Fabrication and Characterization of PCL/GE-based electrospun Nanofibers for Tissue Engineering and Drug Delivery Application

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Abstract. Tissue engineering (TE) provides an alternative option to solve limitation of organ and tissue transplantation issues. Fabrication of scaffold is a major challenge because it needs to provide a suitable medium for cell growing and drug delivery which enhances cell transplantation efficiency. The porosity, biodegradability and biocompatibility are the important properties in fabricating a scaffold. Our previous work had found that electrospinning of Polycaprolactone (PCL)/Gelatin (GE)-based nanofibers in 14\% w/v polymer solution in 18kV showed the best results in morphology, average diameters, average pore size and hydrophilicity. Hence, in this project, we are interested to study the relationship between weight ratio of PCL and GE in the same concentration (14\%) to the morphology, degradation rate and porosity of nanofibers. Experimental results showed that PCL with 1.2g and GE with 0.2g was able to produce better nanofibers. The sample was then further deployed in drug (tetracycline hydrochloride) loading and it was successfully loaded proven by Energy Dispersive X-Ray (EDX) Spectrum. These nanofibers are predicted to be potentially useful for drug delivery and TE application.

Introduction

In the United State, approximately 18 of patients die each day while waiting for a suitable organ donor \cite{1}. The current demand is critical \cite{2}, and some patients even die after organ transplantation due to their own immunity rejection \cite{3}. Hence, TE has developed dramatically as a novel scientific approach \cite{4} and alternative option \cite{5}. It aims to regenerate biological substitutes to repair or replace the damaged organs and lost tissues \cite{6}. However, one of the major challenges in tissue engineering is to design and fabricate a suitable scaffold \cite{7}. Biodegradable nanofibrous polymer scaffolds with an open pore structure have been extensively investigated for TE applications, since they have similar structure to extracellular matrix (ECM) \cite{8}. High porosity provides more structural spaces for cell activities and facilitates efficient exchange of nutrient and metabolic wastes \cite{9}. Biodegradable property is beneficial to enable tissue integration and avoid subsequent surgical removal of scaffold \cite{10}. Therefore, polymeric scaffolds fabricated by electrospinning are gaining a great interest due to ability to provide ideal microenvironments \cite{11}.

Up to now, various research works focus on blend nanofibers to gain the desired properties of scaffold, for example PCL/GE \cite{12,13}. GE, a biological origin natural polymer, rapidly dissolves and disappears under cell culture condition \cite{14} while PCL, a low degradation rate of synthetic polymer, relatively lacks of cell recognition sites \cite{15}. Hence, polymer blending of PCL and GE can control the degradation rate \cite{16}. PCL and GE are approved by U.S Food and Drug Administration (FDA), proving that they are non-toxic polymers and safe to be used in human body \cite{17,18}.

However, most of the recent researches concentrate on fabrication, morphological, characterization and biological investigations. For instance, Gupta \cite{19} and Yanzhong \cite{20} focused on fabrication and characterization of PCL/GE while Laleh \cite{21} emphasized on PCL/GE application on nerve growing.
There are only few papers describing the influences of PCL and GE to the degradation rate and porosity of blended nanofibers. Hence, in this study, we emphasize on how the weight ratios of PCL and GE in same concentration (14%) can affect the morphology, degradation rate and porosity of nanofibers due to their importance to a nanofibrous scaffold. After that, the drug of tetracycline hydrochloride was tried to load inside the nanofibers and spinned. The EDX is used to check the presence of drug in nanofibers after electrospinning.

**Materials and Methods**

**Materials.** GE powder from porcine skin, PCL pellets (Mw = 70,000-90,000), and solvent of formic acid (density: 1.22g/mL) were purchased from Sigma Aldrich. Tetracycline hydrochloride powder was obtained from Calbiochem.

**Preparation of PCL/GE-based solutions.** Two samples of 14% w/v PCL/GE-based solution (Sample A: PCL=1.0g; GE=0.4g and Sample B: PCL=1.2g; GE=0.2g) were prepared.

**Fabrication of 14% w/v PCL/GE-based Electrospun Nanofibers.** The two samples (Sample A and B) of 14% w/v PCL/GE-based nanofibers were fabricated by electrospinning.

**Characterization.** The density and porosity were then measured by liquid displacement method.

**In-vitro degradation study.** The 2 samples of nanofibers were immersed in Phosphate Buffer Saline (PBS) and incubated in vitro at 37°C for 1 to 14 days. Water uptake and weight loss percentages were calculated using formulas below.

\[
\text{Water Uptake} (\%) = \left(\frac{W_d-W_i}{W_i}\right) \times 100; \quad \text{Weight Loss} (\%) = \left(\frac{W_w-W_f}{W_w}\right) \times 100
\]

where \(W_d\) and \(W_i\) are specimen weights before soaking in PBS; \(W_w\) and \(W_f\) are specimen weights after soaking in PBS. The morphologies of nanofibers were observed by Field Emission Scanning Electron Microscopy (FESEM).

**Results and Discussions**

**Average diameter and pore size.** Experimental results (Fig. 1) depicted that sample B had a smaller average diameter and larger pore size.

**Porosity and Density.** The experimental results showed that the porosity of sample A is slightly higher than sample B.

**Degradation Test.** The overall water uptake percentages of sample B was lower than sample A as shown in Fig 2 (I). For sample A, there was a declination after day-2 indicated the starting of degradation of GE in nanofibers due to less amount of hydrophilic GE was available to take up the water. In Fig. 2 (II), the sample B, the percentage of weight loss was a little higher than sample A in day-14. The weight loss can be caused by degradation of GE in nanofibers. It can be demonstrated that the weight loss of nanofibers increases with the increase of pore size in nanofibers. The broken fibers in Fig.3 again emphasized that the degradation was happened in nanofibers after 14-days.
Table 1: Porosity and Density of Sample A and B.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Weight Ratio PCL/GE</th>
<th>Total Volume ($V_T$)</th>
<th>Density (d)</th>
<th>Porosity (Ɛ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1.0g/0.4g</td>
<td>$V_T$ = 0.15ml</td>
<td>d=0.0107</td>
<td>Ɛ =66.67%</td>
</tr>
<tr>
<td>B</td>
<td>1.2g/0.2g</td>
<td>$V_T$ = 0.20ml</td>
<td>d=0.0110</td>
<td>Ɛ =50.00%</td>
</tr>
</tbody>
</table>

Fig 2: The percentage of water uptake (I) and weight loss (II) of sample A and B

Conclusion

In this project, sample B with amount of 1.2g PCL and less 0/2g GE depicted the smaller fiber diameter, larger pore sizes and slightly higher in percentages of weight loss. In the last part of project, tetracycline hydrochloride was successfully solubilized into 14% w/v PCL/GE polymer solution and well loaded inside the nanofibers after electrospinning. Overall, these nanofibers are predicted to be potentially useful for drug delivery and TE application.

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