An Electro-Mechanical Bioreactor for Engineering Diseased Cardiac Tissue Models

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Statement of Purpose: Here we report on the development of a bioreactor capable of controlling the electro-mechanical environment for engineering disease models of cardiac tissue. The bioreactor was designed to allow for non-destructive analyses during tissue culture, which are important for understanding and controlling the effects of bioreactor parameter variation, e.g. of electrical and mechanical stimulation (Radisic M. Philos Trans R Soc Lond B Biol Sci. 2007;362:1357-1368), in the development of cells into 3D tissue constructs that exhibit disease-state characteristics

Methods: A T-75 flask was modified to allow for access to four tissue samples, linear actuation using a LabView controlled stepper motor, and electrical stimulation via a custom-built stimulator. Coverslips were attached to the underside of the T-75 flask to allow for confocal imaging and force sensors were magnetically-coupled to measure force-displacement during tissue culture. Force sensors were characterized for sensitivity, linearity, drift, and repeatability using extension springs to mimic tissue constructs. For preliminary tissue culture experiments, porous polyurethane (PU) scaffolds (n=4) were seeded with human foreskin fibroblasts and subjected to 10% strain for 8 hr/day. Acellular scaffolds served as control. Cells were supplemented with TGF-β on day 8. On day 14, tissue constructs were stained with FDA/PI and tri-labeled for α-SMA, F-actin and nuclei.

Results: The bioreactor was built from off the shelf components, modified off the shelf components, and easily reproducible custom built parts (Fig. 1).

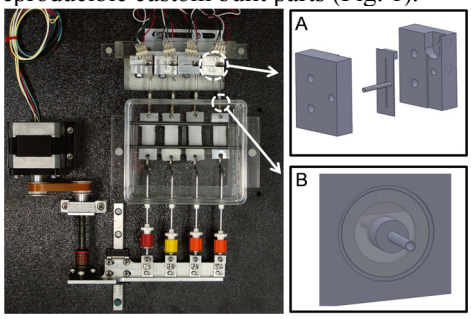

Figure 1. Assembled bioreactor. Sensor housing (A) and magnet-diaphragm assembly (B).

Sensors exhibited high linearity with sensitivities ranging from 7.79 x 10^{-3} to 9.87 x 10^{-3} mV/V/g (Fig. 2). Sensors maintained force readings for extended periods of time and returned to the same force reading for a given displacement (data not shown).

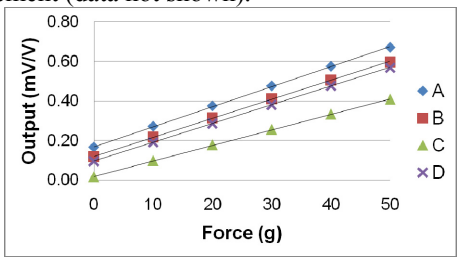

Figure 2. Sensor characterization.

In preliminary tissue culture experiments, constructs exhibited higher stiffness compared to controls ($p<0.05$) on days 10 to 14 (Fig. 3).

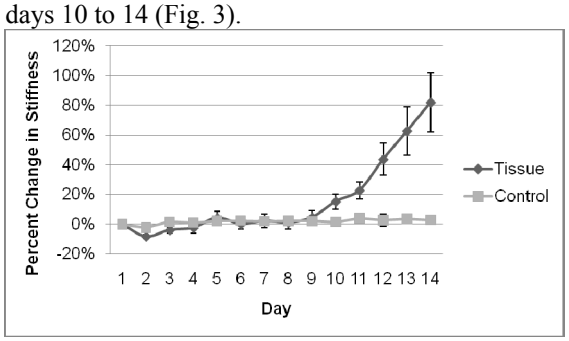

Figure 3. Percent change in stiffness of tissue constructs and acellular controls from day 1. Error bars denote standard deviation.

Scaffolds showed high cell coverage with 100% observable cell viability and fibroblasts stained positive for α-SMA and F-actin (Fig. 4).

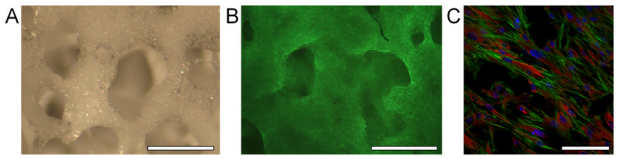

Figure 4: PU scaffold before cell seeding (A). Live/Dead staining (B) showing living cells green and immunofluorescence (C) of α-SMA (red) counterstained with phalloidin (green) and DAPI (blue) after 14 day culture experiment. Scale 400 µm (A, B) and 100 µm (C).

Conclusions: We have developed a bioreactor assembled from a combination of standard and modified off the shelf components and easily reproducible custom parts. The system is capable of controlling the electro-mechanical environment and non-destructively characterizing tissue constructs. In addition to common non-destructive characterization techniques, such as analyzing protein synthesis and gene expression, this system is designed to allow for confocal microscopy of living tissue and measurement of force-displacement relationships. These analyses will allow for real-time manipulation of tissue engineered constructs, which we suggest are important for producing disease tissue models. Benchtop testing and preliminary tissue culture experiments verified the bioreactor's ability to control the mechanical environment while interrogating mechanical properties of engineered tissue to distinguish between experimental groups.

A key feature of the bioreactor system is the ability to translate tissue force measurements from within the bioreactor to an external sensor. To this end, we developed a magnet-diaphragm assembly that allows the transduction of force across a sealed barrier.

Future studies will use confocal imaging techniques as previously described by our group (Lasher RA. IEEE Trans Med Imaging. 2009;28:1156-1164) to non-destructively characterize neonatal cardiomyocytes during development into functional and diseased tissue models.