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Structural characterization of electrospun micro/nanofibrous scaffolds by liquid extrusion porosimetry: A comparison with other techniques

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ABSTRACT

Poly(ε-caprolactone) micro/nanofibrous scaffolds obtained by electrospinning technique from polymer solutions were characterized in terms of fiber diameter (as measured by scanning electron microscopy-SEM), pore size and its distribution (as measured by liquid extrusion porosimetry), and porosity (as determined by gravimetric measurement, liquid intrusion method, SEM image analysis and liquid extrusion porosimetry — LEP). Nonwoven micro/nanofibrous scaffolds were formed by uniform bead-free fibers with mean diameters in the range of 0.4 to 7 μm. The results indicate that pore size and pore size distribution are strongly associated to fiber diameter. Porosity results were analyzed taking into account the accuracy and limitations of each method. LEP resulted as the most suitable technique for measuring through-pore diameter and porosity. In order to compare empirical data of pore size from LEP, a theoretical multiplanar model for stochastic fiber networks was applied. The results predicted by the model were in good agreement with the experimental data provided by LEP for mean diameters higher than 1 μm. The present study shows the potential of LEP as a valuable instrumental technique for characterizing the porous structure of electrospun fibrous scaffolds.

1. Introduction

Biomaterials with micro/nanoscale organizations have been used as controlled drug delivery systems and artificial extracellular matrices for tissue engineering [1–3]. Matrices with a micro/nanoporous structure are believed to be more favorable for tissue scaffold constructs. The traditional tissue engineering strategy uses bioresorbable porous scaffolds with appropriate requirements to regenerate functional tissues. The native extracellular matrix is mimicked by a combination of micro and nanoscale topography.

Micro/nanofibrous scaffolds are finding increasing applications in many bio and nanotechnology fields due to their highly attractive functional characteristics, such as high surface area-to-volume, porous structure, and biomimetic features. Nanofibrous scaffolds are ideal for tissue engineering applications because the highly porous network of interconnected pores provides the necessary pathways for transport of oxygen and nutrients that are crucial for cellular growth, and tissue regeneration [4–8].

Electrospinning provides a versatile technology to produce three-dimensional nanofibrous networks with fiber diameters from micro to nanoscale [9–12]. Processing of polymers through electrospinning has gained much attention in the last decade due to its versatility for producing a wide variety of polymeric fibers as well as its ability to obtain fibers in the submicron range, which is otherwise difficult to achieve by using conventional fiber-spinning technologies. In order to form micro/nanofibers, a stream of a polymer solution or melt is subjected to a high electric field forming a suspended droplet at the tip of a nozzle. The polymer jet is initiated when the electrostatic charge overcomes the surface tension of the droplet. Micro/nanofibers form when the ejection jet stream is narrowed under increasing surface charge density caused by solvent evaporation. A grounded target is used to collect the fibers, forming a non-woven mat. Moreover, electrospinning affords great flexibility in selecting materials (degradable or non-degradable polymers, synthetic polymers, biomacromolecules or natural biopolymers, composites), bioactive agents and drugs for drug delivery applications [4,10].

Since successful performance of many nanofiber-based applications is closely associated with an open porous structure, the study of key factors that determine the pore size and distribution of electrospun materials is important. Theoretical models to study the relationship between fiber diameters and pore size of nanofibrous membranes were reported [13,14]. These models demonstrated that fiber diameter plays a dominant role in controlling the pore diameter of the networks, the mean pore size increasing with fiber diameter. This means that any control of fiber diameter must be done with due care to address its effect on the pore structure, and in the case of tissue engineered scaffolds the ingress and growth of cells.

There is currently little understanding of the pore characteristics of electrospun scaffolds. Since different mean pore sizes can be obtained

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for networks of the same porosity, porosity measurements must be always reported along with pore size and its distribution. With this in mind, and considering the importance of a correct pore size measurement, appropriate techniques for polymeric scaffold characterization must be chosen. Thus, it is essential to have the ability to accurately measure both pore size and porosity in order to develop biologically relevant/successful scaffolds for a chosen tissue type.

Techniques available for characterizing porous scaffolds include mercury intrusion porosimetry [15], capillary flow porosimetry [16], liquid extrusion porosimetry [17,18], gas adsorption, X-ray microcomputed tomography [19], confocal laser scanning microscopy [20], and scanning electron microscopy (SEM) analysis [21,22], each with its own strengths and limitations [19].

Mercury intrusion porosimetry is a well-known and established method used to study porous materials. The technique measures the pressure required to push mercury, a nonwetting liquid, through pores in a material. A maximum pressure of about 60,000 psi (414 MPa) is typical for commercial instruments and this pressure forces mercury into pores down to about 0.003 μm in diameter. The high pressure required to force viscous mercury through small pores could distort flexible nanofiber membranes, the scaffold architecture being completely disrupted. Additionally, mercury is highly toxic, and decontamination is required. Mercury porosimetry is not suitable for fragile compressible scaffolds such as flexible foams (with porosities higher than 90%), textiles and nanofiber membranes [15]. Moreover, Pham et al. reported that pore size of scaffolds with fiber diameters lower than 4 μm could not be determined by mercury intrusion porosimetry due to the application of an initial pressure of 0.6 psi for filling with mercury the void space in the chamber [23].

Liquid extrusion flow porosimetry (capillary flow porosimetry) provides a non-destructive technique that allows rapid and accurate measurement of pore size and distribution [17,18,24]. A non-reacting gas (usually air) flows through a dry sample and then through the same sample after wetting with a liquid of known surface tension. The change in flow rate is measured as a function of the applied pressure for the two processes. Because of the low pressure applied during the process, the porous nanofibrous structure is not distorted [25]. This technique cannot measure pore volume but measures pore throat diameters, the most constricted diameter of a given pore, which are not measurable by mercury intrusion or liquid extrusion porosimetry.

On the other hand, in liquid extrusion porosimetry (LEP) the pores are spontaneously filled with a wetting liquid, and this liquid is extruded from pores by a non-reacting gas. LEP measures the volume, size and distribution of through-pores, surface area, porosity, and liquid permeability, avoiding the use of toxic liquids and high pressure. Thus, LEP is suitable for testing nanofiber scaffolds, which have pore diameters in the range of 1000 μm to 0.05 μm. This technique has been employed in the characterization of complex pore structures of filtration media, particularly in processes involving biotechnology [17]. However, the literature reports of LEP application to the analysis of structural characteristics of biomedical scaffolds are very scarce [26].

Gas adsorption can measure pore size and porosity, however the study is time consuming, and microcomputed tomography has demonstrated various advantages, but depends on the computational capability of the software and hardware [19], is very expensive and/or inaccessible for many countries. Finally, confocal laser scanning microscopy along with 3D image analysis was recently reported as a novel nondestructive approach to investigate 3D pore structure [20], although it has some drawbacks that include making the fiber fluorescent and the difficulty of thresholding.

SEM is a destructive assay that requires physical sectioning, providing qualitative data of superficial regions. The use of image analysis-based methods was also developed for quantitative studies of the pore structure parameters.

In this work, poly(ε-caprolactone) micro/nanofibrous scaffolds with mean fiber diameters in the range of 0.4 to 7 μm were prepared and studied. Electrospun scaffolds were characterized in terms of fiber diameter (as measured by SEM), pore size and its distribution (as measured by liquid extrusion porosimetry), and porosity (as determined by gravimetric measurement, liquid intrusion method, SEM image analysis and liquid extrusion porosimetry). Porosity results were comparatively analyzed, taking into account the accuracy of each method. In order to compare empirical data of pore size obtained from LEP, a theoretical multiplanar model for stochastic fiber networks developed by Eichhorn and Sampson [13,14] was applied.

2. Experimental section

2.1. Materials

Poly(ε-caprolactone) (PCL) with a number-average molecular weight of 80 kg mol^{-1}, chloroform, methanol and dichloromethane were obtained from Aldrich Chemical Co. (St. Louis, Mo, USA) and used without further purification. Liquid medicinal vaseline (Drosanto®, 33.0-35.6 cSt. at 40 °C) was purchased from Drogueria San Antonio (Buenos Aires, Argentina).

2.2. Electrospun scaffold preparation

PCL micro/nanofibrous scaffolds with different fiber diameters were prepared by electrospinning technique. A series of PCL solutions (17–25% wt) were obtained by dissolving PCL pellets in dichloromethane: methanol and chloroform:methanol solvent mixtures with different mixing ratios under magnetic stirring (Table 1). Each of the as-prepared solutions was loaded into a standard 10 mL plastic syringe connected to a polyamide tube, attaching to the open end a blunt 18-gauge stainless steel needle (Aldrich Chemical Co.) as a nozzle. The flow rate was controlled by using a programmable syringe pump (Activa A22 ADOX S.A., Argentina). A high-voltage power source (ES30P, Gamma High Voltage Research Inc., Ormond Beach, FL) was used to charge the solution by attaching the emitting electrode of positive polarity to the nozzle, and the grounding one to the aluminum collector plate. All experiments were carried out at room temperature and relative humidity of 50% in a chamber having a ventilation system.

Electrospun scaffolds were obtained after setting the conditions summarized in Table 1. All samples were collected during a time required to achieve 10 μm thickness. The electrospun scaffolds were dried overnight under vacuum at room temperature to fully eliminate the residual solvent, and finally stored in a desiccator until testing. For characterization studies, circular pieces (10 mm diameter) were punched from the scaffold.

2.3. Methods

2.3.1. Characterization of scaffold microstructure

The electrospun membranes were observed by scanning electron microscopy (SEM) in a JEOL JSM6460 LV instrument. Samples were mounted on the aluminum stub using copper double-sided adhesive tape, sputter coated with gold in a chamber evacuated to 500 mTorr, and examined with an accelerating voltage of 15 kV. The micrographs were processed and analyzed using image processing software (Image Pro Plus) to measure the fiber diameter. In order to obtain a meaningful statistical value, 100 randomly selected fibers per sample were measured on SEM micrographs.

Contact angle measurements were carried out on the center of the scaffold to characterize surface hydrophilicity using a goniometer (model 250, ramé-hart instrument Co., Succasunna, NJ, USA). Drops of 5 μl of liquid medicinal vaseline or distilled water were put onto the scaffold using a micro-syringe pointed vertically downward onto the sample surfaces. Images of the drops (640 × 480 pixels, 256 gray levels) were recorded with a CCD-camera, after adjusting contrast, magnification and focus and after an initial waiting period of 10 s. Six images

Table 1
Experimental conditions used for electrospinning PCL solutions.

Sample	PCL concentration (% wt)	Solvent mixture (% v/v)	Voltage (kV)	Flow rate ($mL.h^{-1}$)	Needle tip-to collector distance (cm)
M1	17	5:1[a]	15	10	18
M2	17	1:1[b]	17	0.8	12
M3	17	1:1[b]	17	0.8	11
M4	20	5:1[a]	11.5	18	15
M5	25	5:1[a]	7.5	8	9

[a] Chloroform:methanol mixture.
[b] Dichloromethane:methanol mixture.

were taken each 20 s. The experiments were performed at room temperature (23 °C). Contact angles were measured with Dropimage Advanced v2.4 software.

2.3.2. Determination of scaffold porosity

2.3.2.1. Gravimetric measurement (GM). Measurements obtained by gravimetric determination are a useful alternative when sophisticated equipment is unavailable. Samples must be carefully prepared and a precise determination of sample dimensions is required to achieve good accuracy. Scaffold porosity was determined according to the following well-known equation:

$$P_{GM} = 1 - (\rho_s/\rho_{bulk}) \quad (1)$$

where ρ_s is the scaffold density, calculated as the ratio between its mass and volume (Table 2). Using the sample thickness, as measured with micro-calipers, and the sample diameter, the scaffold volume was determined. Bulk density of PCL is known ($\rho_{PCL} = 1.145\ g.cm^{-3}$).

2.3.2.2. Liquid intrusion method (LIM). Scaffold porosity was also measured by using the liquid intrusion method. Electrospun scaffolds were weighed and subsequently immersed in vaseline overnight at room temperature in a beaker containing a mechanical shaker to allow the liquid to penetrate into the scaffold voids. The surface of the samples was then blotted dried and weighed again to determine the mass of vaseline present within the scaffold. Measurements were made on five samples of each scaffold type. Porosity was then calculated as follows:

$$P_{LIM} = V_V/(V_V + V_{PCL}) \quad (2)$$

where V_V is the volume of intruded vaseline and corresponds to the total pore volume. It is calculated as the ratio between the observed mass change after intrusion and density of vaseline ($\rho_V = 0.9\ g.cm^{-3}$). V_{PCL} is the volume of PCL, calculated as the ratio between the dry scaffold mass before liquid intrusion and ρ_{PCL}.

2.3.2.3. SEM image analysis. According to the analysis previously reported by the authors [27], the free area (A_V) was calculated as the difference between the total area occupied by fibers (A_F) and the total area of the sample (A_S). Then, porosity was calculated as follows:

$$P_{SEM} = (A_V/A_S) \times 100. \quad (3)$$

Fiber diameters required for area determination were obtained as previously mentioned in Section 2.3.1.

Table 2
Characteristics of electrospun PCL scaffolds.

Sample	$h \pm$ s.d (μm)[a]	$w \pm$ s.d (μm)[b]	$d_{LEP} \pm$ s.d (μm)[c]	$d_S \pm$ s.d (μm)[d]	$\overline{\beta}$ ($g\ m^{-2}$)[e]
M1	9.6 ± 0.4	0.39 ± 0.25	1.06 ± 0.05	0.12 ± 0.01	19.10
M2	9.3 ± 0.5	1.08 ± 0.57	1.42 ± 0.05	0.73 ± 0.02	16.55
M3	10.3 ± 0.7	1.99 ± 0.78	1.68 ± 0.04	1.60 ± 0.03	24.19
M4	10.7 ± 0.1	5.15 ± 0.85	8.09 ± 0.07	6.67 ± 0.22	13.85
M5	9.7 ± 0.8	7.03 ± 0.93	12.01 ± 0.04	10.04 ± 0.05	16.02

[a] Mean thickness measured with micro-calipers.
[b] Mean fiber diameter determined by SEM image analysis.
[c] Mean pore diameter determined by LEP.
[d] Mean pore diameter predicted by Sampson's model.
[e] Mean areal density calculated as sample mass to area ratio.

2.3.2.4. Liquid extrusion porosimetry (LEP). LEP provides the most complete information about pore structure inside a scaffold, and is the appropriate technique for testing polymeric nanofiber mats. In LEP, pores of the sample are spontaneously filled with a wetting liquid which is extruded from pores by a non-reacting gas. A scheme of the sample chamber and placement is shown in Scheme 1. The differential pressure required to displace the wetting liquid is related to pore diameter by the Washburn equation (Eq. (4)), which states that higher pressure is required to remove liquid from smaller pores:

$$p = 4\gamma \cos \theta/D \quad (4)$$

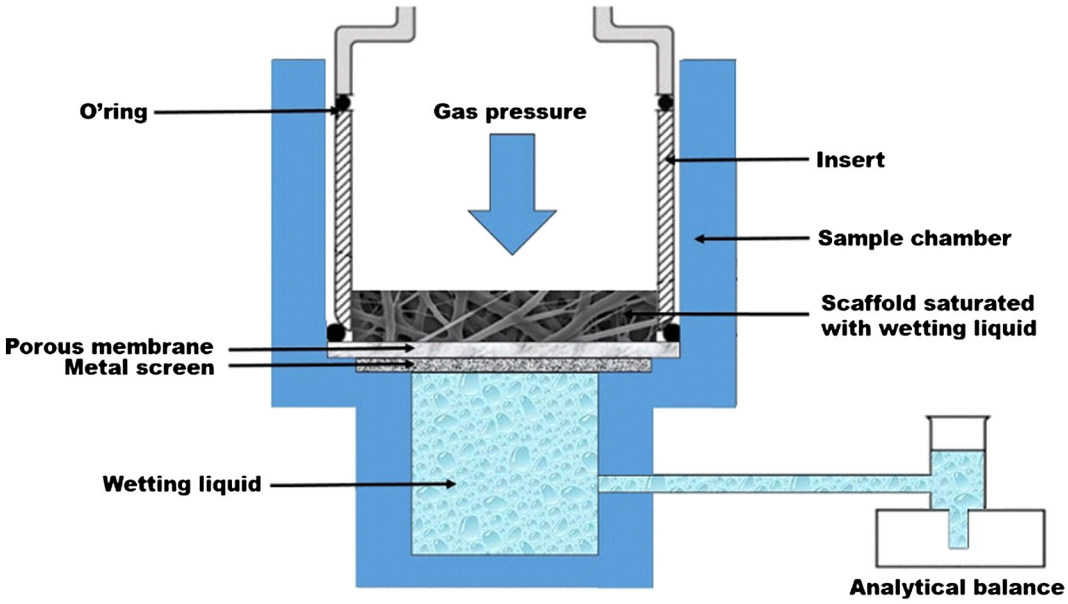
where p is the differential pressure across the length of the pore, D is the pore diameter, γ is the surface tension of the wetting liquid; and θ is the contact angle of the wetting liquid with the sample.

The sample is placed on a membrane such that its largest pore is smaller than the smallest pore of interest in the sample. This is to prevent the gas to escape, because the applied pressure is not enough to displace the liquid from this membrane. The volume of the liquid flowing out of the membrane is collected and weighed in an analytical balance. This volume corresponds to the flow-through pore volume. Differential pressure yields through-pore diameter and variation of volume with pressure yields through-pore surface area [17,25]. In this technique, blind and closed pores are not measured. However, the amount of blind or closed pores in electrospun scaffolds is negligible.

In this work, a liquid extrusion porosimeter (Model 1100-A-X, Porous Materials, Inc., Ithaca, NY, USA) was used to measure the pore size and its distribution. Structural features were calculated by the software provided from Porous Materials Inc. (Ithaca, NY, USA).

Values of liquid density and surface tension, liquid/sample contact angle, sample density and thickness, were introduced as required for instrument calculations. Since water is not a wetting agent for PCL scaffolds, liquid vaseline with a contact angle close to zero and surface tension of 33 dyn cm^{-1} was used as the wetting agent. Liquid vaseline does not swell, shrink nor dissolve the PCL scaffold, and possesses a low vapor pressure which is important to avoid liquid evaporation during testing. Each electrospun sample of specific mass and density was taken and placed on a poly(tetrafluorethylene) membrane type 11806-047N (Sartorius Stedim Biotech GmbH, Goettingen, Germany). This is a hydrophobic membrane with a nominal pore size of 0.45 μm and 47 mm diameter. Thus, pores higher than 0.45 μm were measured.

The test program recorded the initial differential gas pressure and the initial reading of the weighing balance, and increased the gas pressure in small steps, recording the differential gas pressure and readings of the balance. An initial pressure of 0.01 psi was set by the instrument to equilibrate the system, recording the first point at 0.05 psi. The



Scheme 1. Sample chamber and placement used in this study for liquid extrusion porosimetry (LEP).

differential pressure was increased in steps of 0.01 psi up to 22 psi. At the end of the test the report program automatically computed the results. The through-pore volume distribution is given by the distribution function (f_V):

$$f_V = -(dV/d\ \log\ D) \tag{5}$$

where V is the cumulative pore volume and D is the pore diameter. The function is such that the area under the distribution function in any pore diameter range yields the volume of pores in that range. So, scaffold porosity obtained by LEP (P_{LEP}) is determined by integrating the cumulative pore volume distribution in all the pore diameter range.

2.3.3. Statistical analysis

All the fiber diameter and porosity values are expressed as mean ± standard deviation. Statistical differences between the fiber diameters of the scaffolds in the surfaces contacting air and aluminum collector were determined by performing a Student's *t*-test (confidence interval of 99%). For each sample, a factor analysis of variance was performed to determine whether a significant difference existed between the porosity measured using the different techniques with respect to LEP. A Tukey range test at a significance level of 95%, was carried out by using a software package for statistical analysis (SPSS Inc., version 11.5). The mean porosity values were analyzed using Q-test at a significance level of 90%.

3. Results and discussion

3.1. Scaffold preparation and characterization by SEM image analysis

In electrospinning process, the diameter and morphology of the produced micro/nanofibers are determined by a number of factors, including solution parameters (solution dielectric constant, conductivity, polymer type, concentration and surface tension), processing parameters (flow rate of the solution, applied voltage and needle tip-to-collector distance), and environmental parameters (temperature, humidity) [10,28]. The spinning conditions used in this work were selected after a series of screening experiments that led to the production of uniform, bead-free, continuous fibers [27]. Different polymer concentrations, mixed-solvent systems, mixing ratios and electrospinning parameters were explored in a wide range to ensure a stable Taylor cone formation as well as the generation of scaffolds with different fiber diameters. Thus, five poly(ε-caprolactone) micro/nanofibrous scaffolds were prepared by using the parameters showed in Table 1, and selected for characterization studies.

SEM analysis was performed to study the morphology of electrospun scaffolds. Sample surfaces in contact with air and aluminum collector were observed. No significant differences were found in these surfaces. The surface of PCL samples displayed a typical electrospun structure of randomly oriented fibers, exhibiting a highly anisotropic fiber distribution, interconnected open macropores, and pore distribution throughout the structure, which evidenced samples with high porosity (Fig. 1).

Fig. 2 shows the fiber diameter frequency distribution, whereas the mean fiber diameters (w) were found in the range of 0.4 to 7 μm (Table 2). The fiber diameter exhibited unimodal distributions for all scaffolds.

3.2. Pore size distribution obtained by LEP

Pore size measurements were successfully performed by LEP technique for the scaffolds. Fig. 3 shows a representative curve obtained by LEP, in this case from M1 sample. Cumulative pore volume corresponding to flow-through pores was recorded as a function of the applied differential pressure. A maximum pressure of 22 psi was applied to completely extrude the liquid from M1, which is the sample with the lowest mean fiber diameter (0.39 μm). The low pressure applied during all the testing procedure assures that samples were not compressed nor distorted by the measurement technique.

Fig. 4 shows the distribution function of pore volume computed from data shown in Fig. 3, whereas the relationship between pore diameter (estimated from empirical data displayed in Fig. 4) and fiber diameter is shown in Fig. 5. Mean pore diameters obtained by LEP are tabulated in Table 2. Single membranes of similar thickness were measured. The pore size distribution did not depend on the mean fiber diameter, resulting in a unimodal distribution in all cases. LEP produced excellent resolution of peaks and allowed the determination of the complete pore diameter range. The effect of fiber diameter on the pore size and distribution could be discerned in the studied samples. The results indicate that the increase in fiber diameter led to an increase in pore size. This trend was previously demonstrated by Sampson [13].

Samples prepared by stacking multiple layers of electrospun membranes in the test chamber were also tested. This sample configuration

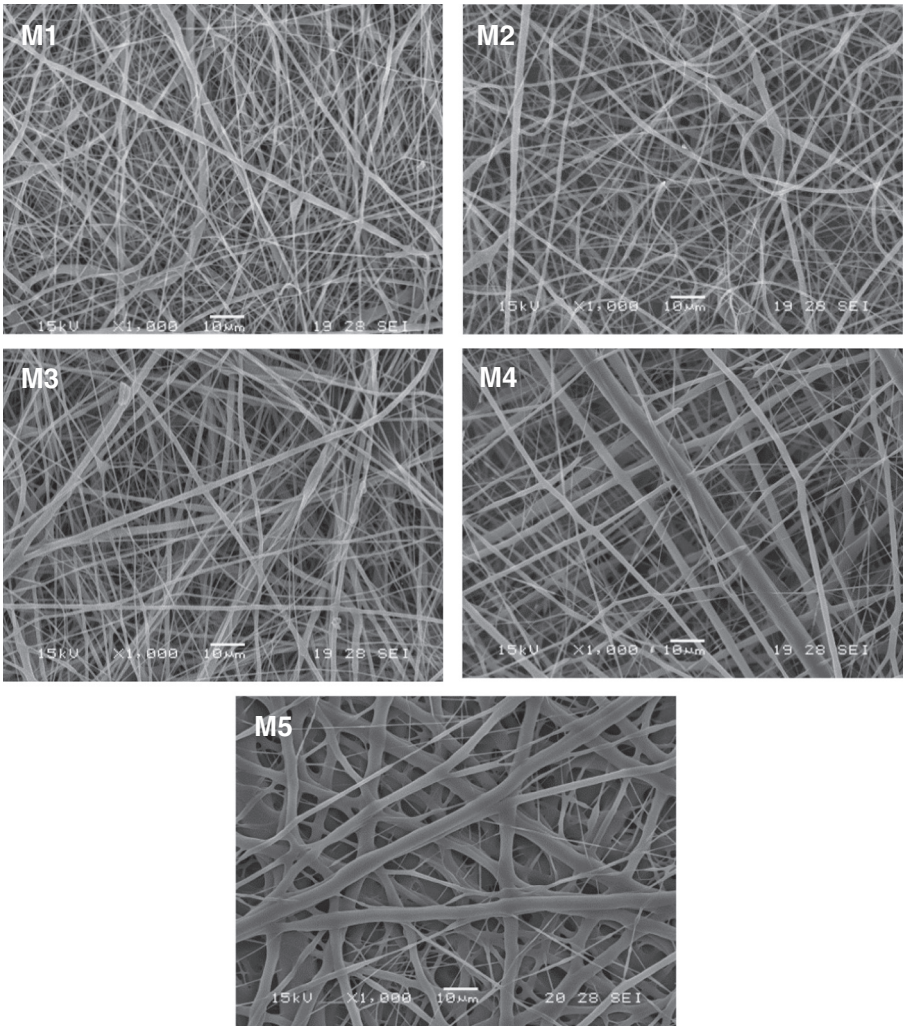


Fig. 1. SEM micrographs of electrospun PCL scaffolds (Magnification 1000×).

led to a decrease in pore diameter when measured by LEP (data not shown). The results are in agreement with the ones reported by Li et al. [16] which demonstrated that pore size and distribution depend on the physical size and amount of fibers. The increase in membrane mass caused a decrease in pore size, shifting the distribution towards lower values.

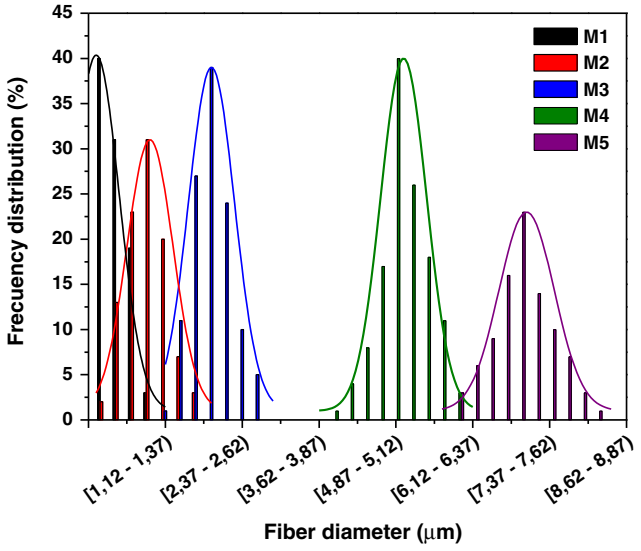


Fig. 2. Fiber diameter distribution for the studied electrospun PCL scaffolds.

3.3. Porosity measurements

Scaffold porosity is defined as the void volume relative to the total volume of the scaffold. Typical porosities for electrospun scaffolds are found in the range of 70-95%. Porosities were measured using four different techniques: gravimetric and liquid intrusion measurements, SEM image analysis, and liquid extrusion porosimetry. Fig. 6 shows the obtained results.

Gravimetric determinations allowed the estimation of an apparent porosity P_{GM} through the theoretical relationship between porosity and density given by Eq. (1). In this technique the overestimation of the sample volume is inevitable, due to the fact that it is calculated from the outward form of the mesh, neglecting the concavo–convexity of the fibrous surface. The overestimation of the volume leads to the underestimation of the P_{GM} values. In fact, P_{GM} were the lowest values for all the tested samples. However, it appears to be dependent on sample composition and measurement technique. Kwon et al. reported porosities of electrospun copolyester with mean fiber diameter close to that reported in this work (0.3, 1.2 and 7.0 μm). They showed that the porosities measured by mercury intrusion porosimetry were lower than that calculated by gravimetric method, P_{GM} [29].

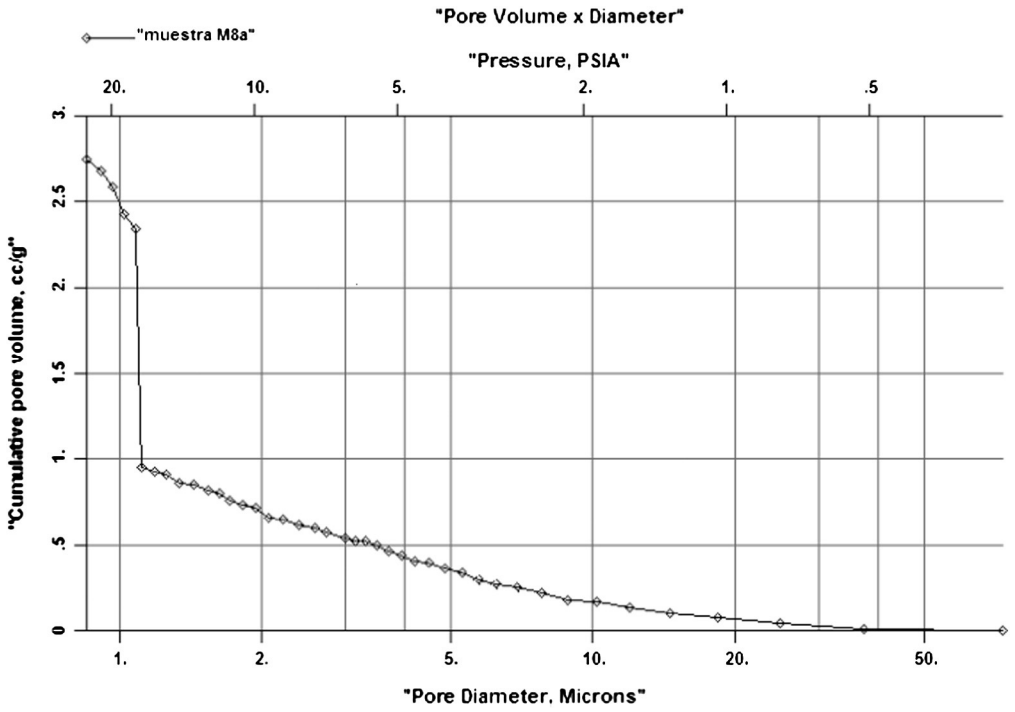


Fig. 3. Typical curve obtained from LEP displaying pore volume as a function of pore diameter. The pore size is calculated by the instrument from the largest to the smallest pores as the differential gas pressure is increased.

The liquid intrusion method determines the porosity via the total pore volume, which is measured indirectly by infiltration of a liquid of known density. Liquid vaseline was used as intrusion liquid due to the fact that it spontaneously filled the porous structure of PCL (contact angle near to zero) without swelling effects. The sample manipulation introduces some difficulties associated to the procedure for complete extraction of superficial liquid. Therefore, LIM technique could have underestimated porosity values due to vaseline removal during the sample surface cleaning.

SEM image analysis complemented these calculations allowing the estimation of porosity values from two-dimensional micrographs. The P_{SEM} values were slightly higher to the P_{LEP} ones, and did not show significant variation with those values. Although this method can analyze only superficial pores without considering scaffolds with high fiber density, surprisingly it provided porosity values close to those obtained by LEP.

There was no significant difference in variability among the porosities at each mean fiber diameter measured by the four techniques used in this work ($p < 0.05$). No significant differences in the mean porosity values were also found ($Q_{0.90} < 0.76$).

3.4. Modeling pore size from fiber mean diameter and scaffold porosity

Pore sizes of electrospun samples can be estimated by using different theoretical and empirical mathematical models reported in the literature. In this work, a theoretical model developed by Sampson [13] and later extended by Eichhorn and Sampson [14] was applied to calculate mean pore diameters. To the best of our knowledge, this work is the

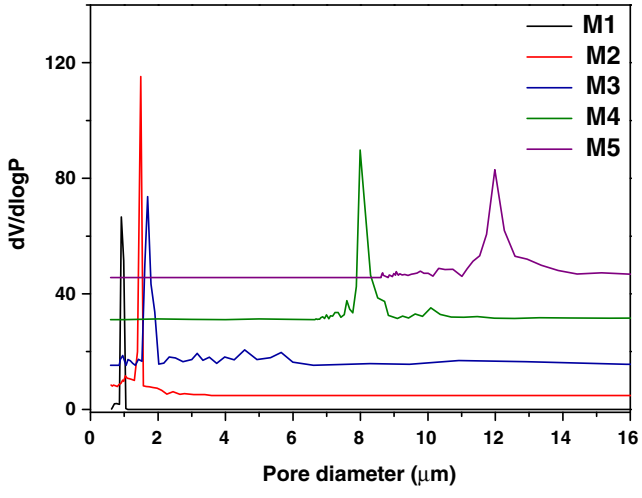


Fig. 4. Distribution function of flow-through pore volume (f_V) as a function of pore diameter for PCL scaffolds, as measured by LEP.

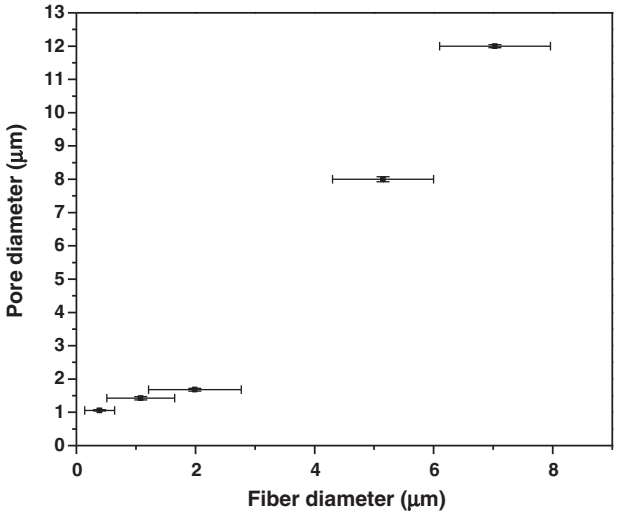


Fig. 5. Pore diameter d_{LEP} (as measured by LEP) as a function of fiber diameter (as determined by SEM image analysis)

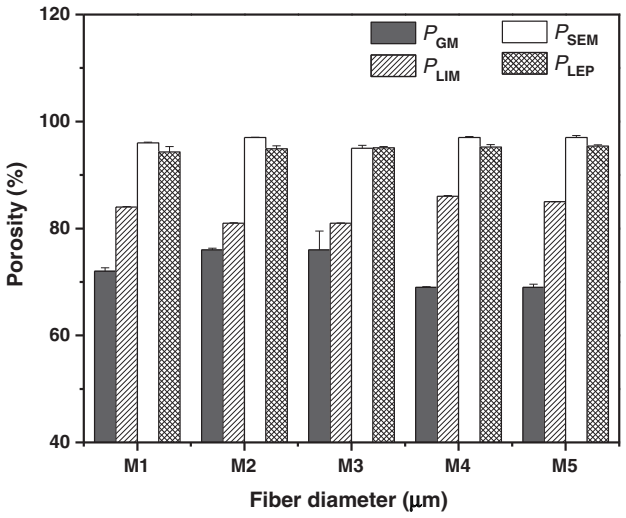


Fig. 6. Porosity of electrospun PCL scaffolds as determined by different techniques plotted for samples with different mean diameters. Data represent means with the error bars showing the standard deviation.

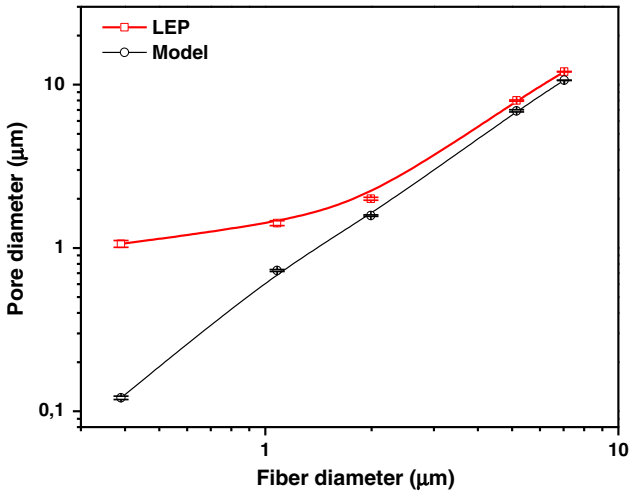


Fig. 7. Relationship between pore diameter and fiber diameter. LEP measurements (red square symbols) and Sampson's model (black circle symbols).

first in reporting a comparison between LEP data and a theoretical model. Most of the studies reported the relationships between mercury intrusion porosimetry/LEP or mathematical models. In Sampson's model, an average in-plane pore diameter for quasi-planar stochastic fiber networks is calculated. Thus, the scaffold is considered as stacked two-dimensional slices composed of random straight rods that represent nanofibers. Fibers of uniform circular cross-section are considered. Mean pore diameter (or radius) was calculated on the basis of the mean fiber diameter (w) and the mean porosity (P).

The distribution of pore radii may be approximated by the gamma distribution (Γ). For a two-dimensional random network with porosities greater than approximately 0.3, the mean pore radius can be approximated by a function of porosity and fiber diameter as:

$$\bar{r}_{2D} = \frac{\sqrt{\pi}}{4}\left(\frac{\pi}{2\log(1/P)} - 1\right)w. \tag{6}$$

The gamma distribution of pore radii has two parameters (k and b) that characterize the distribution, mean $r_{2D} = k/b$, variance $\sigma^2(r) = k/b^2$, and coefficient of variation $CV(r_{2D}) = k^{(1/2)}$. This coefficient was taken as constant, and its value for random networks is $(16 - \pi^2)^{1/2}/\pi$. Sampson [13] derived the probability density function for pore radii in a isotropic near-planar stochastic network formed by the superposition of n two dimensional layers as

$$f(r) = \frac{\pi}{P}\left(\frac{\Gamma(k, br)}{\Gamma(k)}\right)^{\left(n/p\right)-1}\frac{b^k}{\Gamma(k)}r^{k-1}e^{-br} \tag{7}$$

where the number of layers, n, is given by

$$n = \frac{\bar{\beta}w}{\delta\log\left(1/p\right)} \tag{8}$$

where β is the mean areal density and δ is the linear density of fibers (fiber cross-section multiplied by density of fibers).

The mean pore radius for a structure consisting of n layers is determined by the integral

$$r\int_0^\infty rf(r)dr. \tag{9}$$

For each sample, the mean pore radius (and diameter d_S) was computed using Eq. (9), taking the porosity value obtained by LEP and the mean fiber diameter (w) obtained from SEM analysis, assuming a monodisperse fiber diameter. An average porosity value ($P \sim 0.87$) obtained by averaging the values showed in Fig. 6 was also used. Calculations were performed using Mathcad version 14.0 software.

Table 2 shows the mean areal density ($\bar{\beta}$) of the scaffolds. There are few studies that report this property of a nanofibrous scaffold [14]. Values of $\bar{\beta}$ were found in the range of 16 to 24 g m^{-2}. Fig. 7 illustrated the relationship between mean pore diameter predicted by Sampson's model and empirical data from LEP, as a function of fiber diameter, when a porosity value $P = 0.87$ was used. Pore diameters are also tabulated in Table 2. The pore sizes predicted by the model were in good agreement with the experimental data provided by LEP for mean diameters higher than 1 μm. The highest deviation was found in the sample with the lowest fiber diameter (M1). For this sample, a mean pore size of 1.06 μm was obtained by LEP, the predicted by the model being 0.12 μm.

Pham et al. [23] also found that pore size predicted by Sampson's model was smaller than those measured experimentally using mercury intrusion porosimetry. On the contrary, Szentivanyi et al. compared pore sizes obtained from mercury intrusion porosimetry measurements and Sampson's model, observing that the multiplanar model overestimates pore sizes in all the fiber diameter range [30]. Recently, Bagherzadeh et al. [31] developed a theoretical model for characterizing the porous structure of electrospun nanofibrous network by combining the stochastic and stereological probability approaches. Fiber diameter, porosity and thickness of assembly strongly influenced morphological and structural parameters of the network. They found that values predicted by Sampson's model fall far short of both the experimental ones and the predicted by applying their theoretical model.

During the electrospinning process, the repeated fiber deposition over time increases the thickness and areal density of the scaffold. Consequently, a decrease in the mean pore diameter of the scaffold is produced. Thus, even for the same polymer and fiber diameter, it is not possible to establish a direct comparison of pore sizes reported in the literature for a particular scaffold without considering the areal density. As example, the sample with the highest fiber diameter (7.03 μm) exhibited a pore diameter of 12 μm as measured by LEP, and 10 μμm as predicted by the model. However, a pore size of approximately the same value (10.5 μm) has also been reported for scaffolds composed of fibers with 2 μm mean diameter [23].

Although controversy exists regarding the optimum pore size for efficient cell infiltration and tissue regeneration, there is a consensus that pore sizes of few tens to a few hundred micrometers are required for three-dimensional cell diffusion and ingrowth [32,33]. Thus, small pores formed by electrospun scaffolds are a disadvantage for tissue engineering applications where large pore size is needed to allow efficient cell ingrowth. In this context, recent emerging approaches for enlarging pore size of electrospun scaffolds are under progress [33]. These new advanced nanofibrous architectures as well as most of the electrospun scaffolds cannot bear high pressure. Then, based on the results obtained in this work, we envisioned that the structural characterization of such structures could be accurately performed by LEP. Measurements of pore characteristics in scaffolds with increased pore size are under progress and will soon be published elsewhere.

4. Conclusions

Electrospun scaffolds of poly(ε-caprolactone) were successfully obtained by electrospinning technique. Scaffolds consisted of randomly oriented fibers with mean fiber diameters in the range of 0.4 to 7 μm. The structural characteristics of scaffolds were assessed. All samples exhibited a unimodal pore size distribution. Porosity was estimated by different experimental techniques, and did not present significant differences associated to each measurement procedure. LEP demonstrated a high potential for studying the porosity characteristics of electrospun scaffolds as compared to other non-instrumental analysis techniques. Although fine-tuning of LEP is time consuming and depends on each sample and extrusion liquid, it is a suitable technique for characterizing the structural features of micro- and nanofibrous scaffolds. Moreover, the application of low pressure avoids the distortion of pore structure and size, and then LEP could be very useful for studying scaffolds with enlarged pores.

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