THE EFFECTS OF MATRIX STABILIZATION USING GLUTARALDEHYDE AND GLYCATION WITH RIBOSE ON THE MATERIAL PROPERTIES OF PORCINE MENISCUS

*Hunter, SA; +*Butler, DL; **Noyes, FR; ***Haridas, B; +*Levy MS
+University of Cincinnati, Cincinnati, OH

Introduction

Given the limitations of meniscectomy and meniscal repair, allograft meniscus replacement is gaining momentum. Allografts incorporate into the joint and relieve pain in the short term, but arthroscopic or MRI evaluation reveals evidence of arthrosis and allograft tearing [1]. Research into alternative preservation methods is needed since current processes do not deter enzymatic degradation, a likely cause of such deterioration [2-3].

Two crosslinking stabilization methods under study are glutaraldehyde and glycation. Glutaraldehyde stabilization of porcine meniscus using heart valve fixation protocols excessively stiffens the tissue and increases its permeability, which could adversely affect meniscus function in vivo [4]. Decreasing glutaraldehyde concentration and/or exposure period might yield a more biomechanically favorable result while still preventing biodegradation after surgery. Another option is glycation, in which collagen is non-enzymatically cross-linked by reducing sugars. Glycation improves material and mechanical properties and increases resistance to enzymatic degradation in various tissue models [5-6], but it has yet to be used to stabilize the meniscus matrix.

The objective of this study was to evaluate the effects of glutaraldehyde and glycation on porcine meniscus material properties, specifically the aggregate modulus (solid component stiffness, $E_i$), permeability (measure of fluid flow through the tissue, $k$), compressive strain at equilibrium ($\varepsilon_{eq}$), and tissue water content. Our hypotheses were that both glutaraldehyde and ribose stabilization produce dose-dependent and time-dependent changes in meniscus material properties during in vitro testing.

Methods

Cylindrical specimens (3.0mm dia, 1–2mm thick) were obtained from medial and lateral menisci from twelve pairs of porcine knees (male and female mixed breed, 3–4 mos.). Three specimens were removed from the anterior, middle, and posterior thirds of each meniscus. Each specimen was tested in uniaxial confined compression in custom testing systems under one of three compressive stresses (0.066, 0.196, 0.326 MPa) until equilibrium was reached. Specimens were allowed to recover at least 80% of their original height after load removal. For glutaraldehyde treatment, specimens were subjected to either a dual- (0.6% for 1 day followed by 0.2% for 2 days) or single-cycle fixation (0.2% or 0.02% for 1 day). These specimens were incubated in 5mL of glutaraldehyde and phosphate-buffered saline (PBS) at 4°C. For glycation, specimens were placed in 5 ml of PBS containing antibiotics, protein synthesis blockers, metalloproteinase inhibitors, and either 15 or 30mM ribose. Specimens were incubated for either 2 or 10 days at 37°C in the presence of oxygen. After incubation, specimens were rinsed in 1L of PBS for 20 min. and re-tested in confined compression under conditions identical to the initial test. $E_i$, $k$, and $\varepsilon_{eq}$ were calculated by analyzing the creep response with a custom nonlinear least squares optimization program based on the biphasic theory [7]. Water content of the tissue remaining from the coring process and specimens after the second biomechanical test were measured. Statistics were performed using a mixed model one-way ANOVA with contrasts ($\alpha = 0.05$).

Results

Neither specimen thickness nor water content was altered by any stabilization treatment (p>0.11). The level of applied compressive stress significantly affected all material properties (p<0.003), but since the treatment effects did not vary with applied contact stress for any property (p>0.17) the following results are averaged across stress.

Glutaraldehyde

Aggregate modulus of the 0.02%/1d group was not different from untreated control (p>0.25; Fig. 1) but moduli for the dual-cycle and 0.2%/1d groups were at least 131% higher than control values (p<0.001). Similarly, the equilibrium strain of the 0.02%/1d group was not different from control (p>0.10), but in the remaining groups was at least 51% lower than control (p<0.001). Permeability was greater than control by at least 77% for all treatments (p<0.001; Fig. 2), with $k$ of the 0.02%/1d group lower than that of the other treatments (*p<0.047).

Glycation

Aggregate modulus of the 30mM/10d group was 20% lower than that of controls (p<0.001). Permeability was greater than control by at least 154% for all treatments (p<0.001; Fig. 2). Specimens incubated for 2 days had lower $k$ than those incubated for 10 days (**p<0.007).

Discussion

Material property changes depend greatly on fixation conditions. Fixative concentration most influenced the properties of glutaraldehyde-fixed tissue, while incubation time appeared to govern glycation effects. Previous glycation studies have shown that various mechanical properties and resistance to enzymatic degradation increase with increasing incubation time [5-6]. Stabilization techniques must still be optimized for future use. Selected glutaraldehyde and glycation fixation protocols do not alter aggregate modulus and equilibrium strain but still increase permeability. A supplementary experiment showed that incubation in PBS alone for 10 days increased $k$ over control by 133–560%, which may explain the high values observed in the 10-day glycation groups. While these results advise against prolonged incubation in future research, the mechanisms for increased permeability are still not fully known. Glycation may have distinct advantages over glutaraldehyde for future allograft development. Glutaraldehyde is cytotoxic even at lower concentrations, whereas up to 30mM ribose does not affect cell viability [6]. Glycated tissues also show no evidence of calcification that afflicts glutaraldehyde-fixed heart valves. Future research will explore these issues in meniscus and determine whether the stabilization treatments used in this study render meniscal tissue resistant to enzymatic degradation.

References


Listing for additional author affiliation

** Cincinnati Sportsmedicine and Orthopaedic Center, Cincinnati, OH
*** Stress Engineering Services, Inc., Mason, OH