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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