Determining minimum strain which results in activation of the collagen-secreting pathway ERK in engineered-tissue

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Background

- Inducing uniaxial strain in connective tissue strengthens extracellular matrix of artificial valves
- Optimum strain regimen has yet to be determined
- We measure ECM strength which is correlated to collagen deposition, mediated by ERK pathway
- We hypothesize 1% strain will cause similar response as 5% strain (Experiment 1)
- We also must show the bioreactor is capable of 1% strain (Experiment 2)

Methods

Experiment Description

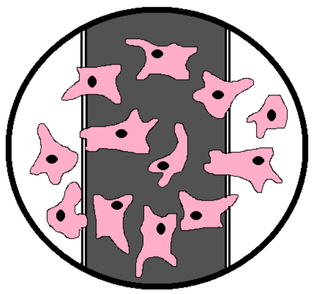


Fig. 1: Neonatal Human Dermal Fibroblasts (nHDFs) grown on Flexcell® mechanical stimulator

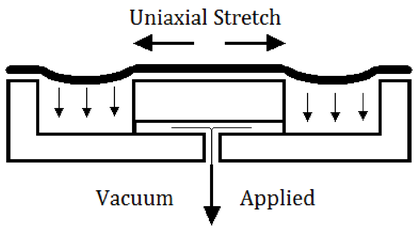
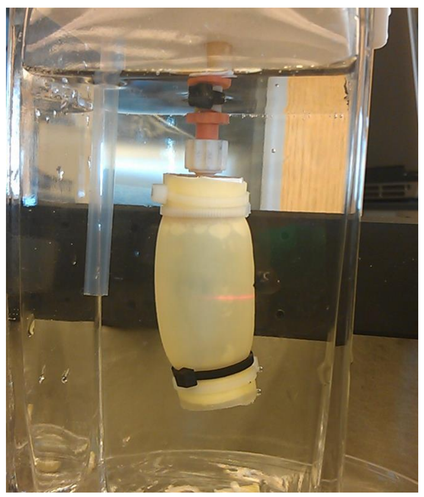


Fig. 2: Flexcell® uses vacuum pressure to induce strain in cells grown on silicone base

Fig. 3: Bioreactor used to strengthen engineered valves. Media pumped into construct distends latex sleeve. Diameter is measured with Laser Scanning Micrometer (LSM)



- nHDFs remodel fibrin to collagen and elastin
- After 15min stretch, tissue was analyzed with Western Blot for activation of signaling protein ERK, involved in collagen secreting pathway
- We also verified 1% strain was achievable using current bioreactor design

Results

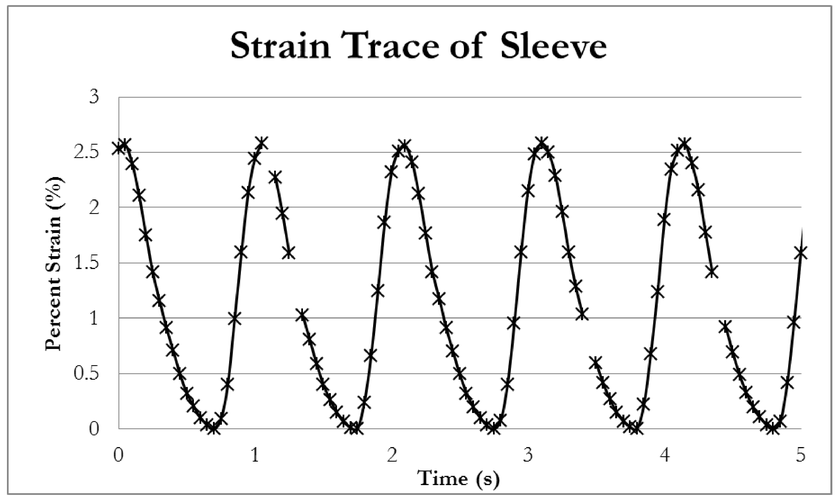


Fig. 6: Map of strain of latex sleeve over time as measured by LSM. Measurements are indicated by 'x's. Strain is a function of the diameter of the stressed construct over the unstressed construct.

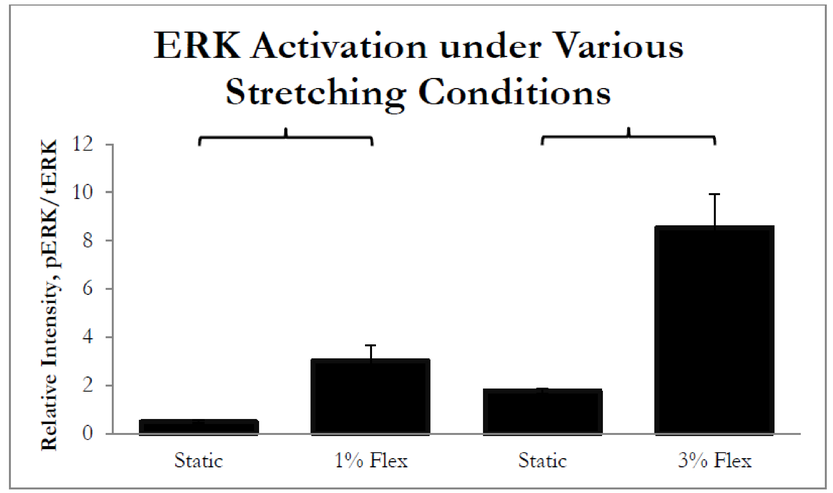


Fig. 5: Ratio of phosphorylated ERK (pERK) normalized to total ERK (tERK), indicating degree of ERK pathway activation. All samples normalized to B-Actin. Each ratio is the average of three samples. Error bars represent standard deviation. Statistical significance is indicated, and was determined by Student's T-Test, $p<0.05$

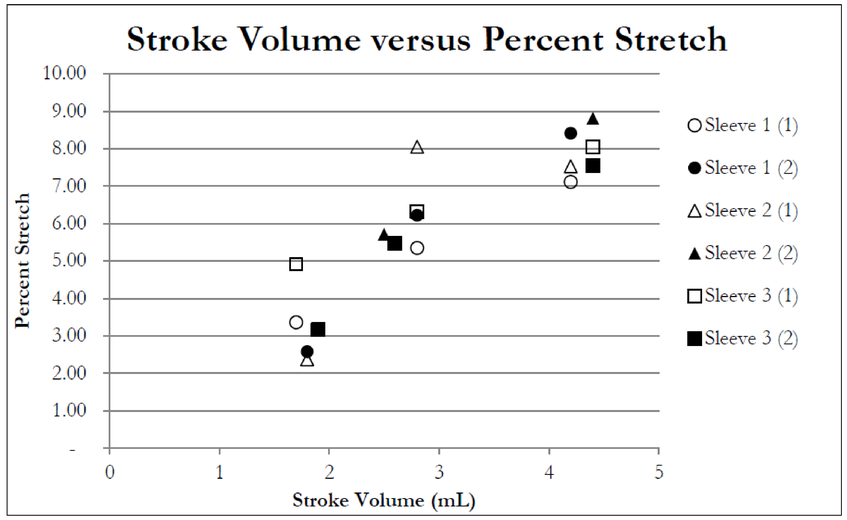


Fig. 7: Maximum strain in each sleeve displayed as a function of the volume pumped into each sleeve. Each dot represents one trial.

Discussion and Conclusions

- Current practice of stimulating engineered tissue with 5% strain is unnecessary, 1% is sufficient to activate collagen-secreting pathway.
- Further experimentation indicates that incrementing strain 1% (e.g. from 4% to 5%) elicits a similar response in the ERK pathway
- 1% strain is achievable by current bioreactor setup. Results also indicate the bioreactor can be relied upon to achieve similar strain on different tissue samples
- In the future, we can use these results to design a better regimen to stimulate collagen secretion, strengthening the heart valves

References

1 Syedain, Zeeshan H., and Robert T. Tranquillo. "Controlled cyclic stretch bioreactor for tissue-engineered heart valves." *Biomaterials* 30.25 (2009): 4078-4084.

2 Papakrivopoulou, Jenny, et al. "Differential roles of extracellular signal-regulated kinase 1/2 and p38MAPK in mechanical load-induced procollagen α1 (I) gene expression in cardiac fibroblasts." *Cardiovascular Research* 61.4 (2004): 736-744.

3 Syedain, Zeeshan H., and Robert T. Tranquillo. "TGF-β1 diminishes collagen production during long-term cyclic stretching of engineered connective tissue: Implication of decreased ERK signaling." *Journal of Biomechanics* 44.5 (2011): 848-855.

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