Electrospun Polycaprolactone/Polylactic Acid Nanofibers as an Artificial Nerve Conduit

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ABSTRACT

Development of conduits made of biodegradable nanofibers is gaining substantial interest due to their suitability for nerve regeneration. Among all polymeric nanofibers PCL (Polyε-Caprolactone) is distinctively found for mechanical stability and PLLA (Poly (L-Lactic Acid)) for relatively faster biodegradability. The aim of this study is to investigate blending compatibility between PCL and PLLA and the ability to fabricate nanofibers conduits via electro spinning. The PCL-PLLA nanofiber tubular made from different blend ratios of PCL-PLLA were electro spun. The electro spun nanofibers were continuously deposited over high speed rotating mandrel to fabricate nanofibers conduit having inner diameter of 2mm and the wall thickness of 55-65μm. The diameters of nanofibers were between 715-860nm. FTIR (Fourier Transform Infrared) spectroscopy used to analyze chemical change in the blends of nerve conduits, which revealed that the PCL-PLLA blend nanofiber exhibit characteristic peaks of both PCL and PLLA and was composition dependent. The crystallinity of PCL-PLLA tubes were studied using WAXD (Wide Angle Xray Diffraction). The morphology of nanofibers were investigated under SEM (Scanning Electron Microscope). The mechanical properties of the conduits were also tested; the Young's modulus obtained for small diameter was 10MPa, twice as high as larger diameter.

Key Words: **Polycaprolactone; PolylacticAcid; Nano-Fiber Conduits; Electrospinning; Artificial Nerve.**

1. INTRODUCTION

The major cause of nerve injury is due to road accidents; a small gap of damaged nerve such as <6mm can be regenerated by implanting an extra nerve conduit (blood vessel) [1]. The clinical treatment requires a second surgery of the patient to obtain a donor nerve. Avoiding the second surgery, many artificial nerves made of rigid channel guides have been proposed in recent years, but that interrupting its motor-sensory pathways and therefore impair patient's movements [2]. Tissue engineering has opened new era in the last few decades that propose nerve conduits made of nanofibrous. Therefore, various attempts have been made to prepare nerve conduits based on polymeric materials for peripheral-nerve regeneration. These materials include degradable and non-biodegradable polymers [3]. Nerve conduits made of biodegradable polymers has a clear advantage because the implanted nerve tends to degrade after the nerve is regenerated and therefore no need to have a

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second surgery for conduit removal as in case of non-biodegradable polymers are used [4].

Electro spun nanofibers based on biodegradable polymers exhibit a unique 3D scaffolds characteristic that favors nerve regeneration [5]. Well known biodegradable polymers in neural tissue engineering are PLA, PGA (Polyglycolic Acid) and PLGA (Poly Lactic-Co-Glycolic Acid). PCL is another polymer used to enhance mechanical properties of nerve conduits [6-7].

Electro spinning is a successful method to prepare nanofibers for tissue engineering purposes, because electro spun nanofibers have an ability to mimic native tissues and organs [8-9]. This report describes preparation of nerve conduits via electro spinning for potential use as an artificial nerve. All electro spun PCL and its blend with PLLA nanofibers were characterized by mechanical properties, SEM for fiber morphology examination, FTIR spectroscopy for chemical structural changes and WAXD analysis fiber crystallinity.

2. MATERIALS AND METHOD

2.1 Preparation of Nanofibers

PCL (Mw. 80,000) of Sigma-Aldrich was used as received. A 12% (w/w) solution of PCL, was dissolved in 1:9 w/w Dimethyl form amide: chloroform. PLLA (Mw. 143,000) was purchased from Sigma-Aldrich. 8% PLLA solution was prepared by dissolving in 3:1 w/w chloroform: Acetone. PCL/PLA blend ratios were prepared as 1:0, 2:1, 1:1, 1:2 and 0:1.

Electro spinning (Har-10012 Matsusada Co., Tokyo, Japan) was used to elctrospin nanofibers. The equipment was modified locally to prepare tubular structure. Stainless-steel wire was installed between a syringe and a metallic-collector. The stainless-steel wires were rotated and reciprocated continuously during electro spinning. The nanofibers were deposited over the stainless steel wire.

Three PCL nanofibers were prepared via electro spinning with three different diameters (0.30, 0.50 and 2mm) whereas, PCL/PLLA blend were electro spun with various blend ratios. A 5mL syringe with a capillary-tip of 0.6mm diameter was used to dispense the PCL or PCL/PLLA blend solution. High power voltage supplied to the polymer solution via a positive electrode (anode), and electrospun nanofibers were deposited over a collector attached to a negative electrode (cathode). The voltage of 20kV was supplied to PCL solution and 10kV to PCL/PLLA blend solutions. The TCD (Total Distance between tip and Collector) was set at 15cm, while the distance between the stainless-steel wire and capillary-tip was 15cm. In order to flow the polymer solution, the plastic syringe was set at 10^{o} angle from the horizontal plane. The PCL or PCL/PLLA nanofibers were collected continuously on rotating stainless-steel wire [10].

2.2 Characterization

The average diameter of nanofibers was measured Keyence-Digital Microscope VH-Z500R using a magnification of 5000x. The tube wall thickness was assessed using Digital-Micrometer MCD130-25 ±1 μm. In order to determine Young's Modulus of nerve conduits, the TENSILON RTC1250A, A&D Company Ltd., Japan was used, where, the crosshead speed was kept at 10mm/min. All specimens were vacuum dried overnight at 25°C before use. The morphology of conduits was examined under Scanning Electron Microscope (SEM; S3000N Hitachi, Japan); all samples were sputtered with Pd-Pt before assessment. The crystalline structure of nanofibers was studied using WAXD in Rotaflex RTP300, Rigaku. Co., Japan; 40kV, 150mA using a scanning speed of $2\theta=2^{o}$/min. The changes in chemical structure for all conduits were analyzed by FTIR Spectroscopy (Prestige21Shimadzu, Japan) as shown in Fig. 1.

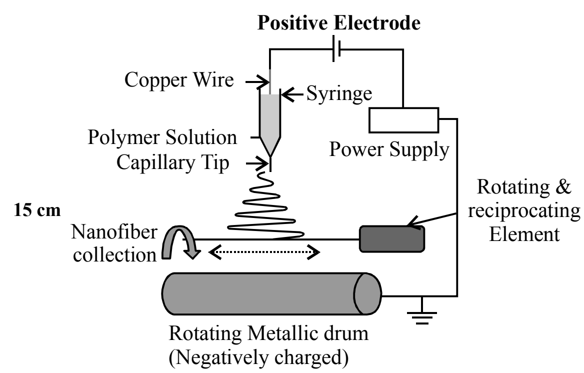

FIG. 1. ELECTROSPINNING SETUP

3. RESULTS AND DISCUSSION

3.1 Nanofiber Diameter and Nerve Wall Thickness

Table 1 shows electro spinning time that is deposition of nanofibers time versus the nerve wall thickness obtained against all types of nerve guides. Results indicated that increasing PLLA ratio such as 1:2, the deposition time was increased to achieve comparable nerve wall thickness. This may be due to PCL nanofibers bulkiness that renders the nerve wall thickness obtained earlier than that of PLLA nanofibers. The small caliber of around 0.5 and 0.3mm in diameter required even less deposition owing to their small circumference. The effect of increasing ratio of the PLLA (1:2) has significant effect on nanofiber diameter that decreases with increasing PLLA ratio.

3.2 Effect of Nerve Conduit Diameter on Tensile Strength

Reducing nerve conduits diameter less than 2.0mm has greater effect on tensile behavior, therefore, we selected two nerve conduits of 0.5 and 0.3mm diameters and compared it with 2.0mm diameter. Owing to the small diameter of nerve conduits of 0.3and 0.5mm, the tensile behavior was one of the key factors to evaluate their mechanical stability. Surprisingly, higher Young's Modulus values of 10MPa obtained for a relatively small nerve guide of 0.3mm, whereas, lower Modulus values obtained for 0.5and 2.0mm nerve conduits. This is mainly due to the arrangement of nanofibers in small diameter conduit having more chance to wrap around the nerve core, which imparts stiffness and resulted higher young's modulus. The tensile strength is also higher about 13MPa for small nerve conduit but lower strain 500% than 0.5and 2.0mm nerve conduits (Fig. 2).

TABLE 1. NERVE WALL THICKNESS

Nanofibers Ratio PCL:PLLA	Diameter of Nanofibers (µm)	Diameter of Nerve (µm)	Nerve Wall Thickness (µm)	Deposition Time (min)
1:0	1.8	0.3	24.50	20
1:0	1.8	0.5	35.66	30
1:0	1.8	2.0	58.83	45
2:1	1,51	2.0	60.10	60
1:1	1.35	2.0	55.50	90
1:2	1.22	2.0	58.42	120
0:1	1.17	2.0	62.08	160

3.3 Effect of Nerve Conduit Diameter on Crystalline Structure

Fig. 3 demonstrates WAXRD pattern for each nerve produced. Two distinctive diffraction peaks of semi-crystalline PCL nerve conduit observed at 2θ=21.4° and 23.8°, which attributed to the (110) and (200) lattice planes respectively. Sharpening of peaks mainly attributed to the more crystalline structure obtained for small diameter 0.3mm nerve conduit. It was observed that crystallinity increased with decreasing nerve conduit diameter. Increasing crystallinity is directly proportional to the tensile strength. It can be seen from the Fig. 2, that the 0.3mm nerve conduit show highest tensile strength due to increased crystallinity. The probable reason for improved crystallinity may be due to wrapping nanofibers around the conduit's periphery.

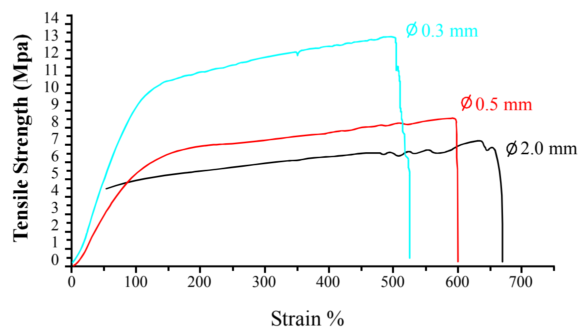

FIG. 2. TENSILE STRENGTH OF PCL NERVE CONDUITS

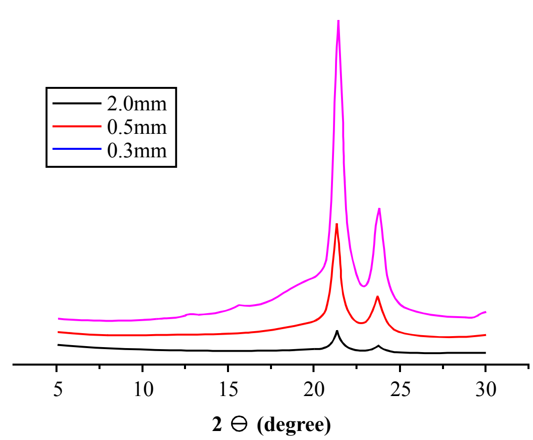

FIG. 3. WAXD PATTERNS OF PCL NERVE GUIDES OF DIFFERENT DIAMETERS

3.4 FT-IR Spectroscopy

The FTIR spectra of PCL/PLLA blend is shown in Fig. 4(a-b). The scans show a carbonyl (C=O) stretch band from the PCL. Fig. 4(a) shows the peak at around 2900cm^{-1} was decreased and finally disappeared when the ratio of PLLA was increased from 1-2. Fig. 4(b). The characteristic peak of PCL at 1700cm^{-1} was gradually disappeared and the characteristic peak of PLLA at 1750cm^{-1} was gradually appeared when the PLLA ratio was increased. This suggests that the blending of PCL and PLLA of 2:1, 1:1 and 1:2 are well in agreement. There were no characteristic obtained that suggest any chemical bonding between PCL and PLLA.

3.5 Morphology of Nanofibers and Nerve Conduit Structure

SEM images for each nerve guide are shown in Fig. 5. From Fig. 5(a-f) are the 3D view of nerve conduit fabricated with different ratio of PCL and PLLA nanofibers. Where PCL:PLLA ratios are (a) 1:0, (b) 2:1, (c) 1:1, (d) 1:2 and (e) 0:1. In general, the PCL nanofibers had relatively good cohesiveness in nanofibers that led the structure compact and formable (Image (h)). Whilst the PLLA nanofiber was more bulky with relatively less cohesiveness in nanofibers (Image (i) and (j)). The nanofibers cohesiveness factor has significant influence over the final structure of nerve conduit, that became softer and less formability as the PLLA ratio was increased (Images (a-e)). The sample (Image (f)) PCL/PLLA/PCL was fabricated by layer-on-layer, in which PLLA nanofibers were sandwiched between two layers of PCL. The image f shows a peeling-off the PCL with PLLA and the structural stability was not as good as PCL/PLLA

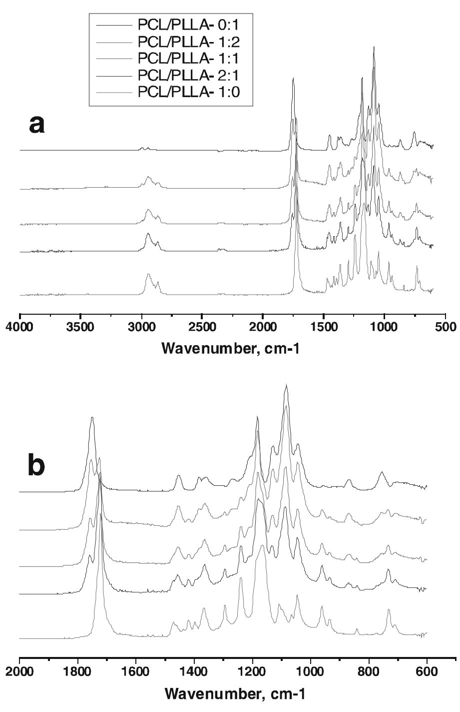

FIG. 4(A). FT-IR SPECTROSCOPY FOR PCL AND ITS BLEND WITH PLLA NANOFIBERS AT VARIOUS RATIOS AND (B) IS A SELECTED WAVELENGTH OF FT-IR SPECTROSCOPY

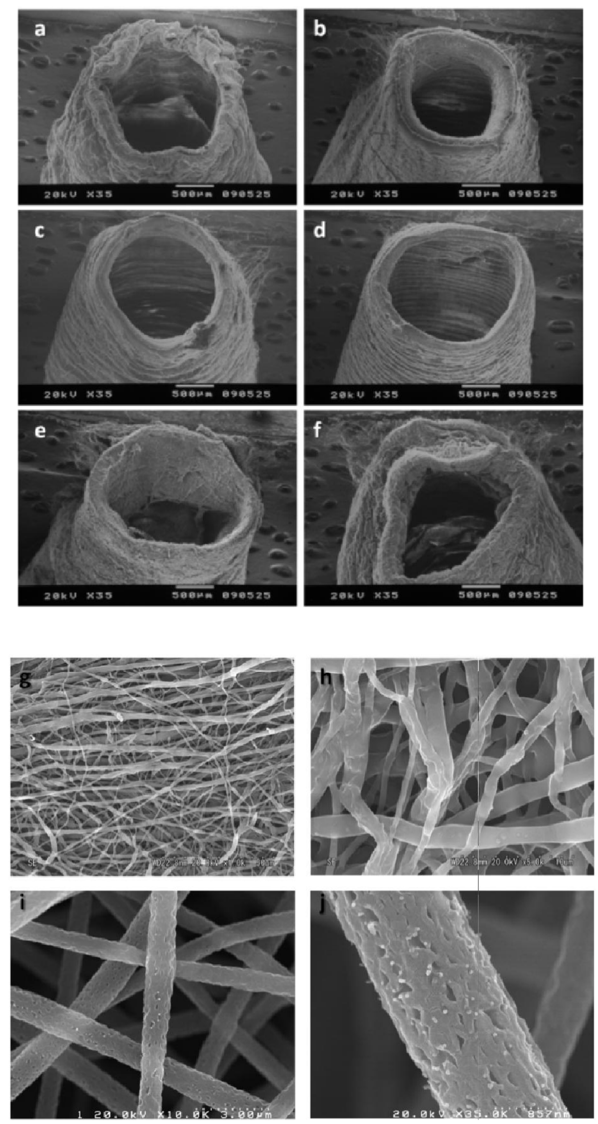

FIG. 5. SEM AND FE-SEM IMAGES FOR PCL AND PCL/PLLA NANOFIBERS NERVE GUIDES

polymer blending. Therefore, the study was focused mainly in the direction of PCL/PLLA electro spinning via polymer blending.

Irrespective to the type of nanofiber composition ratio, the nanofibers alignment was quite prominent and parallel in nerve guide of 2mm diameter (Image (g)). This may be because of the higher circumference speed of 2mm nerve conduit than the smaller nerve (a duit during electro spinning. The 1:1 ratio of PCL/PLLA was the most stable with good formability.

4. CONCLUSION

The nerve conduits of PCL and PLLA with various ratios were successfully fabricated via electro spinning. Morphology suggested that good structural stability and formability was obtained at 1:1 ratio of PCL/PLLA. The Young's modulus of 0.30 mm PCL conduuit obtained as high as 10 MPa than the 0.50 mm and 2 mm PCL conduits.. More deposition time was required to obtain around58 μm nerve wall thickness as the PLLA ratio in the blend was increased. Nanofiber diameter of PCL was relatively larger than that of the PLLA nanofibers. FTIR analysis suggested that there was no chemical interaction between PCL and PLLA. Overall, electro spinning of nerve guides using biodegradable PCL/PLLA has potential to be used to fill the nerve gap for nerve regeneration.

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