Sequential, Differential Solubilization for Antigen Removal in Xenogeneic Scaffold Generation

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INTRODUCTION

The extracellular matrix (ECM) of native tissue is an attractive scaffold for tissue engineering and regenerative medicine applications due to the inherent composition and architecture of ECM components. However, antigens in the biomaterial are the primary obstacle for expanding the use of xenogeneic scaffolds in clinical medicine. Decellularization xenogeneic biomaterials have been shown to elicit an immune response following implantation due to incomplete removal of antigenic molecules from the tissue. This finding has initiated a paradigm shift away from simple decellularization and towards the more clearly defined and clinically relevant goal of antigen removal (AR).

We have demonstrated that AR from bovine pericardium (BP) is enhanced—beyond that achieved by hypotonic solution or 0.1% (w/v) SDS decellularization methods—by facilitating solubilization of hydrophilic, water-soluble proteins (WSPs). However, it has not been investigated whether hydropobic, lipid-soluble protein (LSP) antigens are efficiently removed from BP using methods designed to promote WSP solubilization.

HYPOTHESES & AIM

We hypothesize that (1) promotion of WSP solubilization for AR will not significantly reduce residual lipid-soluble protein (LSP) antigens in BP and (2) a sequential strategy promoting solubilization and AR of WSPs followed by LSPs will reduce total residual antigenicity of BP compared to AR of WSPs alone.

We aim to adapt the principles of differential protein solubility into a novel AR strategy for the generation of xenogeneic scaffolds.

METHODS

Antigen removal. Antigen removal was performed as previously described. Briefly, BP pieces (0.2 g) were incubated in AR buffer at 4°C and 125 rpm for 48 h; adjacent pieces subjected to 1 min incubations served as negative AR controls.

One-step antigen removal. Solubilization and removal of WSPs was performed using basic AR buffer (BARB) or optimized solubilizing AR buffer (opt SARB; 100 mM DTT, 2 mM MgCl2, 100 mM KCl in BARB) containing no additional additive. 134 mM NDSB-256, or 0.1% (w/v) SDS (n=6 per group).

Two-step antigen removal. Solubilization and removal of WSPs was performed using opt SARB. Bovine pericardium samples were then subjected to a second step of AR using no additional additive, 134 mM NDSB-256 and 1% (w/v) SDS to solubilize and remove LSPs. Antigen removal using 0.1% (w/v) SDS and 1% (w/v) SDS in BARB for steps 1 and 2, respectively, served as positive control (n=6).

Assessment of antigen removal. Assessment of AR was performed as described previously. Briefly, residual WSPs and LSPs extracted from minced BP post-AR (BPAR) were subjected to electrophoresis and Western blot, probed with rabbit serum generated against native BP. Residual antigenicity, defined as the ratio of 48 h to 1 m samples, was determined by densitometry and normalized against the negative control (BARB for one-step AR, opt SARB for two-step AR). Means were analyzed using ANOVA and Tukey-Kramer HSD, with significance defined as p<0.05.

Histology. Gross tissue morphology and presence of residual nuclei were assessed for H&E-stained sections of native BP and compared to BP-AR (n=6 per group).

RESULTS

One-step antigen removal. The level of residual LSP antigens in BP-AR generated using no additional additive, 134 mM NDSB-256, or 0.1% (w/v) SDS in opt SARB was not significantly different from that in BARB (Fig 1).

Lipid-soluble protein antigenicity is not significantly decreased following WSP solubilization.

Two-step antigen removal. Residual WSP antigenicity of BP-AR following a second step of AR using detergents, chaotropes, or organics in opt SARB was not significantly different from that after treatment with opt SARB alone (Fig 2A). Use of opt SARB during two-step AR significantly reduced residual WSP antigenicity of BP-AR to 25% of that remaining following two-step AR with 0% (n=0.05) (Fig 2A).

Residual LSP antigenicity of BP-AR following a second step of AR using additives to promote LSP solubilization was significantly decreased compared to that of opt SARB-treated BP-AR by 44% with detergents (p=0.05), 91% with chaotropes (p<0.0001), 33% with organics (p=0.05), or 37% with 1% (w/v) SDS (p=0.01) (Fig 2B). Residual LSP antigenicity present following treatment of BP with detergents or organics was not significantly different from the level that persisted after treatment with 1% (w/v) SDS (Fig 2B). However, a second step of AR with chaotropes resulted in a significant 54% decrease in residual LSP antigenicity compared to with 1% (w/v) SDS (p=0.0001) (Fig 2B).

Water-soluble protein antigenicity is not significantly decreased following sequential, Differential WSP step antigen removal followed by LSP step antigen removal.

Solubilization of protein antigens to facilitate efficient AR is an important concept in xenogeneic scaffold generation. Although we previously demonstrated that solubilization of WSPs with opt SARB significantly reduced residual WSP antigenicity in BP-AR, WSP solubilization did not significantly reduce residual LSP antigenicity in BP-AR. Conversely, LSP solubilization did not significantly alter residual WSP antigen levels in BP-AR. Targeting both WSP (using opt SARB) and LSP (using detergents) solubilization in a two-step AR strategy significantly reduced residual LSP antigenicity and nuclei in BP-AR without damaging gross tissue morphology compared to when only opt SARB was used.

Histology. Gross tissue morphology and presence of residual nuclei were assessed for H&E-stained sections of native BP and compared to BP-AR (n=6 per group).

Antigen subsets for enhanced removal from xenogeneic scaffolds.

We aim to adapt the principles of differential protein solubility into a novel AR strategy for the generation of xenogeneic scaffolds.

CONCLUSIONS & FUTURE WORK

Solubilization of protein antigens to facilitate efficient AR is an important concept in xenogeneic scaffold generation. Although we previously demonstrated that solubilization of WSPs with opt SARB significantly reduced residual WSP antigenicity in BP-AR, WSP solubilization did not significantly reduce residual LSP antigenicity in BP-AR. Conversely, LSP solubilization did not significantly alter residual WSP antigen levels in BP-AR. Targeting both WSP (using opt SARB) and LSP (using detergents) solubilization in a two-step AR strategy significantly reduced residual LSP antigenicity and nuclei in BP-AR without damaging gross tissue morphology compared to when only opt SARB was used.

Taken together, these results underscore the need to account for the spectrum of protein antigen solubilities in a tissue by employing a multi-step strategy to sequentially and differentially solubilize protein antigen subsets for enhanced removal from xenogeneic scaffolds.

Current and ongoing studies aim to characterize the tensile properties, ECM composition, DNA content, and recellularization potential of BP-AR to ensure that a functional scaffold is maintained following sequential, differential AR.

REFERENCES


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