Oriented Pore Scaffolds from an Elastase-Sensitive, Biodegradable Elastomer to Achieve Mechanical Anisotropy and Improved Cellularization

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Introduction: Forming a microporous scaffold with oriented pores would be expected to impart anisotropic mechanical behavior, and through reduced tortuosity in the pore structure, might improve mass transfer characteristics and potentiate higher *in vitro* cell densities. We have previously reported on the development of elastase-sensitive polyurethaneureas that act as thermoplastic elastomers.[1] These polymers undergo ester hydrolysis of the soft segment and elastase cleavage of the hard segment, and readily support cellular growth. In moving these materials toward applications in soft tissue engineering we have investigated the formation of oriented pore scaffolds using thermally induced phase separation and the impact of the resulting microstructure on scaffold mechanical properties and scaffold support of muscle-derived stem cell growth.

Methods: The elastase-sensitive polyurethane was synthesized using poly(caprolactone) and butyldiisocyanate, with chain extension by alanine-alanine-lysine.[1] The polymer was processed into microporous 3-D scaffolds by dissolution in DMSO and injection into a breaker wrapped with insulation tape on the lateral and top surfaces. The beaker bottom was then quenched at different temperatures to thermally induce phase separation after the method of Ma and Zhang.[2] Polymer solution was injected into a breaker without insulation to form randomly oriented scaffolds. Solvent was extracted with ethanol. Murine muscle-derived stem cells were isolated by a pre-plate method and have previously been characterized.[3] Cells ($5x10^6$/mL) were filtration seeded into random or oriented scaffolds and cultured in spinner flasks.

Results and Discussion: Scaffolds with oriented micropores resulted by introducing a heat gradient during the thermally induced phase separation process. Without the heat gradient, a random morphology resulted. Both oriented and random scaffolds had interconnected pores that opened to the surface with morphologies dependent upon the polymer concentration and quenching temperature (**Figure**). Scaffolds porosities were >87%. Oriented scaffolds had pore sizes varying from 31-290um. Oriented scaffolds showed different mechanical properties in the longitudinal and transverse directions. Longitudinal tensile strength was >1.0 MPa and breaking strain >200%, whereas the transverse direction showed mechanical properties similar to the random scaffold with tensile strength >0.12 MPa and breaking strain >50%. Muscle-derived stem cells could be uniformly distributed in the scaffolds with filtration seeding. Over the 14d culture period oriented scaffolds exhibited cellular densities approximately 20% greater than random scaffolds.

Conclusions: Oriented pore scaffolds from an elastase-sensitive biodegradable polyurethane were obtained by controlling thermal gradients in phase separation processing. Scaffolds with oriented micropores exhibited mechanical anisotropy while remaining flexible and strong, and also supported higher cellular densities during *in vitro* culture. Control of pore microstructure, combined with elastomer molecular design, offers increased functionality for soft tissue engineering applications.

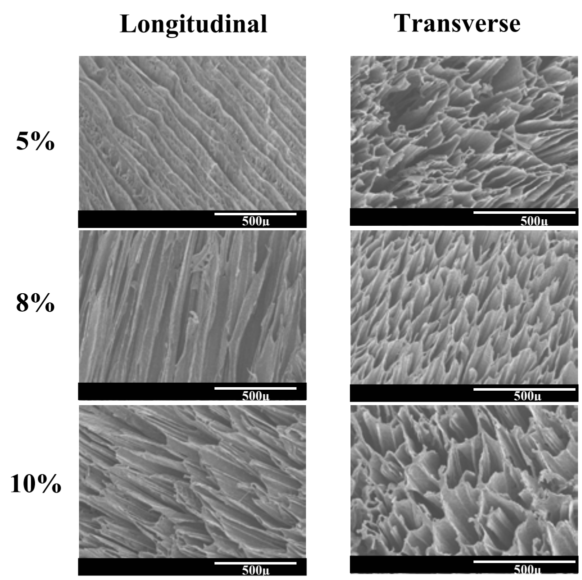

Figure. *Polymer concentration effect on oriented scaffold morphology.*

References:
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3. Qu-Petersen et al. *J Cell Biol*, 2002; 157: 851.