Characterizing Downstream Mechanisms of *LMNA*-Associated Dilated Cardiomyopathy Using a Myocardial Tissue-Slice Model

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Abstract:

LMNA-associated dilated cardiomyopathy (LMNA-DCM) is a genetic cardiac disease caused by mutations in the LMNA gene, which encodes the nuclear envelope proteins lamin A/C. Although LMNA mutations are known to disrupt nuclear structure and mechanotransduction, the downstream mechanisms connecting these defects to cardiac dysfunction remain poorly understood. A common hallmark of LMNA-DCM are misshapen or damaged nuclei, which we hypothesize drives disease pathology. Nuclear damage causes the release of nuclear content, exposure of DNA to the cytoplasm, DNA damage, and cellular stress through a myriad of pathways, but we do not know which specific pathways drive disease progression, if any.

We hypothesize that nuclear envelope damage causes sarcoplasmic reticulum (SR) stress, altering calcium handling and protein homeostasis. Initial experiments investigated the response of isolated cardiomyocytes to SR stress using thapsigargin and tunicamycin. However, a three-hour treatment period was insufficient to produce a robust stress response, highlighting the limited experimental window of isolated cardiomyocytes. To address this limitation, we developed a murine myocardial tissue-slice model that preserves native cardiac environment and cellular interactions while allowing tissue to remain viable for longer periods. This model provides a platform for longer term imaging and controlled stimulation, enabling investigation of disease mechanisms over time. Future studies will use the model to examine calcium dynamics and stress responses in LMNA-mutant tissue and determine whether altered calcium handling and SR stress contribute to LMNA-DCM progression.

Summary of Research:

To investigate potential downstream effects of LMNA-associated cardiomyopathy, we first examined whether inducing SR stress in isolated cardiomyocytes was sufficient to induce pathways previously identified in a transcriptomics study. Wild-type cardiomyocytes were treated with the SR stress-inducing compounds thapsigargin (5 μM) and tunicamycin (5 μM or 10 μM) for three hours, and changes in ER/SR-associated proteins were measured. While the experiment provided an initial indication that SR stress may affect protein expression, the three-hour treatment did not produce a strong enough response to draw definitive conclusions.

This result highlighted an important limitation of the isolated-cell approach. Three hours may not be long enough for downstream changes in protein expression and calcium handling to develop. At the same time, substantially extending the treatment could cause excessive cellular stress and cell death. Isolated cardiomyocytes have a limited lifespan and lose many of the structural and cell-cell interactions present in intact cardiac tissue. Together, these limitations make it difficult to study processes that develop over longer timescales.

To address this, we developed a myocardial tissue-slice model that allows cardiac tissue to be maintained in culture for up to 48 hours while preserving its native architecture. Thin sections (250 μm) of mouse myocardium retain cardiomyocytes and surrounding heterogeneous cellular interactions and extracellular matrix structures, providing a more physiologically relevant environment than isolated cells.

We developed a 3D printed custom mounting system to hold the myocardial slices during culture and imaging. The three-part mounting system is designed to keep the tissue under constant tension, and the addition of electrical pacing lets us mimic the electrical and mechanical environment of the heart. We developed a pacing device to provide electric stimulation to the tissue. The pacing device consists of a 3D printed lid to

a 6 well plate that allows us to hang graphite electrodes down into the tissue media. Because the electrodes need to be extremely conductive but not affect the tissue sample, we coated the graphite in PEDOT:PSS, 5% v/v DMSO, and 1% GOPS. The combination of these chemicals prevents particles from dissociating into the media while retaining maximum conductivity. This platform will allow us to move beyond short, endpoint experiments and instead monitor changes in cardiac tissue longitudinally.

This model directly addresses the limitations of the initial three-hour experiment. Instead of exposing isolated cardiomyocytes to prolonged stress until they lose viability, the tissue-slice system provides a longer experimental window in which we can monitor how cardiac tissue responds to SR stress. This will allow us to determine whether changes that were not detectable after three hours emerge following longer treatment and whether those changes differ between WT and LMNA-mutant tissue.

Ultimately, the model provides a foundation for studying calcium dynamics, cellular stress responses, and disease progression longitudinally in a more native cardiac environment.

Conclusions and Future Steps:

The initial SR-stress experiments demonstrated the limitations of using isolated cardiomyocytes for studying processes that may require longer periods in culture. Rather than treating this limitation as an endpoint, we developed a myocardial tissue-slice model designed to provide a longer experimental window while preserving native cardiac structure and cellular interactions.

The resulting platform establishes a foundation for longitudinal studies of LMNA-DCM. Future work will first optimize tissue-slice culture and imaging conditions to establish consistent tissue viability and measurements over time. We will then perform longer SR-stress experiments to determine whether prolonged treatment produces measurable changes in stress-response pathways and calcium handling.

Future experiments will use Fluo-4 calcium imaging to compare calcium dynamics between WT and LMNA-mutant tissue. Additional markers of ER stress, including phospho-PERK, will help determine whether prolonged SR stress activates cellular stress pathways. The model can also be used to investigate how mechanical stimulation influences these responses.

Ultimately, the goal is to use this platform to determine whether altered calcium handling and SR stress are downstream consequences of LMNA-associated nuclear dysfunction and whether these changes contribute to the progression of LMNA-DCM. By enabling longer-term observation in a more native cardiac environment, the myocardial tissue-slice model provides a new approach for studying disease mechanisms that cannot be adequately captured using short-term experiments in isolated cardiomyocytes.

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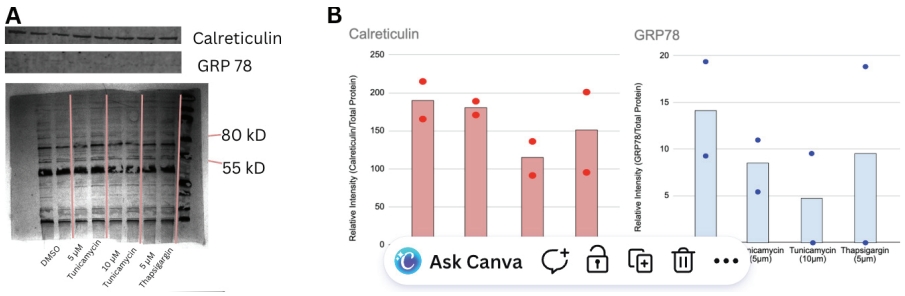

Figure 1: (A) Representative Western blot, proteins of interest on top, total protein below. (B) Analysis showing decreases in calreticulin (left) and GRP78 (right) with higher concentrations of positive control SR stress drugs Tunicamycin and Thapsigargin. Note that all cells treated with 10µM Thapsigargin died before the 3 hour treatment period ended.

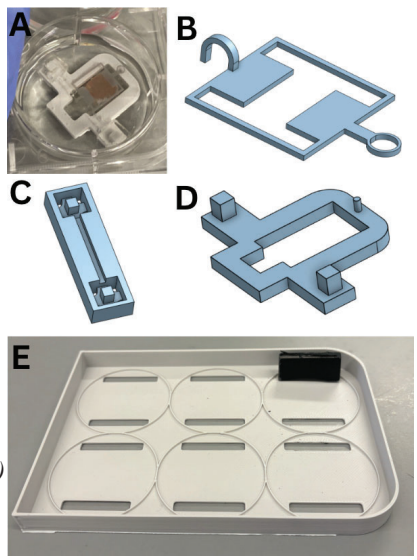

Figure 2: A) Sample loaded into the full device in a 6 well plate. (B) 3D rendering of the tissue mount. (C) 3D rendering of mold used to cast the tissue stretching band. Use 1:10 PDMS to get correct tensile strength. (D) 3D rendering of the base design used to hold the band and keep the mount level for imaging. (E) 3D printed lid for 6 plate well that allows electrodes to hang down into the media. Graphite electrodes are coated in PEDOT:PSS (a highly conductive polymer) with 1% m/v GOPS and 5% v/v DMSO to prevent graphite particles from breaking off into the media.