Novel TRI™ Heart Valve Biomaterial Resists Calcification and Structural Degradation In-vivo

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Introduction: Over 300,000 heart valve transplants are performed annually[1]. Bioprosthetic heart valves (BHVs) are the predominant option for valve replacement because they do not require lifelong anticoagulants, can be implanted minimally invasively via catheter, and offer better biomechanics/hemodynamics[1]. Unfortunately, the implant life of commercially available BHVs on market treated with glutaraldehyde (GLUT) is 10-15 years[2]. GLUT BHVs primarily fail due to two failure modes that are not mutually exclusive. (1) GLUT BHVs heavily calcify and GLUT treatment is partially responsible for calcification[2]. (2) GLUT only cross links the collagen component of the tissue through a reversible Schiff-base reaction thus allowing endogenous and host proteolytic activity to structurally degrade away other components such as glycosaminoglycans and elastin over time.

We have developed a novel, proprietary fabrication method (TRI™) that utilizes carbodiimide crosslinking chemistry, neomycin trisulfate, and pentagalloyl glucose to stabilize other ECM components to resist (1) calcification and (2) structural degradation. In this study, we demonstrate that porcine aortic valve leaflets treated with TRI™ resist calcification and structural degradation *in vivo*, have superior biomechanics, and may be more biocompatible than GLUT valves, the current market gold standard.

Methods: Freshly harvested porcine aortic heart valve leaflets were treated with either 0.6% GLUT or TRI™. Differential scanning calorimetry (DSC) and enzymatic degradation was used to analyze samples for tissue stability. Biomechanical characterization was done by subjecting treated samples to biaxial tension testing at UT Austin. Treated leaflets were subcutaneously implanted into juvenile rats for 90 days to assess *in vivo* biocompatibility and structural integrity. Implanted and unimplanted samples were analyzed for mineralization via inductively coupled plasma mass spectrometry (ICP) and Alizarin red staining. Hematoxylin and Eosin staining (H&E) was utilized to evaluate fibrous capsule thickness, macrophage infiltration, and foreign body giant cell formation. Structural integrity was evaluated through Movat's Pentachrome staining. Lectin histochemistry using biotinylated isolectin B4 was used to detect Galα epitope left in the tissue.

Results: Thermal denaturation temperature Td (DSC) and enzymatic resistance showed tissue stability at par or better than GLUT in TRI™ samples (Table 1). Biaxial tension testing revealed no significant difference in stiffness between GLUT and TRI™ leaflets in the circumferential direction but a significantly more compliant toe region in the radial direction in TRI™ than GLUT. Alizarin red staining showed heavy calcification in GLUT tissues; however, this calcification was absent in TRI™ leaflets (Figure 1). These data are consistent with ICP quantification of calcium and phosphorus mineralization found in explanted tissues. H&E on fibrous capsules of GLUT found chronic persistence of macrophages, formation of foreign body giant cells, and moderate fibrous capsule thickness (Figure 1). TRI™ elicited little to no inflammatory response. Movat's pentachrome staining indicated ECM degradation of important components (such as elastin) in GLUT while TRI™ retained key ECM components post implantation (Figure 1). No significant difference was found between GLUT and TRI™ valves in Galα detection indicating that this highly immunogenic epitope was not recognizable.

Table 1: Tissue stability assessment

	Collagenase Stability (% wt. loss)	Elastase Stability	GAGase Stability (% loss)	Td by DSC (^{o}C)
GLU	1.52±1.07	16.8±2.1	57.3±4.9	89.7±0.9
TRI	0.42±0.42	4.6±1.9*	9.5±0.4*	90.1±1.2

*Indicates significant difference ($p<0.05$) from GLU (GLUT). Elastase stability measured by % wt. loss.

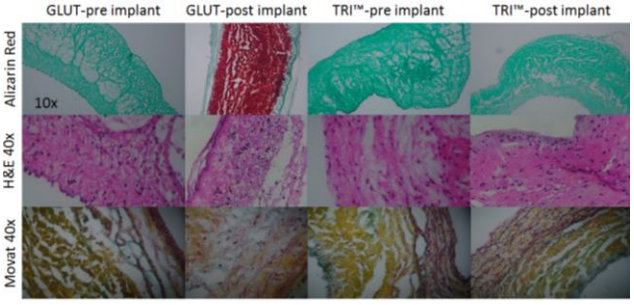

Figure 1. Histology of leaflets treated with GLUT or TRI™ pre or post implantation in juvenile rat calcification model for 90 days. Alizarin red stained calcium deposits red with green counterstain. H&E stained nuclei dark purple and cytoplasm pink. Movat's Pentachrome stained glycosaminoglycans blue, collagen yellow, and fibrous elastin purple/black.

Conclusions: Biomechanical testing suggested a more compliant and durable biomaterial crosslinked by TRI™. Histology corroborated with analytical techniques indicated that TRI™ treated tissues resisted calcification, retained key ECM components, and may be more biocompatible with reduced inflammatory response than GLUT. Accelerated wear testing is currently ongoing and will give insight into stress concentrations, failure points, and fatigue limits of GLUT vs. TRI™. Investigation into cellular toxicity of TRI™ and its leachables *in vitro* in preparation for a large animal study to demonstrate *in vivo* safety and efficacy are underway. Future work will investigate tissue composite biomechanics in collaboration with UT Austin for development of a BHV fatigue model to better design BHVs.

References: 1) Manji R. Am Heart J 2012; 164:177-85. 2) Schoen FJ. Annu Rev Pathol Mech Dis 2012; 7: 161 – 83. **Acknowledgements:** NIH Grant R01HL108330

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