Fabrication of Tissue-engineered Cardiac Patch with Decellularized Porcine Myocardial Scaffold

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Statement of Purpose: Each year, approximately one million Americans suffer myocardial infarctions (MIs) with a 10% in-hospital mortality rate. Engineered cardiac tissue might provide optimal tissue performance maintained by viable transplanted cells, and might also stimulate the formation of vasculature supplying oxygen and nutrients in the patched region. However, fabrication of 3D porous scaffolds that are thoroughly reseeded with cells remains a challenge for making a viable thick cardiac patch. Inspired by major achievement in creating decellularized whole rat heart scaffolds, and successful applications of tissue derived acellular scaffolds such as heart valves, small intestinal submucosa, and urinary bladder, we propose to investigate decellularized porcine myocardial scaffold, which preserves natural myocardial ECM structure, as a potential template for cardiac tissue engineering. In this study, our goal is to create a thick cardiac patch using decellularized pig myocardium scaffold and bone marrow mesenchymal stem cells, and assess its potential for positive tissue remodeling.

Methods: Bone marrow was extracted from femurs and tibias of fetal pigs. Bone marrow mononuclear was characterized one week after primary culture using CD44 mesenchymal stem cell surface antigen. Porcine myocardial scaffolds were decellularized with 0.1% sodium dodecyl sulfate (SDS) with 0.01% Trypsin for 2.5 week. For differentiation, the second passage MSCs were seeded into 175-mm flasks at a density of 5 x 10^4 cells/flask and treated with 3 µmol/L 5-azacytidine for 24 hours, the medium was then changed to complete medium without 5-azacytidine. For recellularization, the decellularized scaffolds were sterilized and reseeded with the differentiated bone marrow stem cells (1×10^6 cells/ml) for 4 hrs under gentle agitation and cultured in a rotating bioreactor. Mechanical and structural properties of the tissue constructs were then characterized by: (i) histology and SEM, (ii) immunohistochemical assessments, and (iii) uniaxial and biaxial mechanical properties.

Results / Discussion: Thorough cell decellularization was achieved (Fig. 2a). Histology and SEM results indicate that SDS decellularization technique successfully removed cells and cell debris, and most closely preserved 3D porous ECM network. Thorough recellularization was verified by Masson's trichrome staining in the 2 weeks and 4 weeks constructs (Fig. 2b, c). In the tissue constructs, positive sarcomeric α-actinin (Fig. 3a) and cardiac myosin heavy chain staining (Fig. 3b) were observed. The vWF positive cells were also observed in the locations showing channel-like morphology (Fig. 3c). Mechanical tests (Fig. 4) show a stiffer mechanical response due to removal of cells. After one week and two weeks tissue culture, constructs show a recovering tendency, which likely resulted from higher cellular content.

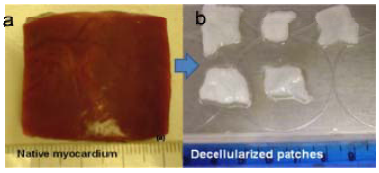

Fig. 1: (a) Native myocardium. (b) Patches after removing heart muscles.

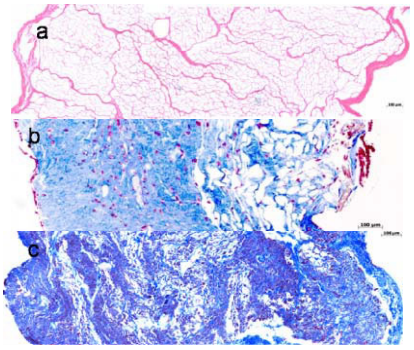

Fig. 2 Edge to edge view of (a) acellular porcine myocardial scaffold (b) tissue construct after 2 weeks and (c) 4 weeks construct

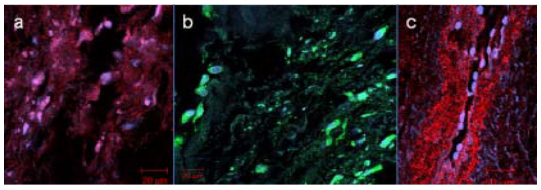

Fig. 3 (a) Sarcmeric α-actinin, (b) MHC, (c) vWF staining of 2 weeks reseeding patch.

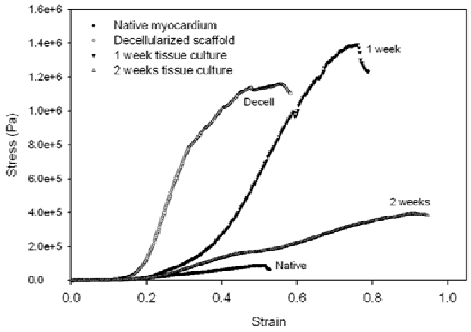

Fig. 4: Uniaxial mechanical property

Conclusion: In this study, we successfully developed acellular myocardial scaffold with well-preserved ECM structure. We observed successful recellularization with good cell viability, ingrowth, and proliferation. Both uniaxial and biaxial mechanical studies demonstrated the positive tissue remodeling in the engineered patch.

Acknowledgements: This study is supported by the Health Resources and Services Administration (HRSA) (DHHS R1CRH10429-01-00) and MSU Research Initiate Program.

Reference:

[1] H. C. Ott, et al. *Nat Med* 14 (2), 213 (2008).