Developmental Mechanobiology of the Epicardium

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Grafting 3D Collagen Scaffolds onto Infarcted Myocardium Improves Cardiac Remodelling and Neo-angiogenesis

This study aims to investigate the influence of multilayered microenvironment, mass transfer properties, and stiffness of the grafted collagen scaffolds in cardiac cell behavior and hence, in cardiac remodeling processes. The ultimate clinical goal is to develop a grafting technique to mount ECM-like matrices on to the heart in patients with acute myocardial infarction (MI).

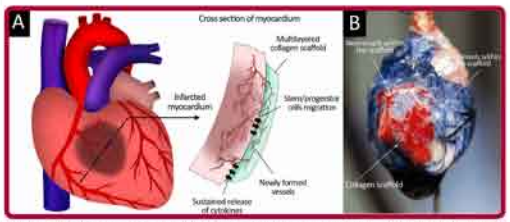


Figure 1. (A) Schematic representation of the incidence of myocardial infarction (left) and application of 3D multilayered collagen scaffold to interfere with remodeling processes post injury (right). (B) Coronary artery perfusion (Evans blue) of infarcted rat heart with collagen scaffold. The neo-vasculature within the scaffold that perfuses the blue stain indicates the connectivity of the coronary arteries (reconstructed from Gaballa et al. J Heart Lung Transplant 2006).

Experimental Methods In vitro reconstituted type I collagen gels undergo plastic compression by applying different compressive stresses for 2 minutes, in order to produce dense, multilayered scaffolds with improved mechanical properties (Figure 2). Mouse models of MI will be produced using left anterior descending (LAD) artery ligation. Immediately after infarction, the scaffold is sutured onto the injured myocardium along the outer boundary of the scaffold. Six weeks post grafting, hemodynamics, LV pressure-volume relationship, vascular density and connectivity, immunohistochemistry, and LV remodeling measurements will be performed. To determine whether the cells within myocardium exhibit a cardiomyocyte-like phenotype, sections including the scaffold will undergo immunostaining using mouse monoclonal IgGs primary antibody. Immunoreactivity will be visualized with a fluorescent secondary antibody. Samples will be also visualized with a laser scanning confocal microscope. Cell migration and proliferation will be assessed using Trypan blue exclusion and AlamarBlue assays. Cardiac cell-induced matrix contraction will be investigated by measuring normalized surface area of the grafted collagen gels during culture for up to 6 weeks.

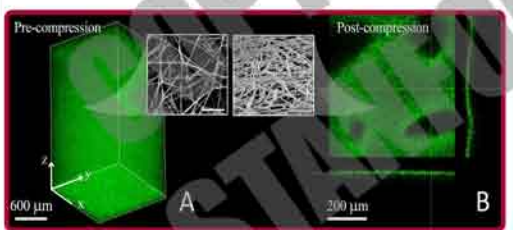


Figure 2. Confocal laser scanning microscopy of collagen Immunoreactivity in (A) highly hydrated, and (B) compressed collagen gel scaffolds. (A) 3D image stack of as cast collagen scaffold. (B) Post compression, a thin dense collagen lamella is formed at the bottom, along with a hydrated layer on top (multi-layered structure). The insets are SEM micrographs revealing the 3D fibrillar structure of collagen matrices pre- and post- compression (reconstructed from Serpooshan et al. Soft Matter 2010).

Stretch Regulated Response of the Notch Pathway in EMCs

Cardiovascular disease (CV) remains the most prolific killer in the United States. Increasing prevalence coupled with an aging population has underscored the need for novel therapies and clinical approaches to CV disease. Congenital heart defects, affecting nearly 1 out of every 100 newborns, are also the leading cause of infant death resulting from a birth defect. The elucidation of mechanisms involved in cardiogenesis on a cellular and molecular level is necessary for the development of potential cell-based CV therapies for both degenerative adult diseases and congenital abnormalities of the heart. The epicardium represents a developmental structure critical to modulating the differentiation of the embryonic myocardium as well as the cardiac conduction system, in addition to secreting factors that promote myocyte proliferation, the epicardium gives rise to the cellular elements of the subepicardium, intermyocardium connective tissue, and coronary vasculature. The mechanical environment of the embryonic heart has recently been shown to be critical in regulating differentiation and determination of cell fate in cardiogenesis. However, investigating the role of mechanical forces in epicardial function during development has been a relatively recent undertaking. Therefore, there is a critical need for a comprehensive understating of the complex interplay of mechanical forces and the role of the epicardium in development. The aim of this study is to further our fundamental knowledge of the epicardium and ascertain how exogenous mechanical stimuli can contribute to cardiogenesis and differentiation.

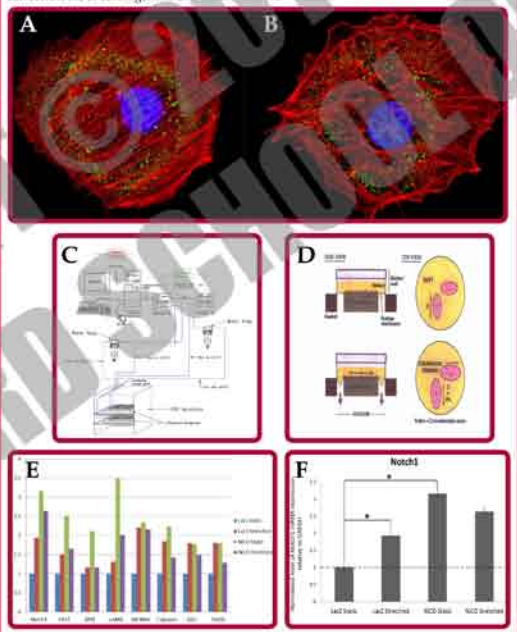
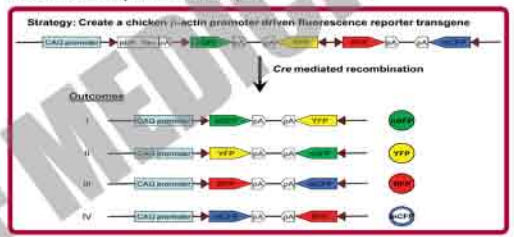


Figure 3. Cyclic Stretching Response of Epicardial Mesenchymal Cells: Using a Flexcell cellular stretching apparatus (diagram, C and stretching schematic, D), EMCs were stretched equibiaxially 10% for 24 hours at 1Hz (A), or statically incubated (B) in Bioflex Collagen Type I 6 well plates. Laser scanning confocal images depicting cell nuclei (DAPI, blue), filamentous actin (F-actin, AlexaFluor 568, red) and alpha smooth muscle actin (α-SMA, AlexaFluor 488 Abs, green). PCR was performed to assess Notch pathway activity in stretched and unstretched EMCs (E,F). Data presented are mean ± SD for n=3 biological replicates, * indicates statistical significance at p≤0.05, using single factor analysis of variance (ANOVA).

Epicardial Specific Rainbow-Cre mouse

Current existing tools to analyze epicardial lineage are based on the analysis of Cre-loxP based technology using a single readout indicator mouse. Here we propose generating a epi-rainbow mouse that will allow the fine study of the genetic and cellular events occurring during epicardial differentiation and map individual cell lineages.



Experimental Methods We have used an adaptation of the Brainbow system (Livet Et al. Nature 2007), which has been used successfully in the neural system, to generate a transgenic mouse that drives the expression of multiple copies of fluorescent proteins flanked by variations of different loxP sites (above). We will use the rainbow combination downstream of a strong ubiquitous promoter (chicken β-actin) which along with appropriate controls will be used in combination with a set of epicardial promoter (G5) to map the subset of lineages that are derived from Cre-expressing cells. This mouse line will allow the fine study of the genetic and cellular events occurring during epicardial differentiation and map individual cell lineages. This labeling strategy will allow us to visualize and trace large number of progenitor cells and their final destination. This method may revolutionize our current understanding of cardiac cell lineages.

This labeling strategy will allow us to visualize and trace large number of progenitor cells and their final destination. This method may revolutionize our current understanding of cardiac cell lineages. We crossed the CAG-rainbow line with Gata5-cre line (FG-cre) to generate epi-rainbow line and collected heart from P11 neonates. The heart was cryosectioned and the sections were analyzed by fluorescence microscopy. As shown below , we detected cells that were expressing YFP and CFP. The YFP and CFP labeled cells can originate only after Cre-mediated recombination events suggesting that these labeled cells were FG-Cre derived. This CAG-Rainbow line is now being used to further characterize the localization of epicardial progenitor population.

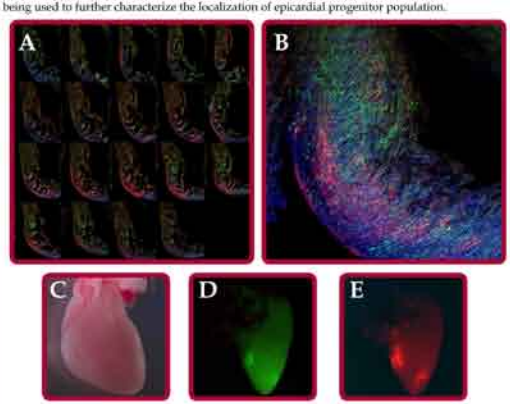


Figure 4. Laser scanning confocal images of 50 µm thick frozen sections (A) from epicardial-rainbow transgenic mice heart right ventricle and a projected image (B) depicting fluorescence of ~1mm² of tissue. Total red (E) and green (D) channel fluorescence from a widefield fluorescence microscope from a heart seen ventrally (C)