ELECTROSPUN POLY (L-LACTIDE-CO-Ɛ-CAPROLACTONE) (PLCL) NANOFIBERS STRUCTURAL EVOLUSION IN SERIES OF BINARY SOLVENT SYSTEMS

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ABSTRACT

Solvent is one of the important factors which has great influence in obtaining fibrous materials from the electrospinning technique. The use of an appropriate solvent for the preparation of spinning solution before undergone electrospinning process is the fundamental aspect that one must have in producing good quality fibers. For that reason, selective solvents including dicholoromethane (DCM), N-N-Dimethylformamide (DMF) and acetone were employed in this study to generate series of binary solvent systems. Poly (L-lactide-co-ɛ-caprolactone) (PLCL) solutions of 11% (w/v) were prepared for series of binary solvent systems which are DMF/acetone, DCM/DMF and DCM/acetone in selected ratios of 7:3, 1:1 and 3:7. The prepared PLCL solutions were electrospun at 10-14 kV with the solution flow rate of 1.00 mL/hr. Scanning Electron Microscope (SEM) analysis on the morphology of the PLCL nanofibers obtained from the DCM/DMF binary solvent produced the nonporous fibers at the optimum solvents ratio of 7:3. The substitution of DMF to acetone for the DCM/acetone binary solvent system on the other hand resulted to the formation of highly-porous PLCL fibers at the optimum solvents ratio of 7:3. The AFM characterization has revealed the differences in the surface roughness and pores depth of both non-porous and highly-porous PLCL electrospun fibers fabricated.

A well interconnected open pores polymer nanofibers has been proven as the promising materials for tissue culture substrates which allow high density cell seeding and tissue growth [1]. Poly (L-lactide-co-ɛ-caprolactone) (PLCL) is an example of synthetic biodegradable polyester which is commonly used as the scaffolding material for biomedical application [2]. PLCL is a copolymer of $_L$-lactide and ɛ-caprolactone monomers building block, which possess good biocompatibility, low rate of degradation with minimum harmful by-products, and is widely utilized for the regeneration of bone and nerve tissues [3, 4]. Electrospinning has special ability to produce diverse form of nanofibers. The remarkable of highly porous nanofibers developed the interest to mimic the architecture of native tissue at nanoscale. Efforts have been made to modify nanofibers surface with several techniques [5]. According to Park *et al*. [6], the technique known as the "gas foaming" method using an effervescent salt as a gas foaming agent, was carried out. The porosity of the nanofiber was controlled by the gas evolved from the acid-base reaction between the ammonium bicarbonate and citric acid used. However, the resulted pores generated on the surface of the nanofibers were not uniformed. The technique was then incompetent. Another research conducted by McCann has reported on the effect of solvent used in the electrospinning technique on the morphology of the nanofibers produced [7]. Yet, the process needs another post-treatment by calcinations process further. They do confirm that the composited poly (vinyl) pyrrolidone (PVP) nanofibers had a highly porous structure after the calcinations of the nanofibers in air at high temperature. The solvent is certainly the prominent component in the spinning solution and therefore it has a major influence on almost aspect of the electrospinning process. Wannatong *et al*. [8] explained that both the density of the electrospun nanofibers and their morphology were influenced by the choice of solvent. The chosen solvent must have the specific criteria such as low surface tension and high dielectric constant value. It is mentioned that the vapour pressure of the solvent should be suitable so that it evaporates sufficiently for the electrospun nanofibers to retain its shape when it reaches the targeted collector. Successful experiments in producing PLCL nanofibers via electrospinning have used solvents such as methylene chloride (MC), 1,1,1,3,3,3-hexafluoro-2-propanol(HFIP) and acetone. The results by both Mo *et al*. [9] and Xu *et al*. [10] have reported the non-porous bead-free PLCL nanofibers were successfully obtained. Nevertheless, we found the limitation in the publication on porous structured PLCL nanofibers being reported. The objective of this study is to produce porous structured PLCL nanofibers via exchanging the solvents used in electrospinning process, as the novelty of this work. Polar solvent which is dichloromethane (DCM), N-N-Dimethylformamide (DMF) and acetone were chosen as the solvent medium in preparing PLCL solution. The effect of the chosen selected solvents on the morphological appearance of the resultant PLCL nanofibers was observed by Scanning Electron Microscope (SEM) and Atomic Force Microscope (AFM) analysis.

Materials. Poly (L-lactide-co-ε-caprolactone) PLCL (70:30, molecular weight 150kDa) was purchased from Boehringer Ingelheim Pharma (Ingelheim, Germany). Dichloromethane (DCM), N,N-dimethyl formamide (DMF) and Acetone were purchased from Merck (Germany), Sigma Aldrich (USA) and QRec (New Zealand), respectively. All chemicals were of analytical grade and were used as received.

Electrospinning of nanofibers. The PLCL solutions (11% w/v) were prepared by dissolving PLCL in binary solvent mixture of DMF/acetone, DCM/DMF and DCM/acetone in (7:3), (1:1) and (3:7) respectively. The prepared PLCL solution was loaded into plastic syringes equipped with blunt 25-gauge needles. The syringe then was attached to the vacuum pump (KDS 100, KD Scientific, Holliston, MA). During electrospinning, high voltage power (Gamma high voltage research) was applied through the metallic clip attached to the needle end tip of the plastic syringe containing PLCL solution. The current voltage was maintained at 10 to 14 kV, with electrospinning solution flow rate at 1.00 mL/hr. The nanofibers formed were collected on an electrically grounded aluminum foil attached to the jet collector. The distance between jet and end tip needle is approximately 8 cm.

Scanning electron microscope (SEM). The surface morphology characterization of the electrospun PLCL nanofibers were conducted by scanning electron microscope (JSM – 6390, JEOL, Japan) after gold coated with Autosampler coater (JEOL JFC-1200 fine coater, Japan) with an accelerating voltage of 10kV. Diameters of the electrospun nanofibers from SEM micrographs were analyzed using image analysis software (Image J, National Institutes of Health, USA).

Atomic force microscope (AFM). In addition to surface morphology analysis, AFM also offers a powerful tool for measuring surface roughness of nanofibers. 2cm x 2cm size of nanofibers film was placed on the sample stage and nanofibers sample was characterized using AFM model SP A300HV instrumentation. The cantilever tip used in the AFM detector is non-contact mode and the scan area for each sample is 3000 nm, with 1.00 Hz speed. The SPIWin software was used to analyse the surface roughness, pore size and pore depth measurement of nanofibers.

The electrospinning process of 11 % (w/v) PLCL nanofibers were carried out by using binary solvent mixture of DMF/acetone, DCM/DMF and DCM/acetone at ratios of 7:3, 1:1 and 3:7, respectively. The SEM micrographs of the electrospun PLCL nanofibers at different ratios of the binary solvent systems were shown in Figure 1. While having PLCL in DMF/acetone binary solvent mixture, the representative SEM micrographs showed the appearance of sticking and irregular shape of electrospun PLCL nanofibers were produced. The stretching of the polymer droplet were observed, however it is clearly shown the stretched were not enough to produce an appropriate fibrous form nanofibers. The size of fibers diameter is in the range of 50 to150 nm with the beaded size of 2 μm. The morphology of PLCL nanofibers at all ratios of DMF/acetone solvent mixture reflects the unsuitable of the solvent for producing good quality of PLCL nanofibers. For DCM/DMF binary solvent mixture, the representative SEM micrographs depicted the electrospun PLCL nanofibers morphology were optimized at the ratio of 7:3 with the electrospun PLCL nanofiber were observed in dense, bead-free highly interconnected nanofibers. The diameter size of the non-porous PLCL nanofibers formation is in the range of 350 to 450 nm. In contrast, the DCM/DMF solvent mixture at ratios of 1:1 and 3:7 gave a mixture of fibers with irregular beads morphology of the PLCL nanofibers. The size of fibers diameter is in the range of 50 to 80 nm with the beaded size of 2 to 4 μm. It is explained that the charged polymer droplet was not fully stretched for both of the spinning solution in the DCM/DMF 1:1 and 3:7 ratio, resulting to the beaded fibers formation. In contrast, there is a significant difference on the nanofibers morphology while using DCM/acetone binary solvent mixture. The SEM analysis showed the highly porous structured nanofibers were obtained. According to the representative SEM micrographs, the optimum ratio for DCM/acetone binary solvent mixture is 7:3 which resulted to the formation of good quality nanofibers in term of their homogeneity of nanofibers size, bead-free and highly porosity PLCL nanofibers. The diameter size of porous PLCL nanofibers is approximately 800 nm, quite larger compared to the rest of nanofibers produced from other solvent mixture in this study. It is concluded that the optimized binary solvent systems for the PLCL nanofibers formation were DCM/DMF 7:3 and DCM/acetone 7:3 for dense and porous structured PLCL nanofibers, respectively.
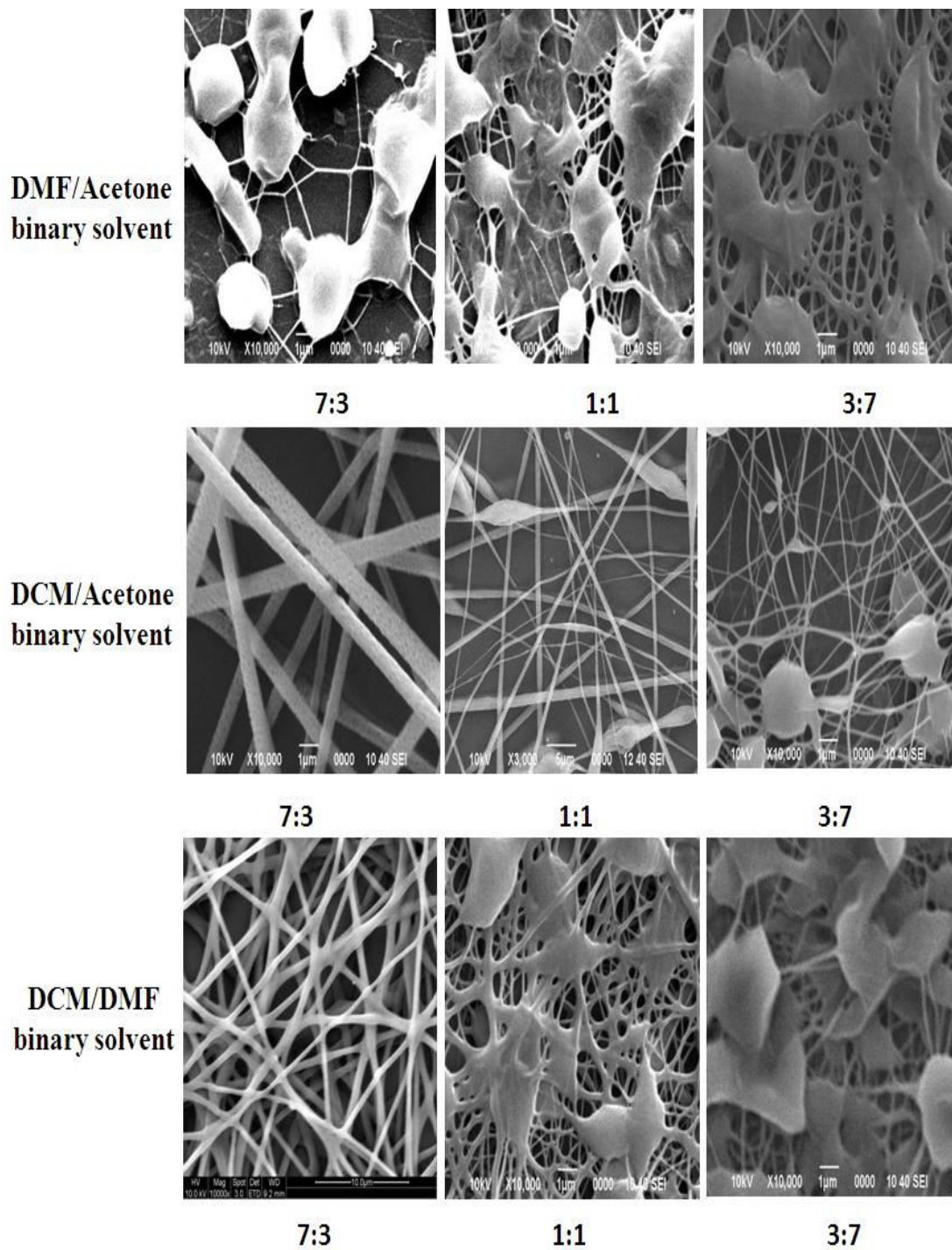
Figure 1. SEM micrographs of electrospun PLCL nanofibers composed from three series of binary solvent systems.

AFM structural characteristic. The surface roughness of the electrospun PLCL nanofibers from the AFM images were evaluated using SPIWin software. In this study, the distinguish structural characteristic of two different surfaces of PLCL nanofibers composed from DCM/DMF (7:3) and DCM/acetone (7:3) which represent the dense and porous structured PLCL nanofibers, respectively were determined. The surface roughness of the tested PLCL nanofibers is shown in Table 1.

Table 1. Surface roughness of dense and porous PLCL nanofibers

Sample	AFM Images	Surface roughness
PLCL nanofibers DCM/DMF (7:3)		Pore size (nm): 0.36-0.50 Pore depth (nm): 1.85-2.23
PLCL nanofibers DCM/Acetone (7:3)		Pore size (nm): 180.92-250.37 Pore depth (nm): 55.60-69.98

The employed solvents systems in this study have shown the important of the solvent in the evolution of the electrospun PLCL nanofibers morphologies. The transformation of the fiber morphology from beads to beaded fibers, then to dense bead-free fibers and finally to porous bead-free fibers were clearly observed during the electrospinning process by exchanging the solvents used. In the case of solvent mixture composed of DMF/acetone, the electrospinning of PLCL solution resulted in the formation of spherical beads and irregular beaded nanofibers and similar behavior were observed for the rest of the ratios of DFM/acetone mixture used in this study. Bending instability during the spinning process strongly suggested the mixture of DMF and acetone is not suitable to create good PLCL solution for spinning. These findings were supported by Reneker *et al*. [11] who reported the formation of spoilt structures of PCL fibers in acetone while, Hsu and Shivkumar [12] reported the formation of spherical beads upon increasing the DMF amount in spinning solution. Table 2 showed that both DMF and acetone have high dielectric constant values. By adding mixture of DMF and acetone, higher dielectric constant is exist in the spinning solution mixture thus resulting in the instability of the spinning solution once high current voltage is introduced during the spinning process. When the binary solvent mixture component is changed to DCM/DMF, the spinning solution gave an irregular beaded fibers and bead-free fibers formation upon the spinning process, depending to the DCM/DMF ratios. By increasing the DCM ratio in the DCM/DMF mixture, the morphology of PLCL nanofibers were slowly changed from beaded to bead-free fibers. However, higher proportional ratio of DMF destroyed the morphology of PLCL nanofiber since DMF is known to have high dielectric constant properties. Therefore it is necessary to control over the polarity of the solvent mixture as the criteria for producing good nanofibers. The combination of DCM and DMF at ratio of 7:3 has created synergistic effect in stabilizing the formation of jet during the spinning process [13, 14].

18

Table 2. Basic properties of DCM, acetone and DMF

| Solvent	Mol. Weight [g/ml]	Dielectric constant [ε]	Dipole moment [D]	Boiling point [°C]	Melting point [°C]	Density [g/cm³]
DCM	84.9	8.93	1.60	40	-95	1.317
Acetone	58.1	20.7	2.88	56	-95	0.790
DMF	73.1	36.7	3.82	153	-60	0.949

The highly porous structure of PLCL nanofiber was spun from binary solvent system of DCM to acetone at the ratio of 7:3. The formation of porous nanofibers was due to the rapid evaporation of highly volatile acetone. The rapid evaporation of acetone gives rise to polymer-solvent phase separation, and the solvent-rich regions in the polymer composite transformed into pores during the electrospinning process [15]. The variation in the formation of PLCL nanofibers morphology was influenced by the dielectric constant of the spinning solution. Troy and Beringer [16] provided the mathematical formula to calculate the dielectric constant (ε) approximation as shown in (Eq. 1). The calculated of dielectric constant for all set of the binary solvent mixture in this study was shown in Table 3.

$$\varepsilon \text{ (mixture)} = \text{(fraction ratio)} (\varepsilon \text{ solvent A)} + \text{(fraction ratio)} (\varepsilon \text{ solvent B)} \qquad \text{(Eq. 1)}$$

Table 3. Calculated dielectric constant of binary solvent mixture

Binary solvent mixture	Fraction ratio	Dielectric constant [ε] approximation
DCM/Acetone	7:3	12.67
	1:1	15.05
	3:7	17.43
DCM/DMF	7:3	17.77
	1:1	23.55
	3:7	29.33
DMF/acetone	7:3	32.90
	1:1	29.50
	3:7	26.10

Throughout of this study, the suitable dielectric constant of the spinning solution for the PLCL spinning is from12.67 to 17.77. The increment in the dielectric value of the binary solvent mixture solution would only producing beaded nanofibers instead of smooth bead-free nanofibers, which are an unwanted phenomenon in the nanofibers production. Further investigation using AFM has proven that the PLCL nanofibers obtained from DCM/acetone 7:3 gave greater surface roughness and pores depth compared to the PLCL nanofibers obtained from DCM/DMF 7:3. The depth of the pores is between 55.60 to 69.98 nm for PLCL nanofibers accomplished from DCM/acetone 7:3 which is significantly greater than the PLCL nanofibers obtained from DCM/DMF 7:3, with the pores depth around 1.85 to 2.23 nm . Highly porous fiber with the interconnected pores generated on the PLCL nanofibers floss is an indication of the rapid evaporation of acetone during the spinning process. In contrast to DMF, acetone with lower boiling point properties has resulted in rapid release of the acetone molecules from the polymer composite subsequently creates interconnected pores on the spun nanofibers. The evolution on the morphology of the PLCL nanofibers obtained from series of DCM, DMF and acetone binary solvent systems via electrospinning process were depicted in Figure 2. Generally, the combination of DMF and acetone will only resulted in the fragmented structure of nanofibers due to the high dielectric constant properties possessed by both solvents. Thus, to control the PLCL nanofibers, a moderate dielectric constant of the solvent should be employed in the electrospinning process and DCM has been identified in this work as the main solvent towards the production of good quality of PLCL nanofibers. The combination of DCM and DMF in the binary solvent system is able to vary the high dielectric constant property of DMF towards the production of bead-free PLCL nanofibers. DCM/DMF binary solvent system with the ratio of 7:3 has been established as the optimum ratio in obtaining dense PLCL nanofibers. Hence, the substitution of DFM with acetone resulted in the production of highly porous PLCL nanofibers with an optimum DCM/acetone binary solvent system ratio of 7:3.
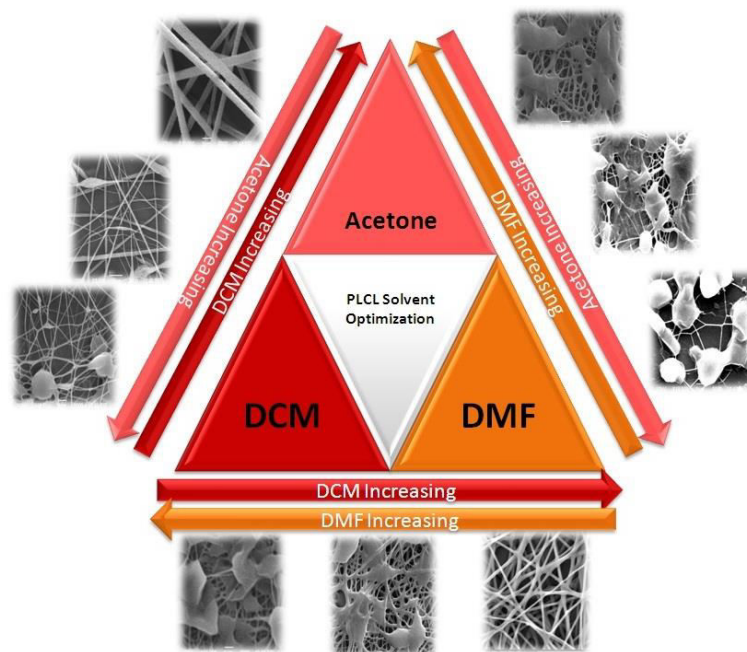
Figure 2. The electrospinnability of PLCL in series of binary solvent system of DCM, DMF and acetone

In this study, we revealed the evolution of the PLCL nanofibers morphologies which composed from the three different binary solvent mixtures. DCM which has moderate dielectric constant was identified as the prominent solvent that controls the PLCL nanofibers formation. DMF and acetone which is in the high dielectric constant group of solvent should be in an appropriate amount to control the surface roughness of the PLCL nanofibers. The optimized binary solvent mixture composed of DCM/DMF in 7:3 ratio provided a bead-free dense type of PLCL nanofibers whereas DCM/acetone ratio of 7:3 yielded a bead-free porous type of PLCL nanofibers.

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