Effect of vitro preservation on mechanical properties of brain tissue

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Abstract. To develop the protective devices for preventing traumatic brain injuries, it requires the accurate characterization of the mechanical properties of brain tissue. For this, it necessary to elucidate the effect of vitro preservation on the mechanical performance of brain tissue as usually the measurements are carried out in vitro. In this paper, the thermal behavior of brain tissue preserved for various period of time was first investigated and the mechanical properties were also measured. Both reveals the deterioration with prolonged preservation duration. The observations of brain tissue slices indicates the brain tissue experiences karyorrhexis and karyorrhexis in sequence, which accounts for the deterioration phenomena.

1. Introduction
The ever rising concerns on traumatic brain injuries caused by such impact loading conditions as car accidents, blasts, sports or even daily falls have stimulated the intensive mechanical modeling of brain tissue. The simulations may also find applications in computer-integrated and robot aided surgery, surgeon training and development of protective facilities[1,2]. There is no doubt that accurate mechanical property data of brain tissue is of critical importance to validate the constitutive models and therefore to ensure the reliability of simulation results. One question facing mechanical property characterization is how to preserve the brain tissue after the dissection. The common method used is to keep the brain tissue at 0-6°C[3-5]. Nonetheless, how this vitro preservation will affect the mechanical property has not been fully investigated. In this paper, the mechanical performance of brain tissue (both white and grey matter) preserved at 1°C in vitro for various duration was studied and Differential Scanning Calorimetry (DSC) measurements were also conducted to explore the thermal behavior of brain tissue. Finally, the brain tissue slices were observed using optical microscope with the attempt to study the mechanisms of variation of mechanical property and thermal behavior with prolonged preservation duration.
2. Experiments

2.1 Preparation of samples
Small tailed han sheep (kindly provided by Dalian Shun Feng Farm) were used. These sheep are at the same age (1.5 y) and are raised in the same manner. After the euthanasia, the sheep were dissected and the brain was kept for further measurements. All these were done with the permission and monitoring of Biological and Medical Ethics Committee of Dalian University of Technology.

2.2 DSC measurement
Differential Scanning Calorimetry (DSC) measurement were conducted on a Netzsch DSC-204, for each brain, one white and one grey matter specimens were taken from the corona radiate and thalamus, as illustrated in Figure 1. Brain tissue samples (5-10mg) were sealed in standard aluminum pans (40μl) and an empty pan was used as reference.

![Figure 1. The schematic of brain tissue](image)

2.3 Mechanical property measurement
The brain tissue were preserved at 1°C for 0 h, 2 h and 14h. Using the method we developed previously[6], pure white and grey samples with the size of 5 mm×5 mm×5 mm (±0.1 mm) of were obtained, as illustrated in Figure 1.

2.4 Preparation of brain tissue slice
The slices of brain tissue were prepared in reference to Cheng’s method[7]. For the wax slices, steps includes selecting the materials then fixing, washing, dehydrating, transparent, dipping in wax, wrapping in wax, slicing, sticking, dewaxing and dying.

3. Results and discussion

3.1 DSC measurement
To characterize the effect of preservation condition on the thermal behavior, brain white matter of 5-10 mg were sealed in aluminum pan and then were kept at 1°C for 0 min and 5 min. Then the DSC curves were recorded, given in Figure 2. Likewise, the DSC curves of grey matter were also recorded as given in Figure 3.
Figure 2 indicated that for as-obtained white matter the phase transition takes place at 17.67°C and the corresponding entropy is 0.76J/g, whereas after kept for 5, the phase transition temperature and entropy decreases to 13.87°C and 0.64J/g, respectively. Analogous trend can be observed for grey matter, see Figure 3. After preserved at 1°C for 5 min, the phase transition temperature drops from 17.87°C to 13.83°C and entropy reduces from 0.79J/g to 0.72J/g.

Skrzyński et al [8] concluded that the high transition temperature can be related with the cross links of bio-molecules and consequently the mechanical properties of tissue. The higher the transition temperature, the higher density of cross linking and the better mechanical strength. As reflected by Figure 2 and Figure 3, the transition temperature drops with the prolongation of preservation time, indicating the reduction of mechanical strength of brain tissue. In terms of entropy, it also decreases with prolonged preservation, see Figure 2 and Figure 3. As entropy is indicative of capacity of absorption of energy, the decrease of entropy may suggest the deterioration of mechanical strength.
3.2 Mechanical performance in compression
To measure the low temperature preservation on the mechanical properties, white matter were preserved at 1℃ for 0 h, 2 h and 4 h and then the engineering stress-strain curved in compression with a strain rate of are given 0.01/s in Figure 4.

![Figure 4](image1)

**Figure 4.** Engineering stress-strain curves of white matter in compression. 6 repetitions with ±standard deviations

Figure 4 demonstrates that at the strain of 0.3, the engineering stress decreases from 1.81 kPa of as obtained white matter to 0.89 kPa after preserved for 2h and then to 0.28 kPa after preservation of 14 h, indicating the pronounced decrease in mechanical strength with the prolongation of preservation time. Similar trend can be observed for grey matter as exhibited in Figure 5. At strain of 0.3, the engineering stress of grey matter of as-obtained tissue, preservation of 2 h and preservation of 14 h are 1.11 kPa, 0.66 kPa and 0.27 kPa, respectively.

![Figure 5](image2)

**Figure 5.** Engineering stress-strain curves of grey matter in compression. 6 repetitions with ±standard deviations

3.3 Observation of brain tissue slice
To investigate the mechanism of deterioration of mechanical strength with prolonged preservation time at low temperature. The brain white and grey matters were made into slice and observed on optical microscope. The images were taken using the ISCapturesoftware and are shown in Figure 6 and Figure 7.
Figure 6. Cell nucleus of white matter: preserved at 1 °C for (a) 0 h, (b) 1 h, (c) 2 h, (d) 4 h, (e) 14 h. Scale bar represents 15 μm.

Figure 7. Cell nucleus of grey matter: preserved at 1 °C for (a) 0 h, (b) 1 h, (c) 2 h, (d) 4 h, (e) 14 h. Scale bar represents 15 μm.

Figure 6 exhibits that for the as-obtained white matter, the nuclei are large and the color is light. After preserved at 1 °C for 1 h, the nuclei becomes smaller and the color becomes dark, indicating the pyconsis of cells. After preserved for 2 h, the nuclei turns out to be larger, the shape approaches to a circle and the color is light, implying the transition of nuclei from karyopycnosis to karyorrhexis. After preserved for 4 h, the shape of nuclei turns from a circle to an irregular one. After 14 h, the nuclei becomes larger and the shape becomes indistinct, suggesting the karyolysis.

For the as-obtained grey matter, see Figure 7, the nuclei is a ellipse and its color is light. After preserved at 1 °C up to 2 h, the shape of nuclei turn to be a circle and no significant change can be observed in terms of color strength. After 4 h, the nuclei becomes smaller and the color turns to be much darker, indicating the karyopyknosis of nuclei. After preservation of 14 h, the nuclei becomes larger and the color becomes light, indicative of karyorrhexis.
Figure 6 and Figure 7 demonstrate that with the prolonged preservation at 1 °C the nuclei of both white and grey matters experiences karyopycnosis and then karyorrhexis, which can explain the corresponding deterioration of mechanical strength with increasing preservation duration. This may be further ascribed to effect of enzyme and the further investigation is ongoing.

4. Conclusion
The mechanical strength of both white and grey matters deteriorates with the preservation duration at 1°C. This is in agreement of the DSC measurements, which indicates the decrease of both the phase transition temperature and entropy of white and grey matters. Slice observation implies cell experiences karyopycnosis and karyorrhexis in sequence, which may takes responsibility for the deterioration of mechanical strength.

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