**Nanoporosity of PEOT/PBT Electrospun Scaffolds Enhances Cell Proliferation and Influences Cell Morphology.**

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**Introduction:** Electrospinning (ESP) has lately shown a great potential as a novel scaffold fabrication technique. Scaffolds are produced by spinning a polymeric solution in fibers through a spinneret connected to a high voltage electric field. The fibers are then collected on a support, where the scaffold is created. Scaffolds can be of different shapes, depending on the collector geometry, and have high porosity and high surface per volume ratio, since the fibers are in the micro- and nanoscale range [1]. Fibers can display a nanoporous ultrastructure depending on the solvent used. This can influence cell morphology, which is known to affect tissue formation.

Within this scenario, the aim of this study was to characterize electrospun PEOT/PBT block copolymers nanoporous fibers and to assess the influence of fiber diameter and nanoporosity on cells.

**Methods:** PEOT/PBT block copolymers were obtained from Isotis S.A. A 300/55/45 composition was used, where, following an a/b/c nomenclature, a is the molecular weight of the starting PEG blocks, and b and c refer to the weight ratio of PEOT and PBT blocks, respectively. PEOT/PBT solutions were prepared in different concentrations with chloroform ($CHCl_3$), or a mixture of $CHCl_3$ and hexafluoroisopropanol (HFIP). A voltage of 12 kV was applied to process the fibers. Human mesenchymal stem cells (HMSC) obtained from two donors who had given an informed consent were harvested, expanded in vitro, and then seeded and grown for a maximum of two weeks on discs (Ø=6 mm; h=0.1 mm) with an average fiber diameter of 1 µm, 4 µm, 10 µm, 21 µm, and 10 µm with nanopores approximately 200 nm in size. Three dimensional (3D) scaffolds with a fiber diameter of 270 µm fabricated with a 3D fiber deposition technique were also cultured as a reference [2]. Cell activity was determined with an alamar blue assay and their morphology analyzed by SEM.

**Results & Discussion**: ESP scaffolds were fabricated with a wide range of fiber diameters, between 50 nm and 30 µm, depending on the solution flow rate and concentration. Nanopores on the fibers were dependent from the solvent, the solution concentration, and the air gap between the syringe and the collector, and varied from 70 nm to 7.25 µm (figure 1). This might be explained by the colloidal nature of PEOT/PBT solutions, comprised of polymer rich and solvent rich phases. During the fast solvent evaporation occurring in electrospinning [3] the solvent rich domains of the solution are replaced by air, thus allowing the formation of nano and micro pores.

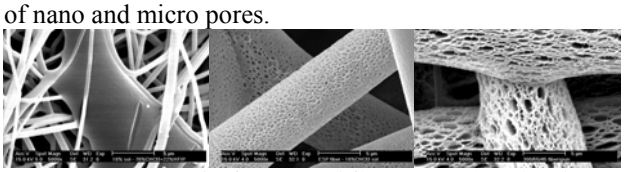

*Figure 1: SEM micrographs of non porous (left), nanoporous (center), and microporous (right) fibers in ESP scaffolds.*

HMSC attached and proliferated on all the scaffolds. A significantly higher number of cells was found in the scaffolds with nanoporous 10 µm fibers as compared with the scaffolds with smooth fibers, suggesting a positive influence of nanometer surface topology on cell proliferation. 10 µm fibrous scaffolds also showed a significant higher number of cells than those with smaller (4 µm and 1 µm) or larger diameter (21 µm and 270 µm) fibers. This suggests a specific capacity of the cells to recognize an optimal fiber size for attachment and proliferation (figure 2).

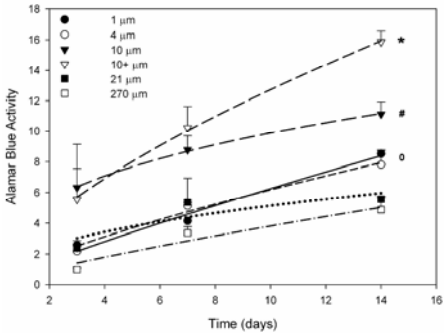


*Figure 2: Effect of the fiber diameter and surface topography on cell proliferation. (*) indicates significant difference between nanoporous and smooth surface; (#) shows significant difference of 10 µm fiber diameter with respect of the other fiber diameters; (o) depicts significant differences between 1 µm and 4 µm fiber diameter with respect of 21 µm and 270 µm. Significant level $p < 0.05$.*

SEM analysis revealed that cells aggregated on all scaffolds with smooth fibers, while they tended to spread over the fibers when nanoporosity was added (figure 3). This difference can be very useful when influencing cell aggregation or spreading is desirable, for example, in cartilage or bone tissue engineering.

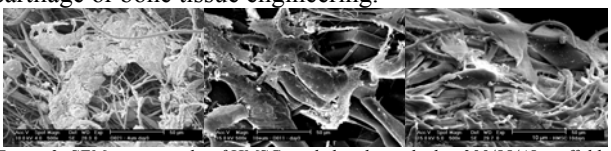

*Figure 3: SEM micrographs of HMSC seeded and attached to 300/55/45 scaffolds at day 3. (left) cell aggregates on smooth fibers; (center) cells spreading on nanoporous fibers;(right )scaffold cross section at day 14, showing cell penetration. Scale bar: 50 µm.*

**Conclusions:** A wide range of ESP scaffolds can be fabricated with PEOT/PBT copolymers. Scaffolds are comprised of porous and/or non-porous fibers, depending on the electrospinning setup and on the physicochemical properties of the polymer solution. HMSC have been cultured on these scaffolds and fibers with a diameter of 10 µm were found to have optimal cell attachment and proliferation. Smooth or nanoporous fibers resulted in rounded aggregated or spread cells. Ultimately, the presence of nanopores can suggest the use of such scaffolds for a specific tissue formation.

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**References:** [1] Doshi J, et al. J of Electrostatics 35 (1995): 151-160. [2] Moroni L, et al. Biomaterials 2005, doi:10.1016/j.biomaterials.2005.07.023. [3] Bognitzki M, et al. Adv. Mater. 2001, 13, No.1, January 5: 70-72.