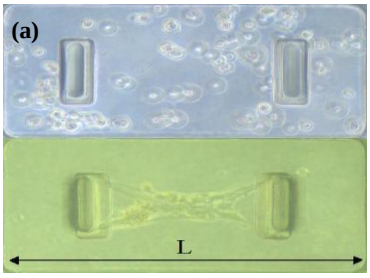
Introduction: Central to wound healing is fibroblast cells, which once activated to the myofibroblast phenotype are capable of contracting the wound size and repairing the tissue, though when unregulated, can cause pathological fibrosis.[1] Matrix stiffness and transforming growth factor beta (TGF-β) are known to regulate fibroblast activation to the myofibroblast phenotype; however, few studies have explored the effects of these two factors in concert, especially in a 3-dimensional (3D) environment. The aim of this study is to characterize the interactive effects of stiffness and TGF-β in a 3D collagen gel model system. It is hypothesized that the two factors will have a synergistic effect.

Materials and Methods: Small fibroblast-populated collagen gels were cultured between two flexible polydimethylsiloxane (PDMS) posts where the stiffness could be modulated by altering the dimensions and/or material properties. Micro-wells, each containing two cantilever posts with heads on top of the posts, were made by creating the negative construct of a template, generously donated by Dr. Christopher Chen (University of Pennsylvania), as described by Legant.[2] Different stiffness levels were attained by varying the PDMS base to curing agent ratio and baking time. For this study, two stiffness levels were used (0.811μN/μm and 4.27μN/μm) for two TGF-β levels (0ng/mL and 10ng/mL). The stiffness values chosen represent the largest possible range within the limits of the device and measurable deflections, and the TGF-β concentrations were chosen from relevant studies of myofibroblast activation in collagen gels.[1] Each of the constructs was filled with collagen gel containing fibroblast cells (final concentration 0.75mg/mL and 200,000 cells/mL, respectively). Pictures were taken as soon as the collagen gel polymerized at t = 0 hrs, and again at t = 41 hrs when the cells had formed a tissue around the posts and reached maximum contraction (Figure 1a). The post deflection was measured and used along with the stiffness value, and cell number (obtained by performing a Hoechst stain and counting the applicable nuclei) to calculate the force per cell using the following bending equation: $F=6\delta EI/a^2(3L-a)$. Myofibroblast presence was confirmed by staining for alpha smooth muscle actin, characteristic of the myofibroblast phenotype.[1]



(b) Force/cell for varying Stiffness & TGF-β

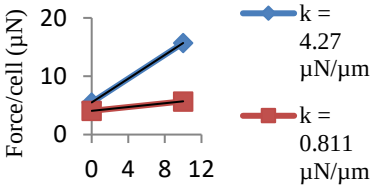


Figure (1a) (left) a well at time t = 0 hrs; (right) the same well at time t = 20 hrs (L = 800μm)
Figure (1b) Graph showing the relationship between stiffness, TGF-β, and the resulting force/cell

Results and Discussion: The cells generated the greatest force when treated with TGF-β in the stiff construct and the smallest force when not treated with TGF-β in the soft construct. The fact that the force/cell for the stiff construct containing cells treated with TGF-β was more than twice that for the cells in either the stiff construct without TGF-β or the soft construct with TGF-β implies that stiffness and TGF-β do have a synergistic effect, confirming our hypothesis (Figure 1b). There was no significant difference between the stiff construct without TGF-β and the soft one with TGF-β. Higher sample numbers will be achieved by improving well manufacturing techniques to yield more usable wells.

Conclusions: Increasing stiffness and TGF-β together increases force/cell much more than either factor alone, creating a powerful mechanism for generating the myofibroblast phenotype in tissue repair. Future experiments will include intermediate stiffness values and TGF-β concentrations to further quantify the relationship between the two factors providing data that could be used to manipulate the rate of fibroblast activation.

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References

1. Desmouliére, et al., 1993, *JCB*, v. 122.
2. Legant, et al., 2009, *PNAS*, v. 106.