A Novel Biodegradable System Based on BSA/PCL Core-shell Structured Nanofibers for Controlled Drug Delivery

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ABSTRACT

Electrospun core-shell structured nanofibers encapsulating BSA were evaluated as a potential drug delivery model in a biological system in the present study. These nanofibers were prepared by coaxial electrospinning method with PCL as the shell and BSA as the core. During electrospinning, graded and constant feed rates were set for the core and shell components, respectively. These fibers were characterized for their structure, *in vitro* release, and interactions with human dermal fibroblasts (HDFs). The core-shell structure was confirmed by TEM and ATR-FTIR spectroscopy. Release kinetic studies of these nanofibers without HDFs showed a gradual release of BSA instead of a burst release profile. Faster release was observed when HDFs were cultured on these fibers. These results suggest that the core-shell structured nanofibers could possibly have great potentials as a novel controlled drug delivery system, as well as tissue engineering scaffolds encapsulating bioactive molecules for tissue repair and regeneration.

Keywords: core-shell nanofiber, scaffold, PCL, controlled drug delivery, HDF

1 INTRODUCTION

Many bioactive drugs, like proteins and peptides, have their own specific physiochemical and biological properties, e.g. molecular size, conformational stability, solubility, dose dependence and site specificity. These properties often need to be modified when the drugs are applied with conventional delivery systems or administration methods, which might lead to the weakening or even failure of the drugs' pharmacological effects in the body. Additionally, since the drugs are delivered throughout the body instead of specific areas, the risk of side effects on other tissues will be highly increased.

To solve this problem, many new delivery systems have been developed and tested so far [1-7]. Among those, nanofibers are promising for the delivery of drugs and other therapeutics to specific cells, even to the specific compartment within those cells, or specific tissues, wherever they are meant to have effects. By directing drugs primarily to their targeting sites, lower dosage will be needed and exposure of other body tissues to the drugs will be reduced. This would in turn reduce undesirable side effects of the drugs.

By far, electrospinning is the most prevalent method used for the production of nano/microscale fibers because of its simplicity, versatility, low cost, and scale-controllability. During electrospinning, an electric field generated by high-voltage sources cause the polymer jet to elongate and spray into fibers. These fibers are then collected onto a grounded surface [8].

To incorporate drugs into the nanofibers, three major methods are used: (1) surface modification of the nanofibers after electrospinningm [9,10]; (2) use of drug and polymer mixed solution in electrospinning to produce blended nanofibers [5,6]; (3) utilizing of co-axial electrospinning technique to generate core-shell structured nanofibers [7,11-13]. The co-axial electrospinning method has many advantages over the other two. For instance, many drugs are only water-soluble and will likely lose their bioactivity after dissolving in organic solvents. Therefore, co-axial electrospinning seems to be the optimal method for incorporation of these drugs into the nanofibers. Recently, co-axial electrospinning has been explored for its potentials in surface functionalizing of nanofiber scaffolds for skin tissue engineering [14] and release of bioactive agents in the absence of cells [7]. However, there are no reports of cells influence on the release of the drugs encapsulated inside the fibers. In addition, drugs solubilized in water have never been attempted in co-axial electrospinning as the inner core component of the core-shell structured nanofibers.

In this study, we fabricated core-shell structured nanofibers and studied the kinetic release of the core component from the fibers with and without cells. A model protein, bovine serum albumin (BSA) and Poly (caprolactone) (PCL) were selected as the core and shell component, respectively. In a novel approach, BSA was dissolved in phosphate-buffered solution (PBS) and electrospun as the core component of the core-shell nanofibers. Human dermal fibroblasts (HDFs) were used to investigate the cell effect on fiber degradation and BSA release.

2 MATERIALS AND METHODS

2.1 Materials

Poly (caprolactone) (PCL, Mw=80,000), bovine serum albumin (BSA), FITC conjugated BSA (FITC-BSA) were purchased from Sigma, USA. The solvents 1,1,1,3,3,3-hexafluor-2-propanol (HFIP, purity>99%) and phosphate-buffered solution (PBS, pH 7.4) were obtained from Aldrich, USA. Human dermal fibroblasts (HDFs) were obtained from the National University Hospital, Singapore

2.2 Fabrication of Core-shell Structured Nanofibers

Core-shell structured nanofibers were prepared using coaxial electrospinning method as described previously (Fig. 1) [13]. Briefly, a spinneret consisting of two coaxial capillary tubes was used to electrospin two immiscible liquid solutions into a compound jet directly. After evaporation of the solvents during jet propulsion, core-shell structured bi-component nanofibers were produced.

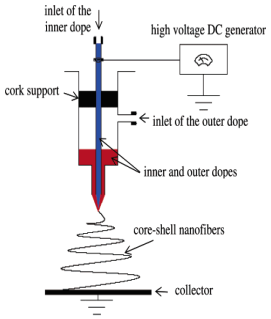

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Figure 1: Experimental setup for the co-axial electrospinning setup.

For protein release study from these fibers independent of cells, BSA and PCL were used as the core and shell components, respectively. The protein released was determined by UV absorbance at 280 nm. For cell-induced release study, HDFs were cultured on core-shell nanofibers with growth medium which contains various proteins. Thus measuring the amount of BSA released by UV absorbance at 280 nm of the culture medium is no longer feasible. To overcome this problem, fluorescein isothiocyanate conjugated BSA (FITC-BSA) was used as the core with PCL as the shell component. When performing coaxial electrospinning, the flow rate of shell component PCL was set at a constant of 2.0 ml/hr. In contrast, graded flow rates were set for the core component BSA and FITC-BSA at 0.06 ml/hr, 0.10 ml/hr, and 0.14 ml/hr, to obtain fibers encapsulating different amount of proteins.

Pure PCL nanofibers were used as the control of all release and fiber-cell interaction studies, which were prepared with the conventional electrospinning [15].

2.3 Core-shell Structure Confirmation

The core-shell structure of the nanofibers was characterized by transmission electron microscopy (TEM) and attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy. TEM images were obtained with a JEOL JEM-2010F FasTEM field emission electron microscope (JEOL, Japan) operated at 100keV. Based on the TEM microphotographs, fiber diameters and the corresponding wall thickness were analyzed by an image visualization software ImageJ developed by the Upper Austria University of Applied Sciences. Surface composition of the core-shell structured nanofibers was analyzed using AVATAR 300 ATR-FTIR spectroscopy (Thermo Nicolet, USA). ATR-FTIR spectra of core-shell structured nanofibers, pure PCL nanofibers, as well as finely powdered BSA were collected using Nicolet OMNI-Sampler ATR Smart Accessory (Thermo Nicolet, USA).

2.4 Morphology and Size Distribution Measurements

The morphology of electrospun core-shell structured nanofibers was observed by a Quanta FEG 200 field emission scanning electron microscope (SEM) (FEI, Netherlands) at an accelerating voltage of 10kV. Fiber diameters were also analyzed by ImageJ, based on the field emission SEM microphotographs. For each of the three different nanofiber samples, more than 200 individual fibers were analyzed.

2.5 *In vitro* Release Study

In vitro protein release study of the core-shell structured nanofibers with and without cells was performed in triplicate. For the release study without cells, a known weight of BSA/PCL core-shell structured nanofibers was immersed in PBS and incubated in a shaking water bath at 37°C. At predetermined time intervals, BSA released in the PBS was removed and replaced with equal amount of fresh PBS. The BSA released in the PBS was analyzed by a UV-spectrometer at the wavelength of 280 nm. The amount of BSA released was determined from a calibration curve. Pure PCL fibers under the same conditions were used as the control. Similar treatment was performed with the FITC-BSA/PCL core-shell structured nanofibers for release study with cells.

3 RESULTS AND DISCUSSION

3.1 Core-shell Structure Verification

The core-shell structure of the nanofibers was confirmed by transmission electron microscopy (TEM) and attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy. The TEM results are shown in Fig. 2. For fibers with inside feeding rate of 0.06 ml/hr, 0.10 ml/hr, and 0.14 ml/hr, their average wall thickness to diameter ratio were 0.2271(SD=0.056), 0.2938(SD=0.057), and 0.3537(SD=0.031), respectively. This shows that as the inside feeding rate increased, the wall of the fibers also increased.

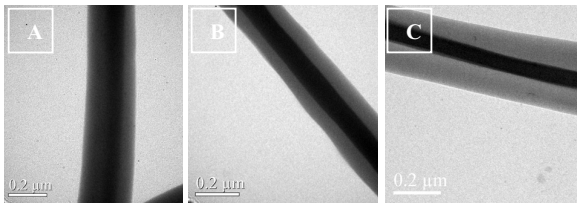

Figure 2: Representative TEM images of FITC-BSA/PCL core-shell structured nanofibers with inner feed rates set at 0.06 ml/hr (A), 0.10 ml/hr (B), and 0.14 ml/hr (C) when performing coaxial electrospinning. Mag. 15000x.

Fig. 3 shows the results of ATR-FTIR which analyzed the surface composition of the core-shell structured nanofibers. As can be seen from the figure, the core-shell structured nanofibers have the same surface composition as the pure PCL fibers. The two typical peaks for pure BSA were not present in the spectra of core-shell nanofibers. Thus we could conclude that the BSA was encapsulated inside the fibers. Similar results were also obtained in the ATR spectra of FITC-BSA/PCL core-shell structured nanofibers.

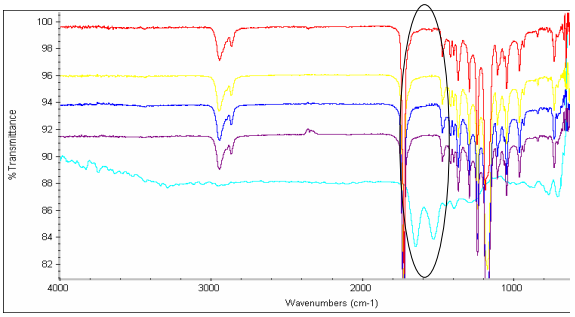

Figure 3: ATR-FTIR spectra of pure PCL fibers, the three different core-shell structured fibers (with inner feed rates of 0.06 ml/hr, 0.10 ml/hr and 0.14 ml/hr), and pure BSA.

3.2 Morphology and Size Distribution Measurements

The morphology of the electrospun core-shell structured nanofibers was observed by SEM (Fig. 4). Fiber diameters were analyzed by ImageJ, based on the field emission SEM microphotographs.

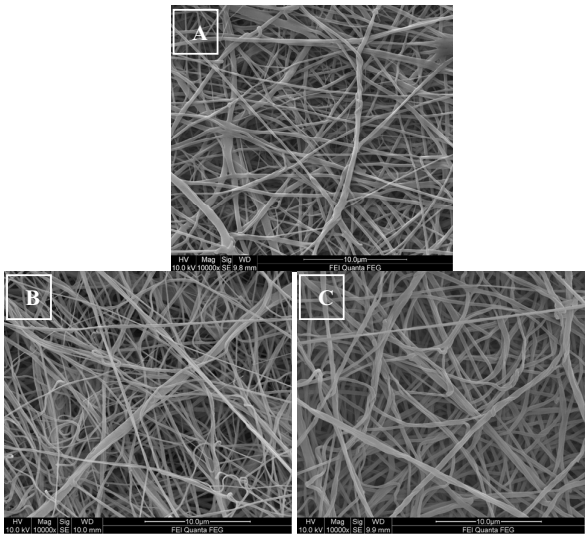

Figure 4: SEM images of BSA/PCL core-shell structured nanofibers with inner flow rates set at 0.06 ml/hr (A), 0.10 ml/hr (B), and 0.14 ml/hr (C) when performing electrospinning. (A-C) Mag. 10000x.

According to the size distribution results (Fig. 5), the fibers with the fastest inside feed rate, i.e., 0.14 ml/hr, have the biggest fiber diameter. As the inside feeding rate decreased, the diameter also decreased.

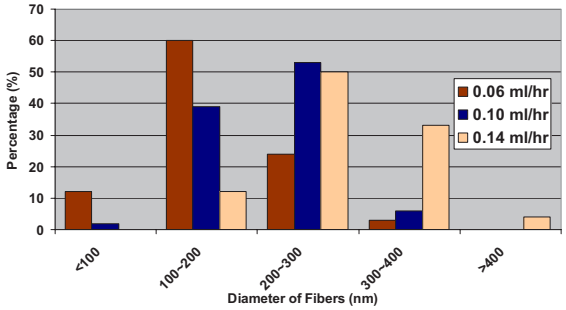

Figure 5: Size distribution of the core-shell structured nanofibers with inner feed rates set at 0.06 ml/hr, 0.10 ml/hr, and 0.14 ml/hr when performing coaxial electrospinning.

3.3 *In vitro* Release Study

Release kinetic studies of these nanofibers without HDFs showed a gradual release of BSA, instead of a burst release profile (Fig. 6). Faster release was observed when HDFs were cultured on these fibers. This is likely due to the degradative enzymes secreted by the cells, which potentially increased the polymer degradation rate of the polymer.

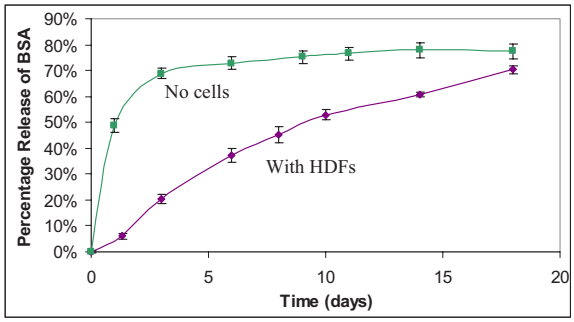

Figure 6: Percentage BSA release from the core-shell structured fibers (inner feed rate set as 0.06 ml/hr) vs. time with and without cells cultured on the nanofibers. Similar results were observed from core-shell fibers with the inner feed rates set as 0.10 ml/hr and 0.14 ml/hr.

4 CONCLUSIONS

BSA/PCL core-shell structured nanofibers were fabricated by co-axial electrospinning. Release kinetic studies of these core-shell structured nanofibers in the absence of HDFs showed a gradual release of BSA instead of a burst release profile for around twenty days. Faster release of BSA could be observed when HDFs were cultured on these fibers. This release profile is critical for regulating cell growth in tissue engineering applications in which bioactive molecules need to be encapsulated and slow passive delivery is preferred. These results suggest that the core-shell structured nanofibers could possibly have great potentials as a novel controlled drug delivery system, as well as tissue engineering scaffolds encapsulating growth factors, enzymes, bioactive molecules, drugs, and even antibiotics for tissue repair and regeneration.

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