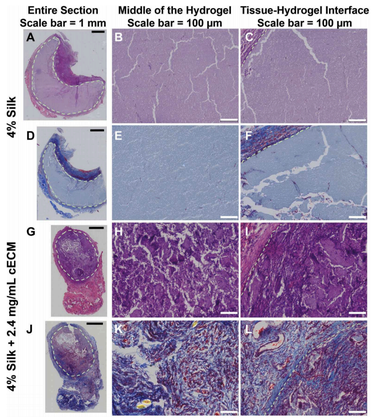
Using Physiologically Relevant Electromechanical Stimulation to Investigate Biological Mechanisms Responsible for Cardiomyocyte Hypertrophy

Ross Bretherton / Mentor: Whitney Stoppel / Black Lab

Intro / Clinical Need

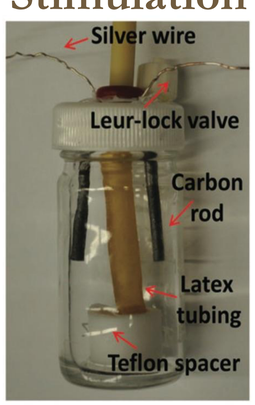
- Cardiovascular disease is the leading cause of death in US, has recently become the leading cause of death in the world as well
- During a myocardial infarction (heart attack), over 25% of the heart's cardiomyocytes (muscle cells) can die in only a few hours
- Scar formation post-infarct is initially beneficial, but leads to a cascade of compensatory mechanisms that eventually lead to Heart Failure (HF) and subsequently death
- There is a dire need for CVD treatments that are regenerative, allowing for the *in vivo* replacement of damaged tissue
- Realistic *in vitro* heart tissue models play a key role in elucidating cellular responses to the altered electrical and mechanical environment of diseased tissue

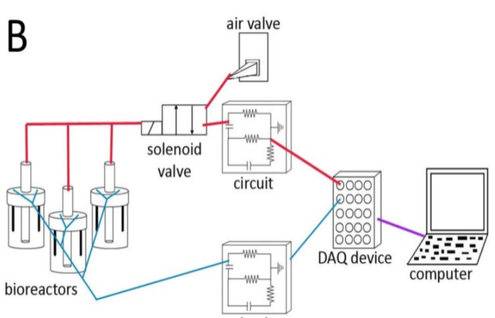
Silk cECM Hybrid Hydrogels for Cardiac Tissue Engineering



- Silk hydrogels provide a mechanically tunable, biocompatible and degradable platform for encapsulating cells
- Di-tyrosine crosslinking of silk fibers in the gel provide cellular attachment points
- The addition of cardiac extracellular matrix (cECM) to the hydrogels enhances cell infiltration and gel remodeling

Existing Bioreactor for Combined Electromechanical Stimulation

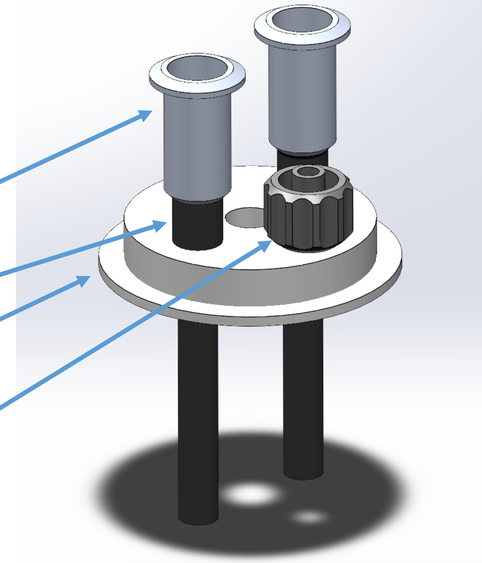




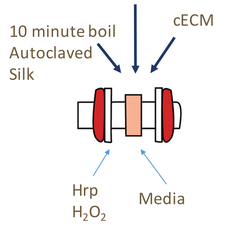
- 3D dynamic cell cultures can provide a more accurate in vitro model of cardiac disease, due to the effect of physical and electrical stimuli on a cell's phenotype.
- An existing bioreactor design in the lab provides radial stretch through a ring-shaped gel, and has been shown to affect cell communication and growth signaling in fibrin gels

Bioreactor Redesign

- Existing system was unfit for the longer-term experiments required to model heart disease.
- Redesign addressed two major problems with the existing bioreactor: lack of sterility, and ease of use
- Electrical connector caps increase durability, ease of use for carbon electrodes
- Threaded carbon electrodes lock into threaded ports in the redesigned cap, reducing risk of contamination
- Custom fabricated Teflon insert sits below existing thread, allowing the new cap to be used in the current system.
- Threaded air filter port doubles as a media exchange port, allowing researcher to change the media without removing the entire cap from the bioreactor.



Integration of Silk / cECM Hydrogels into Bioreactor

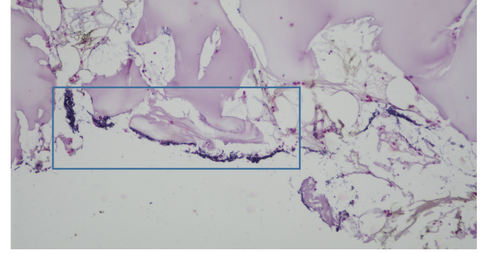


Conditions

- Static
- Mechanical or electrical stimulation only
- Combined M&E stimulation
- cECM added
- No cECM
- Consistent stimulation regime
- Variable stimulation regime

Output

- Western Blot
- Immunofluorescence
- Dynamic Mechanical Analysis
- Twitch force



Progress

- Heterogeneous gelation produces poor cell encapsulation in molds
- Difficult to control pH
- Need to optimize gelation time for proper cell encapsulation

Future Work

- Develop a protocol for differentiating induced Pluripotent Stem Cells (iPSCs) within the bioreactor system
- Fabricate new bioreactor parts
- Incorporate a variable stimulation regime to mimic periods of activity and rest in the heart
- Model cardiovascular disease by disrupting the frequency/amplitude/delay of the bioreactor

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References

Engler, A. J., Sen, S., Sweeney, H. L., & Discher, D. E. (2006). Matrix elasticity directs stem cell lineage specification. Cell, 126(4), 677-689. doi:10.1016/j.cell.2006.06.044

Laflamme, M. A., Zbinden, S., Epstein, S. E., & Murry, C. E. (2007). Cell-based therapy for myocardial ischemia and infarction: Pathophysiological mechanisms. Annual Review of Pathology-Mechanisms of Disease, 2, 307-339. doi:10.1146/annurev.pathol.2.010506.092038

Segers, V. F. M., & Lee, R. T. (2008). Stem-cell therapy for cardiac disease. Nature, 451(7181), 937-942. doi:10.1038/nature06800

Rubart, M., & Field, L. J. (2006). Cardiac regeneration: Repopulating the heart. Annual Review of Physiology, 68, 29-49. doi:10.1146/annurev.physiol.68.040104.124530
The Top Ten Causes of Death. (2012). Fact Sheets. Retrieved from http://www.who.int/mediacentre/factsheets/fs310/en/

Mozaffarian, D., Benjamin, E. J., Go, A. S., Arnett, D. K., Blaha, M. J., Cushman, M., . . . Stroke Stat, S. (2016). Heart Disease and Stroke Statistics-2016 Update A Report From the American Heart Association. Circulation, 133(4), E38-E360. doi:10.1161/cir.0000000000000350

Huebsch, N., Arany, P. R., Mao, A. S., Shvartsman, D., Ali, O. A., Bencherif, S. A., . . . Mooney, D. J. (2010). Harnessing traction-mediated manipulation of the cell/matrix interface to control stem-cell fate. Nature Materials, 9(6), 518-526. doi:10.1038/nmat2732

Stoppel, W. L., Gao, A. E., Greany, A. M., Partlow, B. P., **Bretherton, R. C.**, Kaplan, D. L., & Black, L. D., III. (Accepted 2016). Elastic, silk-cardiac extracellular matrix hydrogels exhibit time-dependent stiffening that modulates cardiac cell response. Journal of Biomedical Research part A.

Kundu, B., Rajkhowa, R., Kundu, S. C., & Wang, X. (2013). Silk fibroin biomaterials for tissue regenerations. Adv Drug Deliv Rev, 65(4), 457-470. doi:10.1016/j.addr.2012.09.043
Stoppel, W. L., Hu, D., Domian, I. J., Kaplan, D. L., & Black, L. D., 3rd. (2015). Anisotropic silk biomaterials containing cardiac extracellular matrix for cardiac tissue engineering. Biomed Mater, 10(3), 034105. doi:10.1088/1748-6041/10/3/034105

Kim, U. J., Park, J., Li, C., Jin, H. J., Valluzzi, R., & Kaplan, D. L. (2004). Structure and properties of silk hydrogels. Biomacromolecules, 5(3), 786-792. doi:10.1021/bm0345460