SHORT COMMUNICATION
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Effect of Postmortem Time and Preservation Fluid on the Tensile Material Properties of Bovine Liver Parenchyma

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ABSTRACT –The purpose of this study was to quantify the effects of postmortem degradation on the tensile material properties of bovine liver parenchyma when stored in DMEM or saline. Fourteen fresh bovine livers were obtained from a local slaughter house and stored in either DMEM or saline as large blocks, small blocks, or slices of tissue. Multiple parenchyma dog-bone samples from each liver were tested once to failure at three time points: ~6hrs, 24hrs, and 48hrs postmortem. The results showed that the failure strain decreased significantly between 6hrs and 48hrs after death when stored as large blocks in saline, while the cellular disruption increased. Preliminary results indicated that reducing the tissue storage size had a negative effect on the material properties and cellular architecture for both fluid types. Overall, this study illustrated that the effects of postmortem liver degradation varied with respect to the preservation fluid, storage time, and storage block size.

INTRODUCTION

The liver is one of the most frequently injured abdominal organs in motor vehicle collisions (MVCs) (Lamielle et al., 2006; Yoganandan et al., 2000; Klinich et al., 2010). Anthropomorphic test devices (ATDs) and finite element models (FEMs) are commonly used to predict injury risk during MVCs. However, current ATDs lack the instrumentation necessary to assess individual abdominal organ injury risk (Hardy et al., 2015). FEMs can be used to assess abdominal organ injury risk in MVCs, but they must first be validated using biomechanical data from material and structural testing. Although there have been a number of biomechanical studies performed on liver tissue, it is currently unknown how the failure properties of the liver change between time of death and 48hrs postmortem. Since abdominal organ tissue can degrade quickly after death, it is important to evaluate the effectiveness of preservation techniques in minimizing material and cellular changes between time of death and testing. Therefore, the purpose of this study was to quantify the effects of postmortem degradation on the tensile material properties of bovine liver parenchyma when stored in DMEM (Dulbecco's Modified Eagle Medium) or saline.

METHODS

Tension Testing

Uniaxial tension tests were conducted on isolated parenchyma samples of fourteen bovine livers at three time points after death; 6hrs, 24hrs, and 48hrs. Tissue was stored in one of two different preservation fluids (DMEM or saline), and in one of three different storage sizes (large block, small block, or slice). Each liver was obtained from a local slaughterhouse immediately after death. During transport to the lab, each liver was sealed in a plastic bag and placed on wet ice in a cooler. The livers were not frozen at any point during transport, testing, or storage. Upon arrival at the lab, a portion of each liver was used immediately for fresh specimen preparation and testing, i.e. 6hr time point. The remaining liver tissue was stored in the assigned preservation fluid until testing at one of the latter two time points. The stored tissue was divided into sections and placed into containers. The containers were filled with the assigned preservation fluid so that the tissue was completely submerged, sealed, and kept cool by means of refrigeration until the 24hr or 48hr time points.

A previously developed procedure was followed to obtain multiple dog-bone samples from each liver (Kemper et al., 2010). A small rectangular block of tissue was cut from the liver and sliced into thin rectangular slices using a custom slicing jig. Each slice was then laid on a stamping base and a template was used to position the slice so that the final sample would not include visible vasculature or defects. Finally, a custom stamp was used to cut a dog-bone sample from the tissue slice. In order to assess the effect of preservation time and fluid type on the cellular architecture, tissue samples were taken at each time point for histological analysis. Histology samples were stained with Hematoxylin and Eosin, and then graded by a certified veterinary pathologist on a scale of 0 to 3 based on the level of cellular disruption (0= normal;

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1= mild cell swelling; 2= moderate cell swelling and dissociation; and 3= moderate cell swelling, dissociation, and nuclear dissolution).

A testing procedure was developed to maintain consistency in initial specimen slack and alignment (Kemper et al., 2010). First, the top grip assembly was laid flat on a table top. The specimen was positioned and clamped into the top grip so that the long axis of the specimen aligned with the main axis of the grip and load cell. White circular paint markers were applied to the front surface of the specimen. Then, the top grip assembly was reattached to the testing device. The sample was allowed to hang under its own weight before being clamped into the bottom grip (Figure 1).

Figure 1. Mounted sample.

Side and front view pictures of the mounted sample were taken to obtain initial specimen width and thickness. During the test, the top and bottom grips moved away from each other at a constant velocity, applying tension to the specimen at a rate of ~1strain/s. High-speed video of the front face was recorded at 500fps. Load cell, potentiometer, and accelerometer data were sampled at 20kHz.

Data Processing

A test was considered successful if the failure tear occurred in the gage region of the dog-bone specimen. For each successful test, the white optical markers were tracked using motion analysis software. The vertical distance between the dots was best fit with a 6th degree polynomial from the time the grips began moving until the failure tear initiated. The distances between the markers surrounding the failure tear were used to calculate the stretch ratio and Green Strain. The inertia compensated force was fit using a 6th degree polynomial from the approximate time the grips began moving until the failure tear initiated. The stretch ratio, initial cross-sectional area at the tear location, and inertia compensated force were then used to calculate the 2nd Piola Kirchhoff Stress.

The failure stress and failure strain were determined for each sample to allow for comparisons and statistical analyses. The values of each variable were averaged among the successful tests within each time point for each liver, then compared across the three time points for each liver and across all livers. A Linear Mixed Effect Model with a random intercept effect and a fixed time effect was used to determine significant changes in the variables of interest with respect to postmortem time. Significance was defined as $p \leq 0.05$.

RESULTS

A total of 243 tests from 12 livers (6 DMEM, 6 saline) were conducted from tissue stored as large blocks, resulting in 112 successful tests (Figures 2-3). The failure strain decreased significantly with respect to storage time for tissue stored as large blocks in saline ($p = 0.009$), while the failure stress did not change significantly ($p = 0.694$). Neither the failure stress nor strain changed significantly with respect to storage time for tissue stored as large blocks in DMEM ($p = 0.782, 0.872$).

A total of 71 tests from two livers (1 DMEM, 1 saline) were conducted from tissue stored as small blocks or slices, resulting in 36 successful tests (Figures 2-3). The failure strain decreased between the 6hr and 24hr time points, then plateaued or increased between the 24hr and 48hr time points, for tissue stored in both fluids. The failure stress decreased between the 6hr and 24hr time points, then plateaued between 24hr and 48hr, for all tissues except those stored as small blocks in DMEM. No statistical analysis was conducted on the small block or slice data since only one liver was tested per fluid under these conditions.

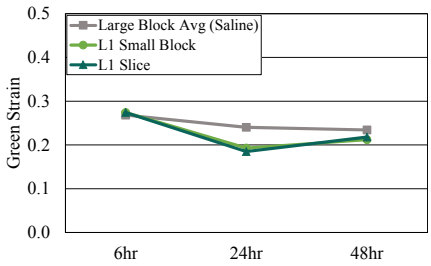

Figure 2. Average failure strain versus postmortem time point for tissue stored in saline.

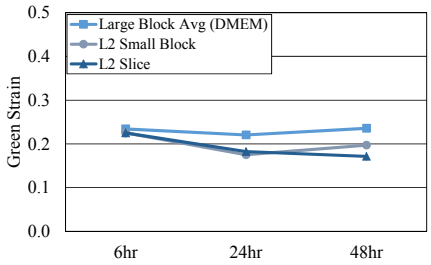

Figure 3. Average failure strain versus postmortem time for tissue stored in DMEM.

Histology

Figures 4 and 5 show an increase in cellular disruption with an increase in postmortem time for tissue stored in both fluids. The disruption seen at each time point was more evident when using smaller tissue storage sizes. Also, tissue stored in saline showed more cellular disruption than tissue stored in DMEM for a given storage block size and time point.

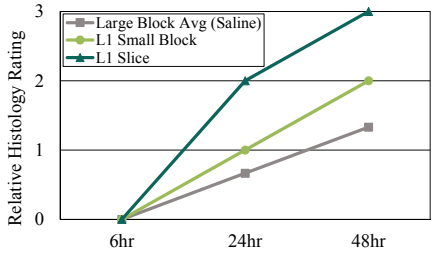

Figure 4. Cellular disruption versus postmortem time for tissue stored as large blocks, small blocks, and slices in saline.

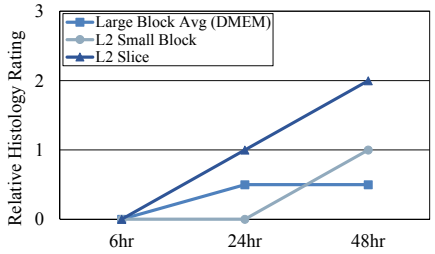

Figure 5. Cellular disruption versus postmortem time for tissue stored as large blocks, small blocks, and slices in DMEM. Note: Histology available from two livers stored as large block.

DISCUSSION

The data from the current study showed that both preservation fluid type and storage block size affected the tensile failure properties and cellular architecture of bovine liver parenchyma between 6hrs and 48hrs postmortem. The failure strain decreased significantly with respect to postmortem time for tissue stored as large blocks in saline, but not for tissue stored as large blocks in DMEM. In addition, cell disruption was more severe for large block tissue stored in saline than in DMEM. The results also showed that storage block size may have an effect on the preservation of the liver's cellular structure and failure properties. The downward trend in failure strain across the time points was more pronounced for smaller tissue storage sizes for both fluids types. Additionally, the disruption seen at a cellular level was more evident when using smaller tissue storage sizes. However, this observation was based on a very limited sample size and no statistical analysis. Therefore, future studies should further investigate the appropriate tissue storage block size by performing additional tests with matched histology and statistical analyses.

The changes in the failure properties appeared to be related to changes in the cellular structure. There was a clear association between a decrease in failure strain and an increase in disruption grade (Figure 6). It is possible that cellular swelling and dissociation decreases cell-to-cell bindings and disrupts connective tissue, thus adversely affecting the tensile properties. Therefore, the lack of change in the failure strain with postmortem time for tissue stored as large blocks in DMEM was likely due to the lower amount of cellular swelling and dissociation.

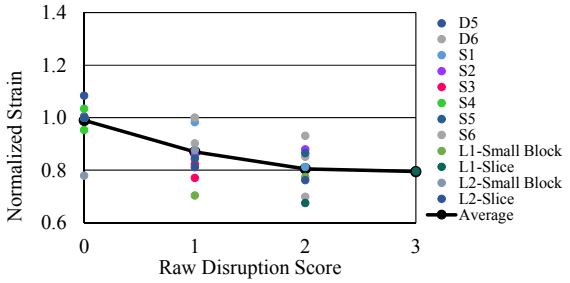

Figure 6. Association between cellular disruption and failure strain.

CONCLUSION

Uniaxial tension tests were conducted on bovine liver samples at three time points after death (6hrs, 24hrs, and 48hrs) to quantify the effects of postmortem time on the tensile failure properties. There was a significant decrease in failure strain with increased postmortem time for tissue stored as large blocks in saline, but not for tissue stored as large blocks in DMEM. Preliminary results indicated that reducing the tissue storage size had a negative effect on the material properties for both fluid types. Histological analysis showed that cellular disruption increased with postmortem time, and that the severity of disruption appeared to increase when stored in saline or a smaller tissue storage size.

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