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FABRICATION OF POROUS MEMBRANES FOR ARTIFICIAL BLOOD VESSELS

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INTRODUCTION

The crosstalk between endothelial (ECs) and smooth muscle cells (SMCs) plays an important role in the physiology of a blood vessel wall. Many cell culture systems have been developed to study these interactions. Selection of appropriate scaffold with suitable mechanical strength and porosity has been long recognized as the key factor that may determine extracellular matrix (ECM) remodelling and tissue generation.
A scaffold in a form of a membrane that we developed here would be a part of a co-culture system that mimics the topographical situation of the vessel wall in which ECs are separated from SMCs by a fenestrated elastic lamina. Such scaffold would act like a semi-permeable internal elastic lamina of native vessel in allowing interactions and fluid exchanges between the two cell types through its pores, similar to their native conditions (Weber E at al. 1986).
For our approach we have chosen polycaprolactone (PCL), a biocompatible polymer that has been tested in many studies with cells of various origins, including ECs and vascular SMCs (Serrano MC 2006). To create permeability, we used two types of porogens: inorganic salt – sodium chloride (NaCl); and water soluble biocompatible polymer – polyethylene glycol (PEG).

MATERIALS AND METHODS

Membrane fabrication.

PCL membrane scaffolds were fabricated by means of solvent casting. To obtain pores two approaches have been employed in the preparation process. In the first method PCL granules (MW 70-90 000) were mixed with NaCl crystals of two size ranges (either below 63 µm or between 63-125 µm, previously prepared by using a mechanic sieve) at a weight ratio 1:1. In the second method the PCL granules were mixed with PEG (MW ~20 000) at various weight rates: 1:2, 1:1.75, 1:5, 1:25, 1:1, 1.25, 1:1.5, 1:1.75 and 1:2. In both approaches two types of organic solvents were used; dichloromethane and chloroform, giving the final concentration of 16.5% of PCL/NaCl or PCL/PEG (w/v). Obtained solutions were cast onto Petri dishes and left to dry for 24h. Then, the membranes were placed on a shaker in distilled water for 48h. At the final stage the membranes were left in oven at 37°C for complete dry up.

Membrane evaluation.

All types of membranes were observed using scanning electron microscopy (SEM) (Pegasus X4M). The membranes were cut into square specimens (1 cm^2), glued with carbon tape to copper supports and sputtercoated with gold to a thickness 10-15 nm. Images were acquired using SEM operating at accelerating voltage of 5 kV.

RESULTS AND DISCUSSION

An apparent difference was observed in terms of the surface topography, pore sizes and distribution in case of the scaffolds prepared using NaCl crystals. Membrane's surface that had contacted with glass during casting process maintained its flat morphology (fig.1A&C), while the topography of upper surface which was in the gas phase, was affected by the salt crystals, or rather the crystal's aggregates (fig. 2B&D).
In the samples where PEG was used as porogen we could observe the change of the pore size while the PCL/PEG ratio was modified. The smallest pore size was obtained when the PCL content was highest (fig. 2A). Then, gradually, the pore size (but also its rounded shape and uniform morphology) was directly related to the increase of PEG content (fig. 2 B & D). Such

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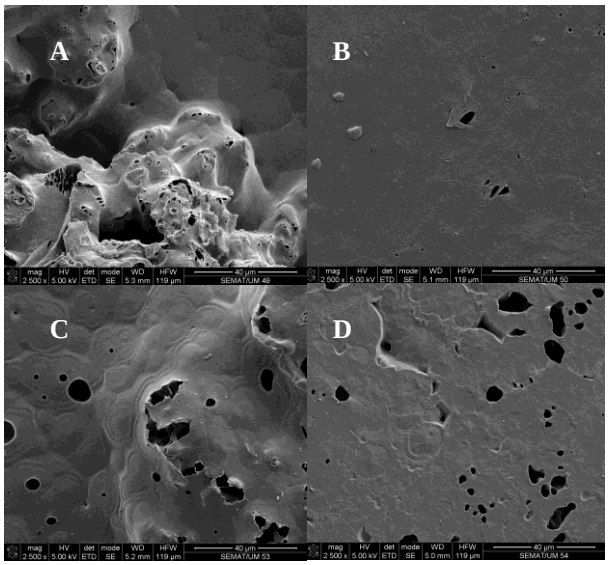

Figure 1: SEM images of PCL scaffolds where NaCl crystals of the size smaller than 63 µm (A&B) or in range of 63 – 125 µm (C&D) were used as porogen. A&C represent surfaces that were in contact with air phase during casting while B&D the surfaces attached to the glass.

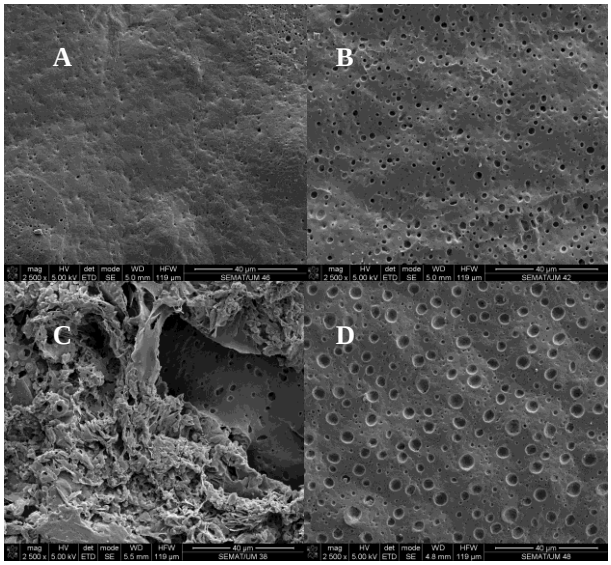

Figure 2: SEM images of PCL scaffolds after washing out the water soluble PEG co-polymer. The images represent scaffolds' surfaces where various PCL/PEG rates were applied: A – 2:1, B – 1:1, C – 1:2 and D – 1:1.25 respectively.

relationship was present up to the crucial PCL/PEG rate of 1:1.25 (fig. 2D). After crossing this ratio the membrane´s surface topography was significantly affected (fig. 2C).

The selection of solvents used in the sample's fabrication did not significantly affect the scaffold parameters in any of the cases. Also, there was no significant difference in the appearance of the upper and lower surface for the membranes prepared with PEG.

CONCLUSIONS

By using NaCl crystals as porogen we were not able to obtain satisfactory results in terms of the pore distribution and size (fig.1). Also, the scaffold's topography was affected by the crystal structure, resulting in alterations of the membrane topography (fig.1A&C).

By controlling the PCL/PEG ratio, we were able to obtain membranes with equal distribution of small pores in the range diameter of 1 - 7 µm (fig. 2B&D). Such porous surface would be suitable for generating uniform coverage of ECs to act as internal elastic lamina, yet allowing for interactions with vascular SMCs that could be seeded on the opposite side of the membrane.

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