Phase II – Biomechanics of Smooth Muscle Cell Differentiation: Experimental Study Using an Innovative *In Vitro* Mechanical System

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Smooth muscle cells (SMCs) controls involuntary contractions and express different genotypic and phenotypic traits on specific organs such as blood vessels, bladder and stomach. However, studies have shown different SMC lineages tend to gradually lose specific characteristics due to a static milieu without exerting forces that they would experience naturally when cultured *in vitro*. The research provided *in vivo* conditions are mimicked effectively *in vitro* by applying controlled mechanical loading, SMCs should express their differentiated characteristics. We have validated an innovative mechanical device that simulates the pulsatile stretching SMCs undergo in their *in vivo* environment. Using the new system and cell and molecular biology techniques, we are evaluating cell differentiation and strain induced alignment when phenotypically modulated SMCs undergo cyclic mechanical loading at 10 and 20 percent strains, for 4, 6, or 8 hours at physiological frequency. We collected proteins after stretch experiments and analyzed via western blot, α-actin, γ-actin, transgelin, and calponin protein expression changes in: coronary SMCs strained 10% and 20% at 4, 6, and 8 hours, bladder SMCs strained 10% at 4, 6, and 8 hours, and BAECs for varying intensities and durations. In order to improvise the machine capability, LabVIEW code is been developed as the user interface providing advantageous of Graphical Approach instead of Cool Muscle Language code. Developed coding provide a complete coverage of acquisition, analysis, reporting, and display features to create modern applications that can scale as system requirements change over time. The next phase of this experiment enable analysis of gene expression using quantitative RT-PCR (qRT-PCR). This facet of research may prove valuable in the analysis of the effect of mechanical stress on maintaining SMC lineage as well as the study of how pathological stretch conditions affect SMC and endothelial cell gene and protein expressions.

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