

Tensional Homeostasis in an Invitro Wound Healing Model

Chelsea Spencer, and Melville Vaughan Ph.D.

University of Central Oklahoma

ABSTRACT

In normal wound healing conditions myofibroblasts, contractile cells, are found. During the aging process, healing of wounds is slow which could be from the inability of myofibroblasts. The tension generated from this is necessary in the healing process. Collagen lattices mimic the environment produced from cells that are under tension in a matrix. My prediction was that tension would be generated all the way to a maximum and then drop back down to match the days before. I used collagen lattice models to test day by day how tension homeostasis changes when given different amounts of time to generate. I have ran this experiment and it agreed with the results that were generated elsewhere. More experiments will be done to reassure these results. The results from the experiment coincides with the predictions made earlier. By understanding the changes in the tension generation by using collagen lattice models, we can understand the aging process and how long it could take for the aging generations to heal and also the way that this could occur.

INTRODUCTION

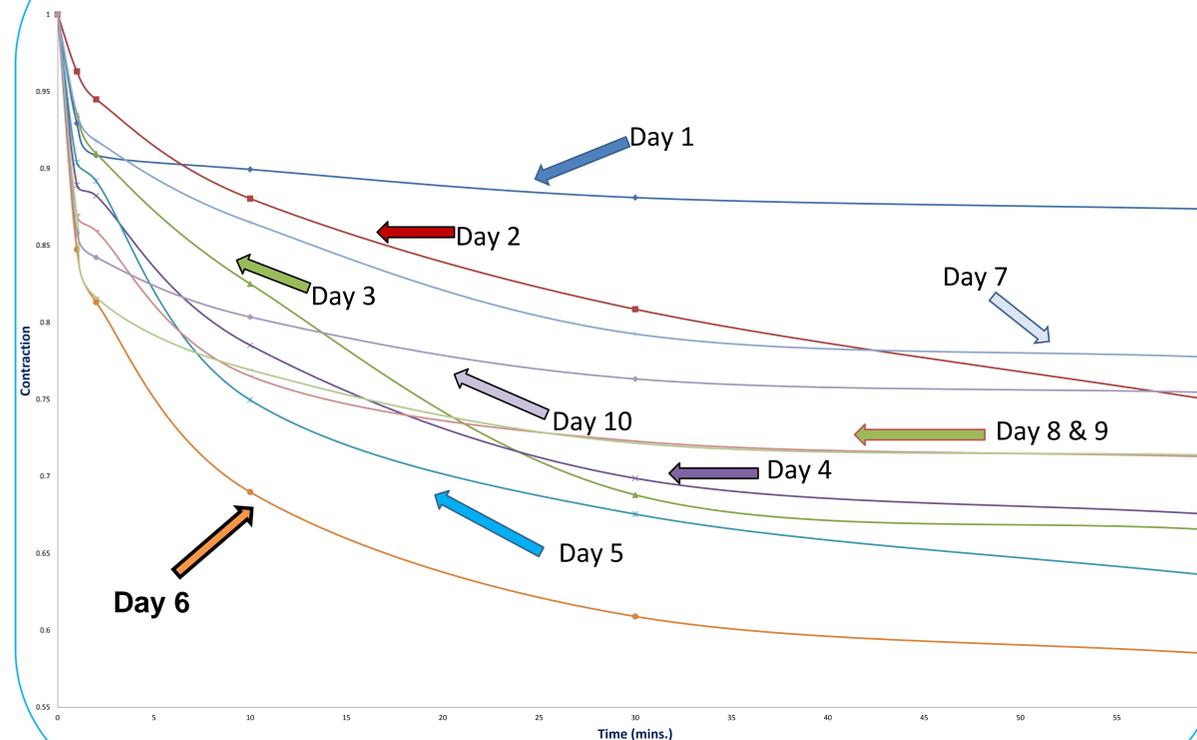
Fibroblasts can generate and maintain tension which can be measured indirectly by contraction after tension has been produced. Tension is important for the myofibroblast phenotype. The myofibroblast is important for wound healing, and might play a role in the reduced wound healing associated with aging. Wound healing is further complicated with age and tends to be decreased in older individuals; therefore there is great interest in how cellular aging may contribute to this difference. I plan to assess the tension that these cells make by performing experiments that generate a predictable amount of tension, as would be produced in the dermal of the skin, that will reach a maximum. Procedures that are used for this research are cell cultures and collagen lattice assays. The attached collagen lattice simulates a wound healing environment. I did not directly study aging cells, just a method that in the future can be used to predict how a cell can generate tension which could then be used in studying aging and wound healing. Predicting would healing ability would be crucial because then we can understand how long it takes an aging patient to heal from a wound and also how the healing process works. This could give head way to new predictions of how a person can heal and also determine the correct treatment to treat them with.

Materials and Methods

Collagen lattice assay: A mixture of Dupuytren's fibroblasts and type 1 collagen were plated on tissue culture plates. After one hour of incubation culture, media was added to the plates. Lattices were then incubated for 1, 2, 3, 4, 5, 6, 7, 8, 9, & 10 days. The initial lattice diameter was measured. Each lattice was released and measurements were taken at various time points (see graph below).

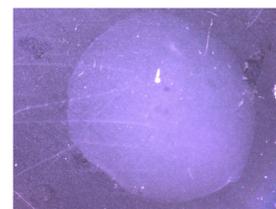
Contraction Graph

Contraction vs. Time

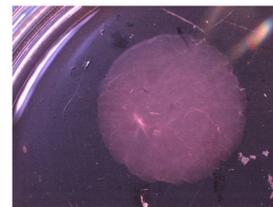


Timed Release Lattices

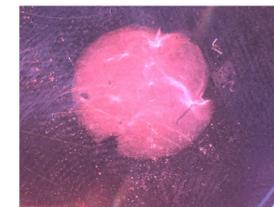
•These pictures are all after an hour of contraction. As you can see the day 6 lattice has contracted the most out of them all which shows that it has reached maximum tension.



1 Day



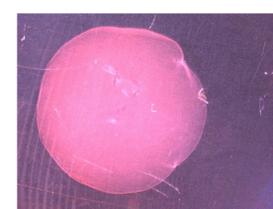
3 Days



6 Days



7 Days



9 Days



10 Days

Discussion and Conclusion

- Based on the contraction data as shown to the left, you can see that Day 6 lattices contracted the fastest in the least amount of time. This happened because 6 days is, we believe to be, the maximum amount of time for tension generation. As the experiment went from day to day, a visual observation was taken that in fact that lattices were getting smaller as the days went on.
- Another observation made was that at day 7 the lattice contraction jump to the same numbers as day 2 had. And after that, Day 8, 9, & 10 followed the same pattern of being around the same as day 2.
- Future studies will be to see how TGF- β affects the contraction from day to day.

References

- Ashcroft GS, Mills SJ, and Asworth JJ. "Ageing and wound healing." *Biogerontology* 3. (2002):337-345
- Eastwood, Mark, Rebecca Porter, Umraz Khan, Gus McGrouther, and Robert Brown. "Quantitative Analysis of Collagen Gel Contractile Forces Generated by Dermal Fibroblasts and the Relationship to Cell Morphology." *Journal of Cellular Physiology*. 166. (1996): 33-42. Print.
- Grinnell, F. "Fibroblasts, myofibroblasts, and wound contraction." *J. Cell Biology*. 124. (1994): 401-404.
- Grinnell, F. "Fibroblast biology in three-dimensional collagen matrices." *Trends Cell Biology*. 13(5). (2003):264-9.
- Grotendorst, Gary R., Hamed Rahmanie, and Matthew R. Duncan. "Combinatorial signaling pathways determine fibroblast proliferation and myofibroblast differentiation." *FASEB Journal*. 18. (2004): 470-479. Print.
- Peterson, Joanne L. "The Effects of Replicative Senescence and Telomerase on Contraction and Motility of Fibroblasts." MS thesis. University of Central Oklahoma, Edmond, 2009. Print
- Tomasek, James, Carol Haaksma, Robert Eddy, and Melville Vaughan. "Fibroblast Contraction Occurs on Release of Tension in Attached Collagen Lattices: Dependency on an Organized Actin Cytoskeleton and Serum." *Anatomical Record*. 232. (1992): 359-368. Print.
- Tomasek, J.J., G. Gabbiani, B. Hinz, C. Chaponnier, R.A. Brown. "Myofibroblasts and mechano-regulation of connective tissue remodelling." *Nat Rev Mol Cell Biology* 3. (2002):349-363.
- Vaughan, M.B., E.W. Howard, J.J. Tomasek. "Transforming Growth Factor- β 1 Promotes the Morphological and Functional Differentiation of the Myofibroblast." *Experimental Cell Research* 257. (2000):180-189.

Acknowledgements

- The authors would like to thank :
- Jessica Goddard and Jessnie Jose and the lab of Wei Chen.
 - OK-LSAMP Program for funding.
 - RCSA Grant Program for funding.