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Production Of Poly(L–Co–D,L Lactic Acid) Porous Fibers By Electrospinning

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Abstract: The production of porous scaffolds has been widely investigated by the scientific community due to its suitability for tissue engineering. Among techniques that allow the fabrication of porous materials, electrospinning is appealing for being robust and versatile. This research investigated the pore formation in poly (L–co–D,L lactic acid) fibers obtained by conventional electrospinning and the influence of chloroform as a single solvent on fiber morphology. Random and highly porous fibers with a mean diameter of 2.373 ± 0.564 µm were collected. Chloroform affects the fiber morphology, mainly for its fast evaporation and low density of charges. The solvent on the surface evaporates quickly, and the low stretch of the jet does not help the polymer to reorganize over the length of the fiber, forming pores. In conclusion, the low dielectric constant and boiling point of chloroform induce pores formation along the PLDLA fibers.

Keywords: Porous scaffold. Nonwoven. PLA. Chloroform.

Introduction

The fabrication of scaffolds from nanofibers has been widely investigated by scientists. Among all possible structures, porous nonwoven made of micro and nanofibers arouses great interest in the scientific community and technology industry. Pores might be formed between adjacent fibers, the inter–fibers pores, or within a fiber, the intra–pores. In a membrane, the interconnected pores might be classified by the length, where there is macro (> 450 nm), meso (2 – 50 nm), and micro–pores (< 2 nm).[1,2]designing such anisotropic functional textiles that allow fast penetration of sweat through one direction but prevent its movement in the reverse direction is still a challenging task. In this regard, fabrication of a novel Janus membrane with multi–scaled interconnected inter– and intra–fiber pores for enhanced directional moisture transport designed by a rational combination of superhydrophilic hydrolyzed porous polyacrylonitrile (HPPAN

An appeal of intra–pores in nonwovens is its capacity to mimic the extracellular matrix and its high surface area, which favors cell attachment and proliferation over the scaffold. Meanwhile, the existence of intra–pores is a key factor for the material's performance towards wicking and permeability.[1,3,4]applied voltage, spinning distance

Several techniques allow the production of porous structures production such as electrospinning, electrospraying, chemical vapor deposition, and nanoimprinting, among others. Nevertheless, electrospinning has an appeal for being a robust and versatile technique. By changing the polymer solution properties and the several processing variables, a range of nanofibers morphologies may be assembled. The use of electrospinning to obtain artificial materials with different porous hierarchies is also possible. However, mastering the design and fabrication of porous materials according to the demand of the application desired, containing intra or inter–fiber pores, with micro, meso, and micro–pores, is still a challenge.[4]

The capacity of processing several types of materials is also an advantage of electrospinning. Natural and synthetic polymers processed with this biomedical technology have been successfully reported, as for the use of collagen–gel nanofibers for endothelial cell guidance[5], for bone critical–sized defects repairing with poly (ε–caprolactone) and poly (rotaxane)[6], for the construction of tubular tissue of poly(trimethylene carbonate–co–(L)–lactide)[7]"ISBN":"1742–7061","ISSN":"1878–7568","PMID":"23416575","abstract":"The growth of suitable tissue to replace natural blood vessels requires a degradable scaffold material that is processable into porous structures with appropriate mechanical and cell growth properties. This study investigates the fabrication of degradable, crosslinkable prepolymers of l–lactide–co–trimethylene carbonate into porous scaffolds by electrospinning. After crosslinking by ε–radiation, dimensionally stable scaffolds were obtained with up to 56% trimethylene carbonate incorporation. The fibrous mats showed Young's moduli closely matching human arteries (0.4–0.8MPa, for produce bone and fibrous tissue with poly(L–co–L,D lactic acid)[8,9], and many others.

Poly(L–co–D,L lactic acid) (PLDLA) is a remarkable polymer: it is bioabsorbable, biocompatible, have high mechanical stability and resistance, and very good processability. In brief, it has several advantages and high potential to be widely used in medical devices and biological applications.[8,10]

This research aimed to produce poly(L–co–D,L lactic acid) nonwovens with intra and inter–fiber pores for biomedical applications through conventional electrospinning, and to investigate the influence of the solvent on the formation of intra–fiber pores.

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Methods

Preparation of PLDLA solutions

The polymer used was poly(L–co–D,L–lactic acid) (Purasorb PLDL 7038), obtained from Corbion (USA), and the solvent used was chloroform 99,8%, from Acros (USA). The polymeric solutions were prepared using chloroform as a single solvent, and the polymeric concentration was 5 % w/v, determined in previous tests. The solvent was added to the polymer and it was magnetically stirred overnight at room temperature. The solutions were used shortly after preparation.

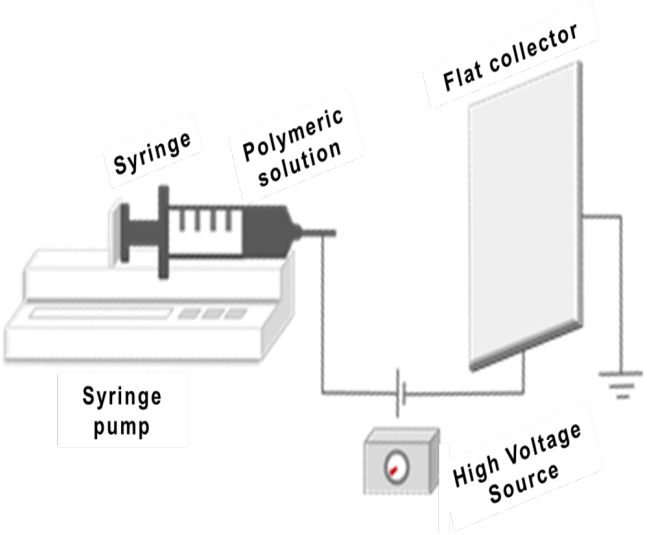

Figure 1. Schematic diagram of the conventional electrospinning set up in a horizontal plane.

Electrospinning process

The solution was transferred to a 20 mL plastic syringe, attached to a metallic flat–end needle (22 G). It was used quickly and for a short time to prevent degradation of the syringe by chloroform. Conventional electrospinning was performed and the fibers were collected in a grounded, static, and flat silicon plate (Figure 1).

The electrospinning parameters were set according to previous tests. The work distance (space between the collector and the needle tip) was 20 cm, the polymer solution flow rate was kept constant at 1.0 mL/h by a syringe pump (Fisher Scientific, USA), and the power applied in the needle (the positive pole) was 15 kV by a high voltage source (Gamma, USA). Samples were produced in triplicate, at room temperature and atmosphere conditions.

Scanning electron microscopy (SEM)

The fibers collected on silicon were coated with a thin layer of platinum by plasma metallization (Hummer® 6.2 sputtering system, Anatech LTD, USA). Afterward, the morphology of the fibers was observed in a JEOL JSM–6010 Plus scanning electronic microscopy (JEOL Ltd., Tokyo, Japan).

The images taken were analyzed with the software Image J (Image Processing and Analysis in Java, National Institutes of Health, Bethesda, MD) for measuring the diameter of the fibers. The software Action® (Estatcamp, São Carlos, SP) was used to statistical analysis, as descriptive statistics, normality test (Anderson – Darling), and a normalized histogram.

Fourier transform infrared (FTIR) spectroscopy

FTIR spectroscopy is a powerful non–destructive method for material identification and a well–established tool in polymer analysis (Kazarian & Chan, 2016). The chemical structure of pure PLDLA, pure chloroform, polymeric solution, and fibers was verified using a Spectrum 100 FTIR spectrometer (Perkin Elmer, Billerica, MA, USA). All materials were scanned from 650 cm–1 to 4000 cm–1, and its transmittance (%) was measured.

Results and discussion

The experiment intended to obtain porous fibers of PLDLA and discuss the effect of chloroform on membrane and fiber morphology and topography. Chloroform is a nonpolar solvent with low dielectric constant (4.80), low electrical conductivity (1.0E–04 μS cm–1), and highly volatile (boiling point of 61°C).[11]the productivity, and the morphology of nanofibres. In this study, poly lactic acid (PLA Its properties have caused some difficulties during the electrospinning process. The low boiling point leads to early solvent evaporation, which has promoted frequent needle clog. The low conductivity, which means reduced charges density in the solution, allowed fibers scattering and low yield (the fibers were sparsely collected in the silicon plate). Despite the adversities, it was possible to collect PLDLA fibers on the silicon plate.

The SEM images obtained (Figure 2) shows continuous fibers, without beads and merged areas, and with a rough surface. The production of a nonwoven with intra–fibers pores was achieved. The absence of beads suggests that the polymer concentration used was adequate since the balance between solution viscosity, surface tension, and electrostatic repulsion resulted in a continuous jet.[11,12]and the formation mechanism of the membranes was determined in this study. The PLA fibrous morphology, including the fiber diameter, bead size, number of beads, and surface structure of the beads, could be closely controlled by regulating the solvent compositions and the concentrations of the PLA solutions. The filtration performance, which was evaluated by measuring the penetration of sodium chlo– ride (NaCl

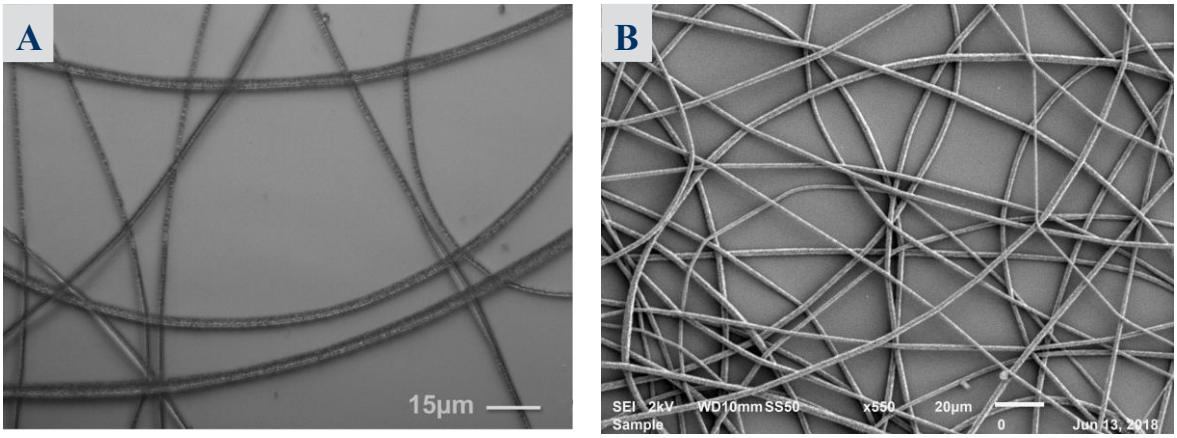

Figure 2. Morphology of the electrospun PLA fibers, taken with (A) optical microscope with scale bar of 15 µ, and (B) SEM image with 550 x magnification and scale bar of 20 µ.

According to descriptive statistics of the fiber diameters data (N=50), the mean diameter is 2.373 ± 0.564 µm, higher than the values reported for electrospun PLDLA with other solvents, as the beaded fibers measuring 0,352 ± 0,197 µm, produced with N,N–dimethylformamide.10 However, the fibers were a lot smaller than PLDLA fibers obtained by other methods, as polymerization and condensation: 110 ± 10 µm.9 The fiber diameter matters because it is inversely proportional to the number of pores and porosity.[13]

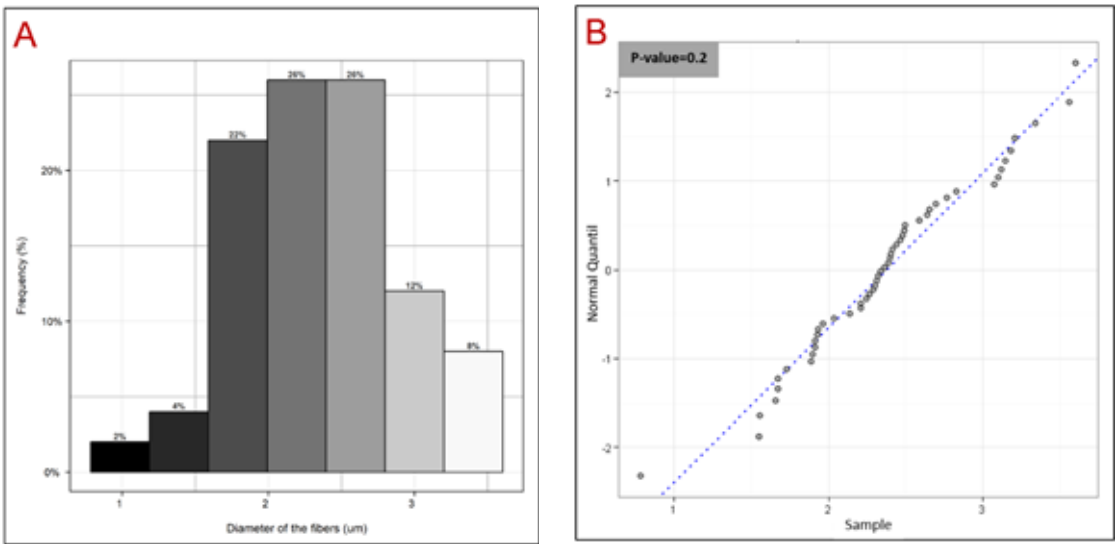

Figure 3. (A) Histogram and (B) normal probability plot of fibers diameter measurements.

The wide distribution of the histogram (A – Figure 3), and the large standard deviation (about 20% of the mean diameter) suggest that the forces on the jet were not strong enough to elongate the fiber and reduce the diameter variation as well as the mean diameter. Despite that, the shape of the histogram suggests that the measurements of the diameter have a Gaussian distribution, with a narrow peak. It indicates that the sample has homogeneous morphology and stability during

the process.[14] The normality test (Anderson – Darling) confirmed the normality of the population at 95% of confidence, with a P–value of 0,1971 (higher than 0,05), and by the normal probability plot (B – Figure 3), where the empirical data are positioned over the values of a standard quantile of a normal distribution (dotted blue line).

The central aspect of the fibers is the irregular surface and intra–fiber porosity, easily observed in the SEM images (Figure 4). Mo et al.[15] obtained porous nonwoven with polymethacrylate, and the surface of the fibers was irregular and porous as well. Their tests proved that this fiber aspect leads to a high capacity to absorb oleophilic material. As this characteristic persisted in the PLDLA fibers, this material might be used as an intermediate layer between different phases and promote better integration between it.

The use of a nonsolvent is appropriate to obtain highly porous fibers because it promotes phase–separation (solvent and polymer).[16] However, as chloroform is indeed a good solvent for PLDLA, this mechanism does not explain the pores in PLDLA surface. In this way, phase–separation is not the cause of pore formation.

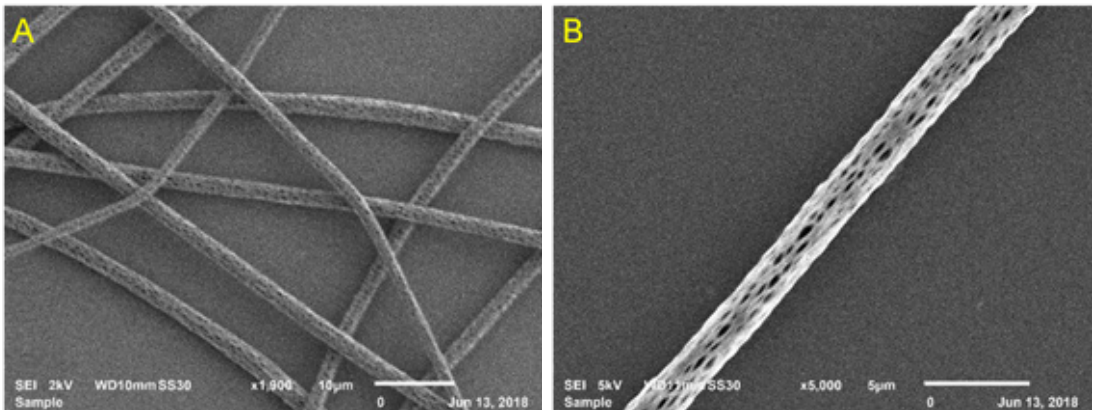

Figure 4. SEM images of porous fibers obtained by electrospinning of PLA in chloroform at 5% w/v concentration at (A) 1900 x magnification, with scale bar of 10 µ, and (B) 5000 x magnification, with scale bar of 5 µ.

The low boiling point of chloroform suggests that the pores on the fiber were formed for the fast evaporation of the solvent. In addition to it, the polymeric solution with pure chloroform has low charges density, and the jet stretch promoted by the electrical field is weak. Thus, the chloroform evaporation results in gaps in the jet, and the polymeric chains do not redistribute along it because the electrical field is low. As a result, the polymer chains do not redistributes itself and fulfill the gaps generated by the solvent evaporation, and a porous fiber is formed.

When the boiling point of the solvent is low, but the electrical conductivity is high, this phenomenon does not occur and the fibers present homogeneous and smooth surface, as for PLLA in pure acetone, observed by Casasola et al.[11] This fact corroborates the idea that the specific chloroform properties, low boiling point allied to low conductivity, promote the formation of porous fibers with a large diameter when compared with other electrospun PLDLA fibers.

Another mechanism for pores formation in the fibers is due to high humidity during the electrospinning. As there is no information on relative humidity, it is not possible to assure that the pores and roughness of the fiber surface were aggravated by it, although it is possible.[17]

Conclusion

The electrospinning of poly(L–co–D,L acid lactic) was conducted using chloroform as a solvent to obtain porous fibers for medical device production. Chloroform changed the morphology of the fibers, highlighting its low boiling point and dielectric constant. Its fast evaporation induced the formation of the pores along the fibers, while the low dielectric constant of the chloroform prevented the polymeric redistribution along the jet in a way that maintains the pores. The low charges density also caused a wide diameter distribution and thicker fibers compared to previously reported PLDLA fibers produced with other solvents. In this way, this research contributed to improve knowledge of the events that promote inter and intra–fiber pore formation for PLDLA.

Acknowledgments

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