Human iPSC-derived Cardiomyocyte Response to 3D Fetal and Adult Decellularized Hearts

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Statement of Purpose: The classic paradigm of the mammalian heart as a post-mitotic organ has been challenged by the demonstration of a partial-to-complete histofunctional restoration of fetal-neonatal myocardium upon injury [1, 2]. However, the cellular and molecular mechanisms regulating this ontogenic-dependent cardiac regeneration are not completely understood. Since the extracellular matrix (ECM) regulates heart morphogenesis and growth, we hypothesized that fetal ECM provides specific cues for cardiomyocyte differentiation and/or maturation. Recent reports have highlighted the relevance and distinct modulatory potential of age-specific ECM on *in vitro* cardiomyocyte behavior in different experimental settings [3, 4]. However, a complete understanding of cardiomyocyte interactions with ECM in a 3D context mimicking the *in vivo* scenario remains unknown. In the present study, we evaluate how 3D fetal and adult cardiac decellularized ECM (dECM) impacts hiPSC phenotype at different stages of cardiomyocyte differentiation (cardiac progenitor cells vs early cardiomyocytes).

Methods: Fetal and adult heart explants were decellularized using an SDS-based protocol as previously described [5]. Adult lung explants were decellularized as a non-cardiac tissue control in parallel. Prior to seeding, dECM tissues were equilibrated in basal medium. An hiPSC line harboring a GCaMP6 calcium reporter was differentiated into cardiomyocytes using a defined small-molecule based protocol [6]. Cells were singularized at the cardiac progenitor stage (day5 of differentiation) or early cardiomyocyte stage (day10) and seeded onto dECM tissues ($0.1x10^6$cells/cm^2 and $0.2x10^6$cells/cm^2, respectively). dECM tissues were monitored every three days by light microscopy to assess matrix remodelling and the emergence of beating foci. Calcium imaging data was recorded at day 12, 18, 24 and 30 of cardiac differentiation. At day 30 of culture, calcein staining was performed to assess cell viability and the repopulated dECM tissues were processed for paraffin embedding. Relative cell numbers and phenotypes were assessed by histological analysis on cardiac and non-cardiac samples.

Results: Post decellularization fetal and adult cardiac matrices retained distinctive biochemical and structural features. Fetal dECM contained a coiled fiber network abundant in fibronectin, whereas adult dECM contained a dense network of thin nanofibers rich in collagen type I. In contrast, adult lung dECM contained a fine mesh network enriched for collagen type I (unpublished data). In order to explore how these different dECM affect cardiomyocyte phenotype, cardiac and non-cardiac dECM were seeded with cardiac progenitor cells and early cardiomyocytes differentiated from hiPSCs. Light microscopy imaging revealed a progressive dECM compaction during culture and enlargement of cardiomyocyte beating areas. An increase in cardiomyocyte calcium flux amplitude (F/F0) was observed overtime. Better dECM repopulation was achieved by more primitive (day 5) differentiated cells compared to early cardiomyocytes (day10), especially on fetal heart and adult lung dECM. After 30 days of culture, troponin T+ (cardiomyocytes) and vimentin+ (fibroblasts/ endothelial cells) were found on all matrices. Interestingly, cardiomyocytes were organized in large clusters throughout the fetal cardiac dECM and to a less extent in lung dECM, whereas troponin T+ cardiomyocytes were largely restricted to the periphery of adult heart dECM (Figure1).

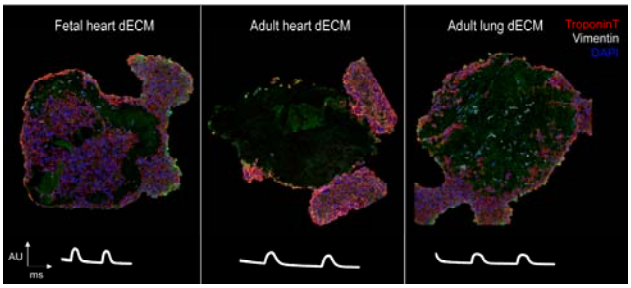

Figure1. Central section of fetal and adult heart dECM and adult lung dECM repopulated with hiPSC-derived cardiomyocytes and corresponding plot of calcium flux.

Conclusions: In summary, the present work indicates that fetal cardiac dECM exhibits a more amenable microenvironment for hiPSCs-derived cardiomyocyte culture. Future work will be focused on gene expression analysis of hiPSC-derived cardiomyocyte cells repopulating the dECM to evaluate their specific response to ontogenic cardiac microenvironments.

References: [1] Porrello ER. Science, 2011. 331(6020): p. 1078-80. [2] Herdrich BJ. Eur J Cardiothorac Surg, 2010. 38(6): p. 691-8. [3] Williams C. Acta Biomater, 2014. 10(1): p. 194-204. [4] Fong AH. Tissue Eng Part A, 2016. 22(15-16): p. 1016-25. [5] Silva AC. Biomaterials, 2016. 104: p. 52-64. [6] Lian X. Proc Natl Acad Sci U S A, 2012. 109(27): p. E1848-57.