INTRODUCTION

Myocardial infarction (MI) accounts for 1 in every 6 deaths in the U.S. Following MI, myocardial ischemia results in pathological cardiac remodeling and ultimately heart failure.

Tissue engineered myocardial patches (MPs) utilizing xenogeneic tissue represent an attractive regenerative strategy for repair of MI lesions due to limited tissue availability. However, xenografts present in the tissue mediate an aggressive immune-mediated rejection response upon implantation. Conventional cardiac tissue engineering approaches utilize a decellularization paradigm for production of xenogeneic tissues or whole organ scaffolds. However, failure of commonly reported sodium dodecyl sulfate (SDS) based decellularization methods to adequately address tissue xenogenicity has been reported to result in significant graft-specific in vivo immune response3, emphasizing the critical need to improve antigen-removal (AR) from xenogeneic tissues. We have previously reported a novel AR paradigm which utilizes the principles of protein chemistry to enhance the solubility of both water- and lipid-soluble antigens from bovine pericardium for heart valve tissue engineering4,5. We have adapted this AR approach to produce a xenogeneic cardiac muscle ECM scaffold by targeting the depolymerization and solubilization of individual sarcomeric component to facilitate removal of antigens from neonatal and adult rat myocardial tissues.

While many ECM proteins have been identified, little is known regarding the ECM scaffold as a whole and such “niches” surrounding the cells at various post-natal developmental stages. The terminally differentiated adult cardiac tissue retains capacity to undergo remodeling when induced by stress stimuli. Similarly, the ECM of developing neonate tissue provides cues to facilitate myocyte differentiation and maturation. Therefore, important differences exist in the inherent adult and neonate cardiac ECM to provide developmental-specific, structural and functional cues for healthy tissue functions. We aim to examine the recellularization potential our innovative AR method to generate a biologically active xenogeneic adult and neonate ECM for use in future cardiac regenerative applications.

HYPOTHESIS

We hypothesize that our innovative AR method will reduce xenogenicity and maintain better structure/function properties of xenogeneic cardiac ECM scaffolds than SDS-decellularization. We further hypothesize that the preserved neonate cardiac niche in AR-scaffolds will result in enhanced fetal cardiomyocyte recellularization compared to adult scaffolds.

METHODS

Rat hearts – Adult (>12d) or neonate (3d) Sprague Dawley (SD) rats were sacrificed, hearts were explanted and stored in DMEM+15% DMSO solution at -80°C (Charles River Laboratories).

Antigen removal – AR protocol for MPs was adapted from our previously published work2,3. Briefly, in addition to our previously published steps designed to solubilize hydrophilic and lipophilic antigens, sarcomeric disassembly and solubilization steps were included to achieve removal of cardiomyocytes macromolecular components. Additionally, both adult and neonatal MPs were generated using a 1% (w/v) SDS solution to serve as a literature control4.

Fetal cardiomyocytes isolation – SD female fetal rats (18 d.p.c.) were explanted by C-section and hearts excised using previously described methods7 according to UC-Davis IACUC-approved protocol. Briefly, cardiomyocytes were isolated by mechanical mincing and enzymatic digestion. Single cell preparation was pre-plated for 45 min to enrich for cardiomyocyte (~65% myocyte purity at the time of recellularization).

Recellularization study – freshly isolated fetal cells were plated using Hank’s MEM with 5% serum at a density of 1,500 cells/cm² onto vehicle, AR or SDS-treated MPs in a 48-well plate for 24 hours. Media was then replaced with serum-free media supplemented with insulin and transferrin for the remaining culture period. The MPs seeded with fetal cells were cultured at 37°C with 1% CO2 for 2 more days until harvest. Harvested MPs were fixed in 10% formalin for histology analysis or were enzymatically digested for flow cytometric analysis. Remaining cells within each well were trypsinized, fixed and analyzed similarly.

Flow cytometric analysis – Cells were fixed with formalin and stained with 7AAD, a DNA intercalating agent that marks nucleated cells, and an anti-β-myosin heavy chain (MyHC) or anti- total MyHC antibody to enumerate fetal and total cardiomyocytes respectively. The percentage of MPs expressing β-MyHC and total MyHC were measured on both the recellularized MPs and vehicle controls, with cells seen to penetrate deep into the scaffold.

RESULTS

Flow Analysis of β-MyHC and Total MyHC Expressions in Recellularized Neonate Cardiac MPs

Figure 1. Representative images of gross morphology of adult and neonate native MPs and MPs treated with 1% SDS or AR solution.

Figure 2. Representative H&E images of adult and neonate MPs recellularized with fetal rat cardiomyocytes before and after seeding for 3 d. (n=3 per group) Scale bar denotes 1 μm.

Gross Morphology of Adult and Neonate Cardiac MPs

Histology of Recellularized Adult and Neonate Cardiac MPs

CONCLUSIONS

- AR treatment yielded both adult and neonate MPs with little to no cytoplasmic or nuclear remnants, whereas these components were retained following 1% SDS treatment.
- Neonate MPs showed more repopulation of fetal cardiomyocytes than adult MPs. In addition, AR-treated neonate MPs resulted in more recellularization than either 1% SDS or vehicle control scaffolds.
- Notably, β-MyHC marker analysis of AR-treated neonate MPs indicated its ability to preserve fetal cardiomyocyte phenotype similarity to freshly isolated cells and cultured cells. AR-neonate MPs promoted survival of fetal cardiomyocytes within the scaffolds itself (~3-fold over SDS-MPs) and around it (~5-fold over SDS-MPs).
- Total MyHC analysis also indicated that AR-neonate MPs maintained a higher amount of total cardiomyocyte repopulation within the scaffold (~2-fold over SDS-MPs) and around the scaffold (~8-fold over SDS-neonate MPs).

REFERENCES