In Vitro Three-Dimensional Engineered Cardiac Tissue Mechanical Stimulation Platform

CNF Project Number: 2619-17
Principal Investigator(s): Jonathan Butcher
User(s): Gaetano Scuderi

Affiliation(s): Biomedical Engineering Department, Cornell University
Primary Source(s) of Research Funding: National Institutes of Health
Contact: jtb47@cornell.edu, gjs95@cornell.edu
Website: http://www.butcherlab.com/
Primary CNF Tools Used: Objet30 Pro 3D printer, Parylene coater, VersaLaser engraver/cutter tool

Abstract:

People who suffer from heart failure have low survival rates since no clinically available cardiac regeneration therapy exists. However, one promising approach is the implantation of tissue engineered myocardium, but due to their immaturity they do not lead to any significant heart function improvements. To improve the maturity of the engineered cardiac tissues (ECT), researchers have attempted to mimic *in vivo* mechanical stimuli. However, current systems have major limitations that lead to only modest improvements in ECT maturity. Therefore, our objective was to develop a superior *in vitro* cardiac tissue platform and mechanical stimulation bioreactor system to ultimately study how various mechanical stimuli affect ECT maturity. Here, we demonstrate the successful development of a novel *in vitro* platform and bioreactor system. We hope to apply this novel system to develop more mature ECTs that can be translated clinically as a viable cardiac regeneration option.

Summary of Research:

In the United States, 750,000 Americans suffer from heart attacks annually with 16% of all cases leading to mortality [1]. Due to the inability for adult mammalian hearts to regenerate, the infarcted region becomes a fibrotic, non-contractile tissue which often leads to congestive heart failure [2]. The gold standard treatment option is heart transplantation, which has major limitations such as an inadequate supply, high risk of donor heart rejection/failure, and need for lifelong immunosuppressants.

Currently, no viable clinical cardiac regenerative therapy is available to restore heart function following a heart attack [3]. One promising cardiac regeneration approach involves the implantation of tissue engineered myocardium to restore heart function. Current tissue engineered myocardium, however, has been unsuccessful at restoring heart function due to the tissue's immature phenotype that has low survivability and poor host integration in adult hearts [3].

Therefore, researchers have turned to investigating ways to improve tissue engineered myocardium maturity that will better integrate with the host tissue. Mechanical stimulation utilizing mechanical anchorage to induce passive tension, passive stretch, and dynamic cyclic stretch have all shown to lead to modest improvements in engineered cardiac tissue (ECT) maturity [4]. However, the current bioreactor systems have many limitations. All systems that dynamically stretch their ECT to mimic *in vivo* mechanical stimuli utilize stiff anchorage points, which prevents the tissues from producing work and can lead to an upregulation of pathological fibrosis/hypertrophic signaling [5]. Likewise, these bioreactor systems often use non-physiological stretching regimens that do not accurately recapitulate normal cardiac development, where a progressive increase in cardiogenic stretch patterns occur to lead to intrinsic maturity improvements [3].

For this project, our objective was to develop a high-throughput *in vitro* ECT platform system and mechanical stimulation bioreactor that allows for the following: 1) anchorage points that allow for contraction and work production, 2) mechanically stretch over a wide range of strain magnitudes (0-50%) and rates (1-5 Hertz), and 3) high-throughput stimulation of 48 ECTs with varying strain magnitudes simultaneously.

Our *in vitro* ECT platform required precise manufacturing of an intricate geometric mold and therefore we turned to Cornell NanoScale Facility's Objet30 Pro 3D printer. This design provided the ability to cast polydimethylsiloxane (PDMS) post constructs that serve as nearly perfectly

elastic bioinert anchorage points. PDMS serves as a reliable candidate for ECTs due to its elastic properties, which allows the tissues to contract/deflect the material and therefore produce work [3]. Our ECTs can be cultured on these PDMS constructs (Figure 1).

This PDMS post design allows us to control the bending stiffness that the tissue experiences by restricting any z-direction movements during contractions and serves as a means for the tissue to be stimulated by deflecting the post from above the tissue using a customized mechanical stimulation bioreactor. We validated our platform's ability to create functional ECTs by culturing primary fetal chick cardiac cells on our post platform and compared them to unloaded (non-anchored) control tissues. The loaded ECTs demonstrated aligned cardiomyocytes and non-cardiomyocytes along the axis of tension while the unloaded controls had no inherent alignment (Figure 2).

Our custom high-throughput uniaxial mechanical stimulation bioreactor system for stimulating 48 tissue constructs on a standard 48 well plate was developed using the Objet30 Pro 3D printer (Figure 3). The system uses a hybrid external stepper motor linear actuator. As the linear actuator translates, it moves a customized 3D printed part attached to a steel rod that is attached inside the customized 3D printed box that sits on top of the 48-well plate. A tab grid system then slides along the 3D printed box's linear track and deflects the PDMS posts just above the tissues to apply a stretch. The amount of stretch can easily be modulated using an Arduino Uno system. Likewise, different tab grid systems have been developed that are offset by certain lengths and thus can apply various strains ranging from 0-50% across the 48-well plate simultaneously.

Our next steps are to test out our design by culturing various ECTs and stretching our tissues with varying stretch regimens to determine what types of regimens provide the greatest improvements in cardiac tissue maturity.

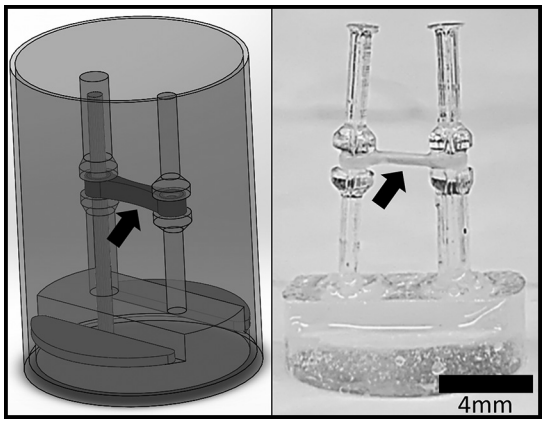

Figure 1: SolidWorks schematic of PDMS construct (left) and fixed ECT after one-week culture on PDMS construct (right). Black arrows point to ECTs.

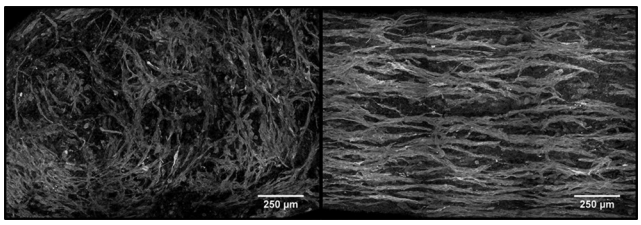

Figure 2: Whole mount immunohistochemical staining of unloaded (left) and loaded (right) ECTs stained for non-cardiomyocytes (red), cardiomyocytes (green), and nuclei (blue). (See pages vi-vii for full color version.)

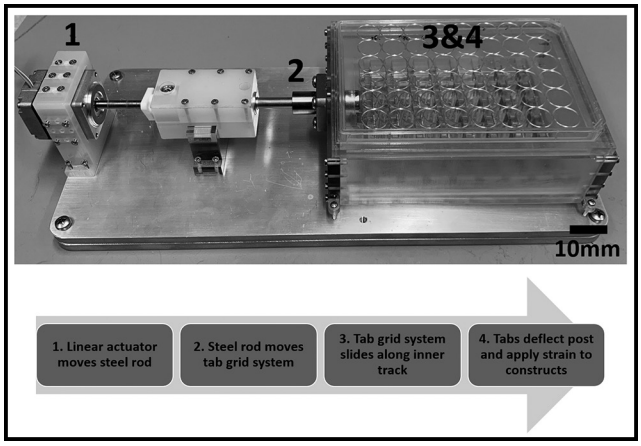

Figure 3: Bioreactor (top) and flow chart of bioreactor operation (bottom).

References:

[1] Mozaffarian, D., et al. Heart disease and stroke statistics—2016 update a report from the American Heart Association. Circulation 133, e38-e48 (2016).

[2] Alcon, A., Cagavi Bozkulak, E., and Qyang, Y. Regenerating functional heart tissue for myocardial repair. Cellular and Molecular Life Sciences 69, 2635-2656 (2012).

[3] Scuderi, G., and Butcher, J. Naturally engineered maturation of cardiomyocytes. Frontiers in Cell and Develop. Bio. 5, (2017).

[4] Radisic, M., et al. Biomimetic approach to cardiac tissue engineering. Philos. Trans. R. Soc. B Biol. Sci. 362, 1357-1368 (2007).

[5] Leonard, A., et al. Afterload promotes maturation of human induced pluripotent stem cell derived cardiomyocytes in engineered heart tissues. J. Mol. Cell. Cardiol. 118, 147-158 (2018).