# Prevention of Bioprosthetic Valve Calcification by Triglycidylamine (TGA) and Ethanol (EtOH) Pretreatment

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**Introduction:** Heart valve disease affects millions of patients per year and is treated by valvular replacement when repair cannot be done in symptomatic individuals. Bioprosthetic valves fabricated from either glutaraldehyde (Glut) fixed porcine aortic valves or bovine pericardium are now used in the majority of cases, and most fail due to calcification within 10 years. We have previously shown that substitution of TGA for Glut as the crosslinker in preparation of bioprosthetic heart valves shows promise in both prevention of calcification *in vivo* (1) and yielding favorable biomechanical properties (1). However, of the three biomaterials commonly used for bioprostheses, TGA alone only optimally inhibits calcification of porcine aortic leaflet, not porcine aortic root or bovine pericardium. Glut pretreated aortic leaflets also pretreated with EtOH calcify less than Glut alone (3); however, Glut-ethanol does not significantly inhibit porcine aortic wall calcification. **Objective:** To investigate the combination of TGA with ethanol as an anti-calcific strategy for preparing bioprosthetic heart valves.
**Methods:** Glut and TGA pretreatment of porcine aortic leaflet and root, and bovine pericardium, were carried out as previously described (1). In addition, 24 hour pretreatments with 95% EtOH with following either Glut or TGA fixation were also investigated. Physical characteristics were assessed using differential scanning calorimetry (DSC), Fourier transform infrared spectroscopy (FTIR), lipid analysis and hydrodynamic testing. Calcification *in vivo* was assessed by both the 90-day rat subdermal implant model (n=12 per group), and after fabrication of clinical-grade trileaflet porcine aortic heart valves (St Jude Medical), by 150-day mitral valve replacement in sheep. Pathological evaluation upon explantation was followed by Ca and P quantitation.
**Results:** DSC showed no significant change in shrink temperature after EtOH pretreatment of TGA crosslinked leaflet or pericardium versus TGA. FTIR revealed a shift in peak heights in the region of 1650-1645 nm, a region otherwise identical between Glut and TGA pericardium without EtOH treatment. Cholesterol was more effectively extracted by EtOH from TGA-treated samples than from Glut ($p<0.001$). Hydrodynamic testing showed a significantly lower pressure gradient across TGA-EtOH valves than Glut valves ($p<0.05$). Calcification analyses of 90 day rat subdermal implants (Figure 1), using leaflets, showed reduction with EtOH pretreatment of either Glut or TGA materials ($p<0.001$), but that TGA-EtOH treatment was significantly superior ($p=0.02$). Duplicate experiments using pericardium demonstrated comparable reduction in calcification with TGA-EtOH treatment ($p=0.026$).

**Figure 1. Calcium content in 90 day rat subdermal porcine aortic leaflet explants**

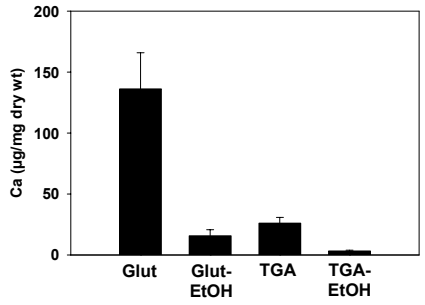
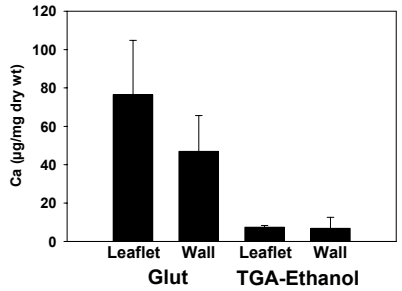



**Figure 2. Calcium content of explanted 150 day sheep porcine aortic valve bioprosthesic mitral valve replacements**




Results of 150 day sheep mitral valve replacements (Figure 2), using Glut (n=7) or TGA-EtOH (n=8) trileaflet valves, showed that TGA-EtOH reduced both leaflet calcification ($p=0.001$), and aortic wall calcification ($p=0.006$), with no mortality or morbidity after surgical recovery. Pathologic examination of TGA-EtOH explants revealed an absence of microscopic intrinsic cuspal calcification. However, two of the cusps (on separate bioprostheses) demonstrated isolated calcific nodules. Microscopic calcification was noted in 11/24 aortic wall segments (6 of 8 explants).
**Conclusions:** TGA crosslinking, followed by EtOH pretreatment, provides protection from calcification of porcine aortic leaflet and aortic wall, and bovine pericardium *in vivo*. These data suggest that clinical use of TGA-EtOH pretreated bioprosthetic materials may enhance bioprosthetic valve durability.
**References:** 1) Connolly JM. Am J Pathol 2005;166:1-13. 2) Rapoport HS.Biomaterials2007;28:690-699. 3) Clark JN. 2005 Ann Thorac Surg 79:897-904.