INVESTIGATION OF POLYMER CONCENTRATION ON PHYSICAL AND MORPHOLOGICAL PROPERTIES OF PLLA BASED FIBROUS STRUCTURES

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Abstract. The selection of the raw materials is one of the most important factors for tissue scaffolds to function as native tissue. In this regard, the usage of biopolymers is crucial because these polymers are biocompatible, biodegradable, and non-toxic. Poly (l-lactic acid) (PLLA) has high biocompatibility that promotes cell attachment and proliferation, also it has suitable biodegradation time to allow cells to generate their own extracellular matrix (ECM) without creating any toxicity. On the other hand, polycaprolactone (PCL) has some mechanical advantages that can contribute to the function of the designed scaffolds when using as a blend. In this study, PLLA-based fibrous structures are produced by electrospinning method. Different concentrations of PLLA (10%, 14%, and 18%) are dissolved in chloroform solvent while PCL/PLLA blends (8% wt.) with different ratios (5/5, 6/4, 7/3, 8/2, and 9/1) are dissolved in 8/1/1 chloroform/ethanol/acetic acid solvent systems. The physical and morphological analyses are accomplished to determine the effect of concentration and blend ratio on the web structure.

Keywords: Electrospinning, fibrous structures, polycaprolactone, poly(L)lactic acid, tissue engineering

1. Introduction

Tissue engineering is an interdisciplinary subject that aims to design, produce and improve the biological substitutes which can mimic the native tissue by using the principles of engineering and medicine [1]. These materials must promote the cell attachment, migration and proliferation and degrade in a certain period of time for the body to produce its own tissue. For this reason, the design parameters of these constructions should be determined clearly [2].

The determination of the raw material to be used is an important factor for the designed scaffolds to function as native tissue temporarily [3]. Therefore, biopolymers are suitable and advantageous materials to be used in these structures because they have outstanding properties such as biocompatibility, biodegradability and non-toxicity which can be modified both physically or chemically [4,5]. The biopolymers can be divided into 2 groups which are natural and synthetic biopolymers. Natural biopolymers like collagen, keratin or elastin are widely used in tissue engineered scaffolds; however, these polymers have some mechanical disadvantages and they degrade in a short time when used individually [6]. Synthetic biopolymers such as polycaprolactone (PCL), polylactic acid (PLA), poly-L-lactic acid (PLLA), poly Lactic-co-Glycolic Acid (PLGA), or polyhydroxyalkanoates (PHAs) are generally preferred for these constructions because they have superior mechanical properties, and they can be mixed in different ratios to obtain optimum biodegradation time [7]. PCL is a synthetic biopolymer that has long biodegradation time but also superior mechanical properties which improve the strength and flexibility of the tissue engineered scaffolds [1]. On the other hand, PLLA is a biopolymer which shows high biocompatibility that supports cell migration and proliferation; moreover, it is widely used to adjust the biodegradation time by blending or creating co-polymers with other biopolymers to allow cells to have enough time for generating their own tissue without causing any toxicity [8].

In addition to the material selection, the production method of the designed scaffolds is also very crucial. There are several methods to construct tissue engineered scaffolds such as solvent casting, gas foaming, freeze drying, phase separation and electrospinning. Also, 3D bioprinting techniques such as inkjet, laser-assisted, and extrusion bioprinting are other methods that have been used for constructing biostructures like blood vessels, organs, cell cultures, drug delivery devices as a state-of-the-art technology. It is possible to produce the 3D computer-based models of tissue engineered scaffolds by using wide range of biomaterials with desired structural properties [9,10]. Apart from the methods mentioned before, electrospinning is an efficient technique to obtain these scaffolds with desired porosity levels by creating fibers based on natural or synthetic biopolymers; therefore, it is possible to construct 3D surfaces which can mimic the native tissue and allow cells to attach and proliferate on themselves [3,11]. In this regard, production parameters are significant for designing and producing scaffolds with desired properties. In this study, it is focused on the effect of the polymer concentration and the polymer blend ratios on the morphologies of the produced samples.

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In a study of Muniyandi et al., porous (PLLA)-based scaffolds were designed for using in cardiac tissue engineering which were modified with natural proteins. PLLA fibers were produced at a concentration of 11.5 wt% and the solution was prepared dissolving PLLA in chloroform/N, N-dimethylformamide (DMF) (9/1 v/v). PLLA surfaces were produced consisting of fibers with an average diameter of 1.58−3.03 μm and the average pore size of 40−50 nm; therefore, it was confirmed that these scaffolds allowed cells to attach, proliferate and create the extracellular matrix (ECM) [12]. Fiqrianti et al. produced vascular grafts by using Poly-L-Lactic acid (PLLA) blended with chitosan and collagen in different concentrations. For preparing the solutions, 10% PLLA was dissolved in chloroform while collagen and chitosan were dissolved in 0.5 M acetic acid with different concentrations. Fibrous surfaces were obtained with a pore size of 20-30 nm and fiber diameters ranged between 89.33 nm to 246.7 nm [13]. Hasan et al. aimed to produce tissue engineered heart valves made from PCL/PLLA blends by using electrospinning method and compared blended scaffold with the surfaces that the pure PCL and PLLA were used. The PCL/PLLA (30/70 wt.) solutions were prepared at a concentration of 10 wt% by using dichloromethane as a solvent due to its superior mechanical and biological properties. Fibers with diameters ranged between 1.85−2.4 μm were obtained. As a result, it was seen that PCL/PLLA blended surfaces were stronger than pure PLLA; moreover, these surfaces had better cell attachment and proliferation properties than pure PCL surfaces [14]. Bolbasov et al. aimed to compare the scaffolds made from PCL, PLLA, PCL/PLLA. The PCL/PLLA solution (70/30 wt., 4% polymer ratio) was prepared by using hexafluoro-2-propanol as a solvent. It was observed that PCL/PLLA blended surface had fibers with an average diameter of 1.2 ± 0.4 μm and pore area of 18.7±1.2 μm². It was concluded that blended surface had larger pore size, better wetting angle and higher cell viability level [8].

In this study, PLLA-based and PCL/PLLA blended fibrous structures are obtained by electrospinning method to be used in tissue engineering applications. Different concentrations of PLLA (10%, 14%, and 18% wt.) and PCL/PLLA (8% wt.) blends with different weight ratios (5/5, 6/4, 7/3, 8/2, and 9/1) are dissolved in chloroform and 8/1/1 chloroform/ethanol/acetic acid, respectively. The effect of concentration on the web structure is evaluated by the physical and morphological analyses.

2. MATERIALS AND METHODS

2.1. Materials

In this study, PLLA (Mn 50,000), PCL (Mn 80,000), and the solvents (chloroform, ethanol, and acetic acid) are supplied from *Sigma Aldrich*. PLLA is used both neat and blend form with PCL. The concentrations of neat PLLA solutions are 10, 14 and 18% while the concentration of PCL/PLLA blend is kept as 8%. On the other hand, the PCL/PLLA blend ratios are fixed as 50/50, 60/40, 70/30, 80/20, and 90/10.

2.2. Scaffold Fabrication

PLLA is dissolved in chloroform while PCL/PLLA polymer systems are dissolved in chloroform/ethanol/acetic acid (8/1/1) solvents system and stirred by a magnetic stirrer for 2-3 hours, then electrospun immediately. Ethanol and acetic acid, which are used together with chloroform in PCL-containing blends, are added as a result of experience gained from previous studies [3,15] to improve fiber spinnability of high molecular weight (80,000Mn) PCL and reducing the evaporation rate of chloroform.

For the manufacture of fibrous surfaces, the flat collector is used. Moreover, the applied voltage, feed rate and the tip to needle distance are adjusted to 13±1kV, 4±1ml/h and 20cm, respectively. The productions are realized at 24±1°C temperature and 74±3% relative humidity.

The sample codes, polymer concentrations, solvent details and polymer weight ratios are given in Table 1.

Table 1. Sample codes and solution details

Sample Codes	Polymer concent ration (%)	CH* weight ratio	ETH* weight ratio	AA* weight ratio	PCL/PLLA blend weight ratio
PLLA_10	10	1	-	-	-
PLLA_14	14	1	-	-	-
PLLA_18	18	1	-	-	-
PCL/PLLA_5:5	8	8	1	1	5/5
PCL/PLLA_6:4	8	8	1	1	6/4
PCL/PLLA_7:3	8	8	1	1	7/3
PCL/PLLA_8:2	8	8	1	1	8/2
PCL/PLLA_9:1	8	8	1	1	9/1

*CH: chloroform, ETH: ethanol, AA: acetic acid

2.3. Fibrous Web Characterization

Scanning electron microscope (SEM) (TESCAN VEGA3) is used for the morphological characterization of the fibers in the web structure. The samples are coated with gold-palladium (Au-Pd) alloy. Fiber diameters are measured by Image J Software System from SEM images using at least 50 different fibers.

3. RESULTS AND DISCUSSION

3.1. SEM analysis

SEM images of the pure PLLA with different polymer concentrations (10%, 14%, and 18% wt.), and PCL/PLLA fibrous webs blended with different weight ratios (5:5, 6:4, 7:3, 8:2, and 9:1) are illustrated in Figure 1, and Figure 2, respectively.

As it can be seen in Figure 1a, there is a fiber formation in PLLA_10 sample even if the bead formation occurs which can be explained by the low polymer concentration. Beaded structure can be obtained if the polymer solution has low viscosity which hinders the entanglement of the polymer [16]. According to the SEM image of PLLA_14 in Figure 1b, bead formation is reduced by increasing the polymer concentration and it is observed that fibers have thicker regions which is resulted from elongated beads through the fiber length that cause non-homogenous

fiber diameter. This can also be explained by the instability of the Taylor cone resulting from inadequate polymer concentration [17]. On the other hand, PLLA_18 surface has smooth fibers without any bead formation as it is shown in Figure 1c although the fiber diameters are not uniformly distributed. Therefore, it can be said that when the polymer concentration is increased, the smooth fibers are obtained without any bead formation as supported in literature [18]. However, fibers with non-homogenous diameters may be obtained.
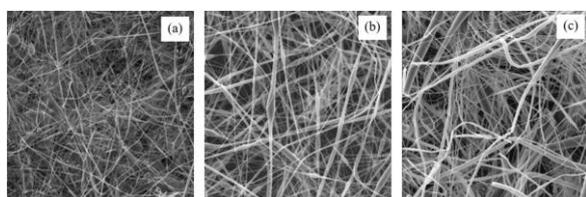

Figure 1. SEM images at 1kx magnification of PLLA samples at different concentrations (a) PLLA_10, (b) PLLA_14, and (c) PLLA_18 (Magnification: 1000x)
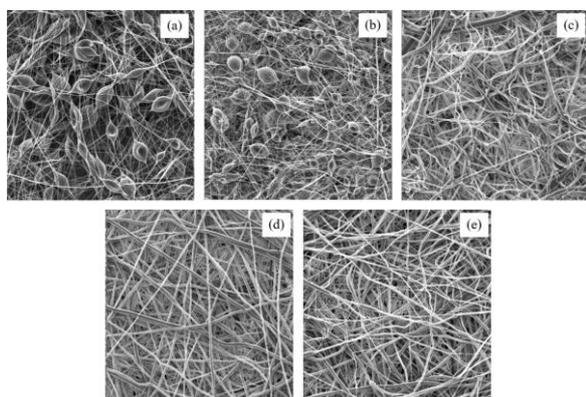

Figure 2. SEM images at 1kx magnification of PLLA /PCL blend-based samples (a) PCL/PLLA_5:5, (b) PCL/PLLA_6:4, (c) PCL/PLLA_7:3, (d) PCL/PLLA_8:2, and (e) PCL/PLLA_9:1 (Magnification: 1000x)

In Figure 2a and 2b, the SEM images of PCL/PLLA_5:5 and PCL/PLLA_6:4 show that the bead formation occurs along the length of the fibers. The dish-shaped and micro-porous beads are observed in both structures. This situation can be explained by the effect of high relative humidity, molecular weight and polymer concentration on the shape of beads. It is stated that when the relative humidity is high, the water in the air evaporates on the fiber surface and this leads to pore formation; moreover, the sizes of the pores are dependent on the molecular weight of the polymers [3,19]. Unlike PCL/PLLA_5:5 and PCL/PLLA_6:4, the bead formation is eliminated in the samples of PCL/PLLA_7:3, PCL/PLLA_8:2, and PCL/PLLA_9:1. It can be explained by the increase in polymer viscosity with the increase of PCL ratio in the polymer solutions.

3.2. Fiber diameter analysis

The average fiber diameters with their standard deviations (SD) and the fiber morphologies are given in Table 2. As it can be observed, when the polymer concentration increases, the fiber diameter increases in pure PLLA samples. In blended samples, when the PCL ratio is increased, the fiber diameter also increases. The fiber diameters in the samples of PLLA_10, PCL/PLLA_5:5 and, PCL/PLLA_6:4 are not reasonable due to the bead formation. When the polymer concentration is low, the polymer solution can be stretched and turned into fibers much easier in the electrical field because of the low viscosity which causes the formation of thinner fibers as supported by the literature [20]. On the other hand, when the fiber diameter distributions are examined, it can be said that the SEM images are compatible with the distribution graphs. For instance, the SEM image of PLLA_18 (Figure 1c) shows non-homogeneous fiber diameters which results in wider fiber diameter distribution (Table 2). It is stated in the literature that obtaining non-uniform fiber diameters is possible because of the bending instability of the charged regions in the polymer jet during electrospinning caused by electrostatic forces [21].

Table 2. The average fiber diameters, fiber morphologies and fiber diameter distribution graphs

Sample codes	Avg. fiber diameter ± SD(μm)	Fiber morphology	Fiber diameter distribution
PLLA_10	0.760 ± 0.210	Beaded structure	
PLLA_14	1.240 ± 0.656	Fiber formation	
PLLA_18	1.683 ± 1.426	Fiber formation	
PCL/PLLA_5:5	0.569 ± 0.187	Beaded structure	
PCL/PLLA_6:4	0.602 ± 0.346	Beaded structure	
PCL/PLLA_7:3	1.172 ± 0.566	Fiber formation	
PCL/PLLA_8:2	1.428 ± 0.523	Fiber formation	
PCL/PLLA_9:1	1.597 ± 0.669	Fiber formation	

4. CONCLUSION

In this study micro fibrous surfaces are produced by using pure PLLA, PCL/PLLA and chloroform based solvent systems to be used in the tissue engineering applications. The effect of different polymer concentrations on the morphology of pure PLLA samples and the blend ratios on the morphology of PCL/PLLA samples are examined. PLLA fibrous webs are successfully obtained from the polymer solutions at the concentration of 18%. On the other hand, PCL/PLLA micro fibers are produced without any bead formation by using the blend ratios of 7:3, 8:2, and 9:1. The PLLA surfaces has fibers with diameters ranged between 0.760–1.683 µm while PCL/PLLA webs include fibers with diameters ranged between 0.569–1.597 µm. Microfibers can limit the end use of these surfaces; however, it can be said that chloroform based solvent systems are appropriate to produce and optimize the PLLA and PCL/PLLA fibrous webs without creating toxic effect.

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