Disease Specific Cardiac Tissue Models for Drug Discovery and Toxicology

Anurag Mathur[1, 2], Peter Loskill[1, 2], SoonGweon Hong[1], Zhen Ma[1, 2], Nathaniel Huebsch[4], Micaela Finnegan[1, 2], Sivan G Marcus[1], Natalie C Marks[1, 2], Bruce R Conklin[4, 5], Luke P Lee[1, 3], Kevin E Healy[1, 2]

1. Department of Bioengineering and California Institute for Quantitative Biosciences (QB3), UC Berkeley, Berkeley, CA, United States
2. Department of Materials Science and Engineering, UC Berkeley, Berkeley, CA, United States
3. Department of Electrical Engineering and Computer Science, UC Berkeley, Berkeley, CA, United States
4. Gladstone Institute of Cardiovascular Disease, Gladstone Institutes, San Francisco, CA, United States
5. Department of Medicine, Division of Genomic Medicine, University of California at San Francisco, San Francisco, CA, United States

Introduction: There is an urgent need in pharmaceutical industry to effectively and efficiently screen potential drug compounds during early stages to assess both effectiveness and toxicity. Now, with the advent of human induced pluripotent stem (iPS) cells[1, 2], we can create a "patient-specific" physiologically functioning *in vitro* model of multiple tissues, including those critical for drug screening and toxicity testing. In this work we present an integrated cardiac system *in vitro*, which mimics critical tissue features of the "minimal tissue element" of the heart, such as anisotropic cellular architecture, tissue perfusion, and coordinated cell-cell associations essential for normal physiologic functions. Our model represents a significant advancement for understanding, studying, and developing new drugs and strategies for treating diseases (e.g., cardiac arrhythmias and other cardiomyopathies).

Methods: A microphysiological platform, consisting of 3 functional components: 2 µm wide "endothelial like" barriers, 30 µm wide "capillary like" media channels, and 100 - 200 µm wide cell culture channels, was fabricated using soft lithography. The fabrication process was done in three steps: i) 2 µm thick SU-8 2001 was patterned on silicon wafer to define endothelial-like barriers that connect media channel with the cell culture channel, ii) 30 µm thick SU-8 2035 was then patterened to fabricate media channel and cell culture channel, iii) finally, a silicone elastomer was poured into the SU-8 silicon master and devices were fabricated using replica molding. The dimensions of the master and the mold were characterized using optical and electron microscopy. Geometry of the endothelial-like barriers ensures diffusive transport through the cell culture channel, whereas a continuous flow through the capillary channel provides convective transport in the media channel. Human iPS cells were differentiated into cardiomyocytes (CMs) by small molecule control over Wnt signaling,[3] and then seeded in fibronectin coated cell channels. Media was continuously perfused through the nutrient channel and brightfield microscopy was performed every 24 hours. For drug screening applications, beating CMs were exposed to different concentrations of a cardiac stimulant, Isoproterenol.

Results: We have developed and characterized a microphysiological platform that organizes the alignment of iPS cell derived CMs into a three-dimensional beating microtissue and can be used for drug screening. The platform is able to create a functional cardiac tissue with physiological beat rates (60-80 beats/min). The alignment and three-dimensional structure of the microtissue was verified using confocal microscopy. Furthermore, using motion tracking software developed in-house, we quantified the alignment of cell contraction. Functionality of the platform was validated by testing the effect of different concentrations (100 nM and 1µM) of Isoproterenol, which yielded a physiological response. Specifically, after 10 min of 1µM Isoproterenol exposure the beat rate doubled from 50 beats/min to 100 beats/min.

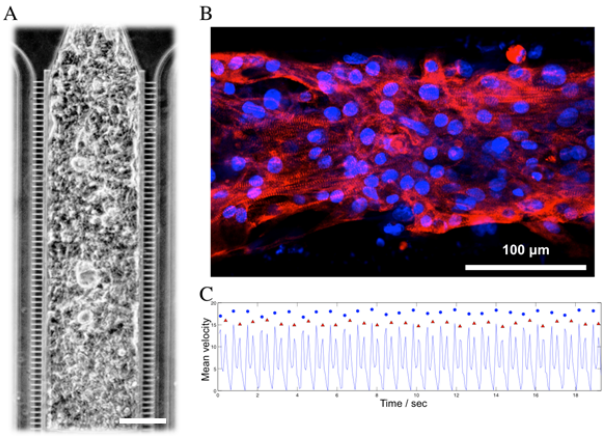

Figure 1. Functional cardiac microtissue in the platform. (a) Brightfield image of iPS derived CMs in the cell channel. Scale bar: 100 µm. (b) Aligned microtissue in the platform stained for sarcomeric alpha actinin (red) and nucleus (blue). (c) Quantification of beat rate in the platform.

Conclusions: We have developed and characterized a microphysiological platform that aligns iPS cell derived CMs into a beating microtissue and can be used for drug screening. Testing the effect of Isoproterenol, a cardiac stimulant, validated the platform. This platform is extremely versatile and can be used to screen drugs for therapeutic and diagnostic applications.

References:

1. (Takahashi K. *Cell* 2007, 131:861-872.)
2. (Yu JY. *Science* 2007, 318:1917-1920.)
3. (Lian XJ. P Natl Acad Sci USA 2012, 109:E1848-E1857.)