Formation of In Vivo-Like Intercalated Discs in a Neonatal Cardiomyocyte-Culture Model

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Keywords: Intercalated Disc, Neonatal Cardiomyocyte, Cell-Culture Model

Research Objectives: The intercalated disc (ICD), the end-to-end abutment between connected cardiomyocytes (CMs), is a unique structure that facilitate mechanical and electrical coupling between adjacent cardiomyocytes. In a working ventricular myocardium of adult higher vertebrates, it has been observed that a typical ICD has a step-like morphology with the "threads" and the "risers", of the stepped structure respectively perpendicular to and in parallel with the longitudinal axis of the cardiomyocytes. Much effort has been made to recapitulate the in vivo step-like ICD structures in an in vitro cell culture to, for example, promote the cardiomyocytes maturation in immature cell-based tissue engineering. This recapitulation has yet to be achieved for obtaining in vivo-like cardiac function in an in vitro cell culture. In this report, we describe a neonatal-cardiomyocyte culture that can facilitate formation and maintenance of in vivo-like ICDs.

Methodology: A microgrooved PDMS substrate (10 μm wide and 1.3 μm deep with a 20-μm repeating period) was fabricated using soft lithographic techniques. The Young's modulus of the PDMS substrate was 130 KPa. Neonatal ventricular cardiomyocytes (NVCMs) were isolated from three-day-old Sprague-Dawley rats using a two-day protocol and seeded to the suspended PDMS substrate coated with fibronectin. Then, cells were stimulated with a biphasic pulse train (1-ms pulse duration and 2-Hz repetition) at 3 V/cm delivered by carbon electrodes that were mounted inside the customized cell-culture chamber. NVCMs were evaluated by immunocytochemistry and second harmonic generation (SHG) imaging. Results were compared with those obtained using the same evaluation methods from random culture on flat PDMS.

Results: Step-like ICDs formed in the culture model with compliance-matched PDMS substrate after the electric stimulation had produced synchronized contraction of the entire cell construct along with the PDMS substrate.

Conclusion and Potential Future Research: The step-like ICDs were formed possibly because the homogeneously distributed stretching force across the entire translational surface; this homogeneous distribution was achieved by matching the compliance of the PDMS substrate and stimulating synchronized cell contraction. We plan to observe sarcomeric addition at ICDs using this cell culture model with static stretch that mimics mechanical overload.