Comparison of Decellularized Left and Right Ventricle Myocardial Matrix Hydrogels and Their Effects on Cardiac Progenitor Cells

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Introduction: Approximately 8.4 in 100,000 live births are diagnosed with Hypoplastic Left Heart Syndrome (HLHS)[1]. Infants with this condition have an underdeveloped left side of the heart which must be corrected via a series of surgeries resulting in the right ventricle (RV) becoming the main systemic pump[1]. Increased afterload from the systemic circulation causes the RV to undergo maladaptive remodeling (hypertrophy, dilation, myocardial apoptosis, modified metabolism, and interstitial fibrosis) that result in RV heart failure (HF)[2]. C-kit cardiac progenitor cells have been shown to mitigate negative remodeling when injected into the RV of both small and large animal models of RVHF[2,3]. It is hypothesized that CPCs promote healing via paracrine signaling that affects pathways such as vascularization[4]. It has further been demonstrated that a myocardial matrix (MM) hydrogel can improve the therapeutic potential of CPCs by enhancing gene expression, increasing proliferation, protecting against reactive oxygen species, and improving the adhesion of CPCs in a 2D *in vitro* environment[5]. We also recently developed a RV MM hydrogel and demonstrated that it has similar properties to our left ventricle (LV) MM, although there were distinct extracellular matrix (ECM) protein signatures. To further assess if there exists a potential therapeutic benefit to combining CPCs with either of our MM hydrogels, we aimed to evaluate cell survival, vasculature gene expression, and angiogenic paracrine signaling *in vitro.*

Methods: LV and RV MM were fabricated according to our previous methods[6]. Protein makeup for both native LV and RV as well as LV and RV MM were evaluated via global and targeted ECM proteomics. All 3D cell assays were performed by encapsulating 200,000 CPCs in either 30 µL of 8 mg/mL LV MM, 8 mg/mL RV MM, or 3.3 mg/mL rat tail collagen (Col), which has the same mechanical properties as 8 mg/mL MM gels and culturing them at 37 °C and 5% CO2. Protection from reactive oxygen species was evaluated via Alamar Blue (n = 6 per group), changes in gene expression were evaluated via qPCR, and paracrine signaling was assessed via tube formation assay, pico green, and trans well migration assay, (n = 4-6 per group per assay). Paracrine assays were performed on conditioned media collected from cells encapsulated in each group (subscript c) and gels alone (subscript nc).

Results: When comparing the LV and RV native and decellularized material, it was noted that the RV consisted of more proteins classified as fibrillar collagen (i.e., collagen I). Conversely, the LV was comprised of more structural ECM proteins, specifically collagen VI. CPCs encapsulated in either MM also had increased paracrine signaling based on tube formation, migration, and proliferation assays. In figure 1A, LVc had a significant

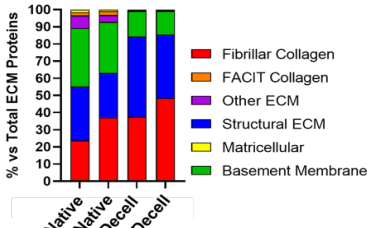

Figure 1. Targeted ECM proteomics revealed that the LV and RV have distinct protein signatures and that they are conserved after decellularization.

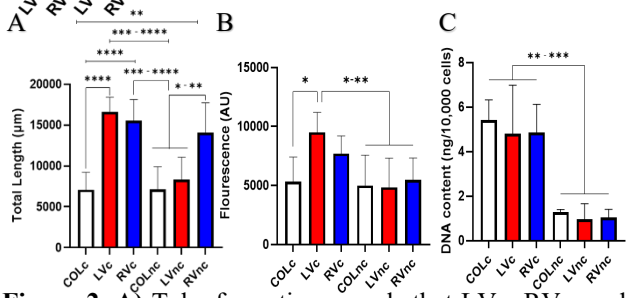

Figure 2. A) Tube formation reveals that LVc, RVc, and RVnc conditioned media produced greater tube length than the collagen encapsulated and other gel alone groups. **B)** LVc media promoted greater migration than COLc and all gel alone groups. **C)** All cell encapsulated groups induced greater proliferation, as indicated by DNA content, than gel alone groups. Letter "c" denotes encapsulated CPC conditioned media and "nc" is gel alone media.

increase in tube length compared to LVnc and COLc indicating angiogenic benefit of the LV MM+CPC combination. RVnc was significantly greater than COLnc and LVnc and was similar in total length to RVc indicating that the RV MM has potential benefit as a standalone treatment. LVc induced greater endothelial cell migration than COLc and all the gel alone groups further suggesting the therapeutic benefit of LV MM+CPC (Figure 1B). Finally, DNA content of endothelial cells was greater in conditioned media from cell encapsulated groups compared to gel alone which indicates the proliferative benefit of combining CPCs and MM.

Conclusion: Quantitative proteomics revealed that the LV MM and RV MM are distinct. Paracrine assays suggest that the combination of the LV MM and CPCs may be the optimal therapy for angiogenesis, but also alluded to the potential of the RV MM inducing significant vascularization on its own.

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