3D Cardiac Microtissues with Integrated Force Sensors for Non-Invasive Readout of Contractile Force

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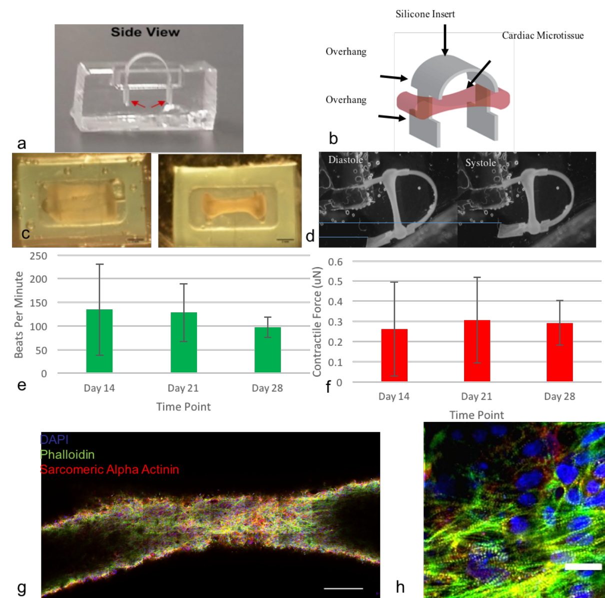
Statement of Purpose: Heart disease is the leading cause of death worldwide, with nearly 2300 Americans dying of cardiovascular disease every day [1]. In cardiac disease, cardiomyocytes either die or become dysfunctional, with the end result being decreased contractile force of the heart and eventual heart failure. Recent efforts have focused on the engineering of 2D cardiac tissues integrated with PDMS elastomer films, termed muscular thin films (MTF), which have enabled development of in vitro disease models [2, 3]. Here we have expanded upon the previous MTF platform by developing a 3D cardiac muscle microtissue with an integrated PDMS strip to serve as a force sensor to noninvasively monitor contractile function. Our preliminary studies demonstrate that the cardiac microtissues integrated with a PDMS strip, formed from human embryonic stem cell derived cardiomyocytes, respond to electrical pacing and chronotropic drugs. Our long-term goal is to use this 3D cardiac tissue system to study how mutations in desmosomes, mechanical junctions formed by plaques between cells, affect cardiac function using patient-derived induced pluripotent stem cells [4].

Methods: PDMS micromolds with rectangular wells were fabricated and designed to hold a laser-cut, PDMS strip around which the cardiac microtissues would compact (Figure a,b). To fabricate the 3D cardiac microtissues, human embryonic stem cells, were differentiated and purified into cardiomyocytes following standardized protocols [5, 6]. Following purification, cardiomyocytes and cardiac ventricular fibroblasts (Lonza: Houston, Texas) were mixed within a Collagen I Gel (Corning: Auburn, Michigan) with 20% v/v Matrigel™ (Corning: Auburn, Michigan) and cast in the wells. Media was changed every two days during culture and cardiac microtissues were removed from wells at days 14, 21 and 28 to monitor beat frequency and contractile force. Tissues were also assessed for their ability to respond to positive ionotropic (epinephrine and isoprenaline) and negative ionotropic (verapamil) drugs. All contractile measurements were performed on a heated stage to maintain the temperature at (37°C±1°C). After functional tests, the cardiac tissues were fixed, cleared and then imaged via microscopy for collagen, nuclei, F-actin and sarcomeric α-actinin.

Results: Phase contrast microscopy was used to image 3D cardiac tissues in the PDMS molds and showed that the tissue compacted rapidly over the first 7 days, forming a cylindrical condensed tissue that was able to contract and visibly deform the PDMS strip (Figure c, d). The spontaneous contraction frequency (Figure e) decreased and contractile force (Figure f) became more uniform with increased culture time (n=5 for 14, n=6 for 21, and n=8 for 28 day timepoint). Cardiac microtissues responded to pacing at 2 Hz and to positive and negative chronotropic drugs. Microscopy demonstrated spreading of cardiomyocytes and fibroblasts within the microtissue (g, scale bar=200 um). High magnification images showed organized sarcomere alignment along the outside of the cardiac microtissue (h, scale bar=10 um).



Conclusions: Cardiac microtissues were reliably formed with integrated silicone inserts. Microtissues were able to significantly bend the small silicone insert and force measurements were obtained. Beat frequency and tissue contractile force appeared to become more uniform with increased culture time. Tissues were also able to respond to pacing as well as positive and negative chronotropic drugs. This suggests that these microtissues can mimic cardiac tissues and that our technology can be used as a platform to model disease in iPS-derived cardiomyocytes.

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