The Effects of Mechanical Stimulation on Controlling and Maintaining Marrow Stromal Cell Differentiation into Vascular Smooth Muscle Cells

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Statement of Purpose: The construction of tissue engineered blood vessels (TEBV) is a promising solution for treating cardiovascular diseases. In order to attain the proper organization and mechanical properties found in native vessels, vascular smooth muscle cells (VSMC) are used as a crucial component of TEBVs. With the growing interest in stem cells, research has shown that marrow stromal cells (MSC) are a potential cell source for VSMCs due to their easier and less invasive isolation procedure, fast proliferation rates, and most importantly, the ability to differentiate into VSMCs. While current studies have provided evidence that mechanical stimulation can induce MSC alignment and differentiation into VSMCs, osteogenesis has also been observed when exposed to mechanical stimuli. A definitive set of lineage-inducing conditions must first be determined in order to successfully control the differentiation and potentially implement mechanical stimulation as a tool for TEBV fabrication. Following the identification of these conditions, a means of preserving the target cell type must also be developed to ensure permanent structural and mechanical properties as well as functionality.

Methods: Human MSCs were seeded at $1.0x10^4$ cells/cm^2 onto elastic PDMS substrates of 254 μm thickness treated with layer-by-layer polyelectrolyte deposition[1] and functionalized with fibronectin. Using a modified design of a uniaxial cell-stretcher device based on our previous work[2], we stretched the samples at 10% strain for 24 hours under the frequencies of 0.1, 0.5, and 1 Hz in the form of single-stage conditions. MSC lineage commitment was evaluated using quantitative RT-PCR targeting lineage specific genes for the VSMC and osteoblast phenotype and verified using immunocytochemistry. To study the potential of mechanically induced transdifferentiation, the two distinct lineage-specific frequencies determined previously were applied to our samples sequentially in the form of dual-stage conditions. Final phenotype evaluation was performed using the same methods.

Results: Our main interest was to determine the specific stretch frequencies that could be used to control MSC differentiation into either the VSMC lineage or the osteoblast lineage. qRT-PCR analysis showed that the expression of VSMC markers SM-α actin, SM-MHC, and calponin were significantly increased at the 1 Hz stretch frequency. Conversely, the expression of osteoblast markers osteocalcin and osteopontin were increased at the 0.1 Hz frequency and decreased at 1 Hz as shown (Fig. 1A). While there were no significant differences in gene expression levels between the 0.5 Hz and the 0.1 and 1 Hz conditions, statistically significant differences existed for all gene expression levels between the 0.1 Hz and the 1 Hz conditions. Fluorescence imaging confirmed these results, with a high expression of SM-α actin and osteocalcin for the 1 Hz and 0.1 Hz conditions respectively (Fig 2). Using both frequencies as lineage-specific conditions, qRT-PCR results for our transdifferentiation study showed a gene expression profile similar to the 1 Hz condition, indicating a VSMC phenotype. Fluorescent images were also similar to those seen under the 1 Hz condition in Fig. 2 and confirmed the qRT-PCR results.

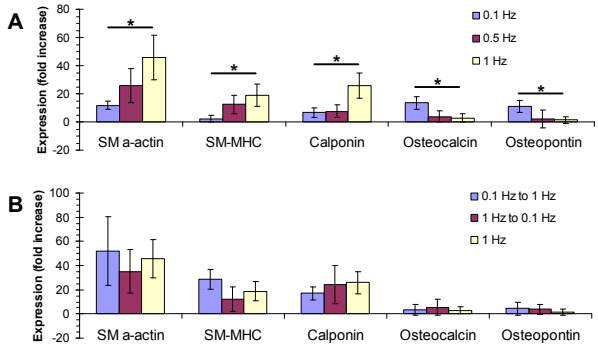

Figure 1. (A) Expression of lineage-specific genes showed MSC lineage commitment to the VSMC and osteoblast lineages under the 1 Hz and 0.1 Hz frequencies, respectively. (B) Gene expression under both dual stage conditions showed no statistically significant differences in all three conditions. Each bar represents the mean ± SD, n = 3.

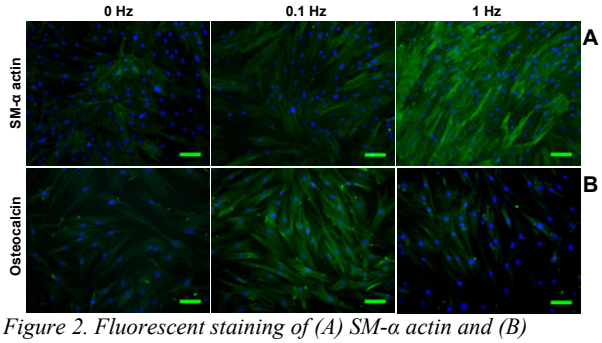

Figure 2. Fluorescent staining of (A) SM-α actin and (B) osteocalcin under single-stage conditions. Scale bar is 100 μm.

Conclusions: We found that at 10% strain, a 1 Hz frequency induced differentiation into the VSMC lineage while a 0.1 Hz frequency induced osteogenesis. Using these results, we proceeded to investigate the potential of MSC transdifferentiation through mechanical stimulation by applying the two distinct lineage-inducing conditions sequentially and evaluating the final cell type. We found that while mechanical stimulation was capable of inducing MSC transdifferentiation, it could only proceed in the direction toward the VSMC phenotype under the applied conditions. Our findings revealed the potential use of mechanical stimulation as a tool for not only controlling MSC differentiation, but also maintaining the cells at the VSMC phenotype for TEBV construction or other tissue engineering applications.

References: (1) Wipff PJ, et al. Biomaterials. 2009;**30**(9):1781-9. (2) Houtchens GR, et al. J Biomech. 2008;**41**(4):762-9.