Contractile abnormalities induced by biomechanical~~Biomechanical~~ mismatch in human iPS-based cardiac microtissues ~~created by laser-fabricated artificial 3D scaffolds~~

Zhen Ma[1], Sangmo Koo[2], Plansky Hoang[1], Nathaniel Huebsch[3], Bruce Conklin[4], Costas Grigoropoulos[2], Kevin Healy[3].
[1]Department of Biomedical & Chemical Engineering, Syracuse Biomaterials Institute, Syracuse University;
[2]Department of Mechanical Engineering, University of California, Berkeley,
[3]Department of Bioengineering, University of California, Berkeley,
[4]Gladstone Institute of Cardiovascular Disease, University of California, San Francisco

Statement of Purpose: The extracellular matrix (ECM) forms the structural backbone of the heart and contributes ~~to~~ passive stiffness ~~to the~~for myocardial function. An important hallmark of maladaptive hypertrophy, myocardial infarction (MI) and heart failure is cardiac fibrosis, which is characterized by an increase in collagens in the interstitial regions of the myocardium. The severity of interstitial fibrosis closely correlates with the extent of ventricular hypertrophy and impaired ejection fraction. Excessive forms of collagen deposition or fibrosis affects myocardial compliance ~~characteristics with a resulting~~by ~~increase in~~increasing myocardial stiffness. Furthermore, the late phase of post-MI remodeling results in ECM deposition that tethers viable cardiomyocytes (CMs) and thereby forms a substrate to resist deformation during the cardiac cycle. This post-MI remodeling process is a clinically significant problem, which leads to ventricular dilation and dysfunction, and finally progression to heart failure.

In this study, we established a 3D filamentous cardiac model based on the reconstitution of synthetic models of human ventricular myocardium with populations of CMs derived from human induced pluripotent stem cells (hiPS-CMs). These synthetic fibers served as the backbone ~~of the~~to organize a 3D cardiac microtissue. ~~that mimicked the perimysial collagen fibers of myocardium . We fabricated synthesized the fibers with different diameters to mimic the collagen thickening during fibrosis.~~ By positioning fibers with different diameters together within one matrix, we created a biomechanical mismatch region that could potentially mimic the biophysical properties of border zone of post-MI tissue remodeling.

Methods: We used two-photon polymerization (TPIP) to create filamentous matrices with three layers of parallel fibers, consisting of synthetic parallel fibers with different fiber diameters (i.e., 5 μm and 10 μm)[1]. To create the biomechanical mismatch region to mimic the border zone of post-MI tissue remodeling, we fabricated the hybrid matrices by positioning the 5 μm and 10 μm diameter fibers one next to each other (x um separation) to create a mismatch region in the middle of each matrix. Because of constant elastic modulus of the fibers, the thicker fibers have a higher mechanical resistance to cardiac tissue contraction than do the thinner fibers. We ~~grew~~ cultured hiPS-CMs on the filamentous matrices to form cardiac microtissues, which could bend the fiber, so that we could calculate the contraction force based on fiber deflection and . ~~We recorded the cardiac microtissue beating at 100 frames per second for 10 seconds using Hamamatsu ORCA-Flash4.0 V2 digital CMOS camera, and~~ analyze the contractile motion~~d~~ using in-house developed motion-tracking software based on MATLAB[2]. ~~The software can automatically output the motion heatmap and contraction waveform for calculation of beat rate and maximal contraction velocity.~~

Results: ~~We seeded the~~The seeded hiPS-CMs ~~into the hybrid matrix to form~~formed a 3D cardiac microtissues across the entire TPIP polymerized matrix (Figure 1a). At Day 20, we found that the cardiac microtissues could synchronously contract with higher contraction velocity within the low stiffness region compared to the high stiffness region (Figure 1b). In contrast, cardiac microtissues ~~growing~~ cultured on hybrid matrices showed higher contraction forces and larger tissue cross-section at the high stiffness region ~~than~~ compared to the low stiffness region ~~(Figure 1c)~~. This ~~phenomenon~~ observation suggested that the entire cardiac microtissues had to self-balance the force generation between high and low stiffness regions for synchronized contraction. Such force equilibrium resulted in the tissue hypertrophy at the high stiffness region with high tissue cross-section (Figure 1c). Furthermore, by calculating the local contraction velocity within the mismatch region, we found that the tissues had higher contraction along the X-axis within mismatch region of low stiffness, whereas higher contraction along the Y-axis within mismatch region of high stiffness (Figure 1d). This indicated that biomechanical mismatch induced the local uncoordinated cardiac microtissue contraction, although no significant difference of sarcomere organization was showed across this region.

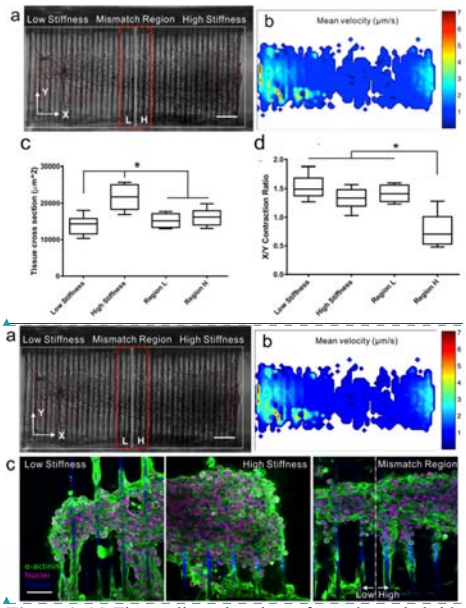

Figure 1. (a) The cardiac microtissue formed on a hybrid matrix fabricated with 5 μm fibers and 10 μm fibers one next to each other. (b) The contraction heatmap showed higher contraction velocity on the region within the low stiffness region. (c) tissue hypertrophy at the high stiffness region resulted in larger tissue cross-section. (de) Region H within mismatch region had lowest ratio of contraction velocity between X-axis and Y-axis~~The confocal microscopic images showed the sarcomere structure of cardiac microtissues within the low stiffness region (left), high stiffness region (middle) and mismatch region (right)~~.

Conclusions: We established an *in vitro* model of 3D human cardiac tissue with a region of biomechanical mismatch that could potentially mimic~~ked~~ the biophysical properties of border zone ~~of~~ in the post-MI fibrosis. We found that biomechanical mismatch induced the local uncoordinated cardiac microtissue contraction, which potentially leads to ventricular dysfunction, and finally progression to heart failure.

References: [1]Ma Z. Biomaterials. 2014;35(5): 1367-77. [2]Huebsch N. Tissue Eng Part C Methods. 2015;21(5): 467-79.

Formatted: Font: 10 pt

Formatted: Font: 11 pt, Bold