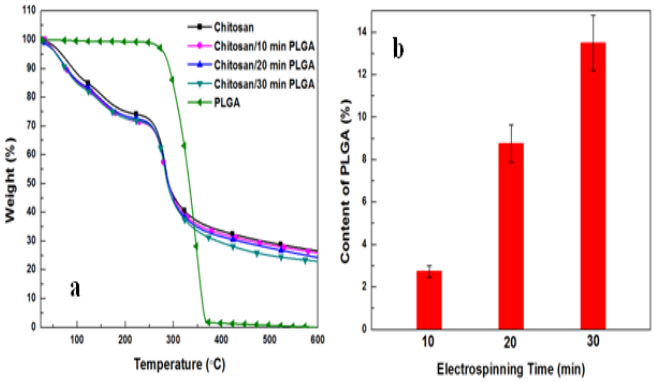

Fig. 2. (a) The TGA curves of pure PLGA and chitosan/PLGA at different electrospinning times. (b) The content (wt%) of PLGA of chitosan/PLGA composites at different electrospinning times.

3.2 Porosity

Fig. 3 shows the effect of chitosan concentration on the porosity of chitosan and chitosan/PLGA scaffolds. For chitosan/PLGA scaffolds, the electrospinng time was set at 30 min. It can be seen that the porosity of all chitosan and chitosan/PLGA scaffolds was higher than 96%, and it almost linearly decreased with an increase in the chitosan concentration. In addition, the porosity of chitosan/PLGA scaffolds was slightly smaller than that of chitosan scaffolds at the same chitosan concentration.

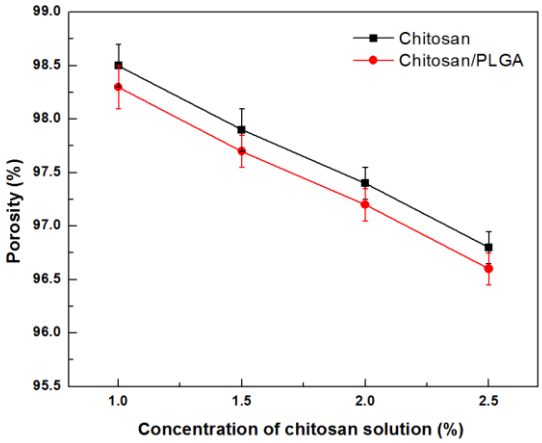

Fig. 3. Porosity of chitosan and chitosan/PLGA scaffolds at different chitosan concentrations.

3.3 Mechanical properties

Fig. 4 presents the compressive modulus of chitosan/PLGA scaffolds made at different electrospinning times with a chitosan concentration of 1.5%. Obviously, the compressive modulus of chitosan/PLGA scaffolds sharply increased with an increase in the electrospinning time. Namely, the compressive modulus increased as the content of PLGA nanofibers became higher. This could be attributed to the fact that the PLGA nanofibers go through and connect to the chitosan marix wall, serving as a bridge between wall layers.

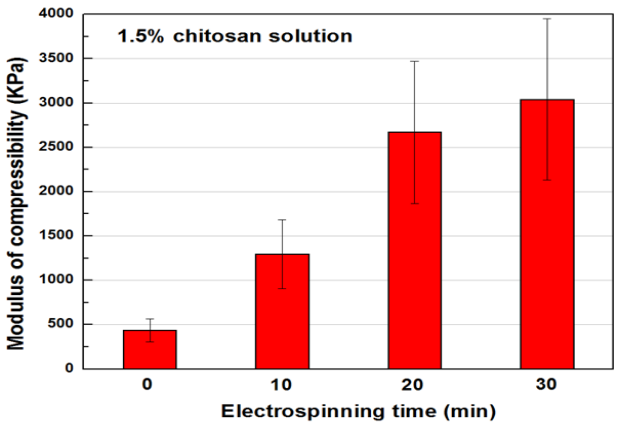

Fig. 4. The modulus of compressibility of the chitosan/PLGA scaffolds at different electrospinning times with a chitosan concentration of 1.5%.

4. Conclusions

In this study, 3-D highly tubular, porous chitosan and chitosan/PLGA nanocomposite scaffolds were produced by combining electrospinning and unidirectional freeze drying technologies. The results showed that both chitosan and chitosan/PLGA scaffolds have high porosity and water

absorption. Compared to the chitosan scaffold, the chitosan/PLGA scaffold had a larger surface area-to-volume ratio due to the addition of the nanoscale architecture (PLGA nanofiber). Moreover, the compressive modulus of chitosan scaffolds increased with an increase in chitosan solution concentration. Furthermore, the compressive modulus of the chitosan/PLGA scaffolds increased with PLGA nanofiber content while the chitosan concentration remained the same.

References

[1] R. Langer, J. P. Vacanti, Science, **260,** 920 (1993).
[2] J. A. Hubbell, Biotechnology, **13**, 565 (1995).
[3] K. B. Hellman, N. Y. Ann, Acad. Sci., **831**, 1 (1997).
[4] M. M. Stevens, J. H. George, Science, **310**, 1135 (2005).
[5] K. Tuzlakoglu, C. M. Alves, J. F. Mano, R. L. Reis, Macromol Biosci., **4**, 811 (2004).
[6] Y. M. Xiao, D. X. Li, X. N. Chen, J. Lu, H. S. Fan, X. D. Zhang, J Appl Polym Sci., **110**, 408 (2008).
[7] V. Gonzalez, C. Guerrero, U. Ortiz, J. Appl. Polym. Sci., **78**, 850 (2000).
[8] K. Sakurai, T. Maegawa, T. Takahashi, Polymer, **41,** 7051 (2000).
[9] S. G. Wang, X. F. Sun, Chemical Engineering Journal, **142,** 239 (2008).
[10] M. Zeng, Z. Fang, C. Xu, J. Membr. Sci., **230**, 175 (2004).
[11] C. P. Chang, C. C. Hsu, Mater. Sci. Eng. A, **433**, 100 \ (2006).

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