VOL. 10, NO 20, NOVEMBER, 2015 ISSN 1819-6608
ARPN Journal of Engineering and Applied Sciences

www.arpnjournals.com

FABRICATION OF LAYER-BY-LAYER ELECTROSPUN COMPOSITE MEMBRANES BASED ON POLYLACTIC ACID (PLA) AND POLY (CAPROLACTONE) (PCL)/CHITOSAN

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ABSTRACT

Tissue engineering is an interdisciplinary field that applies the principles of engineering and life sciences towards the tissue development. Scaffolds can provide physical support and carry growth factors to the target cells. In this study, multi-layered composite scaffolds or membranes were fabricated using poly (lactic acid) PLA and by blending of synthetic polycaprolactone (PCL) and natural Chitosan layer-by-layer with a multilayer electrospinning technique. Scanning Electron Microscopy (SEM), water contact angle measurement (WCA) and water uptake were measured to characterize the multilayered scaffolds. Using optimized solution concentrations and processing parameters, the composite PLA and PCL/Chitosan was successfully fabricated layer-by-layer. It was found that the composite microfibrous membranes were homogenous. No beads were observed in the microfibers of the membranes. The composite layer-by layer membrane had enhanced wettability and water uptake properties than single layered membranes.

Keywords: 3D scaffold, multi-layered composite scaffolds, PCL /Chitosan, PLA, multilayer electrospinning.

INTRODUCTION

Tissue engineering is being developed by applying engineering principles and approaches of material science, as well as the biology of cells and molecules, to the growth the growth of feasible alternates [1]. The aim of tissue engineering (TE) is to restore, maintain, or improve tissue function by applying both biological and engineering principles [2]. The key components of engineered tissues are cell, scaffolds, and growth factors, which are normally referred to as the TE triad [3, 4].

A structure and primary provision by which cells can attach, multiply, and differentiate is provided by the scaffold [5]. An ideally fabricated scaffold should acquire these features: (1) a major linkage of pores to permit cells to transfer, grow, and migrate deep inside the scaffolds; (2) networks that provide elements which are required by cells such as oxygen and nutrients as well as remove unwanted substances from the body; (3) high affinity biocompatibility that allows cells attachment and proliferation; and (4) the strength of the mechanical and biodegradable profile is appropriate [3, 6].

There are different types of scaffolds, including ceramics, glasses, and polymers, the later of which can either be natural polymers, synthetic biodegradable or synthetic non-biodegradable polymers [7]. PLA had Food and Drug Administration (FDA) approval, meaning that it is safe for use in certain human applications; this shows that PLA is biocompatible. The chemical bonding will break down through hydrolysis and the products formed can be moved out of the body in harmless form i.e. carbon dioxide and water. In addition, PLA is hydrophobic[8], while PCL is a biodegradable synthetic polymer that is usually used for the fabrication of nanofibers due to its high mechanical strength[9], flexibility, biocompatibility, and biodegradability[10]. It is safe to be used [11] and can be applied for various biomedical applications. Chitosan has structural properties almost identical to those of glycosaminoglycan and is highly used in biomedical applications such as hemodialysis membranes, systems to transport drugs, materials to coat orthopaedic and dental appliances as well as synthetic skin [12].

Nanomaterials that have a tightly controlled size distribution can be fabricated using an electrospinning process, which is a versatile technique and applicable for numerous organic and inorganic systems [13]. The objective of using high voltage during the electrospinning process is to produce a jet of polymer solution that is electrically charged. Thus, the solution jet congeals before reaching the aluminum foil as a grounded collector. Finally, the connected small fibers are collected [14, 15]. Multilayer electrospinning is an unconventional method in that each polymer is fabricate to form a single layer nanofiber and is successively collected on the same grounded aluminium foil for multilayer electrospinning [16].

Other related research works have also been done on multi-layered scaffolds, such as Wang et al. using a 3 layer scaffold comprised of PLA, PLA/PCL, and PLA/Collagen in brain tissue engineering [17] as well as Sun *et al.* fabricating Segmented Polyurethane (SPU), Styrenated Gelatin (ST-gelatin) and Type I collagen multilayered scaffolds and used them as drug release reservoirs [18].

In this paper, we reported the fabrication of PCL/Chitosan and PLA layer-by-layer microfiber using a multilayer electrospinning method to mimic the native extracellular matrix (ECM). This is due to many tissues and organs consisting of hierarchical layered structures with specific ECM composition and arrangement in each layer. Characterizations of the microfibers were done using SEM in order to study the electrospun microfibers' surface morphology, and by WCA to examine the fibres' wettability.

MATERIALS AND METHODS

Materials

Poly (lactic Acid) (PLA), which had a density in the range of 1.210-1.430 g·cm^{-3}, Poly (caprolactone) (PCL) with a molecular weight of about 70,000-90,000 and Chitosan were purchased from Sigma. Chloroform and dimethyl formamide (DMF) were analytical grade and were used as solvents.

Methods

a) Preparation of polymer solution

In order to prepare pure 12.5% (w/v) PLA polymer solution, 1.25 g of PLA was dissolved in 9 ml of Chloroform and 1 ml of DMF by magnetically stirring at 500 rpm and at 50°C. On the other hand, in order to produce 8.8% w/v PCL/Chitosan polymer solution, 0.8 g of PCL and 0.08 g of Chitosan in 7 ml of Formic Acid and 3 ml of Acetic Acid were dissolved by magnetic stirring at 500 rpm in room temperature.

b) Electrospinning

Pure PLA, PCL/Chitosan, as well as PCL/Chitosan and PLA layer-by-layer microfibers were fabricated by an electrospinning method (NaBond Nanofiber Electro spinning Unit, China). First, pure PLA (1.25g) polymer solution was inserted to a new 6 cm/ml syringe with a needle. After that, the syringe was placed on a syringe pump. An aluminum foil with surface area of 10 cm x 10 cm was prepared before the electrospinning started and used as a collector.

The processing parameters for each microfibrous scaffold were different. Table-1 shows a summary of the parameters used for each microfiber membranes fabrication.

Table-1. Related parameters used for fabrication of pure PLA and PCL/Chitosan through an electrospinning process.

Processing parameters	PLA	PCL/Chitosan
Tip-to-collector distance (cm)	15.00	12.50
Temperature (℃)	22.50	22.50
Humidity (%)	51.00	51.00
Flow rate (ml/min)	40.00	0.02
Voltage used (kV)	17.00	20.00

For the fabrication of PCL/Chitosan and PLA layer-by-layer microfibers, all the steps involved in the electrospinning process were repeated and the parameters were unchanged. The time taken for the electrospinning process (pure PLA) was 1.5 hour. After that, the same collector was used, which meant that the PCL/Chitosan nanofibers were collected on top of the PLA nanofibers. The time taken for the electrospinning process (PCL/Chitosan) was 1.5 hour as well.

c) Characterization

The morphology of pure PLA, PCL/Chitosan as well as PCL/Chitosan and PLA layer-by-layer polymer fiber were observed using Scanning Electron Microscopy (Hitachi 400). A contact angle measuring system (VCA Optima, AST Products, Inc.) was used to determine the hydrophilicities of PLA and PCL/Chitosan as well as PCL/Chitosan and PLA layer-by-layer.

Microfiber membranes collected on the aluminum foil were peeled off and then two-sided tape was used to fix each sample of microfiber on a coin. A titanium coating on the microfibers provided better SEM results. After that, the coins were put in the SEM's sample holder. Then, these samples were visualized under SEM at various magnifications, for example 600x, 1000x, 1500x, 2000x, 2500x, 3000x, 5000x, 6000x, and 8000x. *J image* is image analysis software used to measure the diameters and pore sizes of fibers. At least 25 fiber diameters and pores were measured and the average was calculated.

In order to measure the water contact angle of the membrane surfaces, the electrospun membranes were placed on a sample stage. The water drop was controlled by a measuring system. The sample table was turned upwards and downwards in order to allow water dropped from the machine onto the surface and check the hydrophilicity of the samples. The process was repeated at least five times.

Water uptake by the microfibers was also measured to check their hydrophilicity and porosity. Before the experiment started, the weight of each microfibrous membrane was weighed and recorded as dry weight. Next, the microfibrous membranes were immersed

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in distilled water for 2, 5, 10, 15, 20, 25, 30, 45, and 60 minutes. Then, the microfibrous membranes' weight was recorded for each time period by removing them from the distilled water periodically. In order to get the accurate reading, the microfibrous membranes were roughly dried to eliminate excess water using tissue paper. Then water uptake by the microfibers' composite membranes was calculated using equation 1.

$$\text{Water Uptake } (\%) = \frac{(Ww - Wd)}{Wd} * 100 \quad (1)$$

W_d = Dry weight
W_w= Wet weight of samples obtained periodically

RESULTS AND DISCUSSIONS

Scanning Electron Microscopy (SEM): Figure-1 shows the SEM results. From the micrographs it was observed that PCL/Chitosan and PLA layer-by-layer were successfully fabricated as well as that the membranes produced were homogenous. The membranes did not contain beads. Figure-2 shows the distribution of electrospun fiber diameters of 8.8% w/v PCL/Chitosan and 12.5% w/v PLA layer-by-layer. The average diameter of fibers was 2.85 μm for pure PLA and was 2.95 μm for the PCL/Chitosan and PLA layer-by-layer. The average diameter of the PCL/Chitosan and PLA layer-by-layer fibers was slightly greater than the average diameter of pure PLA fibers. In other words, the double layer polymer fibers increased the average diameter of the pure PLA's fibers by about 3.51%. Table-2 summarizes the morphology of the microfibers and means value of fiber diameter for PLA and PCL/Chitosan as well as double layered PLA and PCL/Chitosan.

Water contact angle: With the multilayer electrospinning technique, two different meshes of fibers can be obtained on the same collector. Sun *et al.* mentioned that the layer-by-layer scaffold generally is less porous than conventional scaffolds. However, high porosity was one of the significant characteristics needed by the ideal scaffolds and further improvement was needed[18]. According to Kumbar *et al.,* skin fibroblasts had a higher proliferation rate on scaffolds with diameters in the range between 350 and 1100 nm[19]. From the SEM results, the average diameter range for the PLA and PCL/Chitosan layer by layer was 2.95 μm.

Table-3 summarizes the water contact angles of microfibers for PLA, PCL/Chitosan as well as PLA and PCL/Chitosan. It was observed that the layer-by-layer PLA and PCL/Chitosan had smaller water contact angles than the single layer PLA membrane and single layer PCL/Chitosan membrane.

Water uptake: According to Sultana *et al.,* the amount of water uptake by microfibrous membranes depends on their thickness and hydrophilicity as well as the fluid taken-in through the pores or capillaries of scaffolds called "capillary water" [21]. Figure-3 shows the percentages of water uptake by single layered PLA and the layer-by-layer microfibrous membranes with immersion time. It was observed that the amount of water taken up by the double-layered scaffold was higher (88%) than by the single layered PLA microfibrous membrane (75%).

PLA and PCL/Chitosan layer-by-layer was more hydrophilic due to the presence of the amino groups on the surface of microfibrous membrane and this chemical group interacted with the surrounding water to form hydrogen bonds [22]. As the number of amino groups increased, the hydrophilicity of the microfibrous membranes also increased. In addition, there are other factors that influence water uptake by the microfibrous membranes. For example, more porous scaffolds have the ability to draw a greater amount of water as well as to retain it. Moreover, the amount of fluid surrounding the scaffold also affects the water uptake by the membranes [21].

Table-2. The diameter range of fibers.

Sample	Diameter range (μm)	Average diameter (μm)	Observation
Pure PLA	1.62 - 4.84	2.85	No beads
PLA and PCL /Chitosan Layer-by-Layer	1.36 - 4.10	2..95	No beads

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ARPN Journal of Engineering and Applied Sciences

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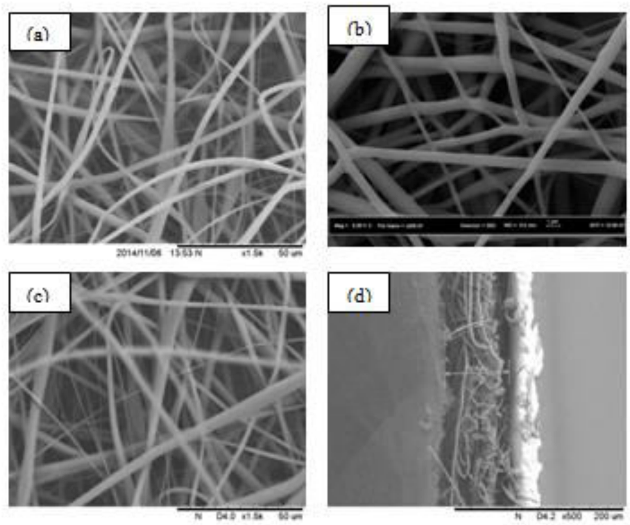


Figure-1. Scanning electron micrographs of electrospun (a) 12.5% PLA using 17kV with magnification 1500, (b) Field Emission Scanning Electron Microscopy (FESEM) Image of PCL/Chitosan; (c) 8.8% PCL/Chitosan and 12.5% PLA layer-by-layer with magnification 1500, (d) Cross Section of 8.8% PCL/Chitosan and 12.5% PLA layer-by-layer with magnification 500.

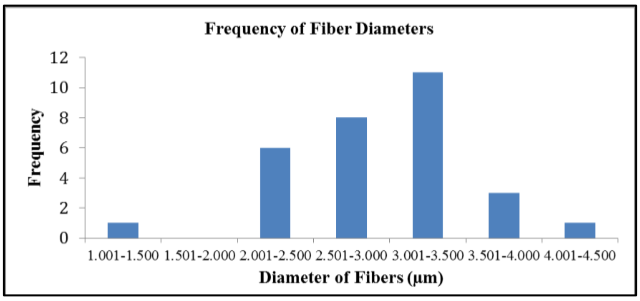


Figure-2. Frequency of electrospun fiber diameters of 12.5% w/v PLA and 8.8% w/v PCL/Chitosan layer by layer.

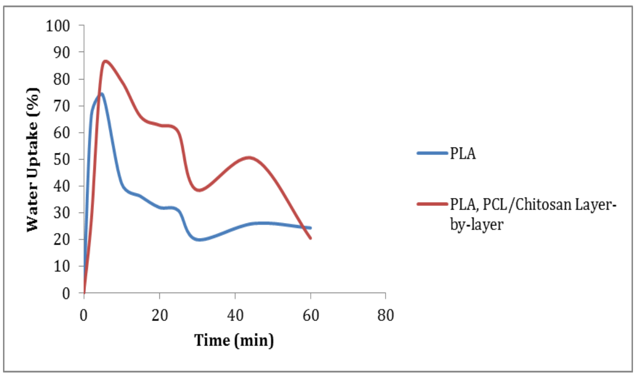
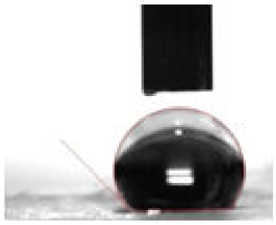
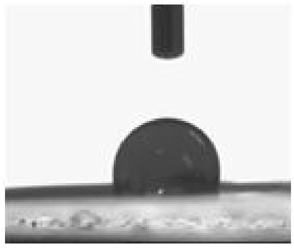
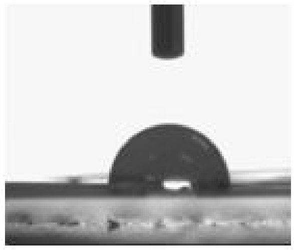


Figure-3. The percentages of water uptake by PLA and layer-by-layer microfibrous membranes versus immersion time.

Table-3. The water contact angle for different samples.

Samples	Contact (°)	Result of Water Contact Angle
12.5% w/v PLA	125.64 ± 5.71	
PCL/Chitosan	115.6± 5.25	
12.5%w/v PLA and 8.8%w/v PCL/Chitosan layer-by-layer	112.1±3.50	

CONCLUSIONS

Composite PLA and PCL/Chitosan layer-by-layer electrospun fibers were successfully fabricated using a multilayer electrospinning technique. Homogeneous membranes without beads were produced using optimal parameters for the composite PLA and PCL/Chitosan layer-by-layer electrospun fibers. Double layered composite PLA and PCL/Chitosan membranes had lower wettability and higher water uptake. Layer-by-layer composite electrospun membranes are expected to mimic the natural extracellular matrix that will enable bottom up assembly of various types of cell into layered electrospun membranes.

ACKNOWLEDGEMENTS

The authors would like to thank the Faculty of Bioscience and Medical Engineering (FBME), Universiti Teknologi Malaysia (UTM), Skudai, Johor Bahru, Malaysia for their support. This work was supported by FRGS (4F507), GUP (06H84), RMC and MOHE.

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