Poly(dimethyl siloxane)-Containing Nanofiber Meshes as a Platform for Studying Stem Cell Behavior

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Statement of Purpose: Cellular mechanotransduction systems are capable of sensing the surrounding microenvironment and transducing these stimuli into biochemical signals, which in turn translate into controlled functional responses[1-4]. However, the complexity and diversity of mechanosensing pathways and processes make it difficult to determine the specific stimuli which affect change in cell behavior. For this reason, development of a platform for studying the effects of physical environmental cues, such as matrix rigidity and nano-/micro-level geometry, on cell behavior is of increasing interest. **The objective of this study** is to develop a novel synthetic nanofiber substrate composed of poly(dimethyl siloxane) (PDMS) and poly(ε-caprolactone) (PCL) for testing the effects of mechanical cues on cell behavior without interference from biological and chemical cues. **It is hypothesized** that the synthetic composition of these meshes will allow for the independent modulation and study of physical matrix properties on cell behavior without the influence of biological and chemical variables on cell response.

Methods: Nanofiber Fabrication: Unaligned and aligned meshes composed of either a 1:1 blend of PDMS (Sylgard 184; Dow Corning) and PCL (Sigma-Aldrich) or PCL only were formed via electrospinning. The PDMS-PCL samples were then left to cure overnight at room temperature to allow for PDMS crosslinking. Characterization: Qualitative analysis of fiber morphology, alignment, diameter and pore size were performed using scanning electron microscopy (SEM; Hitachi, n=2). Scaffold composition was also performed using Fourier Transform Infrared (FTIR) imaging. Mechanical Testing: Elastic modulus, ultimate tensile strength, and % elongation for each polymer blend were tested via uniaxial tensile testing at a strain rate of 5 mm/min (Instron, n=6). Cell Seeding: Scaffolds were seeded with human MSC (21 y/o M; Lonza) at $3x10^4$ cells/cm^2 and cell response was analyzed on days 1, 7, and 21. End-Point Analyses: Cell viability and morphology (n=2) were examined by Live/Dead assay and cell proliferation and fold change in cell number (n=5) were assessed by PicoGreen dsDNA assay. Statistical Analysis: Results are presented in the form of mean ± standard deviation, with n equal to the number of samples per group. ANOVA and the Tukey-Kramer post-hoc test were used for all pair-wise comparisons (*$p<0.05$).

Results: Nanofiber Characterization: SEM revealed that fibers in all meshes were uniform, with fiber diameters on the order of one micron, except for aligned PCL scaffolds, which exhibited a lower fiber diameter. FTIR data confirms that both PDMS and PCL chemical groups were incorporated into PDMS-PCL fibers (Fig. 1). Cell Viability and Proliferation: Cells remain viable in all groups over time (Fig. 2). Cell number on both PDMS-PCL and PCL scaffolds increased significantly by day 21, regardless of alignment. For unaligned scaffolds, fold change in cell number was significantly greater on PDMS-PCL scaffolds compared to PCL scaffolds on day 21. For aligned scaffolds, both total cell number and fold change in cell number were significantly greater on PDMS-PCL scaffolds when compared to PCL scaffolds (Fig. 2).

Discussion/Conclusion: In this study, a novel synthetic PDMS-PCL nanofiber scaffold was developed as a platform for studying the effects of mechanical cues on cell behavior. Proliferation data shows that MSC growth rate is increased on the PDMS-containing scaffolds, when compared to PCL alone. A similar trend was seen for both aligned and unaligned fibers. These differences likely arise from the lower matrix stiffness experienced by the cells on the PDMS-PCL substrate. Future studies will utilize this platform to study the mechanisms behind the observed mitotic increase, including analysis for differences in mechanical properties, surface morphology and substrate chemistry.

References: 1) Discher DE. Science 2005;310:1139-1143. 2) Engler AJ. Cell 2006;126:677-689. 3) Vogel V and Sheetz M. Molec Cell Biol 2006;7:265-275. 4) Guilak F. Cell Stem Cell 2009;5(1):17-26.

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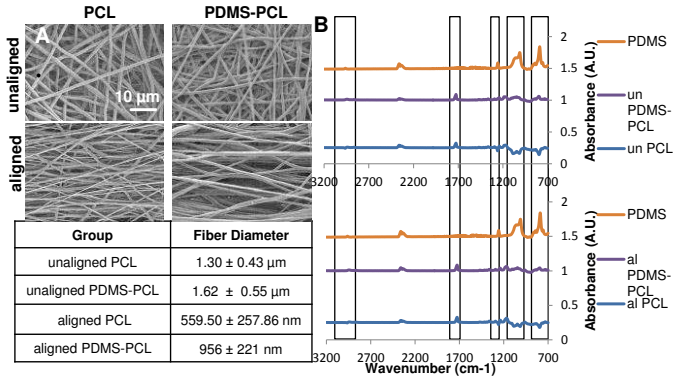

Group	Fiber Diameter
unaligned PCL	1.30 ± 0.43 μm
unaligned PDMS-PCL	1.62 ± 0.55 μm
aligned PCL	559.50 ± 257.86 nm
aligned PDMS-PCL	956 ± 221 nm

Figure 1. A) SEM and associated fiber diameter analysis of unaligned and aligned PCL and PDMS-PCL meshes (n=2). B) Presence of PDMS and PCL in nanofiber meshes was confirmed via FTIR (n=2).

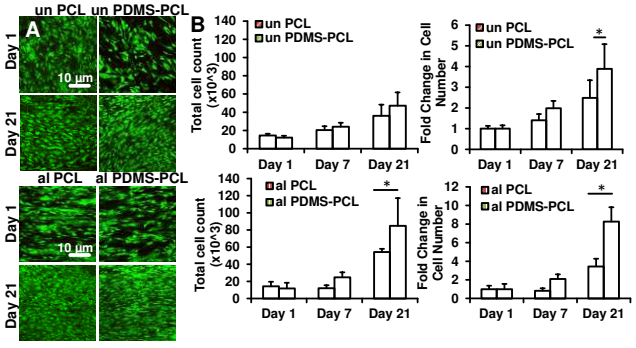

Figure 2. A) Live/Dead images of hMSC seeded on unaligned and aligned PCL and PDMS-PCL meshes after 1 and 21 days in vitro (n=2). B) Fold change in cell number was significantly greater for PDMS-PCL scaffolds after 21 days compared to PCL scaffolds regardless of alignment (n=5).