Effect of Surface Chemistry on Stem Cell Response in 2-D vs. 3-D Cell Culture Niches

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Statement of Purpose: To develop tissue engineered devices for regenerative medicine strategies, key scaffold properties for directing stem cell response must be identified. As focus shifts from 2-D to 3-D culture systems in an attempt to better recapitulate the natural cellular environment, traditional strategies to enhance the cell culture environment may no longer have equivalent effects on cells. The synergistic effects of the new 3-D culture environments are largely undetermined.

In previous studies conducted by our laboratory, we determined that nanofiber scaffold structures provided a 3-D niche to human bone marrow stromal cells (hBMSCs) that induced osteogenic differentiation without osteogenic supplement **(1)**. Nanofibers made from two different polymer materials, poly(ε-caprolactone) (PCL) or poly(D,L-lactic acid), were able to elicit this effect **(1).** In the current study, to more directly investigate the effects of scaffold chemistry in 2-D vs. 3-D cell niches, we have systematically altered the surface chemistry of PCL nanofiber scaffolds and 2-D spun coat PCL films. In this system, scaffold structural properties are kept constant while chemistry is varied by mild hydrolysis.

Partial PCL hydrolysis allows for the generation of hydroxyl and carboxyl groups on the polymer surface. Carboxyl groups can be further functionalized with specific chemistries (e.g. amines, methyls) or biomolecules (e.g. collagens, glycosaminoglycans) for the investigation of varying scaffold chemical properties on stem cells in the 2-D vs. 3-D cell niches.

Methods: PCL nanofibers (NF) with low fiber diameter variablity (< 17%) were fabricated via an electrospinning technique and PCL films were fabricated via spin-coating (SC). PCL NFs and SC films were chemically modified via hydrolysis in 1 mol/L NaOH (7 h, 37° C). Scaffold structure and fiber diameter were visualized using scanning electron microscopy (SEM) and scaffold chemistry was verified with water contact angle (WCA), toluidine blue O (TBO) carboxyl colorometric staining, and x-ray photoelectron spectroscopy (XPS). hBMSCs were cultured on un-modified and NaOH-modified scaffolds and cultures were investigated for proliferation (Picogreen DNA assay), cell shape (immunostaining and confocal imaging) and differentiation (alkaline phosphatase assay). Statistical significance was determined using 1-Way ANOVA (analysis of variance) and Tukey's post test.

Results: Scaffold hydrolysis was optimized to develop a material system with similar structural but significantly varied chemical properties (Fig. 1A). NF scaffolds and SC films both had a significant decrease in WCA (Fig. 1A, $p < 0.01$), with a corresponding increase in surface carboxyl concentration for NFs as determined by the TBO assay. In addition, XPS indicated an increase in surface oxygen:carbon ratio on both NF and SC (data not shown). These results indicate that NF and SC surface chemistry were modified to incorporate carboxyl and hydroxyl moieties by the hydrolysis treatment. Both 3-D nanofiber mats and 2-D spun coat films maintained their structural integrity (Fig. 1A) after hydrolysis and nanofiber diameter was not significantly different between hydrolyzed and un-modified samples (quantitative data not shown). Scaffolds were seeded with hBMSCs and investigated for morphology (1 d), proliferation (14 d) and differentiation (14 d). Cell area was significantly lower on hydrolyzed SC vs. un-modified SC while there was no significant difference in cell shape on hydrolyzed NF vs. un-modified NF (Fig. 1B). Proliferation was not significantly different between hydrolyzed and un-modified scaffolds while alkaline phosphatase activity was significantly increased on hydrolyzed SC films (data not shown). No significant difference was observed in alkaline phosphatase activity when comparing hydrolyzed NF versus un-modified NF.

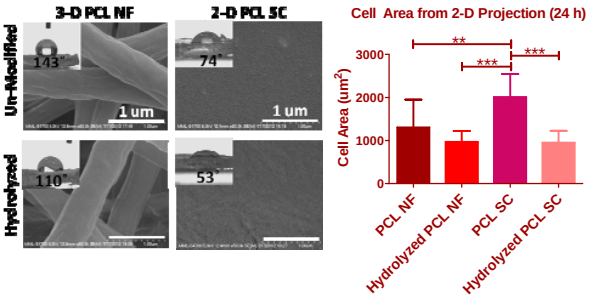

Figure 1: (A) PCL NF and SC before and after NaOH hydrolysis with their WAC. **(B)** Cell projected area was determined for n > 10 cells on each scaffold type from confocal Z-stack images.

Conclusions: We have successfully developed a material system that enables the systematic investigation of the effects of scaffold structure and chemistry in 2-D vs. 3-D cultures. The results show that PCL surface hydrolysis chemistry affects cell shape and differentiation in 2-D culture but not in 3-D culture, indicating that the surface chemistry has less effect on cell function in 3-D than in 2-D.

References: **(1)** G. Kumar, C.K. Tison, K. Chatterjee, P.S. Pine, J.H. McDaniel, M.L. Salit, M.F. Young, C.G. Simon, Jr. The determination of stem cell fate by 3D scaffold structures through the control of cell shape, *Biomaterials*, 2011, **32**, 9188.

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