Antigen Removal for the Production of Immunologically Acceptable Xenogeneic Scaffolds for Myocardial Patch Tissue Engineering

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Motivation
Development of a tissue engineered myocardial patch (TEMP) which could be implanted into a recently infarcted site has the potential to address the negative structural remodeling associated with myocardial infarction and thereby dramatically alter patient outcome. A TEMP utilizing a scaffold derived from xenogeneic myocardium has the advantage of unlimited availability, ideal extracellular matrix structure and mechanical properties. However, xenografts present within the myocardial patch (MP) represent the critical barrier to production of immunologically acceptable, unfixed xenogeneic MP scaffolds. Although effective in solubilizing cellular and tissue components, previously reported decellularization methods utilizing harsh denaturing detergents such as SDS have been repeatedly shown to be detrimental to the extracellular matrix (ECM), and toxic to repopulating cells1-3. We have previously demonstrated that antigen removal (AR), utilizing the principles of differential, sequential solubility, is capable of reducing xenogeneic tissue antigen content of bovine pericardium while retaining native ECM structure, biochemical composition, mechanical properties and recellularization potential4,5. We postulated that our AR approach can be adapted to achieve AR from MPs to produce an ideal cardiac muscle-derived ECM scaffold for production of a TEMP.

Hypothesis and Aims
We hypothesize that promoting protein solubility will be critical for removal of both antigenic proteins and sarcomeric macromolecular structural components from rat myocardium. We therefore aim to exploit protein chemistry principles of differential protein solubility coupled with sarcomeric disassembly to generate MP scaffolds for cardiac muscle tissue engineering applications.

Methods
MP Isolation: Biopsy punches (d=3.5mm) were used to isolate left ventricular (LV) myocardial patches (MPs) from adult Fischer rat hearts. (Fig. 1).

Sarcomeric Disassembly and Antigen Removal (AR): MPs were incubated in hydrophile solubilization buffer (optSARB), 1% SDS (decellularization literature control)6, or stepwise solubilization based AR, hereafter designated as MP-AR. MP-AR consisted of sarcomeric disassembly using 50M Lutrisurf B (acting depolymerization), followed by 0.6 M KCi (myosin solubilization) and 1.0 M Ki (titin solubilization) combined with washes in optSARB for hydrophile antigen solubilization and final lipophile solubilization in (ASB-14 in optSARB)7. For all groups, MPs and scaffolds underwent nucleic acid digestion and 48 h washout.

Assessment of Antigen Removal (AR): Residual proteins were extracted from all groups as previously described8, analyzed with Western Blots probed with mouse anti rat-LV polypeptide anti-serum, and assessed for IgG positivity. Residual antigenicity was defined as the ratio of banding intensity for extracts following each AR approach vs. anatomically adjacent native MP controls.

Statistical Analysis: Means were analyzed using ANOVA and Tukey-Kramer HSD, with significance set at p<0.05.

Results
Phase 1: Removal of cellular components
Actin depolymerization alone or in combination with hydrophilic antigen solubilization buffer (optSARB) was insufficient to facilitate complete solubilization of all macromolecular components of the sarcomere (Fig.2B). Sarcomeric disassembly alone (LatB/KCl/KI) resulted in complete removal of sarcomeric material only in stochastically disperse areas of the patch (Fig.2C). A stepwise targeted three-step AR approach was thus developed, consisting of membrane permeabilization and lipophile solubilization (ASB-14), sarcomeric disassembly and hydrophilic solubilization with optSARB (Fig.2D-E). Three-step AR resulted in MP-AR scaffolds with no residual nuclei, unlike 1% SDS-treated literature controls which retained nuclear debris throughout the patch interspersed with the collagen molecules (Fig.2F). Morphologically, stepwise AR scaffolds retained their structure whereas SDS MPs collapsed into an amorphous mass (Fig.2G-H).

Finally, three-step AR resulted in complete removal of alpha-sarcomeric actin, one of the most prominent proteins found in heart muscle, as confirmed by immunofluorescence (Fig.2G-H) and Western Blotting (Fig.2I).

Phase 2: Antigenicity assessment
Residual antigenicity of three-step MP-AR scaffolds was decreased by >95% for the water-soluble antigens (WSA) compared to native controls and optSARB-treated samples (Fig. 3). Residual WSA were not significantly different between the SDS group and all three MP-AR groups (three-step MP-AR with either 2%, 3% or 4% w/v concentrations of the lipophilic solubilizing agent ASB-14).

Conclusions and Future Work
We utilized the principles of differential, sequential soluble component with sarcomeric disassembly to produce MP scaffolds that display >99% reduction in antigenicity compared to controls without using harsh denaturing detergents commonly utilized in decellularization approaches (e.g., SDS). Additionally, unlike SDS treatment, our AR approach results in complete acellularity, while maintaining ECM structure and gross morphology of the resultant scaffold.

We are currently performing functional tests to assess the biochemical and biomechanical properties of our scaffolds, as well as their recellularization potential. Based on our previous results for bovine pericardium, we anticipate that MP scaffolds generated using this novel AR method will display biochemical and biomechanical properties that mimic native MPs, while remaining compatible with recellularization.

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References

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