Improved Glycosaminoglycan Stabilization in Bioprosthetic Heart Valves

Devanathan Raghavan, Dan Simionescu, Narendra Vyavahare.
Department of Bioengineering, Clemson University, Clemson, SC 29634.

Introduction: Approximately 300,000 heart valve replacement surgeries are performed every year worldwide. About 40-50% of these patients receive bioprosthetic heart valves (BHVs). The new generation of BHVs are significantly better; however, long-term success of these valves will depend upon better extracellular matrix stabilization to prevent degeneration. Commercially available BHV's are fixed with Glutaraldehyde (Glut). Glut is known to be an excellent fixative for the collagenous component of the heart valves. However it does not stabilize the glycosaminoglycans (GAGs) present in the heart valve cusps. It has been shown that GAGs are lost during *in vitro* cyclic fatigue and after animal implantation[1,2]. Clinically explanted BHVs also show GAG depletion[3]. We hypothesized that the loss of GAGs may in part be responsible for degeneration resulting in the ultimate failure of the valves[1]. The objective of this work was to find improved GAG stabilizing chemistries for BHVs. We used two 1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide (EDC) based crosslinking chemistries that would link GAG carboxyl groups to the amine groups of proteins within heart valve cusps.

Methods: Porcine aortic heart valves were obtained from a local abattoir; aortic cusps were dissected along the commissures and rinsed thoroughly in ice-cold saline. Cusps were chemically crosslinked within 3-4 hours of dissection in three fixation groups (n=6 cusps/group)

Group I: Glut (0.6% glutaraldehyde for 24 hrs followed by 0.2% Glut 6 days),

Group II: 30 mM EDC/ 6 mM NHS for 24 h followed by Glut for 6 days as shown in group I (EDC/NHS+Glut),

Group III: GAG stabilizing agent (GSA) for 1 hr followed by EDC/NHS for 24h and Glut for 6 days as shown in Group II (GSA + EDC/NHS + Glut).

Cusps were stored in 0.2% glut after fixation. GAGs in the cusps were quantified by hexosamine analysis and DMMB assay[4, 5]. Stability of GAGs against enzymatic digestion was determined by treating half cusps in 5U/ml hyaluronidase and 0.1 U/ml chodroitinase ABC (Sigma Aldrich Corp, St. Louis, MO) (37^0C for 24hrs at 650 rpm). Remaining half cusps were placed in buffer alone as controls. Protein crosslinking was assessed by determining the thermal denaturation temperature T_d using Differential Scanning Calorimetry (DSC). Cusps were implanted subcutaneously in male juvenile Sprague-Dawley rats (30-45g, Harlan Laboratories, Indianapolis, IN) to determine *in vivo* GAG stability. Cusps were explanted after three weeks, cut in sections for GAG quantification and histology. To analyze the extent of calcification, cusps were digested in HCl and the solution was analyzed for calcium by atomic absorption spectroscopy[6]. For all study groups n=6 was used except for the implantation studies (n=10).

Results and Discussion: After 5 weeks of fixation, cusps in Group III was found to have more GAGs than the other two groups. Cusps fixed with GSA (Group III) showed increased GAG stability against enzymatic degradation after treatment with chondroitinase ABC and hyaluronidase (Group I: 91.67 ± 1.08 μg, Group II: 176.73 ± 4.65 μg, and Group III: 239.04 ± 8.55 μg hexosamine/10mg dry tissue, $p<0.05$). DMMB assays were used to quantify the GAGs released from the cusps into the enzyme solution. More GAGs were released from cusps in Group I and II than from Group III cusps (41.10 ± 1.5, 16.43 ± 0.6, 5.92 ± 0.8 μg GAGs/ 10 mg dry tissue for Group I, II, & III respectively, $p<0.05$). The thermal denaturation temperatures T_d of the cusps were in the acceptable range varying from ~91 – 93° C for all the 3 groups showing adequate collagen crosslinking. After 3 weeks of implantation, the Group III cusps retained more GAGs as compared to Group I and II. (129.55 ± 4.35, 130.87 ± 8.67, 147.42 ± 2.22 μg hexosamine/ 10 mg dry tissue for Group I, II, & III respectively, $p<0.05$). Alcian blue staining for GAGs left in the cusps after implantation showed more staining in the group III than Group I and II and thus corroborated GAG assay results (data not shown). The Glut group showed significantly higher calcification than the other 2 groups (105.11 ± 4.33, 86.17 ± 3.76, 80.15 ± 8.65 μg Ca/ mg dry tissue for Group I, II, & III respectively, $p<0.05$). However, GAG stabilization did not lead to significant decrease in calcification. This was expected as cusps from all groups were finally crosslinked with Glut, which is known to exacerbate calcification. .

Conclusions: Present studies show that GAG stabilization in BHVs is possible by using GAG targeted fixation chemistries prior to Glut crosslinking. Importantly, GAG targeted fixation helps in preserving the GAGs while maintaining adequate collagen stability. Whether such GAG stabilization would improve durability of BHVs is under investigation. Future studies will include *in vitro* and in vivo durability of GAG stabilized valves, and combination of GAG stabilization with anti-calcification treatments.

Acknowledgement: This work was supported by a grant from National Institutes of Health (HL-70969).

References:

1. Vyavahare,N. et al,J Biomed Mater Res.1999;46:44-50.
2. Lovekamp, J. et al, Biomaterials. 2006; 27: 1507-1518.
3. Grande-Allen KJ et al, J Biomed Mater Res A. 2003 May 1;65(2):251-9
4. Blix, G. et al, Acta Chem Scan. 1948;2:467-473.
5. Hoemann, C. et al, Analytical Biochemistry 2002; 300: 1-10.
6. Vyavahare, N. et al. Circulation. 1997; 95: 479-488.