Functional Polymer Scaffolds for Blood Vessel Tissue Engineering

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Summary: Scaffold pore size plays a critical role in the infiltration of the cells into the structure. For engineered blood vessels, co-cultures of endothelial (EC) on the lumen, and smooth muscle cells (SMC) on the external surface of tubular scaffolds are performed. The more adequate pore sizes for EC are, in general, smaller than for SMC. In the present work, poly(ε-caprolactone) (PCL) flat film and hollow fibers are prepared by phase inversion. The influence of polymer and coagulation solution compositions on pore morphology of the films is analysed and the results are applied to obtain, in a one step process, PCL hollow fibers with suitable pore size for both EC and SMC.

Keywords: biodegradable polymers; films and hollow fibers; phase separation; poly(ε-caprolactone); vascular tissue engineering

Introduction

Biodegradable synthetic polymers have been extensively employed in scaffolds for tissue engineering because they show certain advantages over other materials, such as their ability to tailor mechanical properties and degradation kinetics to suit various applications.[1] Among them poly(ε-caprolactone), PCL, is a semicrystalline polymer widely employed in drug release and tissue engineering due to its high permeability to many therapeutic drugs, lack of toxicity, excellent biocompatibility and relatively low price of its monomeric unit (ε-caprolactone).[2–3] Another advantage of PCL is the high elasticity of this material, which is a valuable property for vascular tissue engineering.[4] Typical applications of PCL in tissue engineering are bone and cartilage grafts,[5–6] muscle-tendon (in a dual scaffold with poly(l-lactic acid), PLLA)[7] and vascular grafts.[8–12]

Small-diameter vascular grafts (<6 mm) made of synthetic materials have not been possible yet due to the occurrence of thrombogenesis and intimal hyperplasia.[13] In order to regenerate small-diameter blood vessels, tubular scaffolds are required. There are several techniques employed to create such scaffolds, like i.e. electro-spinning,[8–11] dip coating[14] or wet spinning.[15]

For the engineering of blood vessels, pore size of the vascular scaffold is an important factor as endothelial cells (EC) are attached on the luminal surface of the scaffold and smooth muscle cells (SMC) on the external surface. Although electrospinning provides a fibrous structure that mimics the native extracellular matrix (ECM),[16] the pore size in the scaffold limits the SMC infiltration and colonization of the scaffold.[11] Some alternatives proposed to enlarge the pore size are: salt leaching technique,[17] co-electrospinning with water-soluble polymers (sacrifice polymers)[18] or creation of bi-layered scaffold.[11] The first two alternatives reduce the mechanical properties and dimensional stability under high pressure and flow, while

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Table 1.
Experimental conditions for the preparation of PCL films.

Experiment code	Polymer solution composition		Coagulation bath
	PCL (%w/w)	Solvent	
PCL10/ClF/EtOH	10	Chloroform	Ethanol
PCL15/ClF/EtOH	15	Chloroform	Ethanol
PCL20/ClF/EtOH	20	Chloroform	Ethanol
PCL10/ClF/IPA	10	Chloroform	Isopropanol
PCL15/ClF/IPA	15	Chloroform	Isopropanol
PCL20/ClF/IPA	20	Chloroform	Isopropanol
PCL15/NMP/W	15	N-MethylPyrrolidone	Water
PCL15/NMP/EtOH	15	N-MethylPyrrolidone	Ethanol
PCL15/NMP/IPA	15	N-MethylPyrrolidone	Isopropanol

all above alternatives imply creating the scaffolds in a two step process.

Phase inversion technique provides an easy and tuneable method to make scaffolds with controlled porous structure to achieve optimal cell distribution. In the present study, PCL films were prepared by phase-inversion to analyse the influence of the polymer solution composition (polymer concentration and solvent) and coagulation bath on the scaffold porosity. The results obtained were applied to develop, in one processing step, a hollow fiber by wet-jet phase inversion spinning, aimed at making tubular scaffolds for in vitro co-cultures of EC and SMC for small vascular grafts.

Materials and Methods

Film Preparation

PCL (MW 80 kDa, Sigma Aldrich) solutions were prepared using chloroform (ClF, analytical grade, Merck) or N-methylpyrrolidone (NMP, 99% purity, Across Organics) as solvents at 10, 15 and 20% w/w concentration. The polymer solution was cast at room temperature on a glass plate using a 0.2 mm thickness casting knife and immediately submerged into the coagulation bath composed of either Milli-Q water, ethanol (EtOH, analytical grade, Merck) or 2-propanol (IPA, AR, Biosolve BV). The glass plate with the polymer film was kept in the coagulation bath until the film was spontaneously released from the glass plate. Then, the film was placed into a new coagulation bath to complete solvent exchange during 24 h. In order to ensure complete removal of solvent traces, the films were then immersed in water cleaning baths changed periodically during 48 h. This washing procedure was proved sufficient for complete NMP removal in other works.[19–20] The casting/coagulation procedure was repeated 3 times for each combination of variables polymer concentration/solvent/non-solvent (Table 1).

Hollow Fiber Preparation

A small dimension spinning set-up (Figure 1) was built to process little amount of polymer solution (60 mL syringe).

A dope solution of 15% w/w PCL in NMP was prepared and filtered through a 25 μm metallic mesh filter. The filtered dope solution was left degasifying overnight before spinning. The dope solution was kept at 35 °C in the syringe during the experiment and extruded at a flow rate of 1 mL min^{-1} through a spinneret with outer

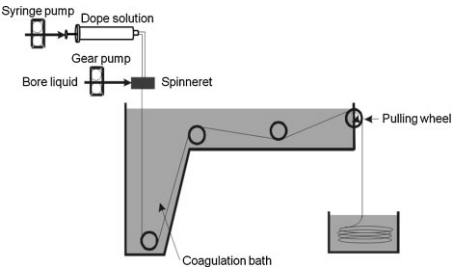

Figure 1.
Small spinning set-up diagram.

and inner needle diameters of 0.5 mm and 0.2 mm, respectively. Milli-Q water was employed as bore liquid at 2.4 mL min^{-1} and IPA was the coagulation bath, both solutions at room temperature (~20 °C). The air gap was fixed at 3 cm and the take up speed was 10 m min^{-1}. The post processing of the fibers after spinning was the same as in the case of the films.

Film and Hollow Fiber Characterization

The morphology of the films and hollow fibers was determined by scanning electron microscopy (SEM, JEOL 5600LV). Surface and cross section samples were prepared. Cross section samples were freeze dried and fractured. All the samples were kept overnight at 30 °C under vacuum and gold sputtered before examination by SEM at a voltage of 5 kV.

In order to measure the water transport through the films, circular sheets (8.0 cm^2 effective surface area) and hollow fiber modules (containing 1 single hollow fiber with lengths between 12 up to 19 cm) were conditioned using 70/30% v/v EtOH solution (sterilization solution) at the maximum pressure tested during 0.5 hour, then Milli-Q water was flashed for another 0.5 hour to clean the films. Water flux through the films or modules was measured as follows: Milli-Q water was pressurized, at different transmembrane pressures, on one side of the film or on the lumen side of the module in a dead-end configuration system and was let to stabilize for 15 minutes. The permeate was collected and weighted over time.

Results and Discussion

Figure 2 shows SEM images of the surface and cross section of the films. Film thickness, δ, was estimated by analysing cross section images and by measuring with micrometer.

In general, film thickness increased and porosity decreased with the polymer concentration (Figure 2), as expected. The solvent has strong influence on the type of porosity obtained. Chloroform leads to a cell-like, non-connected porosity, while NMP gives well interconnected pores when using ethanol and isopropanol as non-solvents (PCL15/NMP/EtOH and PCL15/NMP/IPA). Using water as non - solvent produces films (PCL15/NMP/W) with macrovoids that typically appear in phase separation systems with instantaneous demixing.[21]

Cross sections of PCL10/ClF/EtOH (SEM image not shown) and PCL15/ClF/EtOH (Figure 2) films show that these films are thin (>70% thickness reduction against the 200 μm initial casting thickness) and have low porosity. This can be attributed to compression of the pore structure during polymer precipitation. In general, chloroform affinity for EtOH is lower than for IPA, therefore, when EtOH is employed as non- solvent, delayed demixing occurs creating less porous films, as it can seen between PCL15/ClF/EtOH and PCL15/ClF/IPA (Figure 2). When NMP is employed as solvent, the order of solvent/non-solvent affinity is: water > EtOH > IPA. Therefore, there the fastest demixing occurs between NMP/water, as the macrovoids formation indicates, and the pore size in these films decreases as the solvent/non-solvent affinity decreases.

As mentioned before, one of the major problems in small blood vessel engineering is the occurrence of thrombogenesis. Kikuchi et al.[22] found that a complex microtopography (≤3 μm) in calcium-phosphate endosseous implants affect platelet adhesion and activation. In the present work, when employing chloroform as a solvent, the surface topographies (shown in Figure 2) have grooves and granular texture with both EtOH and IPA as non-solvents (PCL15/ClF/EtOH and PCL15/ClF/IPA). In the case of NMP, the surface topographies for PCL15/NMP/EtOH and PCL15/NMP/IPA are complex while when using water (PCL15/NMP/W), a smoother surface topography is observed. Therefore, it seems better to employ the combination of NMP and water as solvent/non-solvent to minimize platelet adherence and activation. Nevertheless, the surface topography for

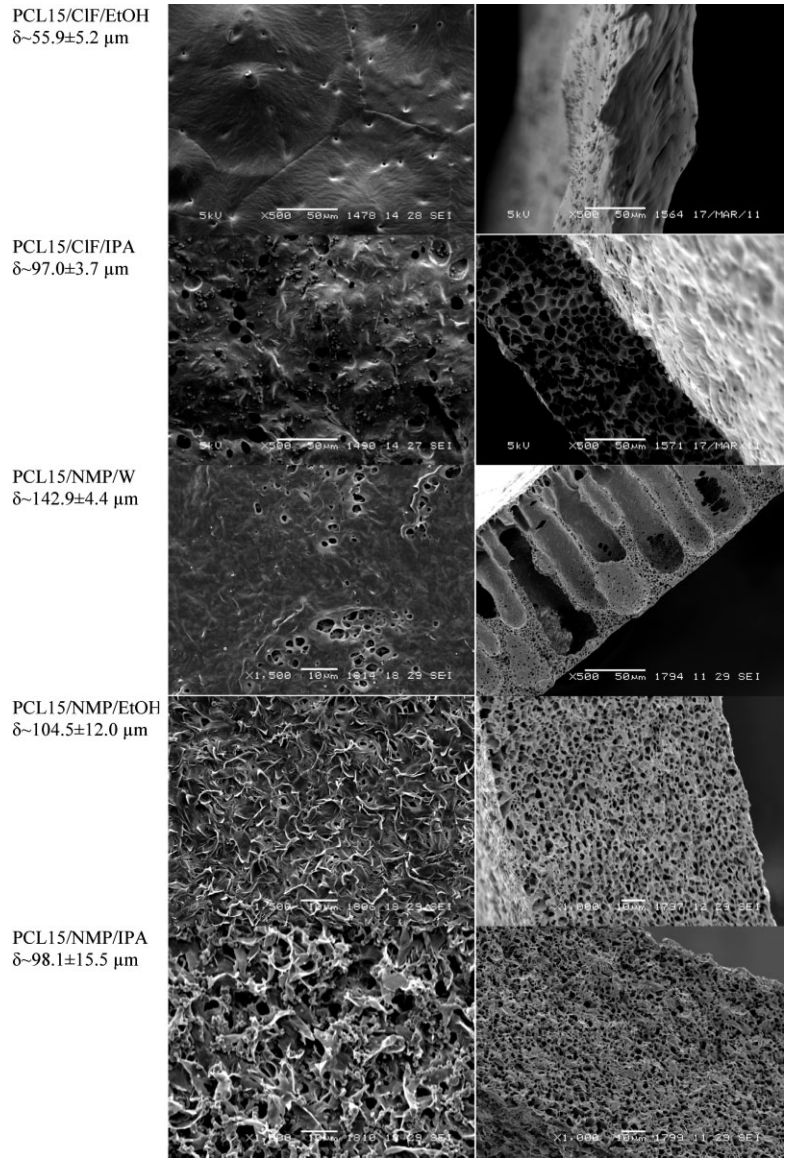

Figure 2.
Surface and cross section SEM images of the PCL films.

the films PCL15/NMP/EtOH and PCL15/NMP/IPA, lacelike and with granular texture respectively, is promising for cell organization.

It is important to note that a strategy that is often followed to overcome thrombogenicity is endothelialisation of the scaffold. There a monolayer of endothelial cells (EC) acts as a thrombo-resistant surface, isolating the foreign material from the blood. Therefore, creating an optimal surface for EC attachment and proliferation will also decrease the probability of thrombocyte activation. Moreno et al.[23] found that in non-woven fibrous scaffolds made from melt-blown polyethylene terephthalate (PET) fiber webs, a narrow pore size distribution was more suitable for Human Brain Endothelial Cells (HBEC) and Human Aorta Smooth Muscle Cells

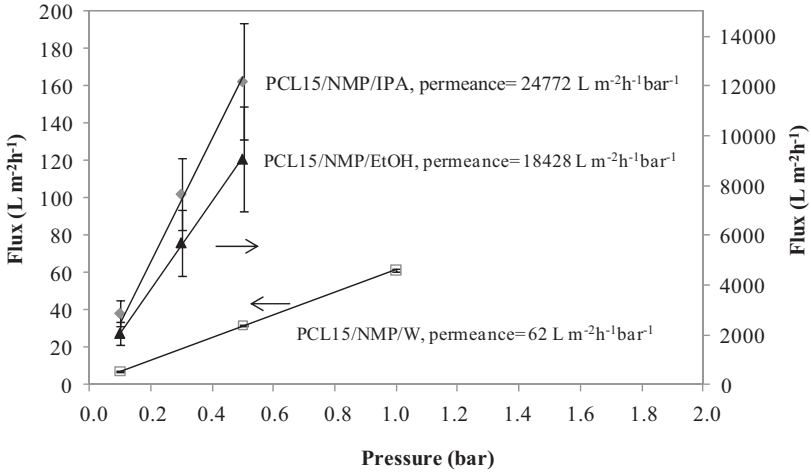

Figure 3.
Clean water flux through PCL flat films at various trans-membrane pressures (data expressed as mean ± SD, n = 3).

(AoSMC). PCL15/NMP/EtOH and PCL15/NMP/IPA pore size distributions are very narrow (~0.5–3.5 μm) and can be controlled well. Further discussion about the optimal pore size will be made below in the hollow fiber analysis.

Figure 3 presents the water transport through PCL15/NMP/W, PCL15/NMP/EtOH and PCL15/NMP/IPA. For PCL15/ClF/IPA films, there is no water flow through them below trans-membrane pressure of 2.0 bar while for pressure between 2.0 to 4.0 bar, filmcompaction is observed leading to almost no water permeance. This result is consistent with the low pore interconnectivity seen in the SEM images (Figure 2).

Despite the presence of macrovoids in the PCL15/NMP/W films, the dense surface skin (see Figure 2) limits water transport. The high porosity of the PCL15/NMP/EtOH and PCL/NMP/IPA films results in very high water permeance. High permeance values are generally desired as they suggest facilitated nutrient transport to the cells. Based on the results of pore structure and morphology obtained with the flat films, PCL hollow fibers for small vascular grafts were prepared, as well. Figure 4 shows a SEM image of the cross section of such hollow fiber and details of the external and internal surface.

Under the working conditions, the PCL hollow fiber has big pores (approximately between 15–50 μm) on the external surface and smaller pores (approximately <1.5 μm) on the lumen side. The water permeance is 333 ± 157 L $m^{-2}h^{-1}$ bar^{-1}. Some literature values of water permeance for biodegradable hollow fibers developed by wet-jet phase inversion spinning using NMP as solvent and water as non-solvent are: 20 L $m^{-2}h^{-1}$ bar^{-1} for Poly(lactic-co-glycolic) acid (PLGA) (50:50) hollow fibers [24] or 238–472 L $m^{-2}h^{-1}$ bar^{-1},[25] while for Poly lactic acid (PLA), water permeance of 120 L $m^{-2}h^{-1}$ bar^{-1} was found.[26] Besides, using 1,4-dioxane as solvent and ethanol as non-solvent and employing porogens (Polyvinylpyrrolidone, PVP and Poly (ethyleneglycol), PEG), PLLA hollow fibers with high water permeance ~ 2100 L $m^{-2}h^{-1}$ bar^{-1} was obtained.[15]

For creating blood vessels, a rather small pore size of the scaffold may not be a major problem for endothelial cells (EC), it however may limit infiltration of the Smooth Muscle Cells (SMC) inside the scaffold.[11] It was found in previous works [27] that, for PLLA scaffolds, vascular SMC preferentially attach to pore sizes between 38–150 μm. In contrast, microvascular EC form a connected multicellular lining on

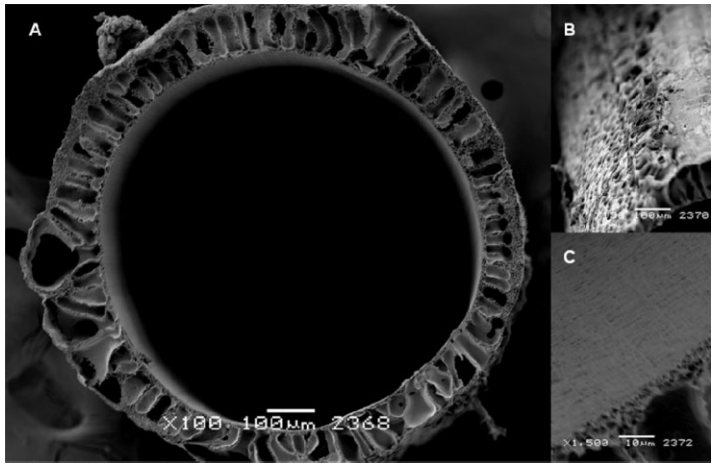

Figure 4.
SEM images of cross section (A), external (B) and internal (C) surface of the PCL hollow fiber (solvent= NMP, bore liquid = Milli-Q water, coagulation bath =IPA).

scaffolds with pores <38 μm. Based on these studies, the obtained PCL hollow fibers appear promising for developing small blood vessels tissue engineering. Further works on tailoring the fiber pore size, mechanical and transport properties of the hollow fiber scaffolds are currently in progress.

Conclusion

In the present work, we have shown the possibility of tuning the morphology and transport properties of flat PCL films by tailoring the phase separation conditions. Based on the results obtained for the films, hollow fibers were prepared, too. Adequate external and internal porosity on the tubular scaffold was observed in order to carry out future co-culture tests with SMC and EC.

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