Protein Solubilization for Antigen Removal from Bovine Pericardium in Heart Valve Tissue Engineering

Maeleen L. Wong1 and Leigh G. Griffiths2

1Department of Biomedical Engineering, 2Department of Veterinary Medicine: Medicine and Epidemiology
University of California, Davis, Davis, CA

Contact information: maewong@ucdavis.edu, lggriffiths@ucdavis.edu

INTRODUCTION

Tissue engineered heart valves (TEHVs) can potentially overcome limitations of current replacement valves. Biomaterial xenogenicity precludes use of unfitted bovine pericardium (BP) as TEHV scaffolds. Decellularization renders tissue acellular, but inadequately removes antigenic material, prompting a need for more efficient antigen removal (AR) techniques. [1-7]

Current AR techniques fail to recognize: (1) AR from xenogeneic biomaterials is largely a diffusion-dependent process and (2) only solubilized proteins are capable of diffusion into the AR solution. [8]

Antigen removal methods yielding immunologically compatible BP are essential for BP scaffold generation. Tissue engineered heart valves developed using these BP scaffolds have the potential to become a new standard in heart valve replacement.

HYPOTHESIS AND AIM

We hypothesize that maintaining protein solubility is crucial for more efficient AR, a concept yet to be explored in tissue engineering applications. We aim to exploit protein chemistry principles to promote protein solubility and facilitate enhanced AR for the generation of a xenogeneic TEHV scaffold.

METHODS

Solubility plot: To assess the importance of maintaining protein solubility for AR, the ability of reducing agent (DTT) and salt (KC1) to enhance antigen removal from BP was investigated. Pieces of BP were incubated in one of six solutions: basic antigen removal buffer (BARB), BARB with 134 mM NDSB-256 (NDSB/BARB), BARB with 0.1% SDS (SDS/BARB), solubilizing antigen removal buffer (SARB, BARB with 1 mM DTT, 2mM MgCl2, and 10 mM KC1), NDSB/BARB, or SDS/BARB (n=6 per treatment and time point).

Solubility study: Ability of increasing DTT and KC1 concentrations to enhance AR from BP was investigated in a pair of two-phase studies. Phase 1 aimed to determine the effect of increasing DTT or KC1 concentration on residual antigenicity of BP-AR. The optimal concentration identified in phase 1 was then tested in phase 2 for its ability to influence AR by BARB, NDSB/BARB, or SDS/BARB.

RESULTS

Fig. 1 – Work flow of BP processing. BARB = 0.1 M Tri-HCl, pH 8.0 with 1% antibiotics-antimycotic solution (AAS), 0.5 mM Pentidine, Macerase solution = 2.5 U/ml, Dhaase 1, 7.5 U/ml, RNase A in 10 mM Tris-HCl, pH 7.4 with 150 mM NaCl, 5 mM MgC12, 1% AAS. Wash buffer = 0.2-buffered saline with 1% AAS, 0.5 mM Pentidine.

Antigen removal: Pieces of BP (0.2 g) were incubated in antigen removal buffer (ARB) for 2 days; adjacent BP pieces subjected to 1 min incubations served as negative AR controls. Nuclear acid digestion (24 hr) and washout (48 hrs) followed AR. All steps were performed at 4°C and 125 rpm.

Assessment of antigen removal: Residual antigens extracted from BP post-AR (BP-AR) were subjected to electrophoresis. Western blot (probed with rabbit serum generated against native BP, and densitometry. [7,9] Residual antigenicity was determined as ratio of day 2 to 1 mm control. Trends and means were analyzed using ANOVA and Tukey-Kramer HSD, with significance defined as p<0.05.

Methods (cont’d)

Solubility study – DTT:

100 mM DTT significantly reduced residual antigenicity of BP-AR compared to 1 mM DTT by BARB, NDSB/BARB, and SDS/BARB containing 2 mM MgCl2.

RESULTS (cont’d)

Fig. 4 – Increasing KC1 concentration resulted in decreased residual antigenicity by NDSB/BARB with 100 mM DTT and 2 mM MgCl2 (p<0.0001). (A) Antigen removal by BARB with 100 mM KC1 resulted in a 36% drop in residual antigenicity compared to BARB with 10 mM KC1 (p<0.05). A 41% reduction in residual antigenicity was observed from NDSB/BARB with 100 mM KC1 over 10 mM KC1 (p<0.05). Residual antigenicity decreased by 20% with SDS/BARB containing 100 mM DTT beyond 1 mM (p<0.05). All ARBs contain 100 mM DTT and 2 mM MgCl2. (B) Treatments not connected by the same letter are significantly different, p<0.05 (n=6 per treatment and time point).

100 mM KC1 significantly reduced residual antigenicity of BP-AR compared to 10 mM KC1 by BARB and NDSB/BARB, but not SDS/BARB. In the presence of 100 mM KC1, residual antigenicity following treatment by BARB, NDSB/BARB, and SDS/BARB were not significantly different.

CONCLUSION & FUTURE WORK

Buffers promoting protein solubility significantly reduced residual antigenicity. Interestingly, differences in AR efficiency by BARB, NDSB/BARB, and SDS/BARB were mitigated by addition of 100 mM DTT and 10 mM KC1, suggesting that factors promoting solubility have a dominant effect on AR beyond that achieved by detergent alone. Taken together, maintenance of protein solubility is critical in the development of more efficient AR strategies and, ultimately, a xenogeneic scaffold with decreased immunogenicity for heart valve tissue engineering.

Tensile testing, biochemical assays, and histological analysis will be performed to assess mechanical properties and ECM structure and function of BP-AR.

Recellularization assays will be performed to determine the biocompatibility of BP-AR.

REFERENCES


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