Effects of Glutaraldehyde Fixation and Cyclic Loading on the Thermoelastic Properties of Aortic Heart Valve Leaflets

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Background: Hydrothermal isometric tension (HIT) testing is a rapid, reliable technique to assess mechanically relevant crosslinking in collagenous tissues. HIT tests measure changes in isometric force exerted by tissue in a polar solution during a course of thermally-induced structural transitions in native collagen: denaturation, scission of thermally labile immature crosslinks, and hydrolysis of the collagen polypeptide chains. We hypothesized that long-term cyclic loading of aortic valve tissue damages collagen at the molecular level, involving collagen denaturation and/or fragmentation. To address this hypothesis, we assessed the thermoelastic properties of porcine aortic valve leaflets following glutaraldehyde treatment and cyclic loading using HIT tests.

Methods: HIT Tests were performed on fresh and glutaraldehyde-fixed circumferential strips cut from porcine aortic valve (PAV) leaflets. One group was glutaraldehyde treated for varying lengths of time (24 hr vs 1 month). Another group (also glutaraldehyde treated) was cycled from 0 to 200x10^6 cycles. Tissue strips were held under isometric constraint in a water bath. Water temperature was increased from room temperature and sustained at 95^{o}C for a 10 hr isotherm. Load, temperature, and time were acquired throughout. In the usual HIT analysis, the temperature at which intrahelical hydrogen bonds are disrupted is the denaturation temperature (T_d) and is an indicator of helical stability and intrahelical collagen crosslinking. The isothermal load decay half-time ($t_{1/2\ control}$), measured as collagen peptide bonds are hydrolyzed, is an indicator of total collagen crosslinking.

Results/Discussion: Fresh tissue exhibited a T_d at 69.5^{o}C, consistent with values reported for other collagenous tissues. Glutaraldehyde treatment significantly increased T_d to ~ 85 ^{o}C (Fig. 1), reflecting an increase in triple helical stability. The length and/or nature of glutaraldehyde treatment also altered T_d, with slight variation in T_d between PAV tissues fixed for 24 hr, 1 month and uncycled control valves (Fig. 1). Furthermore, triple helical stability was reduced in cusps following cyclic loading, with T_d significantly lower in leaflets cycled to as few as 17 x 10^6 from that of uncycled tissue (Fig. 1). Reduced helical stability with cyclic loading may arise from decreases in intramolecular H-bonding and/or covalent crosslinks. These molecular changes may be important initiating events in the fatigue damage to glutaraldehyde treated—and likely native—collagen fibers. During the 95^{o}C isotherm, fresh tissue exhibited the expected load decay which led to tensile failure of the sample. By contrast, glutaraldehyde-treated tissues exhibited a sustained contraction throughout the 95^{o}C isotherm (Fig. 2). Furthermore, the isothermal contraction was significantly *increased* following cyclic loading, with contraction rates approximately doubling in leaflets cycled to as few as 17 x 10^6 cycles (Fig 2.)

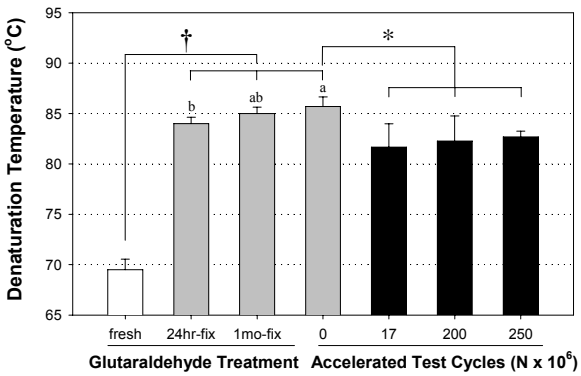

Figure 1. Effects of glutaraldehyde fixation and cyclic loading on T_d. † = significantly different from fresh. ∗ = significanty different from 0 x 10^6 cycles. Tissues fixed under varying conditions were compared using ANOVA; values labelled with the same letter are N.S. different.

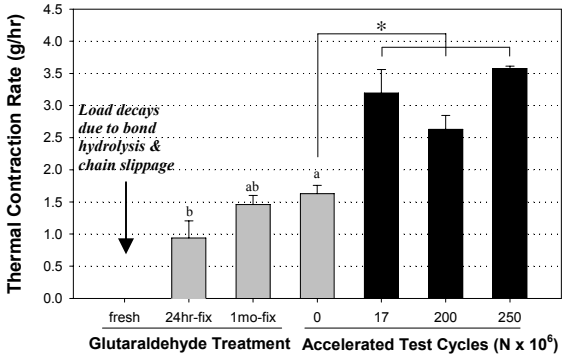

Figure 2. Effects of glutaraldehyde fixation and cyclic loading on isothermal contraction rate. ∗ significanty different from 0 x 10^6 cycles. PAV tissues fixed under varying conditions were compared using ANOVA ; values labelled with the same letter are N.S. different.

Isothermal contraction may result from hydrophobic interactions between glutaraldehyde-treated collagen and water. Water penetration into the dehydrated, hydrophobic fiber may cause contraction as it contracts to expel water. Increased thermal contraction with cyclic loading may reflect (i) increases in collagen hydrophobicity from fiber disruption and greater glutaraldehyde exposure, or (ii) increases in the tissue dehydration, possibly from loss of GAGs.

Conclusions: Long-term cyclic loading greatly alters the thermoelastic properties of glutaraldehyde-treated collagen, revealing disruption or loss of stability at the molecular (triple helical) level. HIT tests provide highly sensitive measures of these changes.