Cellular Orientation Control Using Microcontact Printing and Mechanical Conditioning for Tissue Engineered Blood Vessels for Atherosclerosis

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Statement of Purpose: Atherosclerosis is the chronic inflammatory response in arterial walls that leads to stenosis and ultimately poor nutrition of downstream tissues, which can require blood vessel replacement. Because synthetic vascular grafts often fail (e.g., aneurysm, thrombosis, calcification), cell-based tissue engineered blood vessel (TEBV) alternatives are sought. Highly organized and multilaminate in structure, native blood vessels have complex three-dimensional spatial organization with anisotropic contractile properties. Although cell sheet engineering technologies have been devised to allow cellular alignment and subsequent recovery of the aligned cells without damage, we may also be able to replicate native biomechanical properties using mechanical conditioning. Additionally, aligning cells before mechanical conditioning may influence extracellular matrix secretion, which may affect mechanical properties.

Methods: We modified UniFlex plates (Flexcell, Hillsborough, NC) with thermoresponsive N-isopropylacrylamide (NIPAAm) copolymers as previously described.[1] NIPAAm allows cell attachment at 37°C; following a temperature decrease to room temperature, cells can spontaneously detach without use of damaging enzymatic treatments. Microcontact printing using conformal contact of fibronectin-coated silicone patterned stamps (50 µm-wide ridges and grooves, 5 µm depth) for cellular alignment was performed on NIPAAM-modified UniFlex plates, as previously described for NIPAAm-modified tissue culture polystyrene dishes.[2] Confluent sheets of bovine vascular smooth muscle cells (BVSMCs) on non-patterned and patterned surfaces were stretched at 0% or 8.5% elongation on the Flexcell Tension System for 24 hrs at 1 Hz. Cell sheets were subsequently stained with Alexa Fluor 488 phalloidin (Life Technologies, Grand Island, NY) and Hoechst 33342 (Life Technologies) and imaged. Cell orientation and anisotropy was analyzed using 2D fast Fourier transform and the Oval Profile plug-in in ImageJ as previously described.[2] Two-tailed Student's *t*-test, where p-values ≤ 0.05 were considered statistically significant, were performed.

Results: Cells on patterned substrates had macroscopically visible alignment striations (**Figure 1**), and cell alignment on patterned substrates was significantly higher than in non-patterned substrates. Additionally, cells that were mechanically conditioned demonstrated a significant peak orientation distribution at the preferred direction of alignment on both non-patterned and patterned substrates.

Conclusions: The cellular structure level can influence the functional properties of a TEBV; thus, controlling cellular orientation at the individual cell sheet level is critical. We have shown that we can control and improve cell orientation using substrates that can be both patterned for alignment and mechanically conditioned. Future studies will examine if alignment and mechanical conditioning can improve the biomechanical properties of the cell sheet for use in a TEBV.

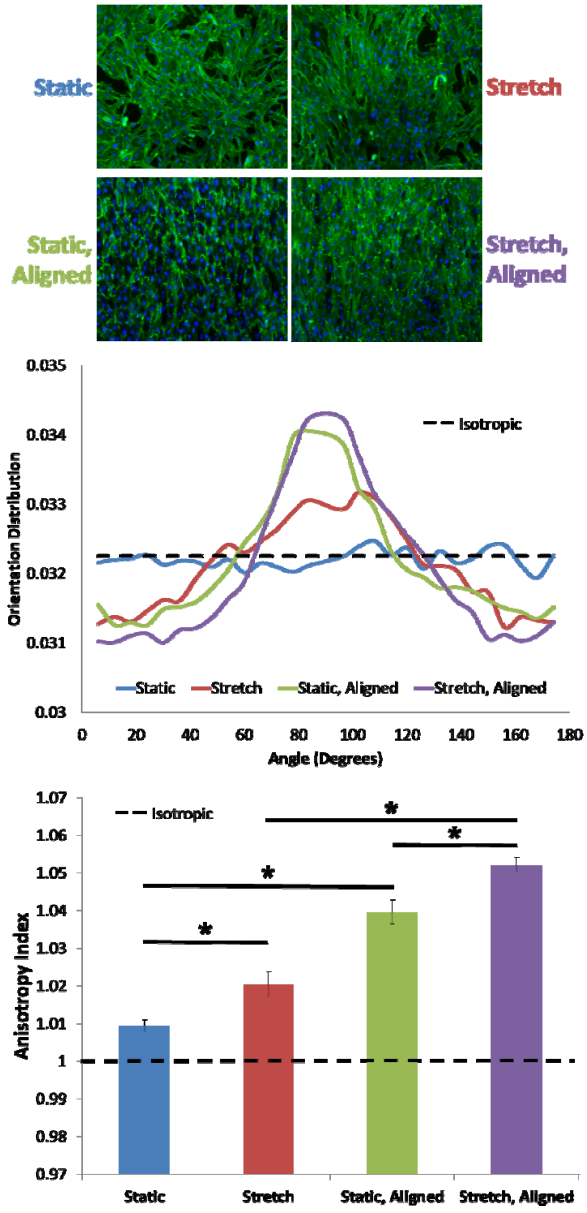

Figure 1. Cells on alignment-patterned or mechanically conditioned substrates had higher anisotropy than those on non-patterned or non-conditioned substrates.

References:

1. Lee EL. J Biomed Mater Res A. 2010;93:411-8.
2. Williams C. Biomaterials. 2011;32:5625-32.

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