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WETTABILITY AND WATER UPTAKE PROPERTIES OF PLA AND PCL/GELATIN-BASED ELECTROSPUN SCAFFOLDS

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ABSTRACT

Electrospun fibrous scaffolds are widely investigated for the regeneration of damaged tissues. In this study, the electrospinning technique was used to fabricate two different kinds of fibrous scaffolds which are poly (lactic acid) (PLA) and polycaprolactone (PCL)/Gelatin (Ge). The scaffolds were then characterized using Scanning electron microscope (SEM) to observe the morphology of fibers. Water contact angle was measured to determine the wettability of PLA and PCL/Gelatin electrospun scaffolds. Water uptake properties of PLA and PCL/Ge were compared. Based on the results obtained, PLA had the average fiber diameter of 2.8µm and it was too hydrophobic. Meanwhile, the diameter of PCL/Gelatin fibers was in the range of 56nm to 473nm. The PCL/Ge scaffolds had lower water contact angle. PCL/Ge had higher water uptake properties than PLA fibrous scaffolds.

Keywords: poly (lactic acid) (PLA), polycaprolactone (PCL)/ gelatin, electrospun fibers, bone tissue engineering.

INTRODUCTION

Metal Implants, allogeneic and autogeneous bone grafting are common medical treatments for the large amount of bone tissue loss due to trauma and severe diseases. However, many restrictions such as shortage of available donor tissue (autogeneous) (Sun *et al*., 2009), cell-mediated immune responses and pathogen transfer (allogenic) (Shafiei, Bigham, Dehghani, & Nezhad, 2009), as well as bio-inert material which limits the active bonding with human tissue (metal implant) (Younger & Chapman, 1989) have constraints its implementation in clinical application. Bone Tissue Engineering scaffold, therefore, holds the promise of therapeutic potential to solve these problems by providing a mimicked bone extracellular matrix (ECM) platform, allowing ingrowth of new bone tissue using the patient own cells. Recent technological researches have facilitated the development of the fibrous scaffold using electrospinning technique in bone tissue-engineered construct application. Electrospinning is widely applied in scaffold fabrication due to its ability to produce high surface area to volume ratio structure, high porosity, and wide polymers selectivity, i.e. poly (lactic acid) (PLA), poly (glycolic acid) (PGA) and poly (ε-caprolactone) (PCL) (Feng, Shen, Fu, & Shao, 2011; Lim, , & Sultana, 2016). Nonetheless, the characteristics of an ideal scaffold such as non-toxicity, biodegradability, hydrophilicity, mechanical strength and biocompatibility are highly depended on the use of polymer (Sultana, Mokhtar, Hassan, Mad Jin, Roozbahani, and Khan, 2015).

Among polymeric materials, PCL and PCL/Gelatin (Chong, Lim, and Sultana, 2015) have been studied for tissues regeneration as these are non-toxic, biodegradable and biocompatible properties (Gautam, Dinda, and Mishra, 2013). Badami et al found that PLA electrospun scaffold able to affect the cell differentiation of bone osteoblast (Badami, Kreke, Thompson, Riffle, and Goldstein, 2006). PCL/Gelatin, on the other hand, was found to be a good penetration for bone marrow stromal cells (BMSCs) within the nanofiber matrix (Jang *et al.*, 2009). Hence, this study aims to compare the characteristics of PCL/Gelatin and PLA, as scaffold materials using electrospinning technique. The fabricated PCL/Gelatin and PLA-based fibrous scaffold were characterized for their morphologies, water contact angles, and water uptake properties. Two different types of polymer-based electrospun fibers were characterized and their wettability and water uptake properties were compared as our next approach of research will be to fabricate a bi-layer scaffold with suitable properties for tissue regeneration.

MATERIALS AND METHOD

Materials

PLA pellets (Density = 1.210–1.430 g/cm3), PCL pellets (Mw = 70,000-90,000), Gelatin from porcine skin as powder form (gel strength 300g Bloom, type A), Chloroform (Molar mass = 119.38 g/mol), Dimethyl formamide (DMF) (Molar mass = 73.09 g/mol) and Formic Acid, (Mw = 46.03; Density = 1.22 g/mL) were purchased from Sigma Aldrich.

Preparation of pure PLA-based polymer solution

A 12.5% of pure PLA-based polymer solution was prepared using chloroform and DMF as solvent in the ratio of 9:1 under magnetic stirrer at 50°C.

Preparation of PCL/Gelatin-based polymer solution

PCL/Gelatin-based polymer solution (70:30) with 12.5% w/v was prepared using formic acid as the solvent under magnetic stirrer at room temperature.

Fabrication of PCL/GE-based electrospun nanofibers by electrospinning

The 12.5% w/v PLA-based polymer solution was delivered through a syringe pump (Veryark TCV-IV) at a constant flow rate of 1ml/h to control the mass flow rate

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during electrospinning. Then, a high voltage (20-25kV) was applied to the needle of syringe. A Taylor cone was form and fluid jet was ejected from the needles to a grounded collector (10cmx10cm aluminum sheet) at the distance of 10cm. The fibrous scaffolds were collected at the collector. All electrospun fibrous scaffolds were kept in desiccator until they were totally dry. These steps were repeated with 12.5% w/v PCL/Gelatin-based polymer solution.

Morphologies observation

Morphologies of the PLA and PCL/Gelatin electrospun fibers were observed using Scanning Electron Microscopy (SEM). The obtained images were further analyzed by J-Image to obtain the fibers' diameter.

Water contact angle measurement

The hydrophilicity of electrospun PLA and PCL/Gelatin fibrous scaffold was studied by a video contact angle instrument (VCA Optima, AST Products, Inc). Deionized water was dropped onto the surface of fibrous scaffold and image of the drop was recorded. The VCA Optima software was used to analyze the drop image and calculate the contact angle.

Water uptake

The 20mmx80mm pre-weighed fibrous scaffolds were immersed in the deionized water for 2, 5, 10, 15, 20, 25, 30, 45 and 60 minutes. The samples were taken out according to the time listed and then dried on filter paper to remove the excess water and weighed. All the percentages of water uptake were measured using the formula below:

$$\text{Water Uptake (\%)} = [(W_w - W_d)/W_d] \times 100\% \quad (1)$$

where Wd and Ww are specimen weights before and after soaking in deionized water.

RESULTS AND DISCUSSIONS

Morphology of PCL/GE-based electrospun nanofibers

Figure-1, Figure-2 and Table-1 give the fibers' diameter data. Based on this study, it was found that both PLA and PCL/Gelatin exhibited bead-free fibers with smooth surface morphology. The only difference between these two fibers was the PCL/Gelatin produced relatively finer fibers in the range of 0.06 μm to 0.47 μm with the average diameter of 0.21 μm. On the other hand, the diameter of pure PLA electrospun fibers was in the range of 1.6μm to 4.8μm with 2.85μm as its average diameter, which was relatively larger than PCL/Gelatin electrospun fibers. It is interesting to note that the diameter of electrospun fibers is able to affect cell morphology and cell proliferation. Electrospun fibers diameters with sizes around 0.14μm to 2.1μm is the optimum for the growth of MC3T3-E1 osteoprogenitor cells (Badami *et al*., 2006) while diameters around 500nm is suitable for the initial adhesion and growth of BMSCs (Jang *et al*., 2009). Thus, in this study, fabricated PLA and PCL/Gelatin scaffolds were suitable for the growth of MC3T3-E1 osteoprogenitor and BMSCs cells.

Table-1. The diameter of PCL/Gelatin and PLA fibers.

Electrospun fibers	Range of diameter (μm)	Average diameter (μm)	Observations
PCL/Gelatin	0.06 - 0.47	0.21	No beads and homogenous
PLA	1.6 - 4.8	2.85	No beads and homogenous

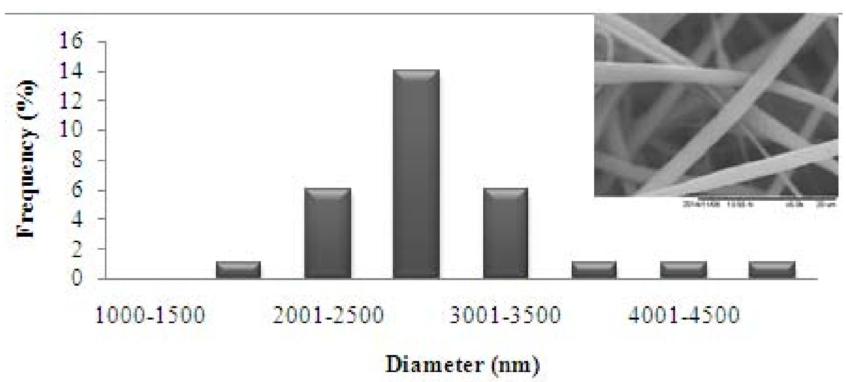

Figure-1. Frequency of diameter and SEM image (inlet) of electrospun PLA fibers.

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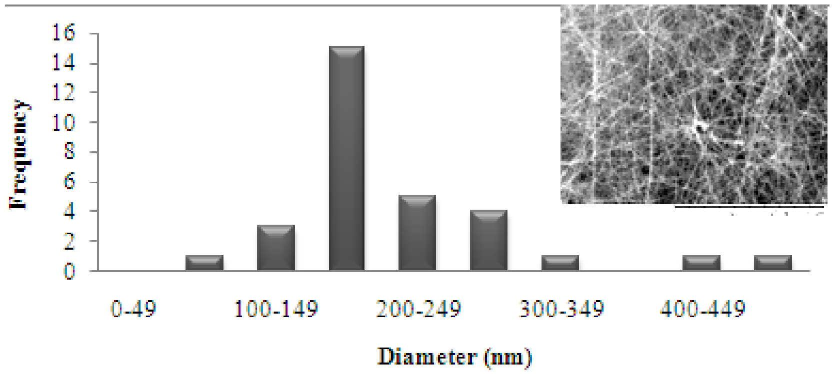

Figure-2. Frequency of diameter and SEM image (inlet) of electrospun PCL/Gelatin fibers.

Water contact angle

The water contact angle measurement was conducted to examine the wettability of the electrospun fibers which was important for cell adhesion. Table-2 depicts the contact angle data on different electrospun fibers. Lower contact angle leads to higher wetting tendency. In this study, water contact angle of PCL/Gelatin (53.95º) was much lower than pure PLA (131.85º) by 77.9º, indicating the PCL/Gelatin was hydrophilic scaffolds while PLA was hydrophobic in nature. This is mainly due to the presence of amide bone in gelatin (PCL/Gelatin blended electrospun fibers), which is able to form hydrogen bond with water, and thus showing higher wettability than pure PLA electrospun fibers.

Table-2. The water contact angle of fibers.

Electrospun fibers	Contact Angle (º)
PCL/Gelatin	53.95 ± 2.19
PLA	131.85 ± 0.21

Water uptake

Figure-3 shows the percentages of the water uptake between PLA and PCL/Gelatin-based electrospun nanofibers at 37◦C. It was obvious that the overall water uptake of PCL/Gelatin was higher than the pure PLA-based electrospun fibers. As expected, the results indicated that the hydrophilic PCL/Gelatin electrospun fibers were able to attain more water than pure PLA electrospun fibers. The interesting part in this study was the degradation rate. After 2 minutes, approximate equilibrium reached for PCL/Gelatin nanofibers indicated that the starting of degradation of nanofibers while PLA electrospun fibers did not show any degradation.

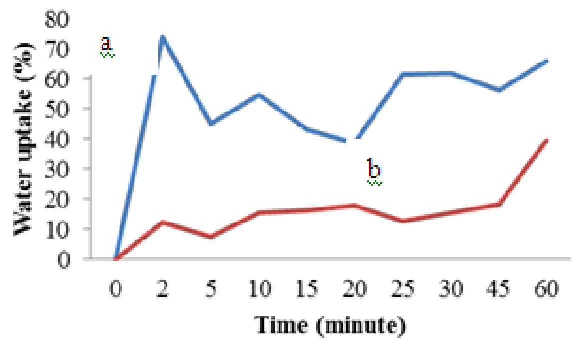

Figure-3. The water uptake properties of (a) PCL/Ge and (b) PLA electrospun fibers.

CONCLUSIONS

In this study, bead-free and smooth PLA and PCL/Gelatin-based electrospun fibers were successfully fabricated using electrospinning technique. The results of water contact angle and water uptake demonstrated that the pure PLA electrospun fibers were hydrophobic in nature. Meanwhile, PCL/Gelatin-based electrospun fibers were hydrophilic and had higher water uptake property. However, further analyses and investigations are needed to be applied for the tissue engineering applications.

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