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In Vitro Studies of the Synergy Between Mechanical Loading and Genetics Within Human Induced Pluripotent Stem Cell Derived Micro-Scale Engineered Heart Tissues

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Introduction: Human induced pluripotent stem cell (iPSC) and genome editing technologies offer the promise of understanding the molecular basis of disease and developing personalized therapies. However, mechanical cues are important in disease pathophysiology, particularly in the heart. For example, sarcomere mutations and blood pressure are both major risk factors for heart disease. Tissue engineering approaches offer the potential to study tissue-specific effects of mechanical loading. In our studies, we used genome editing (TALEN and CRISPR/Cas9) to introduce defined loss-of-function mutations in proteins including the myosin binding protein C isoform 3 (MYBPC3) which are associated with cardiomyopathy. We then used highly polymer-based scaffolds with controlled resistance to cell contractile forces to induce self-assembly of heart muscle from iPSC-derived cardiomyocytes (iPS-CM). Although a complete loss of MYBPC3 should result in a very severe phenotype in the human heart, MYBPC3 null iPS-CM only exhibited defects in force when cultured on rigid scaffolds. This is consistent with observed contributions of both biophysical and genetic cues to cardiac disease progression and suggests that it is important to consider mechanical cues within *in vitro* disease models.

Materials and Methods: We used two-photon polymerization with the UV curable organic–inorganic hybrid polymer (ORMOCLEAR®, Micro resist technology) to create filamentous matrices with three layers of parallel fibers (Fig. 1A). We created different filamentous matrices by fabricating synthetic parallel fibers with different fiber diameters (i.e., 5 μm and 10 μm). Because of their constant elastic modulus, the thicker fibers have a higher mechanical resistance to cardiac tissue contraction. We grew iPS-CM on the filamentous matrices to form microtissues, which could bend the fiber during contraction. Micro-tissue beating videos were recorded to calculate the contraction velocity, based on a motion-tracking algorithm [1], and contraction force based on fiber deflection (Fig. 1B). Potential molecular mechanisms were identified with Taqman gene arrays.

Results and Discussion: The boundary condition set by rigid glass plates resulted in anisotropic contraction (ref. [2]; Fig. 1C). Wild-type (WT) cardiac microtissues growing on 10 μm matrices adapted to the higher mechanical resistance by producing higher contraction force than on 5 μm fibers. In contrast, MYBPC3 null microtissues failed to adapt to the higher mechanical loading (Fig. 1D-E) after 10 days of mechanical exercise. This was concurrent with an increase in calcium handling abnormalities (EADs), along with expression of the hypertrophy marker brain natriuretic peptide (BNP) and the epigenetic modifier gene and protein EP300, for MYBPC3 deficient micro-tissues contracting against the 10μm fibers (ref. [2]; Fig. 1F).

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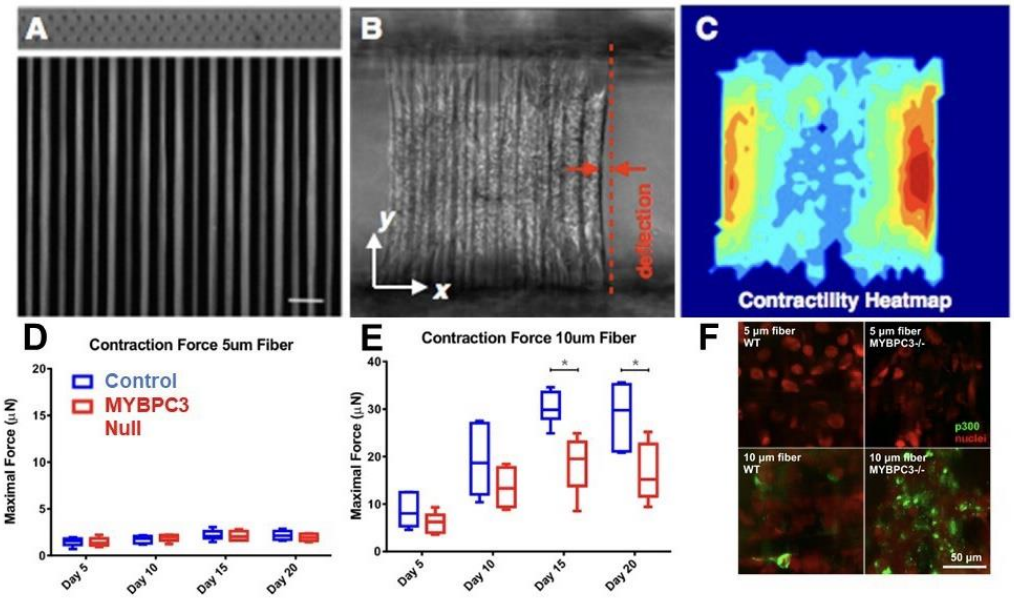

Figure 1: Tissue engineering approach to study synergy of genetic and physical cues in cardiac disease. **A-B)** Micrographs of A) scaffold without cells and B) scaffold seeded with cells. **C)** Heat-map indicating contractility is greatest near outermost fibers. **D-E)** MYBPC3 null iPS-CM exhibit contractile force defects only on rigid (10μm) fibers. **F)** P300 is selectively upregulated on rigid fibers in MYBPC3 null iPS-CM.

Conclusions: WT cardiac microtissues were able to adapt to the mechanical environment with increased contraction force and enhanced calcium influx dependent on the stiffness of the fiber matrices. In contrast, MYBPC3 cardiac microtissues exhibited impaired force development compared to WT tissues only when grown on matrices with higher fiber stiffness, suggesting that biomechanical cues facilitated contractile deficits due to the MYBPC3 gene KO. Our current efforts are aimed at controlling simplified, scalable systems for studying specific effects of preload and afterload on the physiology of iPS-CM micro-tissues [3].

References:

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