Biowire™ II Matured Human Engineered Cardiac Tissues Have Adult-Like Responses to Inotropic Agents

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ABSTRACT

Heart Failure (HF) can be induced by a range of factors including genetics and acquired risk factors. It can also be an unwanted consequence of treatment by various drugs in clinical usage (REF). The need remains for novel model systems to support HF drug discovery and cardiac safety testing of candidate drug therapies.

The Biowire™ II platform was designed to use human induced pluripotent stem cell (hiPSC)-derived cardiomyocytes to generate 3D engineered cardiac tissues that faithfully mimic human physiology and enable assessment of contractility and electrophysiology. Following a 10-week electrical and mechanical stimulation protocol the electrical, calcium handling and contractile machinery of the tissues was evaluated.

The Biowire™ II tissues displayed adult-like properties. The shape of the action potential was ventricular-like, the resting membrane potential decreased to -80mV and the upstroke velocity increased to ~180mV/ms. We assessed post-rest potentiation and found the force increased as the rest period increased from 0-60s, suggesting appropriate calcium handling. A positive force-frequency relationship was observed from 1-4Hz.

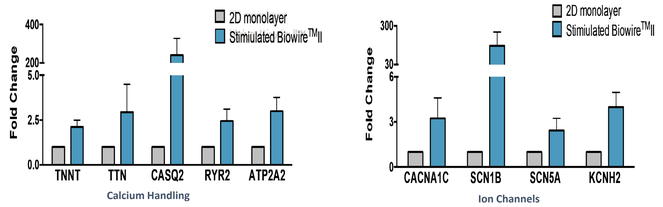
Signaling pathways known to elicit inotropic responses in the human heart were present in the Biowire™ II tissues. Isoproterenol, a β-adrenergic agonist, elicited a 7- to 10-fold increase in contractile force; milrinone, a phosphodiesterase-3 inhibitor, a 4-fold increase; pituitary adenylate cyclase-activating polypeptide, an adenylyl cyclase activator, a 2-fold increase. Nifedipine and FPL64176 L-type calcium channel effectors elicited inhibition and activation respectively. Increased contraction was also observed with digoxin, an inhibitor of the Na^+-Ca^{2+} exchanger, the myosin activator omecamtiv, the troponin-C Ca^{2+} sensitizer levosimendan, endothelin-1 or insulin growth factor-1. Finally, treatment with doxorubicin and H_2O_2 decreased the contractile force, consistent with their known cardiotoxic effects.

Taken together, we have demonstrated adult-like electrical, calcium handling and contractile function of the Biowire™ II tissues, and a capacity to respond to inotropic agents with a variety of different mechanisms of action. The Biowire™ II platform is therefore capable of generating physiologically-relevant, adult-like contractile measurements for drug discovery applications.

INTRODUCTION

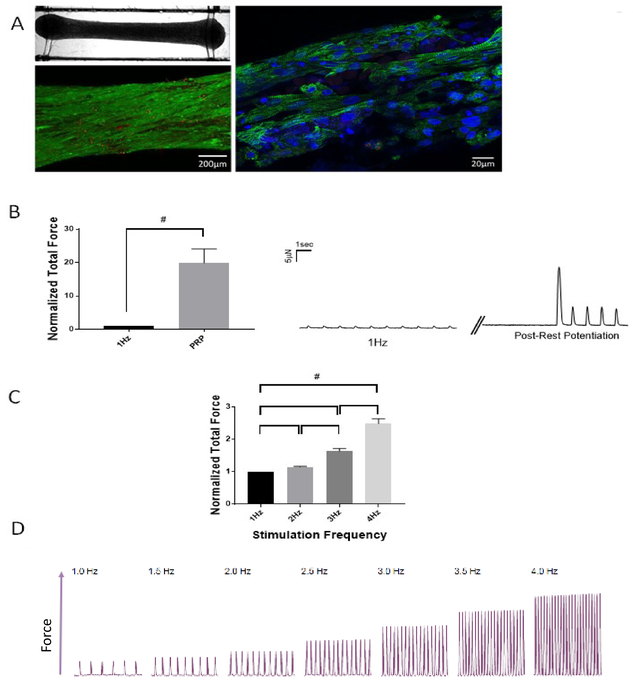
- Unanticipated cardiotoxicity remains a significant cause of discontinuation of drugs in development and clinical use.
- There remains a need for human preclinical models that better assess to the cardiac toxicity of potential drug therapies.
- Recent development of robust cardiac differentiation protocols for human induced pluripotent stem cells (hiPSCs) holds the promise of an unlimited supply of human derived cardiac cells from both healthy and diseased sources. That promise has been tempered by the observation that hiPSC-derived cardiomyocytes (hiPSC-CMs) typically retain a more fetal-like phenotype, raising concerns about the predictability and translatability of results.
- The Biowire™ II platform was designed to generate 3D engineered cardiac tissues from hiPSC-CMs and cardiac fibroblasts. Electro-mechanical stimulation is employed to create tissues that have an "adult-like" phenotype.
- The Biowire™ II platform enables non-destructive contractility measurements of the 3D engineered cardiac tissues.
- The tissues produced have adult-like electrical, calcium handling and contractile properties. In addition, we show that contractility in the tissues can be modulated by a variety of agents know to affect essential intracellular signaling pathways present in the human myocardium.

BIOWIRE™ II-MEDIATED STIMULATION RESULTS IN UPREGULATION OF GENES REQUIRED FOR EXCITATION-CONTRACTION COUPLING



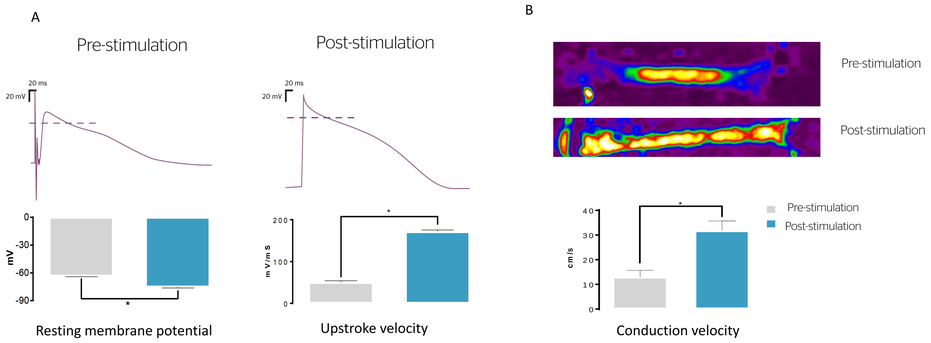
RT-PCR analysis of calcium handling *(left)* and ion channel *(right)* genes in Biowire™ II stimulated tissues or hiPSC-CMs prior to tissue formation. Data represent mean ± standard deviation from 3 independent tissues/cultures (SD; n=3).

BIOWIRE™ II TISSUES HAVE ADULT-LIKE ELECTRICAL, CALCIUM HANDLING AND CONTRACTILE PROPERTIES



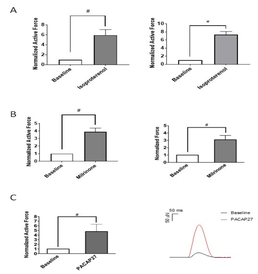
A. A representative image of the suspended tissue in the Biowire™ II platform (*top left*), of live/dead staining of the tissue (green, live, Calcein-AM; red, dead, Ethidium homodimer-1; *bottom left*), and of the sarcomere alignment (green, troponin-T; red, Ki67; blue, DAPI; *right*). **B.** The tissues had a significant post-rest potentiation (PRP) (19.9 ± 4.2-fold, n=4, student's ttest). A representative trace is shown on the right. **C.** A positive force-frequency relationship was observed as a significant increase in force from 1 to 4Hz (2Hz, 1.15 ± 0.01-fold; 3Hz, 1.63 ± 0.08-fold; 4Hz, 2.48 ± 0.15-fold; n=4, one-way ANOVA). **D.** A representative trace of positive force frequency is shown. Data are presented as mean ± SEM. # P < 0.05.

BIOWIRE™ II-MEDIATED STIMULATION RESULTS IN THE DEVELOPMENT OF AN ADULT-LIKE ACTION POTENTIAL AND IMPROVED CONDUCTION VELOCITY



A) Representative intracellular recordings of intact Biowire™II under 1 Hz field stimulation is shown on top. The action potential of the matured Biowire™II tissue is distinguished by more negative mean diastolic potential (MDP) and faster upstroke velocity summarized on bottom. **B)** Representative heat map of impulse propagation in Biowire™II tissue using the voltage sensitive dye di-4-ANEPPS is shown on top with summary data shown on the bottom.

BIOWIRE™ II TISSUES DISPLAY EXPECTED RESPONSES TO CLINICAL STAGE COMPOUNDS WITH DIVERSE MECHANISMS OF ACTION.



	CONTRACILITY RESPONSE
DILTIAZEM	↓ rate, ↓ force
FPL 64176	↑ force
LIDOCAINE	↓ rate, ↓ force
DIGOXIN	↑ force
LEVOSIMENDAN	↑ force
OMECAMTIV	↑ force
MILRINONE	↑ force
EPINEPHRINE	↑ force
ENDOTHELIN-1	↑ force
PITUITARY ADENYLATE CYCLASE-ACTIVATING POLYPEPTIDE	↑ force
INSULIN-LIKE GROWTH FACTOR-1 (96 h)	↑ force
HYDROGEN PEROXIDE	↓ force
DOXORUBICIN (12 h)	↑ force
DOXORUBICIN (96 h)	↓ force
VERAPAMIL	↓ force
ISOPROTERENOL	↑ force, ↑ calcium transient amplitude
NIFEDIPINE	↓ force, ↓ calcium transient amplitude
THAPSIGARGIN	↓ force, ↓ calcium transient amplitude

A. Treatment with 100nM isoproterenol induced a 5.9 ± 1.1-fold increase in contractile force using image analysis (*left*, n=5) and 7.3 ± 0.8 (*right,* n=8) using a force transducer in an organ bath. **B**. Treatment with 100μM milrinone induced a 4.2 ± 0.4-fold increase in force (n=6) using image analysis (*left*) and 3.1 ± 0.3-fold (n=4) u sing the organ bath (*right*). **C**. Treatment with 100nM pituitary adenylate cyclase-activating peptide (PACAP) induced a 4.8 ± 1.7-fold increase in contractile force (n=4). Data are presented as mean ± SEM. # P < 0.05 using paired t-test. **D**. Summary of effects on contractility response to compounds with different mechanisms of action.

Conclusions

- Engineered cardiac tissues formed and subjected to electro-mechanical stimulation in the Biowire™ II platform develop functional characteristics of contractility approaching that seen in adult human myocardium.
- The Biowire™ II platform can detect both positive and negative inotropes.
- Data presented validate the Biowire™II platform as a human model for testing the safety of drugs in preclinical development and for the discovery of novel therapies.