Enhanced Cardiomyocyte Proliferation and Maturation on Fractionated Cardiac Extracellular Matrix Derived from Decellularized Native Ventricular Tissue

Kyle Edmunds[1], Corin Williams[1], Ngonidzashe Madungwe[1], Lauren D Black III[1,2]
[1]Tufts University, Medford, MA 02155 USA; [2]Sackler School of Graduate Biomedical Sciences, Boston, MA 02111 USA

Statement of Purpose: Congenital heart defects (CHDs) are debilitating malformations in cardiac development that currently represent the most common pathological congenital condition in infants and young children[1]. Prognoses of many CHDs, such as hypoplastic left heart syndrome (HLHS) and tetralogy of Fallot (TOF), are severe, as these conditions are fatal if treatment is not immediately available**.** The creation of bioartificial cardiac tissue is therefore of great clinical interest. Previous studies have outlined the utility of naturally-derived cardiac extracellular matrix (cECM) in enhancing c-kit positive cardiac progenitor cell (CPC) proliferation and differentiation[2], and our own lab has shown that cardiomyocyte (CM) proliferation may be enhanced by culturing neonatal CMs on fetal or neonatal cECM[3]. The mechanism of these studies could be related to the presence of cryptic peptides isolated from protease-mediated degradation of cECM – a notion which has been implicated for progenitor cell activation and recruitment[4]. Our hypothesis is that there exists a cryptic peptide or peptides within cECM that enhances CM proliferation and/or maturation, and that these peptides are either promoted in early cardiac development or repressed in aging by other peptides or biological macromolecules. We sought to assess this notion by investigating isolated CMs cultured on native cECM fractionated via SDS-PAGE.

Methods: Sprague Dawley rat hearts were isolated and subjected to decellularization by perfusion (Adult, **Fig. 1A/B**) or immersion (Fetal) with SDS[5]. Ventricular tissue was lyophilized and solubilized using pepsin (10% by weight) in 0.1M HCl[6] and fractionated via SDS-PAGE with 10% polyacrylamide (PAAM) gels. Three regions from these gels were excised (Regions: **1**: >65kDa, **2**: 25-65kDa, **3**: <25kDa), and cECM was eluted (50 mM Tris-HCl, 150 mM NaCl, and 0.1 mM EDTA; pH 7.5) and adsorbed onto either 48-well plates or glass-bottom petri dishes. CMs were then isolated from neonatal Sprague Dawley rats and cultured for 5 days in serum-free myomedia (DMEM, F12, 0.2% BSA, 0.5% ITS, 1% Pen-Strep, 0.1mM ascorbic acid). Afterwards, cells were fixed and stained with Hoechst, Cardiac α-Actin (CAA), and Phosphohistone H3 (PHH3) to discern both the total CM population and the number of proliferative CMs. Additionally, CMs in the glass-bottom petri dishes coated with cECM were stained for sarcomeric α-Actinin (SAA), and sarcomere lengths were measured to ascertain CM maturation[7]. Further investigation involved culturing CMs directly on cECM that was transferred to PVDF membranes. For this work, gels were split in half and one half was transferred to a PVDF membrane, while the other half was stored for future proteomics analysis via LC-MS/MS. Neonatal cells were cultured as above and fixed/stained with Hoechst, SAA, and Ki-67 to obtain precise peptide bands that elicited both enhanced CM proliferation and maturation.

Results: For the gel elution portion of this study, two PAAM gels from the same decellularized rat heart were plated in triplicate for each of the three excision regions. IHC results for PHH3+CAA expression showed an average fold change increase of 2.4±0.23 over TCP control in wells coated with excision region 2 cECM (1.1±0.26 from region 3 and 0.8±0.07 from region 1) (Fig. 1C). Likewise, 14.5±1.8% of cells in excision region 2 expressed CAA, compared to 9.5±1.00% (region1) and 7.5±1.23% (region 3) (Fig. 1D). Additionally, CMs on region 2 cECM exhibited average sarcomere lengths of 2.02±0.2µm, compared to 1.52±0.2µm (region 1) and 1.65±0.1µm (region 3) (Fig. 1E-iii) (N=40 per excision region).

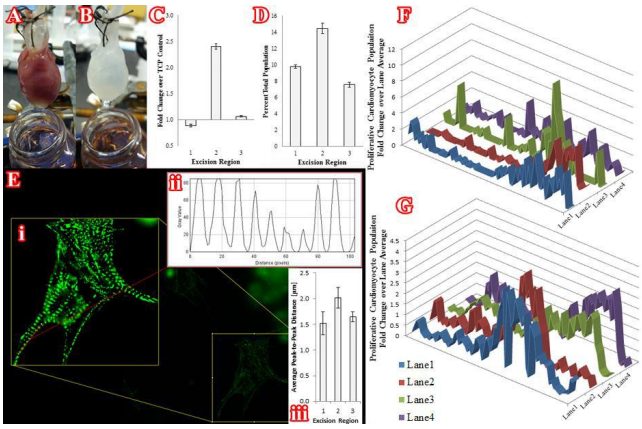

Figure 1 | **A and B,** Initial/Final perfusion decellularization images. **C,** Total proliferative CM's (PHH3+CAA) expressed in fold change over TCP control. **D,** Percent total CM population. **E**, CM maturation study, **i**: SAA stain, **ii**: Line-spread-function to compute sarcomere lengths, **iii**: Average sarcomere lengths by excision region. **F and G**, Fetal and adult cECM (respectively) PVDF study – proliferative CM number expressed in fold change over each lane average.

Furthermore, initial results from both adult and fetal cECM on PVDF membranes have shown a high degree of CM proliferation on peptides less than 65kDa in size (**Fig. 1F/G**). Ongoing studies seek to use LC-MS/MS to identify which peptide sequences are homologous within excised gel pieces corresponding to these regions of high CM proliferation.

Conclusions: The findings of this study have shown both enhanced CM proliferation and maturation as a result of a 5-day culture upon both adult and fetal cECM fractionated between 25 and 65kDa. Likewise, enhanced CM proliferation has been observed on PVDF membranes at corresponding peptide ranges. These data support the presence of the hypothesized cryptic protein or protein combination implicated for driving these phenomena. Future work aims to ascertain the specific peptide sequences that enhance CM proliferation with the eventual goal of utilizing these peptides for biomaterials strategies for cardiac regeneration and tissue engineering.

Resources:

[1] Zimmermann W-H, Cesnjevar R. Cardiac tissue engineering: Implications for pediatric heart surgery. Pediatr Cardiol 2009;30:716-23.
[2] French, KM *et al*, "A naturally derived cardiac extracellular matrix enhances cardiac progenitor cell behavior in vitro", *Acta Biomaterialia*, 2012 Dec, 4357-64
[3] Williams, C *et al*, "Young developmental age cardiac extracellular matrix promotes the expansion of neonatal cardiomyocytes in vitro", *Acta Biomaterialia*, 2013 Sept, S1742-7061(13)00429-7
[4] Agrawal, V *et al*, "An isolated cryptic peptide influences osteogenesis and bone remodeling in an adult mammalian model of digit amputation", *Tissue Engineering Part A*, 2011 Oct, 2435-2443
[5] Ott, HC *et al*, "Perfusion-decellularized matrix: using nature's platform to engineer a bioartificial heart", *Nature Materials*, 2008 Feb, 213-21
[6] Singelyn, JM *et al*, "Naturally derived myocardial matrix as an injectable scaffold for cardiac tissue engineering", *Biomaterials*, 2009 Oct, 5409-16
[7] Jennifer L. Young, Adam J. Engler. Hydrogels with time-dependent material properties enhance cardiomyocyte differentiation in vitro, Biomaterials (2011), 32:1002-1009

Abstract #183