

## Mechanical and Biochemical Cues For Adipose Stem Cell Differentiation Into Heart Valve Interstitial Cells

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**Statement of Purpose:** Thousands of diseased heart valves are being replaced every year with non-living devices that resist no more than 15-20 years after implantation due to coagulation risks, endocarditis, degeneration, calcification and failure to grow and remodel. Our long-term objective is to develop living tissue-engineered valves that will last a life-time, will not be prone to complications, will have the ability to grow and remodel and thus ultimately impact thousands of patients. To accomplish this aim, we propose to develop collagen scaffolds that structurally and functionally mimic the aortic valves and populate them with adipose-derived mesenchymal stem cells. These cells exhibit properties similar to natural heart valve interstitial cells and thus we hypothesize that they may be empowered with the ability to differentiate into mature valve-like cells. Seeding heart valve scaffolds with autologous multipotent mesenchymal stem cells obtained from a convenient source would be extremely advantageous for development of patient-tailored heart valves. Our hypothesis is that adipose-derived stem cells have the capacity to differentiate into valvular cells as a response to mechanical and biochemical cues. The goal of this study was to subject cells to physiologic stretch regimes and transforming growth factors in a FlexerCell setup and evaluate cell differentiation at gene and protein level.<sup>1,2</sup>

**Methods:** Adipose tissue was collected from adult rat inguinal areas and transported to the lab in culture medium plus antibiotics. The FlexerCell system is a computer-regulated bioreactor that uses vacuum to apply cyclic strain to cells cultured on flexible-bottomed transparent culture plates. The vacuum used in the system yields up to 30% substrate elongation and applies a defined, controlled, static or cyclic deformation to cells growing *in vitro*. The system allows programming of multiple frequency, amplitude, and wave changes in one regimen with defined, static or cyclic deformation of cells. Adipose-derived stem cells ( $\sim 2.5 \times 10^5$  per well) will be seeded onto type I collagen and elastin pre-coated BioFlex culture plates<sup>3</sup>. After 3 days of culture, cells will be subjected to square waveforms at 0.6 Hz at forces that generate 14% stretch (physiologic levels for heart valves), using 12 wells for each condition, for up to 4 days. Controls will constitute of same numbers of cell-seeded plates incubated in static conditions (no stretch). Groups will be with or without TGF, collagen or elastin membranes, and static or dynamic for a total of 8 groups. Cell lysates were analyzed by gelatin zymography. Western blotting procedures were performed to analyze type I collagen and  $\alpha$ -actin content.

**Results:** Gelatin zymography studies showed that cells secrete increasing amounts of MMP's when subjected to physiological stretch (Figure 1).

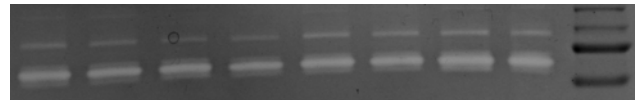
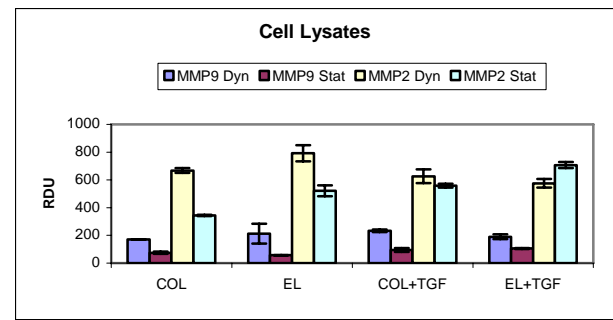


Figure 1. MMP Content

Western blot analysis of  $\alpha$ -actin showed a downregulation in the presence of stretch, but an overall upregulation from TGF with other factors constant (Figure 2).

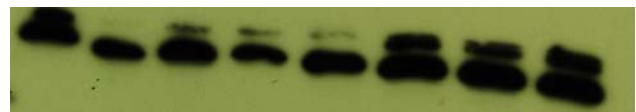
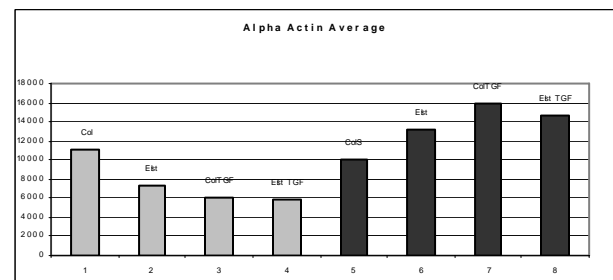


Figure 2.  $\alpha$ -Actin Regulation

Western blotting for type I collagen showed an increase under the effect of stretch.

Cell number was monitored among the groups and it was observed that the static control plates proliferated while the dynamic groups remained constant.

**Conclusions:** The increase of MMP and type I collagen content in the stretched cells indicates remodeling and is an indication of differentiation. Downregulation of  $\alpha$ -actin is also an indication of differentiation. The non-proliferative nature of the stretched groups is further evidence of differentiation. Physiological stretch and the presence of TGF may be the cues needed to differentiate adipose derived stem cells to advantageous interstitial heart valve cells.

### References:

1. Lee, WC et al. *Cells, Tissues, Organs* 183:80-86 (2006)
2. Gimble, JM et al. *Circulation Research* 100:1249-1260 (2007).
3. Ku, CH, Johnson PH, et al. *Cardiovascular Res.* Aug 1 2006;71(3):548-556.