Neomycin-Enhanced Carbodiimide Crosslinking for Glycosaminoglycan Stability in Bioprosthetic Heart Valves

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Statement of Purpose: Current bioprosthetic heart valves (BHVs) suffer from insufficient long-term durability and typically fail within 12 to 15 years after implantation [1]. Although adequate for some, this limitation precludes implantation in younger individuals in order to avoid reoperation. Even in elder recipients, valve dysfunction can still cause death or reoperation that could be avoided with increased durability. Therefore, investigation into methods of increasing the lifespan of BHVs can not only improve the quality of life for BHV recipients, but widen the accessible patient demographic as well.

All BHVs are currently treated with glutaraldehyde (GA), which is used to stabilize collagen through crosslinking as well as reduce tissue immunogenicity. However, the use of GA crosslinking potentiates calcification and leads to undesirable changes in tissue mechanical properties that are conducive to structural degeneration [1]. One phenomenon that can contribute to the accumulation of structural damage is the loss of glycosaminoglycans (GAGs) from BHV cusp tissue [2]. GAGs lack the amine functionalities necessary to react with GA and, under current fixation methods, are lost during fatigue, implantation, and storage [2,3,4]. Carbodiimide crosslinking with EDC and NHS utilizes available carboxyl groups, in addition to amine groups, for crosslinking allowing for both GAGs and collagen to be crosslinked. However, it has been shown that crosslinking alone is not sufficient to fully preserve GAGs, as they are still prone to loss through enzymatic degradation [4].

Neomycin, a hyaluronidase inhibitor, aids in the prevention of enzymatic GAG degradation and contains amine functionalities that enable incorporation into carbodiimide-initiated crosslinks [4]. This study investigates the effects of carbodiimide crosslinking in combination with neomycin, (NEN fixation), on the stability of structural proteins, GAG preservation, calcification, and biomechanical properties.

Methods: Porcine aortic valve cusps underwent three treatments: glutaraldehyde (GLUT), carbodiimide and formalin (EDC), and carbodiimide with neomycin and formalin (NEN). Differential scanning calorimetry was used to determine the collagen denaturation temperature. Collagen and elastin stability were assessed using resistance to collagenase and elastase digestion respectively, defined using % weight loss. GAG preservation was studied after *in vitro* enzymatic digestion, implantation, and storage. GAGs were quantified using hexosamine assay and results were verified visually using alcian blue staining. *In vivo* studies were performed using three-week implantation in male juvenile Sprague-Dawley rats and calcification was quantified using atomic absorption spectroscopy. The stiffness and extensibility of cusp tissues were also evaluated using tensile testing.

Results: Weight losses after collagenase for NEN, EDC, and GLUT tissues were 17.9 ± 1.82%, 22.9 ± 0.92%, and 16.86 ± 0.94%, respectively. Weight loss after elastase for NEN, EDC, and GLUT tissues were 26.69 ± 0.68%, 32.41 + 1.1%, and 27.36 ± 0.62%, respectively. Collagen denaturation temperatures were 93.6 + 0.38 °C, 92.83 ± 0.36 °C, and 86.59 + 0.56 °C for NEN, EDC, and GLUT. GAG quantification data after *in vitro* enzymatic GAG digestion, rat subdermal implantation, and storage is presented in Figure 1. High-strain stiffness for the NEN and GLUT groups were 14.21 ± 1.73 kPa and 28.20 ± 3.32 kPa in the circumferential direction and 1.54 ± 0.13 kPa and 2.22 ± 0.23 kPa in the radial. Low-strain stiffness for the NEN and GLUT groups were 882 ± 111 Pa and 822 ± 53 kPa in the circumferential direction and 173 ± 17 Pa and 137 ± 10 Pa in the radial. Extensibility for NEN and GLUT was 30.78 ± 2.19% and 14.36 ± 0.65% in the circumferential direction and 78.8 ± 5.47% and 39.1 ± 1.89% in the radial.

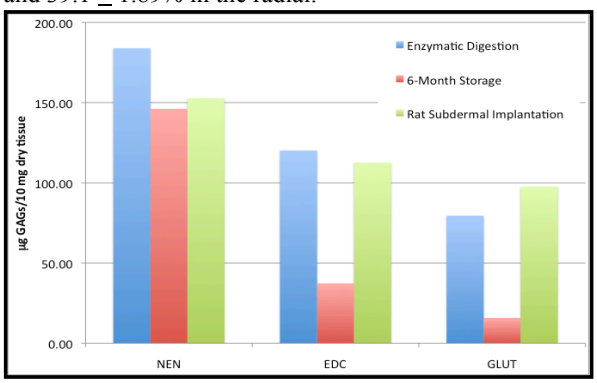

Figure 1. Tissue GAG Contents After Induced Loss

Conclusions: The addition of neomycin improved the resistance of carbodiimide crosslinked tissues to GAGase, collagenase, and elastase. When compared to GLUT, NEN tissues offered similar collagen and elastin stability. The collagen denaturation temperature demonstrated that a sufficient degree of crosslinking was achieved by NEN fixation. The NEN treatment also effectively preserved GAGs better than EDC and GLUT against all modes of loss. NEN tissues demonstrated lower stiffness and increased extensibility when compared to GLUT tissues. All together, this suggests that NEN fixation offers suitable ECM stability while offering superior GAG preservation, possibly improving mechanical function. Basic mechanical properties are also improved using NEN fixation, which may delay the onset of mechanically induced collagen damage. However, further mechanical testing is needed to support this claim.

References: 1. (Schoen FJ. J Biomed Mat Res. 1999;47:439-465.) 2. (Grande-Allen KJ. J Biomed Mat Res. 2003;65A:251-259.) 3. (Raghavan D. Acta Biomat. 2009;5:983-992.) 4. (Raghavan D. Biomat. 2007;28:2861-2868.)