Fabrication and Characterization of Polycaprolactone/Chlorophyllin Sodium Copper Salt Nanofibrous Mats from 2,2,2-Trifluoroethanol Solution by Electrospinning

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T he aim of this study is to investigate electrospinning of polycaprolactone (PCL)-blend chlorophyllin sodium copper salt (CSC) to prepare biodegradable nanofibrous mats for fabricating tissue engineering scaffold. For a suitable biofunctional tissue engineering scaffold, one of the most important factors was that the scaffold should provide enough space for the living cells. Therefore, by adding a non-toxic salt into the nanofibrous mats it was possible to attain this goal. In this study, PCL/CSC nanofibrous mats were tested by scanning electron microscopy (SEM); and the mechanical properties of nanofibrous mats with or without CSC were tested by a universal mechanical testing machine. The crystallized property was investigated by an X-ray diffraction (XRD) instrument. The degradation property and CSC released behaviour were also tested in this study. SEM examinations revealed that the PCL/CSC nanofibres with 20% CSC lose their fibrous structure. For evaluation of the performance of PCL/CSC nanofibrous mats as scaffolds which could provide sufficient space for the cells, the mats were examined for weight loss and CSC release tests. The results demonstrated that the PCL/CSC nanofibrous mats could lose most of the CSC in the first several hours, which may provide more space for cells anchorage and proliferation. We conclude that combination of CSC into PCL nanofibrous mats is feasible for fabricating a novel tissue engineering scaffold.

INTRODUCTION

In contrast to conventional transplantation methods, tissue engineering provides new medical therapy which takes advantage of polymeric biomaterials with or without living precursor. The ultimate purpose of tissue engineering is to re-establish the destroyed human tissues or organs by providing scaffolds for functional cells regeneration [1]. For example, bone tissue repair is one of the most serious problems, which has direct impact on the quality of human life [2]. The effective methods for nerve tissue engineering include fabrication of polymeric scaffolds with bone cells to produce a three-dimensional tissue [2] and biomaterials functional scaffolds with encapsulation of bioactive proteins [3] (e.g., fibroblast growth factor) or DNA [4] suitable for implantation. In human tissue, extracellular matrix (ECM) plays a pivotal role in supporting and controlling living
cells, therefore, the tissue engineering scaffolds should be designed for such purposes. For mimicking the human ECM which has a nanofibrous structure, electrospun nanofibres have been applied in recent years [5-10]. Nanofibrous scaffolds fabricated by electrospinning of biomaterials provide suitable environment for cell attachment and proliferation, because of the similar physical dimension compared with natural ECM.

Electrospinning is a technique that utilizes electric force alone to drive the spinning process and to produce polymer fibres from solutions or melts [11-14]. Unlike conventional spinning techniques (e.g., solution and melt-spinning), which are capable of producing fibres with diameters in the micrometer range, electrospinning is capable of producing fibres with diameters in the nanometer range. Electrospun polymer nanofibres possess many extraordinary properties including small diameters, the concomitant large specific surface areas, a high degree of structural perfection and the resultant superior mechanical properties [15,16]. Additionally, the non-woven fabrics (mats) made of electrospun polymer nanofibres offer a unique capability to control the pore sizes among nanofibres. Unlike nanorods, nanotubes, and nanowires that are produced mostly by synthetic, bottom-up methods, electrospun nanofibres are produced through a top-down nano-manufacturing process, which results in continuous and low-cost nanofibres that are also relatively easy to align, assemble and process into applications. Many synthetic and/or natural polymers including, but not limited to, polylactide (PLA) [17], poly(e-caprolactone) (PCL) [18], poly(glycolic acid) (PGA) [19], poly(L-lactide-co-caprolactone) (PLLA-CL) [20], proteins (e.g., collagen) [21], and polysaccharides (e.g., chitosan) [22] have been electrospun into nanofibrous mats.

The electrospun fibres can be made to form a porous structure that is ideal for drug or protein delivery carrier [23,24]. In electrospinning, many factors are manipulated in order to make scaffolds with different structural properties. These include electrospinning parameters such as the electric field strength, the distance of electric field generated, the length and radius of the spinneret, the solution flow rate and solution parameters such as concentration, viscosity, and conductivity [25]. There is increasing interest towards employing electrospinning for scaffold fabrication because the mechanical, biological and kinetic properties of the scaffold are easily manipulated by altering the polymer solution composition and processing parameters [26]. However, many researchers have focused on fabricating tissue engineering scaffold from two-polymer blends, such as PCL/gelatin [26], collagen/chitosan [27], PEO/collagen [28], etc. Lee et al. have studied a combined nanocomposite technique with electrospinning process, and a robust dual-porosity scaffold structure was developed for a facile transport of metabolic nutrients and wastes through the nano-sized pores and for the cell implantation and blood vessel invasion through the micro-sized pores [29]. A great number of techniques have been developed to fabricate biodegradable tissue engineered scaffolds from synthetic and/or natural polymers [30-33]. These include gas foaming [34], electrospinning [35], 3-dimensional printing [36], phase separation [37], and freeze-drying [38].

In this study, polycaprolactone (PCL) and chlorophyllin sodium copper salt (CSC) were electrospun into nanofibres. PCL was selected for this study because it has been widely investigated for biomaterials applications. CSC has been widely used as additive in food production [39,40]. The morphologies of the prepared nanofibre mats were examined by a scanning electron microscope (SEM); the mechanical properties were tested by a universal mechanical testing machine; the crystallized property was investigated by an X-ray diffraction (XRD) instrument. The degradation property and CSC released behaviour were also tested in this research work.

EXPERIMENTAL

Materials
All reagents and solvents were used without further purification. PCL (Mn = 80000) and CSC in this work were purchased from Sigma-Aldrich (USA). 2,2,2-Trifluoroethanol (TFE) was obtained from Shanghai Darui Finechem Co., Ltd.

Electrospinning of Nanofibrous Mats
PCL was dissolved in TFE under magnetic stirrer at 240 rpm for 1 h at room temperature, and then the CSC was added in the solution. The total solid concentration of PCL/CSC was 8% with different CSC ratios (0, 5, 10, and 20%).
The experimental set-up used for conducting electrospinning included a high voltage power supply (BGG DC high-voltage generator), purchased from the BMEI Co., Ltd. (Beijing, China), and a digitally controlled and extremely accurate syringe pump (KDS 200), purchased from KD Scientific (Holliston, Massachusetts). During electrospinning, a positive high voltage of 20 kV was applied at the tip of a syringe needle with the inner diameter of 0.9 mm. The electrospun nanofibres were collected on a piece of aluminium foil covered on an electrically grounded metal plate, which was placed at a distance of 12 cm below the tip of the syringe needle. The mass flow rate was maintained at 1.2 mL/h. The electrospinning was conducted under the ambient conditions.

Morphologies of Nanofibrous Mats
A JSM-5600 LV digital vacuum SEM, produced by the Japan Electron Optical Laboratory (Jeol), was employed to examine the morphologies of the prepared nanofibrous mats. Prior to SEM examination, the specimens were sputter-coated with gold to avoid charge accumulation. The diameters of nanofibres were measured using an image visualization software (Image-J) developed by the Upper Austria University of Applied Sciences.

Mechanical Properties of Nanofibrous Mats
Mechanical properties of nanofibrous mats electrospun from either pure PCL nanofibrous mats or nanofibrous mats containing different CSC ratios were tested according to the method described by Huang et al. [41]. A computer-controlled universal mechanical testing machine (H5K-S), purchased from the Tinius Olsen, Inc. (Hounsfield, England), was employed to conduct the tests. During the tests, a piece of paper was first cut into templates with width and gauge-length being 10 mm and 50 mm, respectively; and double-stick tapes were then placed on the top and bottom areas of one side. Subsequently, the nanofibrous mats were carefully peeled off from the aluminium foil and placed onto the double-stick tapes. Finally, single-side tapes were used to secure the gripping areas. The prepared specimens had a planar dimension with the width and gauge-length being 10 mm and 30 mm, respectively. Mechanical properties were tested under the ambient conditions with the temperature being 20ºC and a relative humidity being 65%. The crosshead/elongation speed was set at 10 mm/min.

XRD Test of Electrospinning of Nanofibrous Mats
Electrospun nanofibres samples were tested using an X-ray diffraction instrument (D/MAX-2550PC, Rigaku, Japan) under the condition of CuKα1, 40 kV and 300 mA. In this work, CSC powder, PCL and PCL/CSC nanofibrous mats were studied using XRD.

In Vitro Weight Loss Test
In this study, PCL and PCL/CSC nanofibrous mats with different CSC ratios were selected for the degradation measurements. The mats were cut into square pieces of 3.0×3.0 cm, each one was weighed accurately as \( w_0 \), and then it was immersed into glass tube, which contained 10 mL of PBS. The tubes were incubated in a 37ºC water bath for the maximum of 2 weeks. At the predetermined time points, triplicate mats samples were taken out of the tubes, washed with distilled water, and dried in vacuum at room temperature until it completely dried. The weight of sample after retrieving was recorded as \( w_t \). The weight loss was calculated as \( \Delta w\% = (w_0 - w_t)/w_0 \).

CSC Release Measurement
The as-spun nanofibrous mats were cut into pieces with the weight of 0.30 ± 0.05 g. Then, the mat pieces were soaked in 6 tubes filled with 3.0 mL of PBS, respectively. The tubes with nanofibrous mats were incubated at 37ºC. At various time points, 1.5 mL of supernatant was retrieved from each tube and an equal volume of fresh PBS was replaced. The concentration of CSC in the supernatant was then determined by an UV-vis spectrophotometer (WFZ UV-2102 Unique Technology Shanghai) at an optical wavelength of 400 nm.

RESULTS AND DISCUSSION

Morphology of PCL/CSC Nanofibrous Mats
In electrospinning, the travelling jet solidifies through solvent evaporation and the solidified jet turns into a nanofibre. The solvent evaporation during electrospinning occurs under special conditions including: (1) the jet with micron- or submicron-scaled diameter, (2) the jet carrying excess charges, and (3) the solvent evaporation...
Figure 1. SEM images of the PCL/CSC nanofibrous mats prepared by electrospinning with CSC ratios of: (a) 0, (b) 5, (c) 10, and (d) 20 wt%, respectively, and the pictures of (a’), (b’), (c’) and (d’) are the statistical analysis of (a) to (d), respectively.
tion under the influence of a strong electric field. This results in evaporation rate of solvent during electrospinning to be abnormally fast. Nonetheless, the components of solutes still significantly affect the solidification process, and further influence the morphologies of the electrospun nanofibrous mats. Figures 1a-1d are the SEM images showing the representative morphologies of the PCL/CSC nanofibrous mats prepared from TFE solutions with various CSC ratios of 0, 5, 10, and 20%. As a result, it was evident that the high percentage of CSC (20%) in electrospun solution led to less uniform nanofibres. As shown in Figure 1d, the as-spun fibres lost their basic morphology of ultrafine fibre structure, but presented a net form with ultrathin fibres. It is obvious that some of the fibres were ruptured, and the fibres diameters were 300±50 nm. In Figures 1b and 1c, the nanofibres fabricated from PCL/CSC (the ratios of CSC were 5% and 10%, respectively) were generally similar to pure PCL nanofibres. The average diameters of nanofibres in Figures 1a, 1b, and 1c are 950±50, 1050±50, and 1200±50 nm, respectively. As shown in Figure 2, the average diameters of PCL/CSC nanofibres were increased as the CSC ratio increased till the CSC ratio was 10%, however, nanofibres diameter dropped to 300±50 nm sharply when the CSC ratio was 20%. It is noted that the CSC cannot be fabricated into any type of fibres without other polymer materials. Therefore, when the ratio of CSC in polymer/CSC blend increased to a limited value, we believe that there was no fibre collected on the metal plate.

Figure 2. Average diameters influenced by CSC ratio in PCL/CSC nanofibres.

Molecular Structure

Figure 3 shows XRD patterns of the electrospun PCL/CSC nanofibrous mat, PCL nanofibrous mat, and CSC powder. The PCL/CSC and PCL nanofibrous mats both showed a sharp peak at 2θ of 22.3º and a relatively low intensity peak at 24.1º. However, the CSC powder presents no peak in the XRD pattern. The results demonstrated that CSC powder was non-crystalline, sharp and adding of CSC in PCL nanofibrous mats did not change PCL nanofibres basic molecular structure. Therefore, the PCL/CSC nanofibres were just the simple blend of PCL and CSC. There was no chemical reaction or intermolecular action between CSC and PCL. In that case, the new composite of PCL/CSC nanofibres could maintain the PCL properties.

Mechanical Properties of Nanofibrous Mats

For tissue engineering scaffolds, one of the most important properties is the mechanical property. The scaffold should be strong enough to resist the forces from body movement or outer environment. The representative tensile stress-strain curves of the electrospun nanofibrous mats are shown in Figure 4, and were tested till being broken. It was found that the strength of PCL without any CSC nanofibrous mat was higher than other nanofibrous mats, and the average tensile strength of PCL nanofibrous mats was 4.8 MPa. However, the strains of all nanofibrous mats were similar (about 175-
It was also easy to detect that the PCL/CSC nanofibrous mats containing 5% CSC had the greatest initial tensile modulus. Furthermore, the ultimate tensile strength of PCL/CSC nanofibrous mats with 5% CSC was 4.2 MPa, which was only 0.6 MPa weaker than pure PCL nanofibrous mats. These results consisted of nanofibres morphology studies, and the XRD result demonstrated that CSC was not crystalloid. The addition of CSC in PCL nanofibres made no positive changes on the PCL nanofibrous mats mechanical properties, and in fact made them much weaker. This phenomenon can also be explained by the reason that CSC cannot generate any fibre on its own, and its non-crystalloid structure does not allow it as inner enhancement for PCL nanofibrous mats.

**In Vitro Weight Loss Test**

In comparison to PLA, PGA and other aliphatic polymers, the degradation of PCL in buffer solution is very slow because of its semi-crystalline nature [42]. No PCL degradation has been observed in phosphate-buffered saline (PBS) in the absence of a biocatalyst in the first several weeks [43]. Therefore, the weight losses in this study were generally caused by the loss of CSC, because each sample was incubated in PBS at 37°C water bath for the maximum up to 2 weeks. An in vitro weight loss study is carried out by measuring the weights of a sample before and after degradation in PBS. The weight losses of electrospun PCL/CSC nanofibrous mats are shown in Figure 5. Samples used in these experiments were cut into 3.0×3.0 cm pieces and weights were about 105±5 mg. Each time point was given by an average of three samples and the error bars indicated standard deviation. In this study, only CSC ratios of 5% and 10% had been tested, because the fibres from 20% CSC ratio had lost their basic morphology of ultrafine fibre structure like other nanofibres. As shown in Figure 5, significant mass loss was observed only beyond 10 h. Both curves presented a transition point at the second day after incubating in PBS at 37°C. Then, the mass loss of the PCL/CSC nanofibrous mats was gradually increased with increasing incubation time. For 5% CSC in PCL/CSC nanofibrous mats, the

![Figure 4. Representative tensile stress-strain curves of nanofibrous mats (a); and average tensile strength of PCL/CSC nanofibrous mats with 0, 5, 10, and 20% CSC (b).](image)

![Figure 5. Weight loss of PCL/CSC nanofibrous mats with 5 and 10% CSC incubated in PBS at 37°C.](image)
weight loss reached 4.4% at the end of 13 days, while the 10% CSC ratio was 6.7%. There was also some CSC left in nanofibrous, and could continue being released for longer time.

**In Vitro CSC Release Study**

The released behaviours of CSC from electrospun nanofibrous mats are shown in Figure 6. Firstly, we tried to decide the greatest absorbency peak by scanning from 300 nm to 700 nm using an UV-vis spectrophotometer. In Figure 6a, it is obvious to find that the absorbency curve shows a sharp peak around 400 nm. Thereafter, we determined the standard curve by testing the absorbencies of 0.0001, 0.0002, 0.001, and 0.002 g/mL CSC in PBS solution at the wavelength of 400 nm. Then, the released patterns were calculated by the absorbance of released solution retrieved at predetermined time points and standard curve.

As shown in Figures 6c and 6d, both CSC ratios of PCL/CSC nanofibrous mats present the burst release at the first hours after incubating in PBS solution. At the end of tests, both curves achieved 70-80%. During 310 h release process after incubating in PBS solution for 10 h, CSC in PCL/CSC nanofibrous mats only released 10-20%, which were much less than the first 10 h. However, the results were consistent with in vitro fabrication and characterization of polycaprolactone...
weight loss tests. Furthermore, it is also indicated that the weight loss of PCL/CSC nanofibrous mats was caused by the release of CSC from the fibrous mats.

To be used as tissue engineering scaffold, the nanofibrous mats were very dense for the cells to migrate inside the scaffold. However, if the scaffold could maintain its basic structure with loss of some parts in the first several hours, the cells may have enough space to move into the inner sections of nanofibrous mats.

CONCLUSION

The objectives of this study were to investigate electrospinning of PCL/CSC blend for preparation of tissue engineering scaffold, and to explore weather the PCL/CSC nanofibre mats could be utilized as innovative scaffolds. The polymer/salt type of nanofibrous mats electrospun from their blend could possess the property of supporting human cells. In this study, PCL/CSC nanofibrous mats were successfully prepared by electrospinning from TFE solutions. Systematic investigations were carried out to study the effects of CSC content on the morphology of nanofibrous. The incorporation of CSC with PCL nanofibres resulted in lower strength due to the reason that the CSC could not be electrospun along into any fibres, and the existing CSC could make PCL to be fabricated into ultrafine nanofibres. SEM examinations revealed that the PCL/CSC nanofibres with 20% CSC lose their fibre structure. For evaluation of the performance of PCL/CSC nanofibrous mats as scaffolds which could provide sufficient space for the cells, it was examined by weight loss and CSC release tests. The results demonstrated that PCL/CSC nanofibrous mats could be used as an innovative type of scaffolds which potentially provided the appropriate environment for living cells. Further studies are under way to optimize the electrospun nanofibrous scaffold as well as to understand the interaction between cells and the PCL nanofibrous after CSC leaching.

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