In Vitro 3D Wound Platform to Characterize Myocardial Infarct Healing

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Introduction: Myocardial infarction (MI) is a major societal burden affecting ~800,000 Americans each year[1]. It occurs when coronary blood flow is obstructed and downstream myocardial tissue undergoes ischemic damage. As necrotic cardiomyocytes (CM) are degraded and removed, cardiac fibroblasts (cFB) infiltrate the infarct zone and deposit extracellular matrix for wound repair and scar formation. A critical regulatory signal for post-MI cell behavior is the local deformation, but, unfortunately, current in vitro platforms used to investigate cardiac cell physiology do not fully recreate heterogeneous deformation environment of the infarcted ventricle in which the infarct zone undergoes cyclic tension while the remote, uninfarcted myocardium undergoes cyclic contraction. We have developed a 3D coculture system that subjects engineered heart tissues (EHTs) to a cryo-wound, which successfully induces post-MI deformations and cellular distributions.

Materials and Methods: Whole heart tissue was harvested from 1-3 day old Sprague-Dawley rat pups. Following extraction, cFB and CM were isolated through industry standard protocols. A co-culture of CM and cFB were pelleted at a ratio of 10:1 and a final concentration of 1 million cells/EHT. The cells were then resuspended in 112.5ul 0.44 U/mL thrombin and 112.5ul 1.1 mg/mL fibrinogen per EHT. This solution is pipetted into a well containing PDMS pillars and polymerized for 90 mins at 37°C. After 3 days, an electrical stimulus of 5V at 1Hz was applied using a biphasic waveform with a 2ms duration. After 4 days, a 1mm-diameter metal rod was dipped into liquid nitrogen and held against the tissue for 30 seconds, creating a cryo-wound spanning the full depth of the tissue and ~2,000µm wide. At 1h pre/post cryo-wound, a video of the EHT was run through DIC image analysis to create 2-D Lagrangian strain maps. EHTs were fixed at 1h, 1d, 3d, and 7d post-cryo-wound for immunofluorescence staining of CM (cardiac troponin-1 antibody) and cFB (fibroblast-specific protein-1).

Results and Discussion: Prior to wounding, EHTs showed consistent contraction under pacing (Fig 1A). We observed substantial changes in the EHTs following cryo-wound injury, which results in three distinct regions: infarct (wounded center), healthy (between infarct and posts on both sides), and border (transition zone between infarct and healthy). DIC revealed the infarct zone axial deformation changed from contractile to tensile (n=24 EHTs) with concentrated contractile behavior in the healthy region. IF staining of CM (Fig 1C) shows successful region-specific injury post-wound with no recovery over 7 days. CF staining (Fig 1D) shows initial injury successful infiltration into the wound area by 1 day.

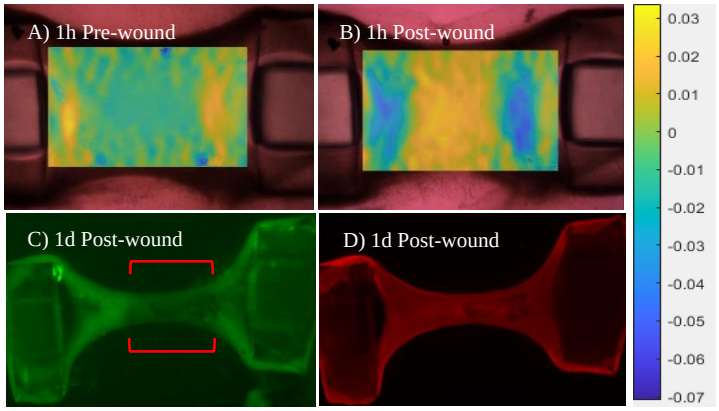

Figure 1: Axial strain map of pre-wound (A) and post-wound (B) EHTs. Immunofluorescence with CM-specific (C) and cFB-specific (D) markers.

Conclusions: We show biomechanical differences between pre- and post-infarct environments, introducing a heterogeneous mechanical environment representative of infarct, border, and healthy remote zones in the infarcted left ventricle. This is paralleled by CM/cFB death and cFB infiltration/remodeling into the infarct zone over several days. Our in vitro wound model allows for investigation into spatial and temporal components of MI at the tissue-level with cell-scale resolution and provides a platform for possible drug screening and therapy applications. Future studies will further characterize tissue remodeling while pacing up to 14 days, expand staining to include secreted matrix proteins, and better differentiate the cell signaling, morphology, and function across the three distinct regions.

1. Centers for Disease Control and Prevention. Underlying Cause of Death, 1999–2018. CDC WONDER Online Database. Atlanta, GA: Centers for Disease Control and Prevention; 2018. Accessed March 12, 2020.