The Effects of Freezing on the Mechanical Properties of Articular Cartilage

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INTRODUCTION

Post mortem tissue testing has many applications in automotive safety research, sports biomechanics, and military restraint design. Much of the current biomechanical research being conducted involves testing of both human and animal tissue. While some studies are conducted in vivo, on living subjects, most studies are performed using post mortem tissue. In many of these research areas, especially those involving human tissue, circumstances may require frozen storage of the tissue for a period before testing (Atkinson, 2001; Duma, 1998). This is largely because cadavers are relatively difficult to obtain. The tissue must also be tested for HIV and Hepatitis requiring that the cadaver be held until these tests are processed. Because of these difficulties, laboratories often choose to procure the tissue when it becomes available as opposed to when it is required for testing. The tissue is then frozen and stored at subzero temperatures, usually around –20C. These specimens are left frozen until they are needed for testing, this timeframe can range from a few days to months at a time.

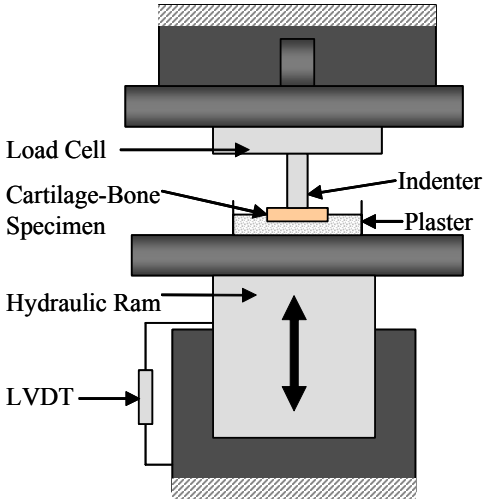
Studies have investigated and dismissed the effect of freeze-thaw cycles on skeletal muscle, trabecular bone, and ligament tissue, but have failed to properly address the effects of these storage methods on the integrity of articular cartilage. This paper addresses this concern with matched pair testing of various low temperature storage techniques against fresh control groups. The purpose of this study is to examine the effects of two freezing methods on the mechanical properties of articular cartilage.

METHODOLOGY

Matched pair testing of bovine articular cartilage was utilized. A total of 20 samples were used from 10 matched pairs. The lateral tibial plateaus of each limb were tested. Dynamic mechanical indention tests were performed on articular cartilage-on-bone specimens using a triangular displacement pulse with a 1 m/s displacement rate. Dynamic testing was accomplished with a hydraulic materials testing machine (Model 810, MTS, Raleigh, NC) similar to methods used by Kerin (1998) (Figure 1). Two freeze-thaw groups were compared to the non-frozen control. One sample from each of five pairs was frozen using a slow freeze conventional freezer (-20°C) at 0.1°C/min. In addition, one sample from each of five pairs was flash frozen using dry ice for a temperature rate of 10°C/min. The peak displacement was set to develop approximately 30 % cartilage strain. Using the impactor area and cartilage thickness measurements, the stiffness, peak stress, peak strain, and loading energy were calculated. Differences between each control sample and the frozen sample were analyzed for statistical significance. Injury analysis was performed using ink staining and histology.

RESULTS

Findings showed that when compared to the matched pair control samples, both the slow freeze and flash freeze regimes caused the articular cartilage stiffness to decrease significantly by 37% ($p=0.01$) and 31% ($p=0.01$) respectively (Table 1 and Figure 2). The peak stress, defined at 20% strain, decreased significantly for the slow thaw by 31% ($p=0.03$) and marginally significant for the fast thaw by 22% ($p=0.07$) specimens. The strain energy density decreased for both frozen methods but not significantly. All specimens showed signs of damage after being stained with ink. Histological inspections showed no obvious differences between fresh or frozen specimens.



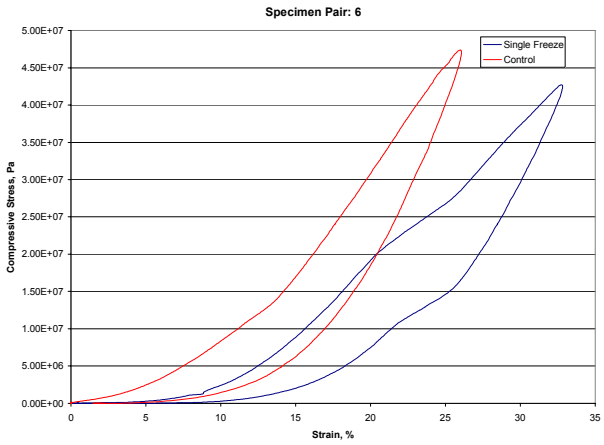


Figure 1: A hydraulic MTS machine was used for dynamic mechanical tests on potted specimens.

Figure 2: Typical stress-strain comparative plot for a frozen and fresh specimens.

CONCLUSIONS

Freezing articular cartilage, either by conventional or flash freezing techniques, affects its mechanical properties. Primarily, this effect is seen in reduced stiffness and peak stresses. These results may be indicative of a weakened extra-cellular matrix structure caused by the freeze and thaw process. However, based on the ink staining and histological examination, it is unclear what effect this has on the global injury prediction when using frozen cadavers. Cryonics research has shown that cell damage occurs as a result of certain types of freezing (Muldrew, 1994. Additional studies have also indicated extracellular matrix damage as a result of freezing (Muldrew 2000). Muldrew reported a greatly disrupted matrix with a large increase in matrix pore size as a result of ice crystal formation in cryopreservation. It is possible that the reduction in compressive stiffness being observed is a result of this damage.

Table 1: Summary of overall differences between frozen and control groups

	Strain Energy Density (Loading to 20%) Mpa	STD DEV	Modulus 15-22.5% strain (MPa)	STD DEV	Modulus 20-22.5% strain (MPa)	STD DEV	Stress at 20% strain (MPa)	STD DEV
SF Controls	1.27	(0.65)	224.00	(65.10)	253.00	(53.70)	21.00	(8.21)
Slow Freeze	1.03	(0.11)	141.00	(52.90)	158.00	(37.40)	14.40	(3.95)
%Difference	-18.9		-37.1		-37.5		-31.4	
p-value	0.11		0.00		0.00		0.03	
FF Controls	1.61E+00	(0.49)	229.00	(27.10)	263.00	(23.40)	23.30	(4.33)
Flash Freeze	1.26E+00	(0.61)	159.00	(54.00)	181.00	(51.00)	18.20	(7.77)
%Difference	-21.7		-30.6		-31.2		-21.9	
p-value	0.14		0.00		0.01		0.08	

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