Glycosaminoglycan Stabilization in Bovine Pericardium

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Introduction: Glutaraldehyde crosslinked bovine pericardium (BP) has been used for fabrication of bioprosthetic heart valves as well as cardiac patches for soft tissue repair. However, calcification and limited mechanical stability result in shortened life for the prostheses. Previous research has shown that glutaraldehyde (Glut) crosslinking does not stabilize glycosaminoglycans (GAGs) and that GAGs are lost from porcine bioprosthetic heart valves[1]. BP tissue is composed of an amorphous network of collagen and elastin fibers, proteoglycans, and GAGs. The GAGs of BP include dermatan sulfate, chondroitin sulfate, and hyaluronan[2].

We hypothesize that Glut does not stabilize GAGs in pericardium and loss of GAGs may play a role in the degenerative failure of pericardial valves. Also stabilizing GAGs in the extracellular matrix of the pericardial valves may improve their function and extend their life. The objective of this study was to quantify GAG content following Glut fixation, and optimize GAG stabilization in bovine pericardial valves. A 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide (EDC) based crosslinking chemistry that links GAG carboxyl groups to the amine groups of proteins was used.

Methods:

Tissue Preparation
Fresh bovine hearts were collected from a local abattoir and transported to laboratory on ice. Pericardial tissue was removed from the heart, trimmed of external fat, and rinsed in saline. Small diameter (0.25 in) samples of pericardium were chemically crosslinked within 3-4 hours of dissection in two fixation groups.

- Group I. Glut (0.6% glut for 24 hrs followed by 0.2% Glut in HEPES buffer at pH 7.4 for 15 days),
- Group II: GAG stabilizing agent for 6 hours followed by 30 mM EDC/ 6 mM N-hydroxysuccinamide (NHS) for 24 hours (Pierce Biotech, Rockford, IL) and Glut (0.6% glut for 24 hrs followed by 0.2% Glut in HEPES buffer at pH 7.4) for 15 days.

GAGs in the pericardium were quantified by hexosamine analysis[3]. Stability of GAGs against enzymatic digestion was determined by treating half cusps in 10U/ml hyaluronidase and 0.2 U/ml chondroitinase ABC (Sigma Aldrich Corp, St. Louis, MO) in 100 mM ammonium acetate buffer (37^0C for 48hrs at 650 RPM). Remaining pericardium samples were placed in ammonium acetate buffer alone as controls. For all groups n=6 was used.

Results and Discussion: Following GAG enzyme digestion GLUT crosslinked pericardium showed a 28% loss in GAG content. Tissue fixed with the GAG stabilizing agent (Group II) showed no decrease in GAG content after treatment with chondroitinase ABC and hyaluronidase indicating a resistance to enzymatic degradation.

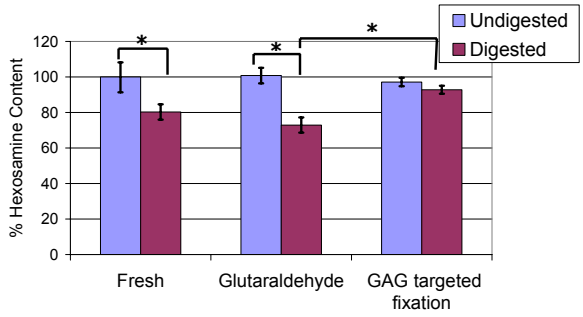

Figure 1: Hexosamine content after enzymatic degradation in Bovine Pericardium. *ANOVA $p<0.05$

Conclusions: Glutaraldehyde fixation does not stabilize GAGs in bovine pericardium against enzymatic degradation. The current study also indicates that GAG stabilization in bovine pericardium can be achieved by using GAG targeted fixation chemistries before Glut crosslinking. Future studies will include in vitro durability testing and in vivo implantation to study degeneration of GAG stabilized pericardium.

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References:

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