Comparison of Glycosaminoglycan-Targeted Fixation Chemistries and their Effects on Bioprosthetic Heart Valve Cuspal Tissue

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Statement of Purpose: Conventional fixation of bioprosthetic heart valves (BHVs) involves the use of Glutaraldehyde (Glut). However Glycosaminoglycans (GAGs) lack the amine functionality necessary for crosslinking to occur. Recent studies have shown that GAGs are chronically lost both clinically and *in vitro*.[1] It is hypothesized that the loss of GAGs, negatively charged, hydrophilic components of the native heart valve leaflets, may contribute to the accelerated degeneration of glutaraldehyde fixed BHVs.[2] Furthermore, fixation of these GAGs within the spongiosa layer of valve leaflets may help to improve the biomechanical function of the valve and improve durability of the implant. Studies have been conducted comparing the ability of Glut, Sodium Metaperiodate ($NaIO_4$), and 1-Ethyl-3-(3 dimethylaminopropyl) carbodiimide / N-hydroxysuccinimide (EDC/NHS) to stabilize GAGs in BHVs.

Methods: Fresh porcine hearts were obtained from a local abattoir; aortic cusps were excised and rinsed thoroughly in ice-cold saline. Cusps are chemically crosslinked within five hours of dissection in three fixation groups (n=6 cusps/group) including Glut (0.6% for 24 hrs followed by 0.2% for 6 days) (Sigma Aldrich Corp, St. Louis, MO), 30 mM EDC/ 6 mM NHS (Pierce Biotech, Rockford, IL) followed by Glut (EDC/NHS + Glut), and 3.25 mM $NaIO_4$ (Fisher Chemical, Fairlawn, NJ) followed by Glut ($NaIO_4$ + Glut). GAGs were quantified by hexosamine analysis.[3] GAG stabilization was determined by incubating half cusps in a solution of 5U/ml hyaluronidase and 0.1 U/ml chodroitinase ABC (Sigma Aldrich Corp, St. Louis, MO) in 100 mM ammonium acetate buffer (37^0C for 24hrs at 650 RPM). Corresponding half cusps were placed in buffer to act as a control. Cuspal tissue water content was measured as the difference between the wet weights (prior to lyophilization) and dry weights (following lyophilization) of the tissue. The rehydration capacity of the fixed tissue was also determined upon soaking the dry tissue in de-ionized water for 24hours at 4^0C and comparing the new wet weight to the tissue's initial wet weight. Collagenase type VII digestion of fixed cusps was used to determine collagen stability. *In vitro* GAG loss was monitored following fixation at specified time points (24 hours, 7 days, 2 weeks, 3 weeks, and 3 months). *In vivo* GAG stability was determined by subcutaneously implanting cusps in male juvenile Sprague-Dawley rats (30-45g, Harlan Laboratories, Indianapolis, IN). Cusps were explanted at two time points (three and six weeks), cut in sections for GAG quantification and histology. For all study groups an n=6 was used.

Results / Discussion: Treatment with chondroitinase ABC and hyaluronidase for cusps stabilized with EDC/NHS + Glut, and $NaIO_4$ + Glut for seven days showed significant GAG retention (146.99 ± 6.49 µg and 110.25 ± 6.98 µg Hexosamine / 10mg dry tissue respectively); however this was not seen in fixation by glut alone (91.67 ± 1.08 µg /10mg dry tissue). Cuspal water content decreased with GAG-targeted crosslinking of cuspal tissue in comparison to fresh, unfixed tissue. Water content of fresh, unfixed, non-GAG digested tissue was approximately 25.84 ± 0.47 mg of water / mg of dry tissue compared to 14.00 ± 0.60, 11.76 ± 0.51, and 10.64 ± 0.17 mg of water / mg of dry tissue for Glut, EDC/NHS + Glut, and $NaIO_4$ + Glut, respectively. However, following enzymatic digestion of GAGs, cusps fixed with GAG-targeted chemistries maintained their water content, whereas the fresh and Glut alone fixed tissue did not. It was also illustrated that cusps fixed with GAG-targeted fixation chemistries were able to rehydrate following GAG digestion. No statistically significant differences in mass loss were seen for Glut, EDC/NHS + Glut and $NaIO_4$ + Glut fixation groups (5.38 ± 0.60%, 3.47 ± 0.25% and 2.13 ± 0.37% respectively) following collagenase digestion showing that the stability of cuspal tissue collagen was unaffected by GAG fixation ($p<0.05$). Following three weeks of implantation, Glut fixed tissue lost 40 ± 2.23% of its initial GAGs while EDC/NHS + Glut only lost 20 ± 6.04% showing significant stabilization of GAGs. $NaIO_4$ + Glut fixed tissue lost approximately 38 ± 3.55% of its initial GAG content and thus this chemistry was unsuccessful in preventing *in vivo* GAG loss. GAG content was confirmed using histological staining procedures for acid muccopolysaccharides (Alcian blue staining). Six week explant results are still pending.

Conclusions: GAG stabilization in valvular tissue is possible using targeted fixation chemistries. Crosslinking of GAGs preserves hydration status while maintaining adequate collagen stability. It is apparent that some *in vitro* and *in vivo* GAG loss does occur over time in all fixation groups to different extents; however GAG stabilization occurs and may be significant enough to improve the biomechanics and longevity of the bioprosthetic heart valve. Future investigations will include determining mechanical properties and *in vitro* durability of mechanically fatigued GAG stabilized valves. It is expected that bioprosthetic heart valves in which GAGs are chemically crosslinked will exhibit increased durability *in vivo*.

References:

[1]Lovekamp, J. Biomaterials.2005; *In press*.
[2] Vyavahare, N. J Biomed Mater Res.1999;46:44-50.
[3] Blix, G. Acta Chem Scan. 1948;2:467-473.