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Research Paper

Effect of storage on tensile material properties of bovine liver

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ABSTRACT

Cadaveric tissue models play an important role in the assessment and optimization of novel restraint systems for reducing abdominal injuries. However, the effect of tissue preservation by means of freezing on the material properties of abdominal tissues remains unknown. The goal of this study was to investigate the influence of frozen storage time on the material responses of the liver parenchyma in tensile loading.

Specimens from ten bovine livers were equally divided into three groups: fresh, 30-day frozen storage, and 60-day frozen storage. All preserved specimens were stored at −12 °C. Dog-bone specimens from each preservation group were randomly assigned to one of three strain rates (0.01 s^{-1}, 0.1 s^{-1}, and 1.0 s^{-1}) and tested to failure in tensile loading. The local material response recorded at the tear location and the global material response of the whole specimen of the liver parenchyma specimens were investigated based on the experimental data and optimized analytical material models.

The local and global failure strains decreased significantly between fresh specimens and specimens preserved for 30 days ($p<0.05$), and between fresh specimens and specimens preserved for 60 days ($p<0.05$) for all three loading rates. Changes on the material model parameters were also observed between fresh and preserved specimens. Preservation by means of frozen storage was found to affect both the material and failure response of bovine liver parenchyma in tensile loading. The stiffness of the tissue increased with increased preservation time and increased strain rate.

In summary, significant changes ($p<0.05$) between the failure strain of previously frozen liver parenchyma samples and fresh samples were demonstrated at both global and local levels in this study. In addition, nonlinear and viscoelastic characteristics of the liver parenchyma were observed in tension for both fresh and preserved samples.

1. Introduction

The liver is the largest abdominal organ in the human body and while it is within a relatively protected location, it is one of the most commonly injured intra-abdominal organs as a result of blunt trauma (Brammer et al., 2002). Abdominal injuries caused by motor vehicle collisions have severe consequences (Arajarvi et al., 1987; Cheynel et al., 2011; Greingor and Lazarus, 2006), and the liver is one of the most frequently injured abdominal organs in frontal vehicle crashes (Elhagediab Rouhana, 1998). In addition, mortality and morbidity rates increase with the severity grade of liver

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injuries (Brammer et al., 2002). While the material properties of liver tissue has been investigated, most of the previous studies tested only fresh human or animal liver tissue (Brunon et al., 2010; Bummo and Jung, 2007; Chui et al., 2007; Kemper et al., 2010). However, the majority of biomechanical tests used to characterize the response and injury tolerance of the human abdomen are performed on fresh, previously frozen post-mortem human surrogates (PMHS) (Crandall et al., 2011; Eichberger et al., 2000; McIntosh et al., 2007; Salzar et al., 2009; Untaroiu et al., 2012). Consequently, there is a need to better understand the potential changes in the material properties of the liver tissue due to preservation by means of freezing.

In the recent years, PMHS have served as invaluable tools for the characterization of human biomechanical responses during impact loading (Crandall, 1994; Crandall et al., 2011). Therefore, the development of reliable preservation methods for PMHS, which minimize the biomechanical differences between living humans and preserved PMHS, has become very important for biomechanics research. During the late 1960s, it was shown that the embalming process changes the response of human tissues (Crandall, 1994). As a result of these findings, the testing of embalmed PMHS was abandoned. From this point, freezing and refrigeration methods have been widely used to store and preserve unembalmed post-mortem tissue. Typically, freezer storage methods are used to preserve post-mortem tissue at temperatures ranging from −10 °C to −70 °C. Various studies have shown that freezing within this range does not have a effect on the biomechanical response of bone or collagenous tissues such as ligaments or intervertebral discs (Frankel, 1960; Griffon et al., 1995; Hamer et al., 1996; Linde and Sorensen, 1993; Sedlin, 1965; Smeathers and Joanes, 1988; Weaver, 1966; Woo et al., 1986). While several studies have reported some comparisons between the mechanical properties of fresh and previously frozen abdominal organ tissues (Hollenstein et al., 2006; Ocal et al., 2010; Tamura et al., 2002), the effect of freezing on the mechanical response of the liver tissue under different loading rates is still largely unknown.

A number of studies have investigated the effects of freezing on the responses of animal livers under different types of loading schemes. Brunon et al. (2010) conducted quasi-static tensile failure tests on both fresh and previously frozen porcine liver parenchyma and capsule samples, and found that freezing affects the failure properties of porcine liver capsule but not human liver capsule. Ocal et al. (2010) conducted compressive impact experiments on bovine liver specimens to investigate the effect of preservation period (1–48 h after harvesting) on the viscoelastic material properties and found that liver tissue becomes stiffer and more viscous as the preservation time increases. Santago et al. (2009) compared the tensile failure stress and failure strain of fresh bovine liver parenchyma and bovine liver parenchyma frozen for 26 days and concluded that freezing decreased the failure strain. However, the study by Santago et al. (2009) was limited to samples obtained from only one bovine liver and the evaluation of a single loading rate. Nguyen et al. (2012) investigated the influence of a freeze-thaw cycle on the stress–stretch curves of porcine liver and reported that the mechanical properties of the liver tissues were almost unaffected by the freeze-thaw cycle. Tamura et al. (2002) performed a series of pre-conditioning compression tests on fresh and previously frozen porcine liver specimens and determined that freezing had no effects on the mechanical response of the tissue. However, it is possible that damage to the tissue during the pre-conditioning may have masked any potential changes in the compressive response resulting from the freezing process. Lu and Untaroiu (2013) conducted indentation tests on fresh porcine liver and porcine liver frozen for 20 days and found that freezing did not change the stiffness of porcine liver.

Although there have been some studies that have investigated the effect of freezing on liver tissue, the conclusions regarding the effect of freezing on the material response of liver tissue varies between studies. The discrepancies between these studies could potentially be due to variability in the composition of the liver tissues between mammalian species, the preservation periods evaluated, the loading rates, or the mechanism of loading. Previous studies have shown that the tensile material properties of bovine liver are not significantly different from those of human liver (Kemper et al., 2010). Therefore, the purpose of the current study was to investigate the influence of freezing on the biomechanical responses of the bovine liver parenchyma in tensile loading.

2. Method

2.1. Sample source and preservation

Uniaxial tensile tests were performed on the parenchyma of 10 fresh bovine livers obtained from Animal Technologies (Tyler, Texas, USA) (Fig. 1). Each organ was sectioned into three equal portions: one was tested immediately upon receipt; one tested after 30 days of frozen storage (Day 30); and one tested after 60 days of frozen storage (Day 60). The bovine livers were received within 24 h after slaughter. During transportation, the livers were sealed in plastic bags, and stored in ice filled containers. Frozen specimens were stored in a sealed container at −12 °C (Lu and Untaroiu, 2013), and then tested under the same testing condition as fresh samples after thawing. The time between the start of the thawing process and the testing was approximately 12 h. Frozen-thawed specimens were evaluated in this study because previously frozen PMHS are commonly used in abdominal impact tests (Untaroiu et al., 2012). The 30–60 day time interval reasonably approximates to the time window required to obtain serology, required approval, and pre-test medical examinations for PMHS.

2.2. Sample preparation and experimental setup

The tissue slicing and stamping procedures described by Kemper et al. (2010, 2012) were used to obtain constant thickness “dog-bone” shaped liver parenchyma specimens (thickness: ~5 mm, gage length: 19 mm, gage width: 10 mm) commonly used for uniaxial tensile testing . The specimens were prepared such that longitudinal axis of each specimen was parallel to the liver surface during the stamping process. All samples were then immersed in a bath of Dulbecco's