Creating and Characterizing Engineered Heart Tissue Strips Using Human iPSC-derived Cardiomyocytes

Human iPSC-derived cardiomyocytes (iPSC-CMs) have emerged as a powerful research tool for modeling human myocardial biology. Applications include basic scientific research, disease modeling, and drug development. Even as the usage of iPSC-CMs continues to grow in these areas, measuring the mechanical function and properties of iPSC-CMs in a physiologically meaningful context remains challenging.

Cardiac tissue engineering methods can be used to form iPSC-CMs into myocardial-like constructs. However, existing techniques require specialized expertise and months of effort to deploy in new laboratories. Mechanical testing of engineered heart tissue requires additional expertise and fragile, multi-component systems.

We report here the development and validation of a new system[1] designed to overcome the traditional challenges involved in making and characterizing iPSC-CM-based engineered heart tissues (EHTs). This system is comprised of two basic components: (1) pre-made decellularized myocardial scaffolds that can be reliably seeded with iPSC-CMs to form EHTs, and (2) an instrument into which EHTs can be placed for comprehensive mechanical testing.

Methods

The process of obtaining contractility data from iPSC-CMs begins with following a published method[2] to produce tissue scaffolds (Fig. 1A). Porcine left ventricular myocardial tissue was cryosectioned, laser-cut, and decellularized using a salt buffer solution and

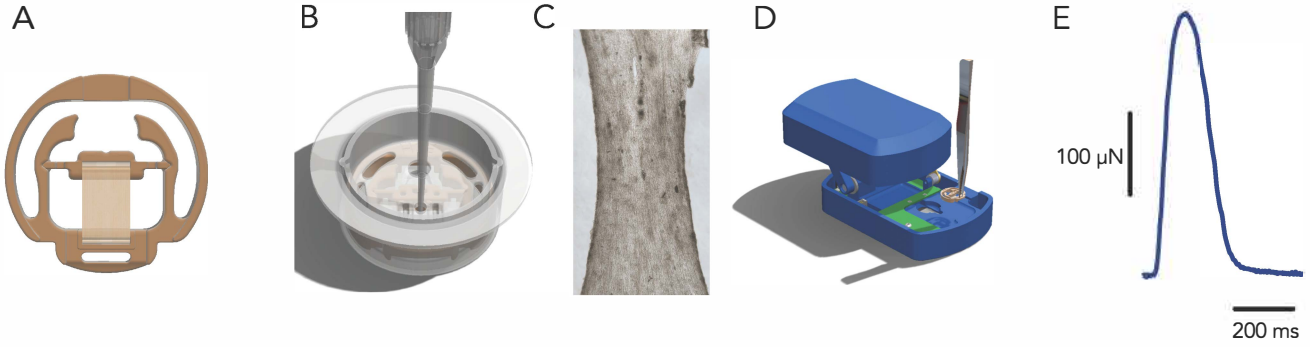

Figure 1: A new system for generating and testing iPSC-CM-based engineered heart tissue (EHT). (A) Porcine myocardium is cryosectioned, laser cut, decellularized, and mounted onto a polymer cassette. (B) The cassette is placed into a specially molded cup. Seeding of the scaffold is performed by pipetting a cell suspension into an opening in the cup. (C) Within days, iPSC-CMs have integrated into the scaffold to form a thin, synchronously beating myocardial construct. (D) The MyoPod is a device that accepts cassette-mounted tissues and measures their biomechanical properties while controlling temperature, stimulus frequency, and tissue stretch. (E) An isometric twitch contraction produced by an iPSC-CM EHT and recorded by MyoLab.

sodium doceyl sulfate (SDS). Each ribbon-like decellularized scaffold was stretched flat between attachment points in its own polymer cassette, specially designed to facilitate construct growth and contractility testing.

The scaffold was placed into a molded cup whose features guide droplets of suspended cells into the scaffold (Fig. 1B). Cells used in this study were iPSC-derived cardiomyocytes (iCell Cardiomyocytes, Cellular Dynamics International, Fujifilm), which were thawed and cultured for 1 week. On the day of seeding, cardiomyocytes were dissociated with TrypLE (Gibco), mixed in a 9:1 ratio with human cardiac fibroblasts (Promocell), and seeded onto the scaffold. Twenty-four hours after seeding, tissues were removed from the seeding apparatus and cultured in a standard 12-well plate in RPMI+B27 (Gibco). Robust, spontaneous beating was observed after three to five days in culture (Fig. 1C). The beating tissues were cultured up to two weeks before being placed into the MyoLab[1] for force testing.

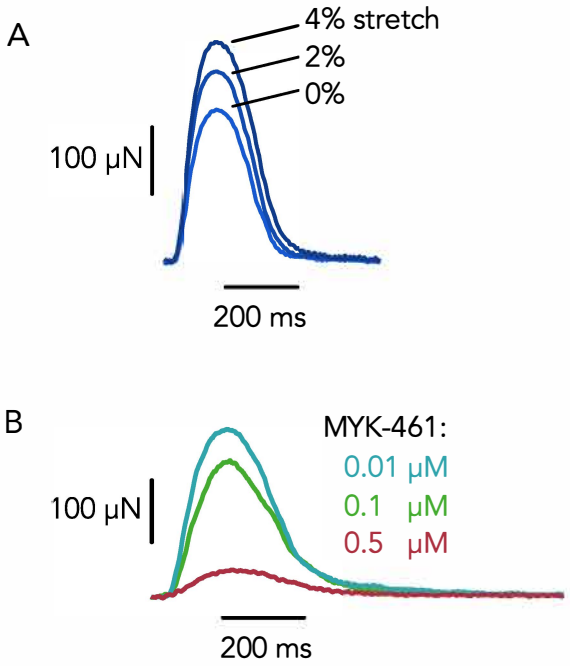

Figure 2: Data obtained from EHTs measured in the MyoPod System. (A) Isometric twitch force measured under increasing stretch. (B) Isometric twitch force after incubation with increasing doses of MYK-461.

Results

The tissue was loaded into the MyoLab, where contractility data was collected in Tyrode's solution (in mM: NaCl 140, KCl 5.4, $MgCl_2$ 1, HEPES 25, glucose 10, and CaCl 1.8; pH adjusted to 7.3).[2] The MyoPod maintained the solution at a temperature of 37°C throughout testing. The contractility testing protocol began with preconditioning the EHT (three slow stretch cycles to 10% in excess of its culture length and back again). After preconditioning was complete, isometric twitch force events were collected while the tissue was field stimulated at 1 Hz. A 'Frank-Starling' type force-length relationship was measured by stretching the tissue to 2% and 4% beyond its culture length, with data collected at each stretch level (Fig. 2A). EHTs exhibited the expected increase in twitch force at increasing levels of stretch. In a second test, the tissue was incubated in Tyrode's solution containing increasing doses of 0.01, 0.1 and 0.5 µM MYK-461. EHTs were permitted to equilibrate for 15 minutes at each drug concentration before collecting force data. The effect of this targeted myosin ATPase inhibitor was to reduce isometric twitch force.

Notes:

1. Patent pending, Propria LLC.
2. Schwan, J. *et al.* Anisotropic engineered heart tissue made from laser-cut decellularized myocardium. *Sci. Rep.* **6**, 32068; doi: 10.1038/srep32068 (2016).