The Influence of Fiber Diameter of Electrospun Poly(lactic acid) on Drug Delivery

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Abstract: Electrospinning is a simple process for the production of fibers with diameters in the range from submicron to micron. Herein we aim to explore the influence of fibrous diameter on the drug delivery. The feasible methods by making choice of solvents and changing flow rate were used to prepare 5-fluorouracil-loaded poly(lactide) (PLA) fibers with a large diameter gap. The drug release behavior in vitro was investigated and analyzed in phosphate buffer solution. The drug distribution and fiber diameter both affected the initial burst release. The results showed that all the asspun fibers could not avoid of burst release. The coarse fibers exhibited slight burst release as compared to fine fibers. During the second stage, the fine fibers released faster than that of the coarse fibers. For the whole release stage, the large-diameter fibers seemed to be beneficial for drug release in the long term and smoothly. The MTT results showed that the cytotoxicity of drugs was maintained.

Keywords: Nanofibers, Electrospinning, Polylactide, 5-Fluorouracil, Drug release

Introduction

Chemotherapy is an effective treatment for cancer and other serious diseases, such as cardiovascular restenosis and AIDS. It carries a high risk due to drug toxicity and problems in drug formulation. There has been an increasing interest in the past decade in developing biodegradable polymer as an effective drug-delivery system for chemotherapy. However, the conventional polymeric drug delivery systems such as nano- or microspheres, liposomes and hydrogels often have the problem of burst drug-release at the beginning and lower drug-loading efficiency. Recently, it was found that drugs could be encapsulated directly into electrospun fibers and used for controlled drug release [1-8]. Varieties of model drugs including cefazolin [1], cefoxitin sodium [2], tetracycline hydrochloride [3], itraconazole [4], ketanserin [4], rifampin [5], paclitaxel [6], doxorubicin [6], lamellar hydrotalcite [7], and ibuprofen [8] have been incorporated into biodegradable polymers such as polylactide (PLA) for drug delivery system, respectively. Luu et al. [9] electrospun polymer/DNA composite scaffolds for therapeutic application in gene delivery for tissue engineering. Wang et al. [10] prepared coreshell fibers used for drug release applications by coaxial electrospinning. Electrospinning is one of the few techniques to prepare long fibers of nano- to micrometer diameter, and great progress has been made in recent years. The use of electrospun fibers as drug carriers would be promising in the future biomedical applications, especially in the postoperative local chemotherapy.

5-Fluorouracil (5-FU) is known as remarkable antitumor activity, but it has high toxic side effects. Several attempts have been done for reducing its toxicity via slow release [11,12]. PLA has drawn great attention and is widely used in various biomedical applications due to its biodegradability, biocompatibility, good mechanical properties and solubility in common solvents for processing.

Several parameters (concentration, solvent, voltage, flow rate, distance between tip and collector) affect the diameter of fibers. Among them, the solvent is principal even though the concentration affects remarkably. Uniform nanofibers less than 500 nm are difficult to obtain, as continued dilution of the polymeric solution results in loss of Taylor cone stability and then electrospaying. Adding dopants can stabilize the polymer jet, yielding fibers as small as 250 nm with a narrow diameter distribution [13]. By using different solvent for electrospinning, the nanofiber with a large gap diameter is available and facile. Previously, Mao group [14] first electrospun poly(ethersulfone) to obtain fibers ranged from 300 to 1500 nm and then explored the influence of fiber diameter on neural stem cell differentiation and proliferation. And so far, some publications have reported the influence of fiber diameter on the drug delivery. We hypothesize that drug release behavior should have a close relationship with the fibrous diameter. In this study, drug-loaded fibers were electrospun using chloroform and hexafluoro-2-isopropanol as solvents, respectively. The fibers were characterized with scanning electron microscope (SEM), Attenuated Total Reflection Infrared Spectroscopy (ATR-IR) and Electron Spectroscopy for Chemical Analysis (ESCA). The in vitro drug release behavior of fibers was studied in the PBS solution. In addition, the cytotoxicity of drug-loaded fibers
was determined by MTT method.

**Materials and Methods**

**Materials**

PLA with an inherent viscosity of 1.822 d/l was donated by Toray-Saehan Co., Korea, and used without further purification. 5-Flourouracil was purchased from Sigma Chem. Co. (St Louis, USA). 1,1,1,3,3,3-Hexafluoro-2-isopropanol (HFIP) was obtained from Aldrich Chemical Co (MO, USA). Dimethylformamid (DMF) and chloroform (CF) were commercially available in China.

**Fabrication of Fibers by Electrospinning**

To obtain nanofiber with largely different diameter, HFIP and chloroform were used, respectively. To get the desired concentration, PLA was added and then stirred in the corresponding solvent overnight. 5-Flourouracil was dissolved in DMF (10 wt %) in advance. Prior to electrospinning, the PLA solution and drug solution were mixed and stirred for 30 min. The blended solution was delivered to a metal needle (19 G) connected to a high voltage power supply. Upon applying high voltage power, a fluid jet was ejected from the needle. As the jet was accelerated towards a ground collector, the solvent was evaporated and a charged polymer fiber was deposited on the collector in the form of nanofibrous mats. The drug loading percentage was 3 % according to the initial polymer weight. In this study, the typical parameters of electrospinning are listed in Table 1.

**Characterization**

The morphology of electrospun nanofibers was observed with field emission scanning electron microscope (FE-SEM, Hitachi S-4300, Japan) after gold coating. The ATR-FTIR spectra of the nanofibers were obtained using a FT-IR spectrometer (Jasco-620, Tokyo, Japan). The electrospun nanofibers were analyzed using electron spectroscopy for chemical analysis (ESCA, VG microtech, UK) equipped with Mg Kα at 1253.6 eV and 150 W power at the anode. A survey scan spectrum was taken and the surface elemental compositions relative to carbon were calculated from peak heights with a correction for atomic sensitivity.

**5-Flourouracil Release in vitro**

The total content of 5-flourouracil in the fibers was determined as follows. About 0.2 g of drug-loaded mats were placed into separate vials filled with 200 ml of 0.05 M phosphate buffer solution (PBS, pH 7.4) containing 50 ug/ml of proteinase K at 37 ºC. For several hours, the mats were degraded into small chips, indicating that 5-FU had been completely released into the buffer solution. The resultant solutions were monitored using a UV-Visible spectrophotometer at the wavelength of 267 nm. The concentration of drug in the release solution was determined according to the calibration curve of 5-FU in the same buffer. The total content of 5-FU in the fibers was easily calculated from the average of the three fiber sheets.

The 5-flourouracil release from the electrospun fibers was determined as follows. About 0.2 g of sheet was incubated in 200 ml of 0.05 M phosphate buffer solution (PBS, pH 7.4) at 37 ºC. At a certain time, 3 ml of the buffer was taken out and then added equal amount of fresh buffer to the incubation solution. The UV absorbance of 5-FU in the buffer was measured and converted to the 5-FU concentration according to the calibration curve of 5-FU in the same buffer. Then the accumulative amount of the released 5-FU was calculated as a function of the incubation time.

**MTT Assay for Cytotoxicity**

In order to examine the viability of cells on nanofibers, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazoliumbromide (MTT, Sigma, MO, USA) assay was used [15]. The circular fiber mats were fitted in a 24-well culture plate and subsequently immersed in a DMEM medium containing 10 % fetal bovine serum (FBS, Gibco) and 1 % penicillin G-streptomycin. 300 ul of NIH 3T3 cell solution (5×10^4 cells/cm^2) was added and incubated in a humidified atmosphere of 5 % CO₂ at 37 ºC. After 48 h incubation, 50 ul of MTT solution (5 mg/ml in PBS) was added to each well and incubated for another 4 h. After removing the medium, the water insoluble formazan product was dissolved with acidic isopropanol (0.04 N HCl-isopropanol) for 30 min at room temperature in the dark and then transferred to a 96 well plate. The absorbance was measured at 570 nm using ELISA plate reader (EL x 800, Bio-Tek Instruments Inc., USA).

**Table 1. The electrospinning parameters and the fiber characteristics**

<table>
<thead>
<tr>
<th>Samples</th>
<th>Solvent</th>
<th>Concentration (wt%)</th>
<th>Voltage (kV)</th>
<th>Distance (cm)</th>
<th>Flow rate (m/h)</th>
<th>Diameter (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLA-F-1</td>
<td>CF</td>
<td>10</td>
<td>10</td>
<td>15</td>
<td>0.5</td>
<td>2.45±0.18</td>
</tr>
<tr>
<td>PLA-F-2</td>
<td>HFIP</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>0.5</td>
<td>0.72±0.12</td>
</tr>
<tr>
<td>Drug-F-1</td>
<td>CF/DMF</td>
<td>10</td>
<td>10</td>
<td>15</td>
<td>0.5</td>
<td>1.02±0.37</td>
</tr>
<tr>
<td>Drug-F-2</td>
<td>CF/DMF</td>
<td>10</td>
<td>10</td>
<td>15</td>
<td>0.3</td>
<td>0.73±0.15</td>
</tr>
<tr>
<td>Drug-F-3</td>
<td>HFIP/DMF</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>0.5</td>
<td>0.53±0.21</td>
</tr>
<tr>
<td>Drug-F-4</td>
<td>HFIP/DMF</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>0.3</td>
<td>0.35±0.11</td>
</tr>
</tbody>
</table>

*The distance is between the spinneret and collector.*