Electrospun Scaffolds with Gradient Morphology for use in Endovascular Aortic Aneurysm Repair

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Statement of Purpose: Current endovascular abdominal aortic aneurysm repair utilizes bioinert stent-grafts to isolate the aneurysmal sac from blood flow[1]. However, these stent-grafts are prone to endoleaks due to migration, improper anastomosis and uncontrolled blood flow into the aneurysmal sac[1]. To address these shortcomings, we have developed a tissue engineering scaffold with mechanical and morphological properties necessary for endovascular aneurysm repair. *In vivo*, the lumen side of the scaffold should encourage cell confluence to reduce unwanted immune response while the rest of the scaffold should aid in structural integrity and vessel elasticity. We developed scaffolds with curved fiber morphology on the lumen side and a linear morphology on the adventitial side to better facilitate the preferred cell organization without an abrupt layer change. This study evaluates the cellular response to such scaffolds.

Methods: Tubular scaffolds were electrospun on an aluminum mandrel using process parameters ranging from 10-14 wt% concentration, 0.012 mL/min or 0.029 mL/min extrusion rates and 10-14 kV applied voltage. The resulting scaffolds were cut into 0.5 cm x 0.5 cm square samples and sterilized with O^2 gas plasma. Human aortic smooth muscle cells were cultured in M231 with SMGS (Invitrogen) and a bank was established so that all studies were seeded with P8 cells. Bare wells in a tissue culture plate and thin films of PCL cut to 0.5 cm x 0.5 cm were used as controls. Samples were placed in ultra-low adhesion well plates with media then cells in suspension were added and allowed to attach for 2.5 hours. After the media was changed, a day 0 time point was recorded for the metabolic assay. AlamarBlue was used to determine metabolic activity of the cells on days 0,1,3,5, and 10 (n=6), fluorescence staining and imaging were used to evaluate the cell density on the surface of the scaffolds (n=3). Cross sections of the scaffolds were used to visualize the infiltration of the cells into the scaffolds (n=3) and characterize the morphological gradient. Scaffolds with and without cells were evaluated by SEM on both the concave and convex surfaces (n=3). ANOVA was used to to determine significant differences ($p<0.05$).

Results: SEM analysis of scaffolds verified curved and linear fibers on the concave and convex surfaces, respectively. After 10 days of cell culture, SEM also indicated sheet-like matrix deposition on the curved fibers while the matrix deposition on the linear fibers was more integrated as shown in Figures 1.

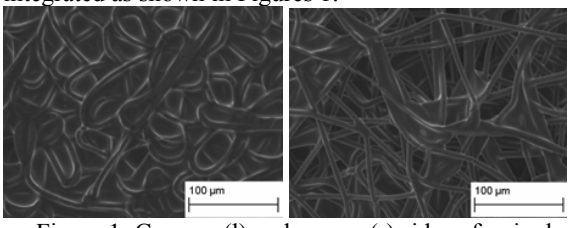

Figure 1: Concave(l) and convex(r) sides of a single scaffold after 10 days of cell culture.

Sectioning the scaffolds as illustrated in Figure 2, verified a gradient as well as cell infiltration.

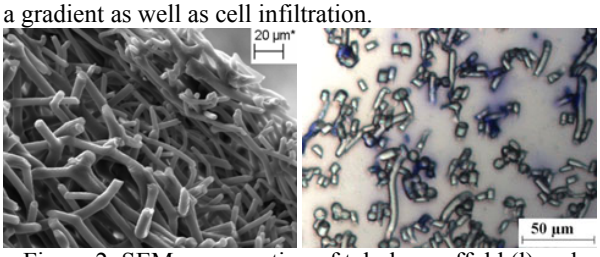

Figure 2: SEM cross section of tubular scaffold (l) and thin section (5 µm) (r) of scaffold with Crystal Violet.

Comparing the cell adhesion on each surface of a scaffold on day 10 as depicted in Figure 3, there are more cells present on the concave surface which supports the SEM images depicting a difference in ECM. However, it is feasible that cells originally attached on the convex surface were more likely to migrate into the scaffold due to the spacing of the more linear fibers.

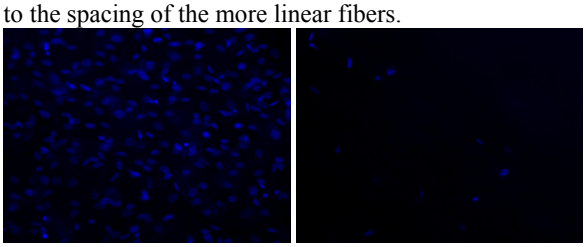

Figure 3: Nuclei on the concave (l) and convex (r) surfaces of a single scaffold.

Metabolic data over 10 days indicates an increase in metabolic activity for all of the scaffolds compared to day 0 as shown in Figure 4.

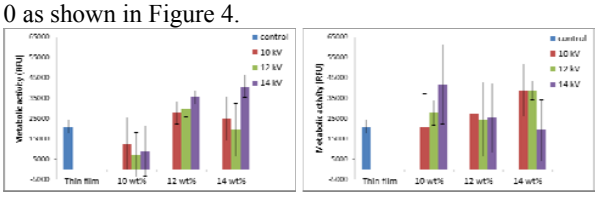

Figure 4: Change in metabolic activity at day 10, normalized to day 0.

Conclusions: A distinction has been exhibited between the concave and convex side of a continuously electrospun scaffold in a manner that affects cell activity including adhesion, spreading and ECM deposition in a 10 day static study of human aortic smooth muscle cells. The cell behavior is favorable for use in endovascular aortic aneurysm repair due to its congruence to native vessel organization and potential to control thrombosis without being inert. Future work will include an endothelial cell study as well as analysis of the scaffolds in co-culture in a bioreactor.

References:

1. Greenhalgh RM. N Eng J Med. 2008; 358:494-501.