Controlling Cellular Orientation Using Microcontact Printing and Mechanical Conditioning to Modulate Contractile Protein Expression

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Statement of Purpose: Following a cardiovascular event, disruption of hierarchical structure at the injury site may cause subsequent mechanical property changes to the remodeling tissue (e.g., scar tissue formation leads to increased stiffness), and a stressed environment may perpetuate maladaptive remodeling. Synthetic grafts lack adaptive structural cues, which impedes integration and regeneration. Cell-based tissue-engineered constructs that mimic native structures can actively adapt to guide remodeling, growth, and self-maintenance at the injury site. Blood vessels are highly organized, multilaminate tissues with complex three-dimensional spatial organization and 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 (ECM) secretion, which may in turn affect mechanical properties.

Methods: We modified UniFlex plates (Flexcell) with thermoresponsive N-isopropylacrylamide (NIPAAm) copolymers (Lee EL. J Biomed Mater Res A. 2010;93:411-8). NIPAAm allows cell attachment at 37°C; following a temperature decrease to room temperature, cells can spontaneously detach without using 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 (Williams C. Biomaterials 2011;32:5625-32). Confluent sheets of bovine vascular smooth muscle cells (BVSMCs) on non-patterned and patterned printed surfaces were stretched at 0% or 10% elongation for 24 hrs at 1 Hz. Cell sheets were subsequently stained and imaged with Alexa Fluor 488 phalloidin and Hoechst 33342 (Life Technologies). Cell orientation was analyzed using 2D fast Fourier transform and the Oval Profile plug-in in ImageJ and sampled every 6° to determine frequency distribution. Data was used to calculate anisotropy index, defined as maximum orientation distribution value divided by the expected distribution value for an isotropic sample based on sampling. Quantitative PCR and Western blotting were performed to determine if pre-alignment and mechanical conditioning increased production of ECM and contractile proteins (e.g., smooth muscle α-actin (α-SMA), calponin). One-way and two-way ANOVA, where p-values ≤ 0.05 were considered statistically significant, were performed.

Results: Patterning and mechanical conditioning both independently induced significant cell alignment (anisotropic index for non-patterned vs. patterned 1.28 ± 0.11 vs. 1.47 ± 0.13, $p < 0.0001$; static vs. 10% stretch conditioning 1.21 ± 0.06 vs. 1.33 ± 0.11, $p = 0.0001$); however, conditioning after patterning did not significantly increase anisotropy ($p = 0.71$). Mechanical conditioning was determined to be a significant independent factor in increasing contractile protein expression of α-SMA ($p = 0.003$), with greater significant influence on cells cultured on non-patterned surfaces ($p = 0.009$). In comparison, expression of calponin (regulatory protein that stabilizes actin) was significantly downregulated after conditioning only for cells cultured on patterned substrates ($p = 0.003$), which supports its role in allowing cytoskeletal reorganization.

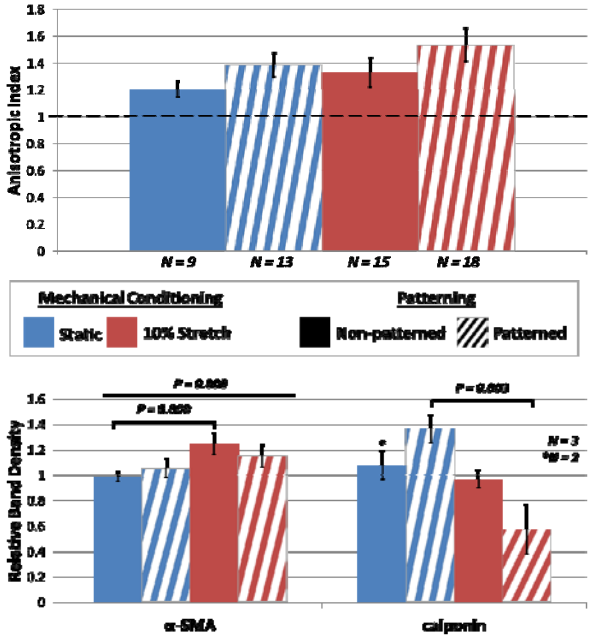

Figure 1. Effects of patterning and conditioning treatments on **(Top)** anisotropic index of cells, where values greater than 1 (perfectly isotropic, dashed line) indicate increased anisotropy, and **(Bottom)** contractile protein expression.

Conclusions: Controlling cell orientation at the lowest hierarchical structure level is critical because the structural organization can influence the resulting functional properties of a TEBV. We have shown that we can enhance control of cell orientation to improve alignment using substrates that can be both patterned for alignment and mechanically conditioned. Moreover, we have shown that contractile protein expression can be differentially modulated with prealignment and mechanical conditioning. Future studies will examine if we can prealign and mechanically condition cells to induce controlled protein and ECM expression and determine its impact on tissue mechanical properties.

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