CHAPTER 3: DEVELOPMENT AND CHARACTERIZATION OF 2D RANDOM NANOFIBROUS SCAFFOLDS OF PLA-PCL PHYSICAL BLENDS

3.1 Introduction

Atherosclerosis associated coronary blood vessel dysfunction is one of the devastating disease with high mortality and morbidity rates globally [1]. Current gold standard approach for the reestablishment of native vessel is the use of autografts which are coupled with the drawbacks such as poor availability; secondary site injury and size mismatch [2]. Further, alternate approaches such as allografts and xenografts are limited due to risk of disease transmission and immune rejection [3, 4]. Hence the ideal vascular substitute should be designed to potentially restore the functional vasculature and overcome existing limitations while providing the structural as well as mechanical integrity for continuous blood supply [3, 4].

Scaffold aided tissue engineering is successful strategy to regenerate and restore the functional vascular tissue through the development of polymeric extracellular matrix analogue [5, 6]. Development of scaffolds with nanofibrous geometry for vascular tissue regeneration has been found to be promising as the ECM of native blood vessels consists of collagen, elastin and fibronectin nanofibers [7]. In addition, nanofibrous topography was found to promote the cell-matrix interaction through the development of focal contacts, thereby establishing contact guidance [8]. Of various techniques to develop nanofibrous geometry, electrospinning receives much attention as it controls the fiber dimension through various solutions and operating parameters such as solution
conductivity, viscosity, volatility, applied voltage, tip-target distance, flow-rate and needle gauge [9].

Choice of the material in the scaffold development play a vital role as material chemistry controls the surface properties such as wettability, biocompatibility, and bulk properties like mechanical strength and degradation, Scaffolds made of natural polymers possess ideal properties such as biodegradation with non-toxic end products, bio-integration, wettability and the demerits of immunogenicity, poor stability and processability with inferior mechanical property [10]. Hence, vascular graft made of synthetic polymers such as polyglycolic acid (PGA), polylactic acid (PLA), poly-ε-caprolactone (PCL) and polyglycerol-sebacate (PGS) has been widely exploited due to its tunable biodegradation, bio-integration and mechanical property with the lack of immunogenicity and ease of processability [10]. For the present study, we have made an attempt to use dual synthetic polymers in scaffold fabrication for the present study as it compensates the drawbacks of parent polymers [11]. Blending of polymers in different ratios for scaffold fabrication can drastically modify the parent properties based on the property of the material, microstructure of the blend and miscibility of the solution [12]. Poly-L-lactide (PLLA) is widely used polymer compared to PGA due to its biocompatibility, slow degradability, hydrophobicity and long-lasting mechanical integrity for tissue engineering applications [13]. However, higher glass transition temperature renders hardness and brittleness to the PLA [14]. The present study aimed to reduce the hardness as well as brittleness of the PLA with the addition of various ratios of elastomeric poly (caprolactone) through blending which could improve the mechanical and biological properties of the scaffold.
In addition, distinct properties such as non-thrombogenicity, non-hemolytic and lesser pore size is indispensable to build-up functional vascular tissue apart from facilitating cell adhesion, proliferation, viability and expression of functional gene markers for the neovascularization and native ECM production [6].

This chapter focused on the optimization and fabrication of 2D random nanofibrous scaffolds of five different ratios of PLA and PCL physical blends namely PLA-PCL (100:0), PLA-PCL (75:25), PLA-PCL (50:50), PLA-PCL (25:75) and PLA-PCL (0:100) by electrospinning. The scaffold properties such as surface topography, average fiber size distribution using scanning electron microscope, quantification of randomization using image processing techniques, hydrophobicity using goniometer, specific surface area using fiber diameter, vibrational characteristics of functional groups in the blend via FTIR, crystallinity by XRD, thermal property by differential scanning calorimeter,

3.2 Materials and Methods

3.2.1 Materials

Poly (L–lactic acid) (M<sub>w</sub> 69,000 g/mol) and poly (ε-caprolactone) (M<sub>w</sub> 65,000 g/mol) were purchased from Lakeshore Biomaterials, USA and Sigma Aldrich, India respectively. Chloroform and Dimethyl formamide purchased from Merck, India are used in the ratio of (7:3) without further processing.
3.2.2 Fabrication and Characterization of 2D Random Nanofibrous Scaffolds

Defect free electrospun nanofibrous matrix were developed from five ratios of PLA-PCL blends (100:0; 75:25; 50:50; 25:75; and 0:100) by optimizing solution parameters such as solvent system (chloroform, THF, and Chloroform : DMF) and polymer concentration (5 - 15 % w/v), and processing parameters such as applied voltage (12, 16 kV) by high voltage source (Zeonics, India); flow rate (0.001-0.003 mL/min) using flow rate controller (Kent Scientific, USA); and tip-target distance (8 -12 cm) Polymer solution of various concentrations were loaded into the glass syringe (Glass van) and aluminium foil on the metal target (20 X 20 cm) was used for the collection of nanofibers. The samples were stored under vacuum for further characterization.

3.2.3 Surface Morphology using FE-SEM

The samples were sputter coated with platinum and imaged under scanning electron microscope (SEM, JEOL FE-SEM 6701F, Japan) at an accelerating voltage of 3 kV. Average fiber diameter was determined by measuring the diameter of 100 fibers randomly from 10 scanning electron micrographs at 3300 X magnification.

3.2.4 Image Processing- Fast Fourier Transform and Orientation,J

Scanning electron micrograph of defect free random fibers of PLA-PCL of five ratios were processed using ImageJ software, version 1.47 (http://rsbweb.nih.gov/ij/index.html) [15]. In short, frequency domain based FFT images were drawn from SEM images in which each fiber leaves sharp peak intensity and determined the direction of the nanofiber orientation.
Further, fiber distribution was assessed by determining coherent coefficient of individual fibers using OrientationJ [16]. The angle of the fibers were represented through the colour code map and histogram was plotted based on the relative angles of random fibers to the horizontal axis.

3.2.5 Specific Surface Area (SSA) Measurement:

The specific surface area of random electrospun scaffolds of PLA-PCL blend were measured using average fiber diameter of each polymeric ratios as reported elsewhere [17]. Briefly, specific surface area (SSA) is the total surface area per unit of bulk volume and calculated using the following formula:

\[
SSA = \frac{4 \sum_{i=1}^{n} D_i f_i}{4 \sum_{i=1}^{n} D_i^2 f_i}
\]

where D is average fiber diameter and f is frequency of average fiber diameter.

3.2.6 Contact Angle Measurement:

Surface wettable property of electrospun scaffolds were determined by measuring water contact angle using Goniometer (Rame-Hart, USA). In short, a drop of deionized water was placed onto the electrospun scaffolds at three different sites and measured the immediate water contact angle. Average contact angle was determined from 30 readings of each scaffold and the images were captured using CCD camera at every initial time point.
3.2.7 Fourier Transform Infrared Spectroscopy (FTIR)
Chemical characteristics of PLA-PCL blend electrospun nanofibrous scaffolds of five different ratios were evaluated by determining the vibration characteristics of functional groups using FTIR spectrometer (Spectrum 100, Perkin Elmer, USA). In brief, electrospun scaffolds were pelletized using potassium bromide (KBr) using hydraulic press and spectra were obtained by recording 25 scans between 4000 and 400 cm\(^{-1}\) with the resolution of 1cm\(^{-1}\).

3.2.8 X-ray Diffraction Pattern
Crystalline nature of the PLA-PCL electrospun scaffolds of five ratios were determined using powder X-ray diffractometer (D8 Focus, Bruker, Germany). X-ray diffraction patterns were recorded at a scanning rate of 10º - 60º (2\(\theta\)) with step size of 0.01 by irradiating samples with Cu-K\(\alpha\) radiation.

3.2.9 Thermal Analysis
Thermal properties and miscibility of the blend nanofibers were assessed using differential scanning calorimeter (Q20, TA Instruments, USA). Shortly, about 2 mg of samples were subjected to two freeze-heat cycles at a rate of 10°C/min from -80°C to +80°C under nitrogen atmosphere.
3.2.10 Mechanical Strength

Dumbbell shaped specimens of five PLA-PCL blend electrospun scaffolds (n=6) with the dimensions of 50 mm length, 10 mm width and thickness of 0.2 mm were used for determining the mechanical property using uniaxial tensile testing machine (INSTRON, USA). Briefly, one end of the specimens was fixed with the mobile grip containing load cell of 500 N and the other end with static grip. Load deformation was recorded at a deforming speed of 10 mm/min until the material fails and stress-strain curve for all scaffolds were constructed.

3.2.11 Statistical Analysis

Statistical significance between the electrospun scaffolds of various ratios (PLA-PCL) was evaluated at p <0.05. One way Analysis of Variance (ANOVA) was used to analyze the statistical significance between the various blend ratios on contact angle, specific surface area and mechanical properties with the confidence limit of 95%.

3.3 Results and Discussion

3.3.1 Fabrication of 2D Random Nanofibrous Scaffolds

Effect of various solvents such as chloroform (100%), tetrahydrofuran (100%) and chloroform: Dimethylformamide (7:3) on the fiber morphology were evaluated (Figure: 3.1). Diameter of the fibers of all ratios of PLA-PCL were found to be heterogeneous from microns (> 1µm) to sub microns (600-900 nm) with the beaded morphology while using CHCl₃ as a solvent (Figure 3.1). For THF, the fibers were observed in all PLA-PCL with spindle shaped defects. 
Figure 3.1: Effect of various solvents on morphology of PLA-PCL nanofiber at Concentration (15 % w/v), Flow rate (0.001 mL/min), Electric voltage (15 kV), Tip to target distance (10 cm) and Needle gauge size (24 G)
On the contrary, fibers spun using CHCl$_3$ and DMF in the ratio of 7:3 were observed to be homogenous with dimension ranging from 100-400 nm. DMF has higher dielectric strength as compared to THF while THF has a higher dielectric strength than chloroform. Defects were observed with the solvent possessing lower dielectric point (chloroform). As the dielectric constant of solvent increased, the defects were transformed into bead on strings for THF. Further increase in the dielectric point of solvent system of CHCl$_3$ and DMF in 7:3 ratio, spinnability of the solution was improved leading to the formation of defect free nanofibers. Thus higher dielectric constant of the CHCl$_3$ and DMF in the 7:3 ratio which could enhance the charge density at the surface of the jet and stretching of the fibers towards the target [18].

At lower concentration of polymer (5 % w/v), defects were observed in both 100:0 and 75:25 of PLA-PCL (Figure 3.2). This may be due to the lesser polymer chain entanglement with higher solvent volume leading to electrospaying rather than electrospinning. As the concentration of polymeric blend increased to 10 % w/v, gradual disappearance of defects with the formation of fibers was evidenced. Further increase in the concentration to 15 % w/v promoted the defect free fiber formation for all ratios of PLA-PCL blends. This may be attributed that the higher solution viscosity increased the polymer chain entanglement and enhances the continuity of the jet. All other ratios of PLA-PCL also found to be producing continuous fibers only in the 15 % w/v.
Figure 3.2: Effect of polymeric concentrations on morphology of PLA-PCL nanofiber at constant Flow rate (0.001 mL/min), Electric voltage (15 kV), Tip to target distance (10 cm), Needle gauge size (24 G)
Effect of applied voltage on the morphology of random nanofibers was shown in (Figure 3.3). For PLA-PCL (100:0), defect free nanofibers were obtained at 12 kV and 14 kV (Figure 3.3). Further, increase in the voltage to 16 kV introduced spindle shaped defects. Hence 12 kV was taken as optimum voltage for 100:0 PLA-PCL blend solutions. Thicker diameter fibers were obtained at 12 kV for (75:25) PLA-PCL (Figure 3.3). Increasing the applied voltage to 14 kV eliminated the defects as well as reduced the fiber dimension (Figure 3.3). However at higher voltage of 16 kV, defects were reintroduced on the morphology of (75:25) PLA-PCL. The defects at the higher voltage for (75:25) PLA-PCL may be because of the jet instability due to the imbalance between the jet initiation and feeding rate resulting in the receding of the taylor cone into the capillary [9]. Thus 14 kV was chosen as an optimal voltage for the PLA-PCL (75:25). For PLA-PCL of (50:50), (25:75) and (0:100), defect free fibers were obtained only at higher voltage of 16 kV (Figure 3.3). Lesser voltage such as 12 kV and 14 kV were found to be not sufficient to break the surface tension of the droplets and stretching of the ejected jet from the capillary. Hence 16 kV was taken as an optimum voltage for the (50:50), (25:75) and (0:100) PLA-PCL blends.

Effect of flow rate (0.001; 0.003; and 0.005 mL/min) on the surface morphology of the various blends of PLA-PCL using scanning electron microscope was shown in Figure 3.4. Flow rate of the solution play a vital role in the Taylor cone stability at a given applied voltage [19]. At higher flow rate, more solution was drawn from the capillary,
Figure 3.3: Effect of Applied voltage in PLA-PCL nanofiber surface morphology at polymer Concentration (15 %w/v); Flow rate (0.001 mL/min); Tip to target distance (10 cm); Needle gauge size (24 G)
resulting in the inability of the voltage to overcome the surface tension. This may lead to the formation of defects or thicker fibers [19].

Figure 3.4: Effect of flow rate in PLA-PCL nanofiber surface morphology at polymer concentration (15 %w/v); Flow rate (0.001 mL/min); Tip to target distance (10 cm); Needle gauge size (24 G)
Figure 3.5: Effect of tip-to–target distances in PLA-PCL nanofiber surface morphology at constant polymer concentration (15 %w/v); Flow rate (0.001 mL/min); Needle gauge size (24 G)
For both PLA-PCL of (100:0) and (0:100), lesser flow rate (0.001 mL/min) facilitated the formation of the continuous fibers (Figure 3.3). Higher feed rate for both the ratios were found to introduce the defects and thicker fibers. Hence 0.001 mL/min was selected as a optimal flow rate for both (100:0) and (0:100) of PLA-PCL blends. As in case of other ratios such as (75:25); (50:50) and (25:75), fibers were observed to be homogenous only at 0.003 mL/min (Figure 3.4). Thus 0.003 mL/min was chosen for further study.

As flow rate, tip to target distance has direct influence on the electric field strength and flight time of the jet [20]. Shorter distance could not allow the jet to evaporate the solvent completely where as higher distance reduces the electrostatic field strength resulting in formation of beaded morphology as well as thicker fibers [20]. Therefore, effect of different tip-target distances (8 cm; 10 cm; 12 cm) were studied on the morphology of the all the PLA-PCL ratios (Figure 3.5). Fibers were observed to be homogenous at 10 cm distance for all PLA-PCL blend solutions which may be due to the presence of optimal electric field strength to stretch the fibers sufficiently across the gap [Figure 3.5].

### 3.3.2 Surface Morphology

Five different blend ratios of PLA-PCL (100:0; 75:25; 50:50; 25:75; and 0:100) was electrospun into defect free nanofibers using optimized condition tabulated in Table 3.1. Mean Fiber diameter and surface morphology of various ratios of blend nanofibers were characterized using FE-SEM (Figure 3.2). Surface morphology of all scaffolds was observed to be smooth and homogenous.
Diameter of the pure PLA and PCL fibers was found to be 129 ± 31 nm and 374±32 nm at the applied voltage of 14 kV and 16 kV respectively. The increase in the diameter as well as higher voltage required for PCL may be due to its viscoelastic nature. Though the high applied voltage improves the jet initiation for viscoelastic nature of PCL solution, it facilitates the rapid splaying of jet leading to the development of higher diameter fibers as the jets have shorter distance for elongation [9]. This was further confirmed by the gradual increase in the fiber diameter from 129 ± 31 nm to 285±40 nm, 360±50 nm, 365±40 nm, and 374±32 nm while increasing the ratio of PCL in the PLA-PCL blend (100:0; 75:25; 50:50; 25:75; and 0:100) respectively (Figure 3.6 A1-E1). From the recent studies of Chen L et al., (2013) it is found that the fiber dimension increased with the addition of PCL in PLA-PCL physical blend as reported in the literature [11].

Table 3.1: The optimized conditions of PLA-PCL 2D nanofibrous scaffolds

<table>
<thead>
<tr>
<th>Experimental Parameters</th>
<th>PLA-PCL (100:0)</th>
<th>PLA-PCL (75:25)</th>
<th>PLA-PCL (50:50)</th>
<th>PLA-PCL (25:75)</th>
<th>PLA-PCL (0:100)</th>
</tr>
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<tbody>
<tr>
<td>Concentration (w/v in %)</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Applied Voltage (kV)</td>
<td>12</td>
<td>14</td>
<td>16</td>
<td>16</td>
<td>16</td>
</tr>
<tr>
<td>Flow rate (mL/min)</td>
<td>0.001</td>
<td>0.003</td>
<td>0.003</td>
<td>0.003</td>
<td>0.001</td>
</tr>
<tr>
<td>Tip to target distance</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>
3.3.3 Image Analysis

3.3.3.1 Fast Fourier Transform (FFT) Analysis

The FFT output of the random electrospun nanofibers from the respective SEM images of the five ratios shows no peak signals at predefined angles rather they were scattered in all of the angles and formed a circular pattern (Figure 3.6. A2- E2) which confirms the randomization of nanofibers [15]. The angular distribution plot also confirms that the peak intensity was scattered at all the angles and not specified to any orientation thus giving the peak intensity in all the regions from 1° to 360° angle (Figure 3.6. A3 - E3).

3.3.3.2 OrientationJ Analysis

Colour code map was drawn from the scanning electron micrographs of various PLA-PCL blend nanofibers using OrientationJ (Figure 1 A4-E4) to determine the distribution and degree of fiber orientation where the fibers of same orientation shares the common colour as they have similar range of localized pixel values [16]. Further histograms were plotted for respective PLA-PCL blend scaffolds using degree of fiber orientation as X-axis and distribution of fiber orientation as Y-axis (Figure 3.6 A5-E5). Colour code map and histogram has confirmed the random distribution of nanofibers. Alignment index is the average value of coherency coefficient was found to be closer to 0 for all the ratios of PLA-PCL tabulated (Table 3.2), thereby confirming the random distribution of fibers.
Figure 3.6: Scanning electron micrographs of optimized PLA, PLA-PCL and PCL nanofibers with their corresponding 2D-FFT image, angular distribution curve, colour code map and histogram for the degree of orientation and distribution
3.3.4 Specific Surface Area (SSA) of PLA-PCL Blend Nanofibers

Specific surface area of PLA-PCL of various blend ratios was determined using the diameter of fiber [17] and tabulated in Table 3.2. Specific surface area for pure PLA and PCL nanofibers were 32.7 µ⁻¹ and 10.69 µ⁻¹ respectively. In addition, various blends of PLA-PCL (75:25; 50:50; 25:75) was observed to possess SSA in between the parent polymeric fibers (Table 3.2). This clearly indicated that the specific surface area was found to be increased by increasing the PCL ratio in the blend which may be due to the increase of fiber dimension.

3.3.5 Physicochemical Properties

3.3.5.1 Wettability of PLA-PCL Nanofibers

Surface wettability of all ratios of blend scaffolds was determined as it allows the passage of blood without inducing thrombosis (Table 3.2). It was observed that the contact angle of pure PLA and pure PCL nanofibers was determined as 109±1.3° and 130±1.3° respectively due to the presence of pendant methyl group in PLA and higher crystallinity of PCL. Hydrophobicity of the polymer solely depends on the material chemistry as well as crystallinity [21]. Higher hydrophobicity of PCL as compared to PLA nanofibers may be attributed that higher crystallinity of PCL may restricts the approach of water to larger extent [22]. It was also noted that the contact angle of various blends of PLA-PCL (75:25; 50:50; 25:75) was increased as 116±1.5°, 118±1.8° and 118±1.3° respectively which lies between the contact angle of 100% PLA and PCL nanofibers.
Table 3.2: Physicochemical characterization of PLA-PCL blend nanofibers

<table>
<thead>
<tr>
<th></th>
<th>Mean fiber diameter (nm)</th>
<th>Alignment Index</th>
<th>Specific surface area (µm(^{-1}))</th>
<th>Contact angle (°)</th>
<th>Glass transition temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLA-PCL (100:0)</td>
<td>129 ± 31</td>
<td>0.08</td>
<td>32.70</td>
<td>109 ± 1.3</td>
<td>17</td>
</tr>
<tr>
<td>PLA-PCL (75:25)</td>
<td>285 ± 40</td>
<td>0.07</td>
<td>14.03</td>
<td>116 ± 1.5</td>
<td>9.69</td>
</tr>
<tr>
<td>PLA-PCL (50:50)</td>
<td>360 ± 50</td>
<td>0.03</td>
<td>11.11</td>
<td>118 ± 1.8</td>
<td>-0.24</td>
</tr>
<tr>
<td>PLA-PCL (25:75)</td>
<td>365 ± 40</td>
<td>0.12</td>
<td>10.95</td>
<td>118 ± 1.3</td>
<td>4.0</td>
</tr>
<tr>
<td>PLA-PCL (0:100)</td>
<td>374 ± 32</td>
<td>0.12</td>
<td>10.69</td>
<td>130 ± 1.3</td>
<td>-52</td>
</tr>
</tbody>
</table>

Although the tissue engineering scaffolds with hydrophilic surfaces enhanced the cell-material interaction, blood contacting scaffolds such as vascular graft should not be hydrophilic as it facilitates the platelet adhesion, thrombosis with the development of "weeping graft" [23]. It was also identified that the biomaterial surface with hydrophobicity possess anti-thrombogenic property through the inhibition of platelet adhesion as its lower surface energy tend to desorbs the plasma proteins for platelet adhesion [24].

3.3.5.2 FTIR Spectrum for Electrospun PLA-PCL Blends Scaffolds:

IR spectra of pure PLA and pure PCL nanofiber was depicted in Figure 3.7 A. PLA fibers showed the CH\(_3\) stretching peak at 2997 cm\(^{-1}\) and 2946 cm\(^{-1}\) and characteristic peak for ester bonds at 1760 cm\(^{-1}\) and 1188 cm\(^{-1}\) corresponding to C=O stretching and O-C=O stretching respectively. Peaks at 1384 cm\(^{-1}\) and 1455 cm\(^{-1}\) assigned to bending vibration
of C-O-H and at 3495 cm\(^{-1}\) confirmed the O-H stretching in the PLA nanofibers. Vibration peaks at 2931 cm\(^{-1}\), 2866 cm\(^{-1}\) (stretching vibration of C-H), 1733 cm\(^{-1}\) (stretching of carbonyl bonds -C=O) and 1192 cm\(^{-1}\) (saturated C-O stretching) were observed for PCL nanofibers. Analysis of FTIR for all PLA-PCL blends of electrospun scaffold (72:25; 50:50; 25:75) was shown in Figure 3.7 B.

Figure 3.7: FTIR spectra of [A] PLA(100%) and PLA(100%) parent polymers; [B] Physical blends of PLA-PCL (75:25), (50:50) and (25:75); [C] Intermolecular hydrogen bond between the carbonyl group of PCL and hydroxyl terminal end of PLA

All polymeric blends showed the characteristic peaks of aliphatic polyesters (PLA and PCL) such as C-H stretching (2840 – 3000 cm\(^{-1}\)), C-O stretching (1163-1210) and C=O stretching (1715-1730), thereby confirming the mixture of parent polymers. Further,
disappearance of O-H stretching at 3500 cm\(^{-1}\) with the addition of PCL and shift in the carbonyl stretching from 1748 cm\(^{-1}\) to 1726 cm\(^{-1}\) at higher ratio of PCL (25:75) confirmed the presence of intermolecular hydrogen bonding between the C=O of PCL and O-H of PLA [Figure 3.7 C]. This has clearly indicated that the higher concentration of PCL in the PLA-PCL blend scaffolds facilitated the molecular interaction between PLA and PCL [25].

3.3.5.3 X-ray Diffraction Pattern for PLA-PCL Blend Nanofibers

![XRD pattern for PLA-PCL blend nanofibers](image)

Figure 3.8: XRD pattern of PLA, PCL and PLA-PCL blend electrospun nanofibers

XRD pattern was investigated for all five ratios of nanofiber scaffolds to evaluate the change of crystallinity based on the addition of PCL in PLA-PCL blend (Figure 3.8).
Existence of broad peak at angle of $\theta = 15.5^\circ$ confirmed the amorphous nature of PLA fibers. In contrast, two prominent crystalline peaks were obtained at $21.5^\circ$ and $23.8^\circ$ corresponding to the plane of (110) and (200) respectively.

Comparing XRD pattern of pure PLA fibers, strong and sharp crystalline peaks at $\theta = 20.8^\circ$ & $23.2^\circ$ for PLA-PCL (75:25) and $\theta = 21.0^\circ$ & $23.3^\circ$ for PLA-PCL (50:50) blend fibers were noticed which correspond to the crystallographic planes of PCL. However, PLA-PCL (25:75) showed a broad and overlapping two crystalline peaks which may be attributed to the reflection of strong interaction between PLA and PCL. Increase in PCL molar ratio increased the crystallinity and shown maximum crystallinity peak for electrospun PLA-PCL (0:100).

### 3.3.5.4 Differential Scanning Calorimeter:

Glass transition temperature ($T_g$) for all scaffolds were presented in Table 3.2. The $T_g$ of PLA-PCL (100:0) and (0:100) nanofibers were evaluated as 17°C and -52°C respectively. It was also observed that the glass transition temperature was found to be lesser with the addition of PCL in PLA-PCL blends. For PLA-PCL (75:25), (50:50) and (25:75), $T_g$ was observed to be 9.69°C -0.24 °C and 4°C respectively. Further existence of single glass transition peak between the $T_g$ of parent polymers indicated the complete homogeneity of PLA-PCL blend which may be due to the physical interaction between PLA and PCL through intermolecular hydrogen bond [25].
3.4.4 Mechanical Strength:

Tensile strength of various electrospun PLA-PCL blend scaffolds were determined using uniaxial tensile testing machine (Table 3.3.). The ideal scaffold should provide required mechanical strength to the vascular grafts initially after implantation though it potentially restores the structural, mechanical and functional characteristics of native vessel gradually [26]. Pure PLA nanofibers was found to possess significantly higher ultimate tensile strength (3.8±0.5 MPa) and higher Young’s modulus (306 ± 87 MPa) as compared to tensile strength (2.1 ±0.8 MPa) and Young’s modulus (51±21 MPa) of 100% PCL nanofibers (p <0.05). Significantly lesser stiffness in PCL nanofibers than PLA may be due to the elastomeric nature of PCL [27].

It was also observed that the tensile strength was found to be gradually reduced while increasing the PCL ratio in the PLA-PCL blend scaffolds (Table 3.3.). However, increase in the ratio of PCL in the PLA-PCL blend significantly reduced the stiffness of the material. Tensile strength of PLA-PCL(25:75) was observed to be comparable with that of saphenous vein and femoral artery in circumferential direction and exhibits significantly lesser stiffness as compared to 100:0; 75:25; and 50:50 scaffolds (p <0.05). In addition, significantly higher elasticity observed in PLA-PCL (25:75) might be due to the intermolecular interaction between PLA and PCL (p <0.05).
Table 3.3: Tensile strength, and Young’s modulus of PLA-PCL Electrospun Scaffolds [100:0; 75:25; 50:50; 25:75 and 0:100] *: # indicates p < 0.05

<table>
<thead>
<tr>
<th>Mechanical Property</th>
<th>PLA-PCL (100:0)</th>
<th>PLA-PCL (75:25)</th>
<th>PLA-PCL (50:50)</th>
<th>PLA-PCL (25:75)</th>
<th>PLA-PCL (0:100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tensile Strength (MPa)</td>
<td>3.8 ± 0.5*</td>
<td>3.6 ± 0.9#</td>
<td>2.7 ± 0.5</td>
<td>2.1 ± 0.3*#</td>
<td>2.1 ± 0.8*#</td>
</tr>
<tr>
<td>Young's Modulus (MPa)</td>
<td>306 ± 87*</td>
<td>255 ± 62#</td>
<td>224 ± 22</td>
<td>75 ± 27*#</td>
<td>51 ± 21*#</td>
</tr>
</tbody>
</table>

3.4 Conclusions

Various ratios of PLA-PCL (100:0; 75:25; 50:50; 25:75 and 0:100) blend solutions were optimized for the fabrication of homogenous nanofibers with the dimensions of 129 ± 31, 285 ± 40, 360 ± 50, 365 ± 40, and 374 ± 32 respectively. Influence of various ratios of PCL and PLA in the nanofiber scaffold was evaluated on physicochemical properties of scaffold. Intermolecular interaction of PLA and PCL in the blend was confirmed using FTIR, DSC and XRD analysis. Hydrophobic nature of PLA-PCL electrospun scaffolds of all ratios could be promising substitute with blood compatibility by preventing the adhesion of platelets and thrombus formation. In addition, PLA-PCL (25:75) scaffold exhibited excellent mechanical properties similar to native arteries with lesser stiffness which is a prerequisite for the vascular graft. Thus the random fibrous scaffolds of PLA-PCL blends have been evaluated further for its influence on the vascular endothelial cell fate processes using cell culture studies.
References


