The development of cardiomyocyte separation
A biochemical and microfluidic approach

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Introduction: Cardiomyocyte (CM) is the functional conducting cells among fibroblast and endothelial in the myocardium. Myocardial infarction results in cell death and scar formation, compromise heart function, and eventually lead to heart enlargement, and death. Cardiomyocyte sources, derived from embryonic and induced pluripotent cells with their virtually unlimited sources due to its self-renewal and directed differentiation capacity, serve as promising resources for cardiac tissue engineering applications. However, intracellular labelling (such as transcription factors or sarcomeric proteins) requires cell permeabilization and results in no viable cell, impeding translational studies. Herein, we developed a microfluidic chip integrated with magnetic field to specifically isolate CMs from heterogeneous cell populations such as heart or embryonic bodies. Viability of cells to oxidant pre-treatment and physical stimuli was studied. Cell responses to magnetic field were measured. And target protein marker in the CM, biochemical induction level, and induced magnetic susceptibility were quantified.

Material and Methods: The microfluidic separation platform was fabricated using soft lithography in PDMS with a single straight channel. Magnetic field is exerted by strong rare earth Neodymium, iron, and boron bar magnets. Magnetic field concentrators are fitted outside the channel. Cardiomyocytes were collected from 2-day-old neonatal Sprague Dawley rats under serial enzymatic digestion or from embryonic stem cell derived embryonic bodies. Cell viability after oxidant, physical stimuli, and separation through the channel was studied using CFDA/PI (live/dead) staining. Target protein was identified using spectrophotomer and quantified using Elisa. Double immunostaining for cardiac troponin T and the target protein was analyzed. CM enrichment quantification was performed using flow cytometric analysis and verified by cytospin immunostaining.

Results and Discussion: Microfluidic devices were fabricated and functional under applied magnetic field of 0.5T validation by a mixture of commercially available model magnetic and fluorescent beads. A purity of 94.8% was achieved in the collection of responding magnetic entities. Cell viability and functionality were preserved up to 50mM oxidant treatment with no significant cell death observed in treated compared to untreated control. Target protein in CM was identified in the absorption spectra with a soret band of 409nm as reported in the literature. Elisa quantification has shown the differential developmental increase in the target protein from neonatal to adult rat (0.11 and 0.73×10^{-14} mole/cell, respectively) and from Day 20 to Day 34 hESC-CMs (1.23 and 5.43×10^{-17} mole/cell, respectively). FACS shown co-localization of the target protein with cTnT as early as Day20 hESC-CMs. With the current concentrator prototype, CM population was enriched through the magnetic microfluidic device compared to non-treated samples shown by image analysis.

Conclusion: The integrated microfluidic device can provide a facile approach to enrich functional cardiac cells. The technology will eliminate current laborious and invasive CM isolation methods. It will provide enriched cardiac cells for opportunities to study CM physiology, and to use them for cardiac tissue engineering applications.