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Featured Letter

Electrospun scaffolds with enlarged pore size: Porosimetry analysis

Pablo R. Cortez Tornello, Pablo C. Caracciolo, Juan I. Igartúa Roselló, Gustavo A. Abraham *

Research Institute for Materials Science and Technology, INTEMA (UNMdP-CONICET), Mar del Plata, Argentina

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ABSTRACT

Electrospun polymeric/composite scaffolds exhibit a micro/nanofibrous structure that resembles the architecture of native extracellular matrix. The unique properties of the interconnected pores and high porosity of electrospun matrices make them very attractive for applications in tissue engineering scaffolding. However, these structures are formed by densely compacted fibers that exhibit pore sizes lower than cell size, leading to poor cell infiltration. In this work, bead-free electrospun poly(ε-caprolactone) fibrous scaffolds were prepared by combining electrospinning and salt-leaching techniques. Non-woven scaffolds were obtained using porogens with different sizes. Liquid extrusion porosimetry was used to accurately determine pore size and its distribution. The structural features of prepared matrices were dependent on the initial porogen size but resulted much smaller than this one.

1. Introduction

Electrospun structures provide an excellent platform for the development of many different bionanotechnological applications [1]. The unique architectures and features of electrospun scaffolds are very attractive in the tissue engineering field, offering a three-dimensional microenvironment that mimics the native extracellular matrix, and facilitates cell functions and nutrient and waste transport throughout scaffolds [2]. Typical electrospun nanofibrous scaffolds have small pore sizes (<1 μm) and they are helpful for skin wound dressings and endothelium regeneration. However, this pore size hinders *in vitro* cell infiltration and *in vivo* tissue-ingrowth into the three-dimensional cellular construct, limiting its clinical performance in other tissues.

It is known that pore size and pore size distribution depend on fiber diameter. The higher the fiber diameter, the larger the pore size. Several mathematical models and their correlation with experimental data have been proposed [3,4]. However, it is possible to manipulate the pore size of nanofibrous structures by using different strategies. Some approaches, such as porogen leaching, sacrificial fibers, gas forming, bath collector, cryogenic collector, patterned collector, laser irradiation, and others, were explored in the last years [5–7].

Particulate leaching is a well-known process to form porous spaces and has even been employed together with electrospinning [8,9]. Then, the architecture of nanofibrous scaffolds can be modified by varying the porogen size and shape as well as porogen/polymer ratio. Moreover, investigations of different particle sizes to elucidate the relationship between electrospinning time, particle deposition and pore size are still lacking.

Although there are many techniques available for characterizing porous scaffolds, little information about pore size and its distribution of electrospun scaffolds is often given [4]. Liquid extrusion porosimetry (LEP) is used to measure the volume, size and distribution of through-pores, surface area, porosity, and liquid permeability of porous scaffolds, avoiding the use of toxic liquids and high pressure. LEP is suitable for testing porous materials with pore diameters in the range of 0.05 μm to 1000 μm and allows the characterization of complex pore structures [10]. However, the analyses by LEP of structural characteristics of biomedical scaffolds are scarce.

In this work, a series of bioresorbable polymeric electrospun scaffolds with enlarged pore size was characterized by LEP. The effect of porogen size on the resulting porous structure was analyzed.

2. Materials and methods

2.1. Electrospinning

Poly(ε-caprolactone) (PCL) solution (20% wt/v) was obtained by dissolving PCL pellets (Aldrich, 80000 g/mol) in chloroform/methanol (5:1 v/v) under magnetic stirring. The solution was electrospun through a blunt 18-G stainless steel needle onto an aluminum collector 15 cm away. A flow rate of 18 ml/h and applied voltage of

* Corresponding author at: INTEMA (UNMdP-CONICET), Av. J.B. Justo 4302, B7608FDQ Mar del Plata, Argentina.
E-mail address: gabraham@fi.mdp.edu.ar (G.A. Abraham).

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Table 1
Electrospun scaffolds studied in this work.

Sample	Salt size (μm)	Mean fiber diameter (μm) (±s.d.)	P_{LEP} (%) (±s.d.)	Pore diameter by LEP (μm) (±s.d.)
S0	–	5.90 ± 1.24	96.35 ± 0.30	8.09 ± 0.07
S53	53–105	6.09 ± 1.13	94.76 ± 0.26	9.03 ± 0.04
S149	149–250	4.84 ± 1.23	94.31 ± 0.18	13.20 ± 0.10
S297	297–350	4.33 ± 1.29	95.16 ± 0.24	15.40 ± 0.08
S350	350–710	3.90 ± 1.21	96.32 ± 0.20	27.65 ± 0.07
S710	710–1000	3.75 ± 1.33	96.35 ± 0.22	41.81 ± 0.10

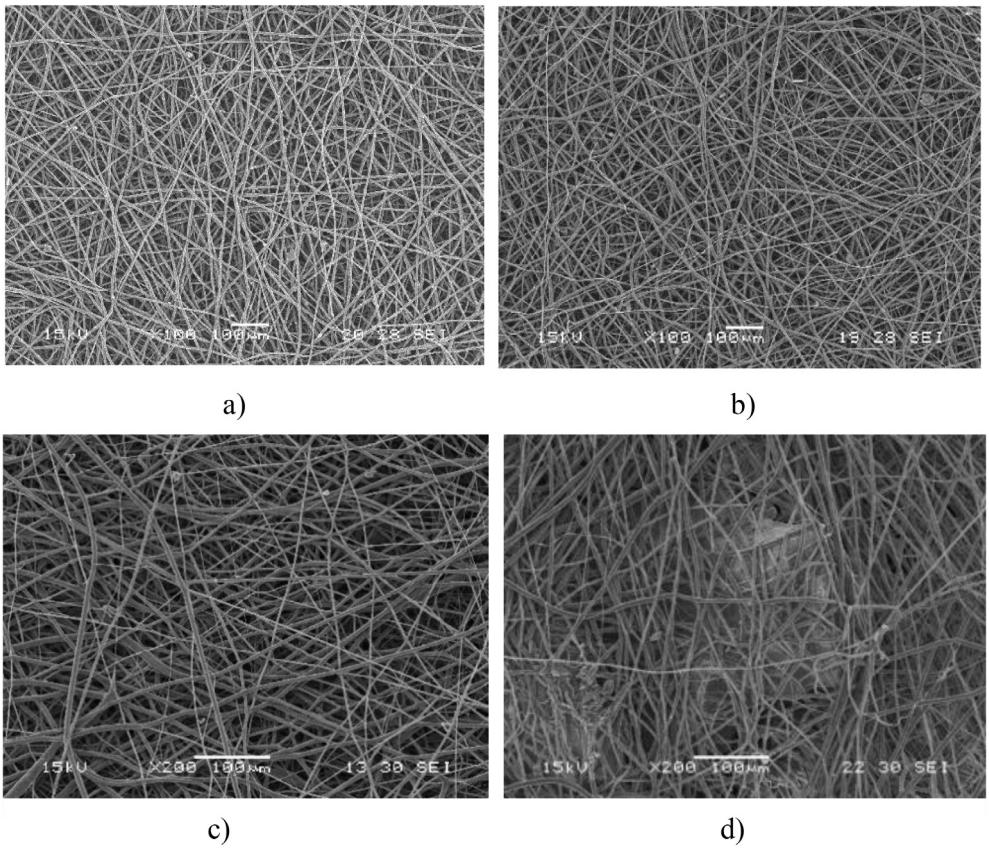

Fig. 1. SEM micrographs of a) S53, b) S297, c) S710 samples after salt leaching, d) S710 sample before leaching showing a crystal salt included in the mat.

11.5 kV were used. Experiments were carried out at room temperature and relative humidity of 50%.

Sodium chloride crystals of predetermined size ranges were used as porogen and homogeneously sprinkled above the collection plate by an automatic vibration-controlled sieving system at a rate of 140 mg/min. Scaffolds without salt addition were also prepared. Samples were dried under vacuum to fully eliminate the residual solvent and placed in deionized water for salt leaching. Water was daily replaced for 10 days for allowing an efficient leaching process. Finally, samples were freeze-dried and stored in a desiccator until testing. Table 1 describes the obtained scaffolds.

2.2. Liquid extrusion porosimetry (LEP)

Discs (10 mm diameter) were punched from each scaffold, weights and thicknesses measured. Liquid extrusion porosimeter (Model 1100-A-X, Porous Materials Inc., USA) was used. Liquid vaseline was used as the wetting agent. An initial pressure of 0.01 psi was set to equilibrate the system, recording the first point at 0.05 psi. The differential pressure was increased in steps of 0.01 psi up to 22 psi. The through-pore volume distribution is given by the distribution function:

$$f_V = -(dV/d\log D) \quad (1)$$

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

2.3. Morphology characterization

Scanning electron microscopy (SEM) was conducted in a JEOL JSM6460 LV microscope after gold sputter-coating. Micrographs were analyzed using Image ProPlus and fiber diameter of 100 selected fibers was measured per sample.

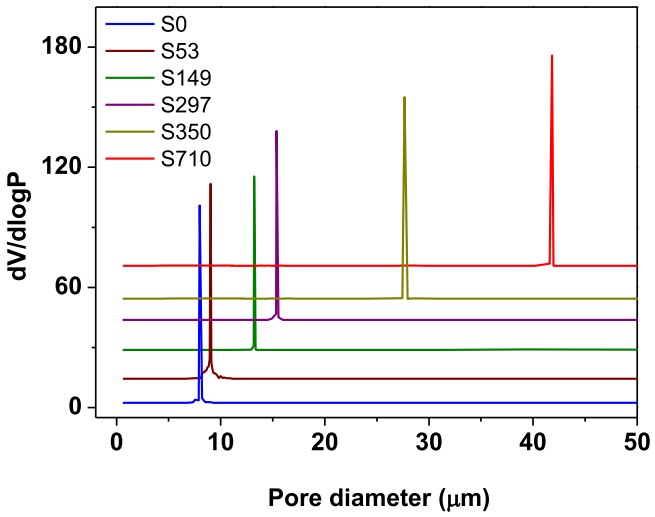

Fig. 2. Distribution function of flow-through pore volume (f_V) as a function of pore diameter for PCL scaffolds, as measured by LEP.

3. Results

Electrospinning parameters used in this work were previously reported [4]. Bead-free electrospun PCL fibrous scaffolds were prepared by combining electrospinning and salt-leaching techniques (Fig. 1). Thickness values were in the range of 550 to 1100 μm. Table 1 shows the mean fiber diameter as measured by SEM analysis, porosity (P_{LEP}) and pore size determined by LEP.

4. Discussion

Typical electrospun nanofibrous scaffolds exhibit pore sizes that inhibit infiltration of most types of cells (5–150 μm). Eichhorn et al. theoretically demonstrated that mats obtained with 50 nm fiber diameter lead to mean pore radius lower than 4 nm for a porosity of 80% [3]. Considering that electrospinning is one of the most efficient strategies to produce tissue-engineered constructs, and that cell infiltration is essential for three-dimensional tissue formation, poor cell infiltration depth is a major concern. Thus, with this concept in mind, Eichhorn et al. stated that control of porosity and mean pore size should be decoupled.

Modified electrospinning techniques by integrating other techniques or designing new setups are among the current strategies to fabricate electrospun scaffolds with enhanced cell infiltration [11].

Yang et al. early reported PLLA scaffolds with mean fiber diameter around 272 nm and pore sizes ranging from several microns to 140 μm without using porogens [12]. However, the wide range of pore sizes appeared to be consistent with samples with very short electrospinning deposition time (few layers and low areal density). Wright et al. reported that the addition of NaCl particles increased the mean pore size from 5.5 μm to 48.7 μm for PLLA mats as determined by LEP, increasing the fraction of pores with diameters above 20 μm [13]. Wright et al. used a rotating drum as collector of fibers and salt particles, the fiber diameter was not reported and porogen size was not stringently controlled. Consequently, a wide range of pore sizes was obtained.

In this work, conventional PCL scaffolds with a mean fiber diameter of 5.90 μm were obtained. Randomly arranged microfibers forming a highly porous and dense mesh were found. The periodical salt incorporation (2-min intervals) led to thicker PCL/salt scaffolds with a slight decrease in fiber diameter (Table 1). After the leaching process, scaffolds kept their structural integrity without signs of delamination. Contrarily, partial delamination with gap sizes in the range of 100 to 200 μm was reported by Nam et al., which could be attributed to higher time intervals between the addition of salt particles [8]. A decrease in thickness was clearly observed for samples with higher crystal size, due to partial collapse of the now-empty pores. This effect was observed in hyaluronic acid/collagen scaffolds [9], but a correlation between particle size and final mean pore diameter has not yet been proposed.

Fig. 2 shows pore size distribution for scaffolds with different particle size. Sharp peaks were displayed. A drastic decrease in mean pore size with respect to the original crystal size was observed in all cases, which is consistent with the observed dimensional shrinking. Clustering of salt particles was not evidenced in the distribution curves. Since the basis of this combined technique lays in creating large pores using a predetermined particle size, this analysis demonstrates that polymeric electrospun scaffolds should be carefully evaluated to assure the appropriate size useful for infiltration of each cell type.

5. Conclusions

Different modifications of conventional electrospinning setups are being used to disperse porogens and enlarge pore size. Dimensional shrinking and pore sizes lower than porogen size were observed. Therefore, pore size and distribution must be carefully studied considering polymer nature, porogen size, incorporation method, electrospinning time, and the porosimetry technique. All these aspects should be considered for designing and preparing electrospun scaffolds and their later correlation with *in vitro* and *in vivo* studies.

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