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Influence of preservation temperature on the measured mechanical properties of brain tissue

Badar Rashid [a], Michel Destrade [a,b], Michael D. Gilchrist [a,*]

[a] School of Mechanical and Materials Engineering, University College Dublin, Belfield, Dublin 4, Ireland
[b] School of Mathematics, Statistics and Applied Mathematics, National University of Ireland Galway, Galway, Ireland

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ABSTRACT

The large variability in experimentally measured mechanical properties of brain tissue is due to many factors including *heterogeneity*, *anisotropy*, *age dependence* and *post-mortem time*. Moreover, differences in test protocols also influence these measured properties. This paper shows that the temperature at which porcine brain tissue is stored or preserved prior to testing has a significant effect on the mechanical properties of brain tissue, even when tests are conducted at the same temperatures. Three groups of brain tissue were stored separately for at least 1 h at three different preservation temperatures, i.e., ice cold, room temperature (22 °C) and body temperature (37 °C), prior to them all being tested at room temperature (~22 °C). Significant differences in the corresponding initial elastic shear modulus μ (Pa) (at various amounts of shear, $0 \le K \le 1.0$) were observed. The initial elastic moduli were 1043 ± 271 Pa, 714 ± 210 Pa and 497 ± 156 Pa (mean $\pm$ SD) at preservation temperatures of ice cold, 22 °C and 37 °C, respectively. Based on this investigation, it is strongly recommended that brain tissue samples must be preserved at an ice-cold temperature prior to testing in order to minimize the difference between the measured *in vitro* test results and the *in vivo* properties. A by-product of the study is that simple shear tests allow for large, almost perfectly homogeneous deformation of brain matter.

1. Introduction

Extensive research has been carried out over the past five decades to characterize the mechanical properties of brain tissue in order to establish realistic constitutive relationships over a wide range of loading conditions. In particular, there is a pressing need to characterize brain tissue properties over the expected loading rate associated with traumatic brain injuries (TBIs). However, the reliable determination of brain tissue properties is a formidable challenge, as it depends heavily on various experimental parameters.

A limited number of studies have investigated the effects of variable temperatures (Brands et al., 2000; Peters et al., 1997; Shen et al., 2006). Hrapko et al. (2008) stored brain samples in phosphate buffered saline (PBS) in a box filled with ice during transportation and maintained at ~4 °C before testing. Tests were conducted at room temperature (23 °C) and at body temperature (37 °C). The measured results were clearly temperature dependent and the dynamic modulus G^* was 60% higher at 23 °C than at 37 °C. This clearly indicates that testing brain tissue at higher temperature accelerates degradation of the mechanical integrity of tissue, thus further deviating from *in vivo* test conditions. However, in a different study by Zhang et al. (2011), brain samples were preserved in ice cold (group A, 10 samples) and in 37 °C (group B, 9 samples) saline solutions. All samples were warmed to a temperature of 37 °C in a saline bath prior to testing. The stress response from brain samples preserved at 37 °C was 2.4 times stiffer at 70% strain, than when preserved at the ice-cold temperature. These findings directly contradict the study by Hrapko et al. (2008), thus leading to inconclusive results and raising important questions: Do higher temperatures lead to a stiffer or a softer response? At which temperature should brain samples be stored prior to testing? In the literature, protocols vary greatly: for instance, Pervin and Chen (2009, 2011) stored tissues at 37 °C, whereas Miller and Chinzei (1997, 2002), Tamura et al. (2008, 2007) and Rashid et al. (2012b,c, in press) stored brain tissues at ice cold/4–5 °C before the tests. The reliability of experimental data obtained from the tissue preserved at higher temperature (37 °C) is questionable based on the contradictory findings of existing studies (Zhang et al., 2011; Hrapko et al., 2008). It is, therefore, crucial to clearly understand the behavior of tissue under different preservation temperature conditions, with a view to achieving reliable material parameters.

* Corresponding author. Tel.: +353 1 716 1884, +353 1 716 1991, +353 91 49 2344; fax: +353 1 283 0534.
E-mail addresses: Badar.Rashid@ucdconnect.ie (B. Rashid), michel.destrade@nuigalway.ie (M. Destrade), michael.gilchrist@ucd.ie (M.D. Gilchrist).

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With this aim in mind, simple shear tests were performed on brain tissue at a strain rate of 30/s (i.e., 3000%/s, *not* 30%/s) and up to 62% engineering shear strain (amount of shear, $K=1$, where K is the ratio of horizontal displacement of the top of a specimen of brain tissue to its thickness, as indicated in Section 2.1 below) under different temperature conditions. It is important to realize for simple shear that the material is being deformed in various directions at different rates, and so the strain rate around a point within a material cannot be expressed by a single number. We took the strain rate tensor to be the symmetric part of the velocity gradient and not the time derivative of the strain tensor. Three groups of brain tissue were stored separately for at least an hour at three different preservation temperatures: ice cold, room temperature (22 °C) and body temperature (37 °C), whereas experimentation was performed at an approximately constant room temperature (~22 °C). The simple shear test protocol is adopted here because of its high reliability due to a global homogeneous deformation field of brain tissue as compared to compression and tension test protocols, which lead to inhomogeneous deformation fields (Ogden, 1997; Rashid et al., 2012a).

2. Materials and methods

2.1. Simple shear experimental setup

A High Rate Shear Device (HRSD) as described in Fig. 1(a) and (b) was used to perform simple shear tests at a dynamic strain rate of 30/s (i.e., 3000%/s). The development and major components of the HRSD have been discussed elsewhere (Rashid, 2012). During tests, the top platen remained stationary while the lower platen moved horizontally to produce the required simple shear deformation in the specimen, as shown in Fig. 1. Force (N) and displacement (mm) signals were captured simultaneously through the data acquisition system at a sampling rate of 10 kHz. The amount of shear is $K=d/y$, where d is the horizontal displacement of the lower platen (maximum displacement is 4.0 mm) and y is the thickness of the specimen (4.0 mm for all tests). Therefore, $K=1$ is the maximum amount of shear or shear strain. The intended velocity of the electronic actuator was 120 mm/s. Hence, the lower platen traveled 4.0 mm horizontally in 1/30 s, to achieve a maximum amount of shear $K=1$ for a 4.0 mm thick specimen. This gave a shear component for the strain rate tensor (symmetric part of the velocity gradient) of 30 s^{-1}, which we note as 30/s; this rate is typical of TBIs. However, the actual loading velocity was slightly higher (130 mm/s) in order to overcome the frictional effects and opposing spring force acting against the striker, which were adjusted during the calibration process.

2.2. Specimen preparation procedure

Nine fresh porcine brains from approximately 6-month old pigs were collected from a local slaughterhouse and tested within 5 h postmortem. The brains were divided into three groups which were preserved for 1 h in a physiological saline solution at three different temperatures (three brains each in ice cold, 22 °C and 37 °C) during transportation. All samples were prepared and tested at a nominal room temperature of 22 °C. Square specimens as shown in Fig. 2, composed of mixed white and gray matter, were prepared using a square steel cutter after removing the dura and arachnoid from the cerebral hemispheres. Two specimens were extracted from each cerebral hemisphere from the medial to lateral direction. The thickness, width and length of specimens before testing were 4.0 ± 0.2 mm, 19.0 ± 0.1 mm and 19.0 ± 0.1 mm (mean ± SD), respectively. 36 specimens were prepared from the nine brains (four specimens from each brain). The time elapsed between harvesting of the first and last specimens from each brain was approximately 18 min. Physiological saline solution was applied to the specimens frequently during cutting and before the tests in order to prevent dehydration.

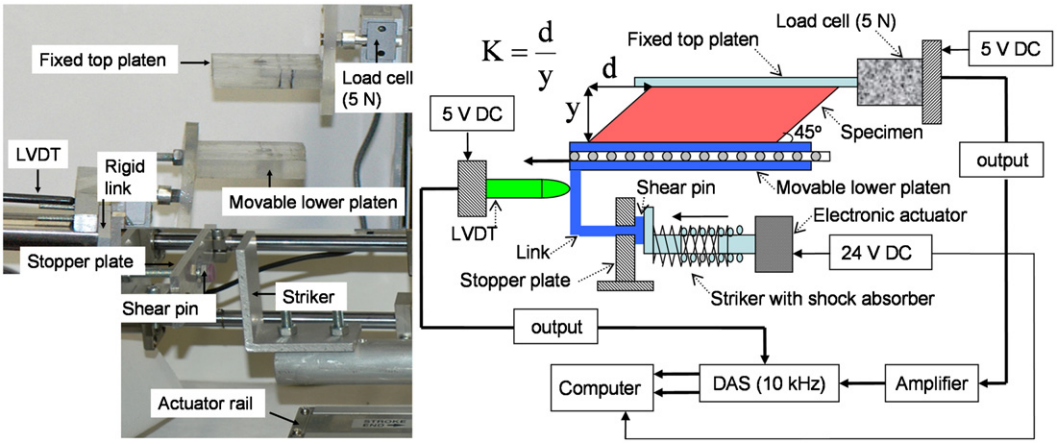

Fig. 1. (a) Major components of high rate shear device (HRSD), and (b) schematic diagram of the complete test setup, with $K=1$ for maximum amount of shear.

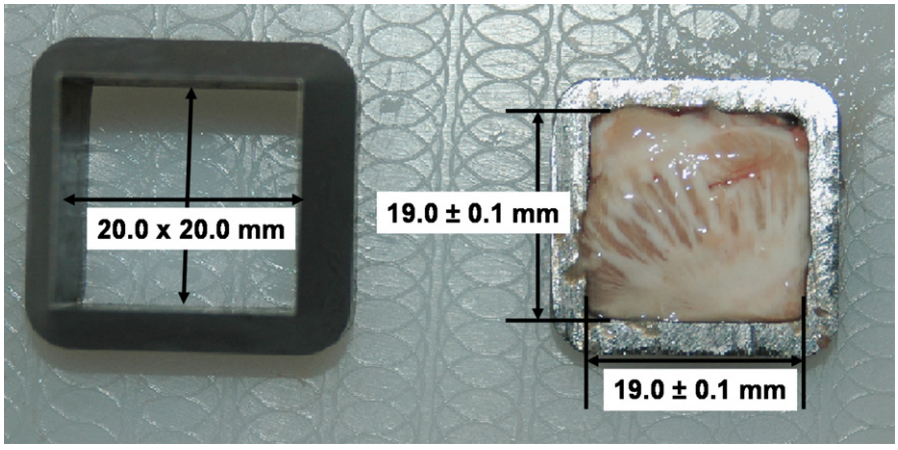

Fig. 2. Square brain specimen ($19.0\pm0.1\times19.0\pm0.1$ mm^2) and 4.0 ± 0.1 mm thick excised from the medial to lateral direction.