Development of 3D bioactive macroporous scaffolds

Hanna Bramfeldt[1,2], Pierre Sarazin[1,2], Patrick Vermette[1,2].

1. Laboratoire de Bioingénierie et de Biophysique de l'Université de Sherbrooke, Department of Chemical Engineering.

2. Research Centre on Aging, Université de Sherbrooke, Sherbrooke, Qc, Canada, J1K 2R1.

Statement of Purpose: The objective of this work was to evaluate the suitability towards 3D scaffold fabrication of two copolymers, one pure PCL-PEG-PCL triblock copolymer, denoted P(100/0), and one polymer made of P(co-DLLA-CL)-PEG-P(co-DLLA-CL) containing 30% D,L-LA in the side blocks, and denoted P(70/30). Two directions of interest were investigated, namely (I) the impact of protein immobilization on these polymers on smooth muscle cell adhesion and (II) the fabrication of highly interconnected, macroporous scaffolds.

Materials and Methods:

I) Proteins were immobilized to the polymer surfaces using a two-step procedure. Polymer films were firstly aminolysed using 1,6-hexanediamine, and subsequently proteins were attached to the functionalized surface using the homobifunctional, aminoreactive dimethyl pimelimidate (DMP). Proteins used to enhance cell attachment on these polymers were different combinations of fibrinogen (Fgn) and fibronectin (Fn), and fibrin through the addition of thrombin on Fn-saturated surfaces. Human umbilical artery smooth muscle cells (SMC) were used to evaluate the ability of these surfaces to support cell adhesion and proliferation.

II) Macroporous structures were fabricated by a co-continuous blend technique using the copolymer and polystyrene (PS) as a porogen material. Pore size was altered by annealing of the blend at 180˚C and 200˚C for P(100/0) and P(70/30), respectively. The final porous structures were obtained by dissolving the PS, and imaged under a scanning electron microscope (SEM).

Results and Discussion:

I) Figure 1 shows the SMC cell count 24h after seeding, indicating generally superior cell-surface interactions for P(100/0)-based surfaces. Immobilizing a mixture of Fn and Fgn on P(100/0) produced superior cell adhesion even compared to Tissue Culture Polystyrene (TCPS). SMC growing on a modified P(100/0) can be seen in Figure. 2.

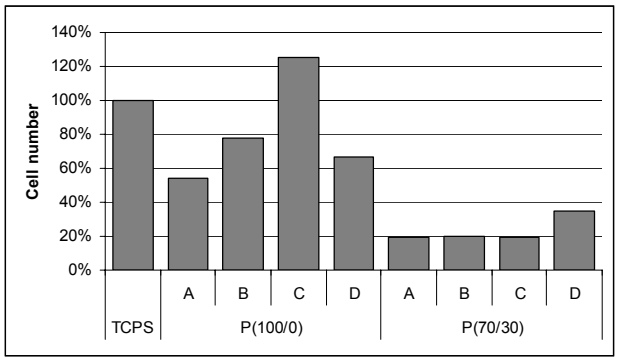

Figure 1: Number of adhering SMC 24h after cell seeding. The proteins immobilized on the polymer films were: 10µg/ml Fn (A); 10µg/ml Fgn (B); a mix of 5µg/ml Fn and 5µg/ml Fgn (C); and thrombin added to Fn-saturated surfaces (D).

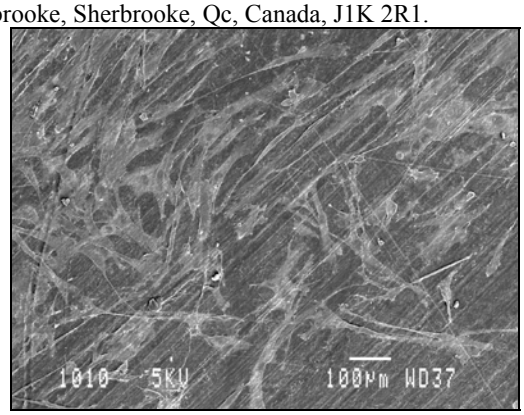

Figure 2: SEM picture of SMC after 48h growing on P(100/0) modified according to scheme D.

II) By annealing copolymer/PS blends, the pore size of the macroporous structures could be controlled. By varying the annealing time from 30 min to 2h, pore sizes ranging from 20-200µm were created in both P(100/0) and P(70/30) samples. This technique generates homogeneous structures of interconnected pores (Fig. 3).

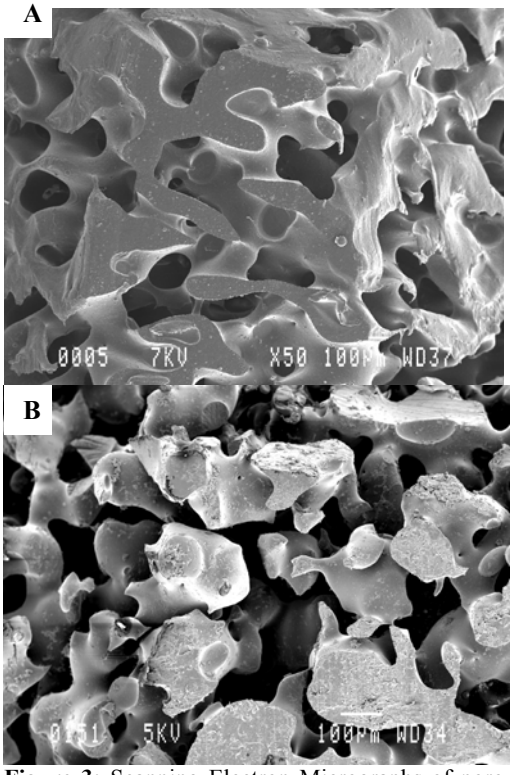

Figure 3: Scanning Electron Micrographs of porous structures following 1h annealing at 200˚C of P(100/0) (A), and 2h at 180˚C of P(70/30) (B).

Conclusions: Both copolymers may be transformed into 3D macroporous scaffolds using co-continuous blends with polystyrene. Also, protein immobilization was used to improve SMC growth on the two copolymers. However, the capacity of modified P(100/0) to support cell growth exceeds that of the LA-containing P(70/30).