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Investigation of the mechanical characteristics of porcine brain tissue in complex environments

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Purpose: Brain tissue immersed in cerebrospinal fluid often exhibits complex mechanical behaviour, especially the nonlinear stress–strain and rate-dependent responses. Despite extensive research into its material properties, the impact of solution environments on the mechanical behaviour of brain tissue remains limited. This knowledge gap affects the biofidelity of head modelling. This study aimed to investigate the effect of solution environments on brain tissue under quasi-static and dynamic loading conditions. *Methods*: Porcine brain tissue was characterized in compression through quasi-static nonlinear testing and Dynamic Mechanical Analysis under various environments: air, physiological saline and artificial cerebrospinal fluid. Frequencies from 0.1 to 40 Hz were applied to determine dynamic behaviour, while brain samples were compressed up to a 0.3 strain level to obtain nonlinear response. The effects of strain, frequency and solution environment on the mechanical response of brain tissue were statistically evaluated. *Results*: As environmental conditions transitioned from air to artificial cerebrospinal fluid, the average stress of brain tissue increased by approximately 1.3, 1.3 and 1.4 times at strain levels of 0.1, 0.2 and 0.3, respectively. A statistically significant increase in dynamic storage and loss moduli was observed between air and artificial cerebrospinal fluid environments. At frequencies above 18 Hz, the tan delta in air was significantly lower. *Conclusions*: The mechanical characterization of brain tissue exhibited a dependency on solution environment under both quasi-static and dynamic loading conditions. Brain tissue showed higher stress levels and dynamic modulus in solution environments compared to an air environment. The results of this study are valuable for improving head simulations and brain material models.

Key words: *brain tissue, mechanical loading, solution environments, porcine*

1. Introduction

The brain model has various applications, including surgical training, traumatic brain injury (TBI) research, and numerous emerging technologies ([7], [15], [23]). It addresses the scarcity of human biological samples by mimicking the mechanical properties of actual brain tissue, which is crucial for evaluating its quality in surgical training and TBI research ([2], [37]). Brain injury symptoms can manifest immediately or several weeks later, necessitating accurate predictive models. Consequently, predictive abilities of brain injuries have been extensively studied, resulting in the development of head simulation models ([3], [35]). However, the accuracy and diversity of these models depend on quantitative data from experiments conducted under various loading conditions.

Brain tissue exhibits nonlinear and viscoelastic mechanical characteristics. Extensive research has focused on this topic, reporting experimental methods and results for brain tissue through compression ([6], [16]), tension ([31], [39]) and shear tests ([4], [20]). Further, testing conditions and protocols in material property research significantly influence experimental results [10]. Factors such as temperature, storage time and specimen location notably impact these outcomes. Most of these experiments were conducted in air environments, with few studies addressing mechanical properties of brain tissue in solution environments.

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Brain tissue is not in an air-like environment inside the skull but is surrounded by cerebrospinal fluid, with a water content of approximately 80% [33]. This high-water content and the ability to exchange ions and solution molecules with the surrounding environment make brain tissue like hydrogel materials. Additionally, brain tissue consists mainly of brain cells and extracellular matrix (ECM). The ECM, a complex network of polysaccharides, proteoglycans and other substances, connects and supports the entire tissue while regulating cell activity [13]. During loading, the ECM bears most of the force and deforms, largely determining the mechanical behaviour of brain tissue [26]. As a result, brain tissue exhibits complex nonlinear mechanical properties, such as strain hardening, where it becomes harder under greater stress or strain. Furthermore, as a highly viscous material typically surrounded by cerebrospinal fluid, the mechanical behaviour of brain tissue significantly depends on the solution environment [11]. The nonlinear mechanical properties, dependence on strain rate [17], [30], and sensitivity to the solution environment [36] make it challenging to develop models that accurately simulate the complex mechanical behaviour of brain tissue.

Dynamic Mechanical Analysis (DMA) is recognized as an effective technique for measuring the bulk mechanical properties of viscoelastic materials [2]. This flexible and powerful method can map the frequency-dependent viscoelastic properties of biological tissues over a range of frequencies, encompassing both physiological and injury loading conditions. In viscoelastic materials, the storage modulus characterizes the ability to store energy in the elastic phase, while the loss modulus characterizes the ability to dissipate energy in the viscous phase [1]. Unlike traditional indentation and stress relaxation tests, DMA employs sinusoidally varying displacement control to analyse the viscoelastic properties of structures, with the resultant force response being out of phase. In addition, large strain testing has been widely applied to investigate the nonlinear response of brain tissue [9], [21]. Thus, it is essential to understand the impact of solution environments on the mechanical behaviour of brain tissue under both dynamic loading and nonlinear testing conditions.

The aim of this study was to characterize the mechanical properties of brain tissue in compression using quasi-static nonlinear testing and Dynamic Mechanical Analysis under various solution environments. The effects of strain, frequency and solution environment on the mechanical response of brain tissue were evaluated statistically.

2. Materials and methods

2.1. Specimen preparation

Fresh porcine brain specimens, approximately 9 months of age, were obtained from a local slaughter. During transit from the slaughterhouse to the laboratory, the brain specimens were maintained at an approximate temperature of 4 °C, and immediate testing upon retrieval was conducted. The entire tests from sample extraction to completion of the experiment were conducted within 10 hours to minimize the effects of postmortem time on the tissue. Cerebrum slices were acquired from brain tissue utilizing a surgical scalpel. The dissection procedure involved the use of a circular trephine measuring 16 mm in diameter to extract brain samples encompassing both white and grey matter. Given the heterogeneous nature of brain tissue, the distribution of meninges, white matter and grey matter varies based on location. To mitigate potential deviations arising from cutting site discrepancies in the experimental data, each specimen was consistently acquired from the parietal lobe. The parietal lobe is often involved in impact and injury scenarios, making it a critical area for understanding the mechanical response of brain tissue [34]. The observed variability in measured dimensions might be influenced by the inherent softness of brain tissue, susceptible to deformation during preparation under its weight. A surgical scalpel and a 16 mm diameter circular trephine were used to extract cylindrical samples with an approximate diameter of 16 mm and a thickness of 7 mm. The precise diameter and thickness of each specimen were measured with a Vernier calliper before testing. The brain samples obtained were in diameters of 16 ± 1 mm and thicknesses of 7 ± 0.5 mm (mean ± standard deviation).

2.2. Experimental setup

The mechanical properties of brain specimens were evaluated using a Bose ElectroForce testing machine. The apparatus was equipped with a 22 N load cell, capable of monitoring force variations with a resolution of 0.002 N and a high-accuracy linear displacement sensor with a range of 8 mm displacement. The force–displacement history was recorded at a sampling rate of 1 kHz. The brain samples were attached to the specimen container with a thin layer of cyanoacrylate adhesive placed on sandpaper. Prior to data

collection, an upper flat indenter was gradually lowered onto the specimen until a preload of 10 mN was achieved, ensuring a zero configuration [16]. A schematic of the mechanical testing system is illustrated in Fig. 1. To minimize the impact of inertia effects during loading, the load cell was installed on the stationary side. The experiment encompassed three groups. For the initial two groups samples were immersed in artificial cerebrospinal fluid (ACF) and physiological saline (PS), respectively, while the third comparative group was conducted in an air environment. The artificial cerebrospinal fluid comprised the following components: 124 mM NaCl, 26 mM $NaHCO_3$, 2.5 mM KCl, 2 mM $CaCl_2$, 1.25 mM NaH_2PO_4, 1 mM $MgCl_2$ and 10 mM glucose. The solution was consistently oxygenated with a gas mixture of 5% CO_2 and 95% O_2. A total of 42 brain samples underwent testing, comprising 14 samples in air, 14 samples in physiological saline and 14 samples in ACF environment.

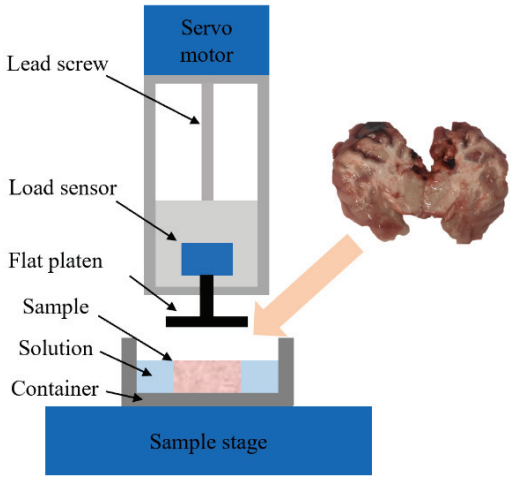

Fig. 1. Schematic of experimental setup for compressive mechanical testing

The compressive characterization was investigated in the quasi-static and the dynamics testing conditions. For the quasi-static tests, the specimens were compressed by the upper plate up to a displacement equal to 30% of their height (nominal strain 0.3). All tests were conducted under room temperature conditions approximately at 25 °C. A speed of 0.07 mm/s was selected for quasi-static loading conditions, corresponding to the strain rates of 0.01/s. The engineering stress was derived by dividing the force by the nominal cross-sectional area of the specimen, while the engineering strain was computed by dividing the actuator displacement by the initial height of the specimen. The stress–strain curves obtained under identical conditions were averaged and considered as the conclusive result.

For dynamic mechanical tests, amplitude sweep tests were conducted at 1 Hz to identify the amplitude range within the material's linear viscoelastic regime. A dynamic amplitude of 1% of the initial sample height was chosen for subsequent dynamic testing. Subsequently, a sinusoidally varying displacement was applied across a frequency sweep spanning 0.1–40 Hz. This frequency range aligns with strain rates observed in prior investigations on porcine and human brain tissue, and represents loading conditions pertinent to both physiological and traumatic scenarios the brain might encounter. The sinusoidal force and displacement data for each frequency underwent recording and analysis using a Fast Fourier Transform (FFT) [19]. The data-set length for force (F^*) and displacement (d^*) at the fundamental frequency were measured to derive the dynamic stiffness (k^*). Following this, the storage (E') and loss (E'') moduli were calculated by transforming the relevant stiffness values using a specific shape factor derived from:

$$k^* = \frac{F^*}{d^*}, \tag{1}$$

$$E' = \frac{k^* \cos\delta}{S}, \tag{2}$$

$$E'' = \frac{k^* \sin\delta}{S}, \tag{3}$$

$$S = \frac{\pi d^2}{4h}. \tag{4}$$

were, h represents the thickness, and d stands for the diameter of the specimen. The phase angle δ denotes the phase lag observed between the applied compressive force and displacement. S denotes the shape factor specifically tailored for cylindrical samples. The damping ratio (δ) of the brain tissue was determined using the storage modulus (Eq. (2)) and the loss modulus (Eq. (3)):

$$\tan\delta = \frac{E''}{E'}. \tag{5}$$

The experimental values were analysed and graphed using Sigmaplot version 14.5 (Systat Software, Inc., London, UK). Storage and loss modulus, as well as phase angle, were compared at each frequency. A one-way analysis of variance (ANOVA) was performed to investigate significant differences. In cases where ANOVA revealed a statistically significant difference ($p < 0.05$), a Tukey's HSD post-hoc analysis was employed for all pairwise comparisons between various testing environments in compressive DMA testing. The results

for all analyses were deemed statistically significant with a probability value of less than 0.05.

3. Results

The compressive stress–strain curves of brain tissue for the three groups of artificial cerebrospinal fluid, physiological saline and air testing environments are illustrated in Fig. 2. It was evident that, within each testing environment, variations among different samples were observed. In Figure 3, the comparison of average experimental curves across three environments is illustrated. Brain tissue exhibited the highest stress levels in artificial cerebrospinal fluid, followed by physiological saline, and the lowest – in an air environment. The statistical results revealed that each testing environment had varying effects on stress levels at strains of 0.1, 0.2 and 0.3. No significant difference was observed ($p > 0.05$) between the groups tested in artificial cerebrospinal fluid and physiological saline environments, as well as between the groups tested in physiological saline and air environments, across all strain levels. A significant effect of solution environments on mechanical behaviour ($p < 0.05$) was found only between the groups tested in artificial cerebrospinal fluid and air environments at a strain of 0.3. As the environmental conditions transitioned from Air to ACF, the average stress of brain tissue rose approximately 1.3, 1.3 and 1.4 times, respectively, at strains of 0.1, 0.2 and 0.3. This disparity could stem from variations in the chemical potentials of the respective environments. Different chemical potentials may lead to distinct external forces acting upon materials. Furthermore, the material itself may undergo alterations when subjected to different environments.

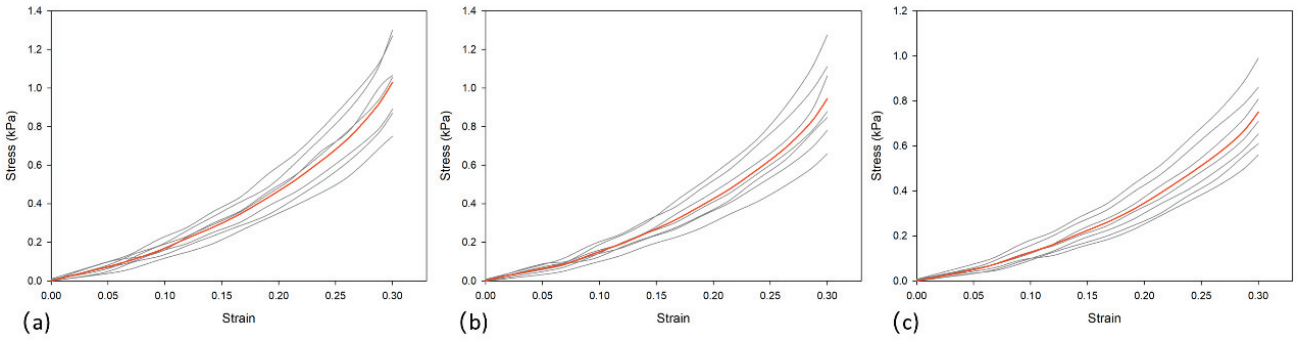

Fig. 2. Experimental stress–strain curves of brain samples in the three groups of (a) artificial cerebrospinal fluid, (b) physiological saline and (c) air testing environments

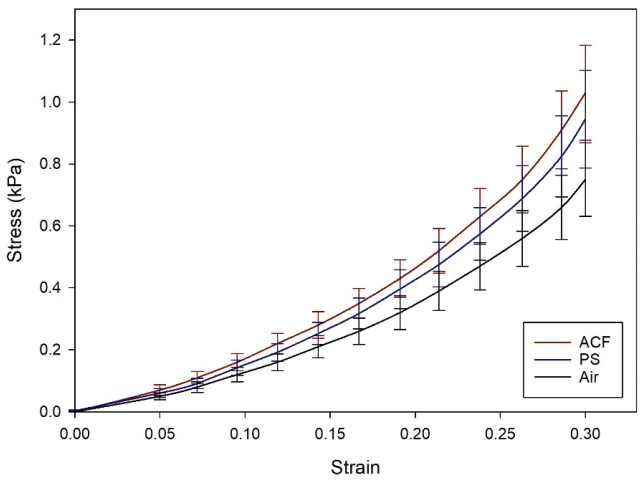

Fig. 3. Variation of stress with strain for brain tissue tested under three different testing environments of artificial cerebrospinal fluid (ACF), physiological saline (PS) and air. Error bars represent 95% confidence intervals

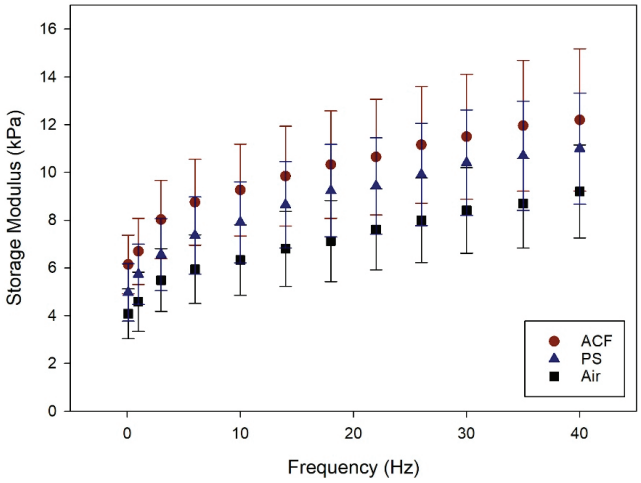

Fig. 4. Variation of storage modulus with frequency for brain tissue tested under three different testing environments of artificial cerebrospinal fluid (ACF), physiological saline (PS) and air. Error bars represent 95% confidence intervals

In specimens subjected to dynamic mechanical conditions, a statistically significant increase in storage modulus ($p < 0.05$) was observed, ranging from 1.3 to 1.5 times through all tested frequencies, as the environmental conditions transitioned from air to ACF (Fig. 4). However, there was no significant difference ($p > 0.05$) observed for the other two pairs (i.e., ACF and PS, PS and air), aligning with the trend observed under quasi-static testing conditions. Across all tested frequencies, the dynamic modulus exhibited an increasing trend, ranging from 6.14 kPa to 12.19 kPa in ACF, 4.97 kPa to 10.99 kPa in PS and 4.08 kPa to 9.21 kPa in air.

The loss modulus exhibited an increasing trend with rising frequency, consistently remaining lower than the storage modulus at each tested frequency (Fig. 5). Notably, as the environmental conditions transitioned from air to ACF a more significantly increasing trend ($p < 0.05$) was observed in the loss modulus, ranging from 1.5 to 1.7 times across all tested frequencies. The significant difference was only discerned between the testing environments of ACF and air. Specifically, the loss modulus demonstrated an increasing trend across all tested frequencies, ranging from 1.76 kPa to 8.33 kPa in ACF, 1.29 kPa to 7.37 kPa in PC and 1.08 kPa to 5.41 kPa in Air.

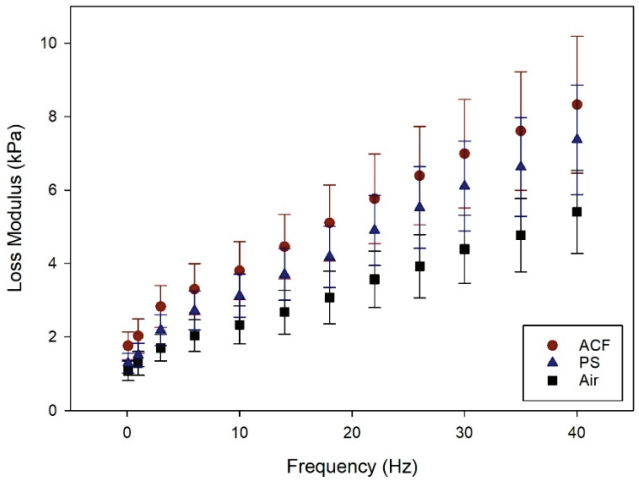

Fig. 5. Variation of loss modulus with frequency for brain tissue tested under three different testing environments of artificial cerebrospinal fluid (ACF), physiological saline (PS) and air. Error bars represent 95% confidence intervals

From the samples tested, the tanδ, which is a measure of energy dissipation in a system, showed an increasing trend with frequencies (Fig. 6). A material with greater tanδ displays the greater proportion of viscous behaviour in the system. The specimens tested in air exhibited the less viscous behaviour with tan δ ranging from 0.27 ± 0.04 (mean ± 95% confidence intervals) to 0.59 ± 0.03; specimens from other testing environments showed a similar ability to dissipate energy with a mean value of around 0.46 across all frequencies. From the frequency of 18 Hz, the tan δ in air was significantly less than in other testing environment.

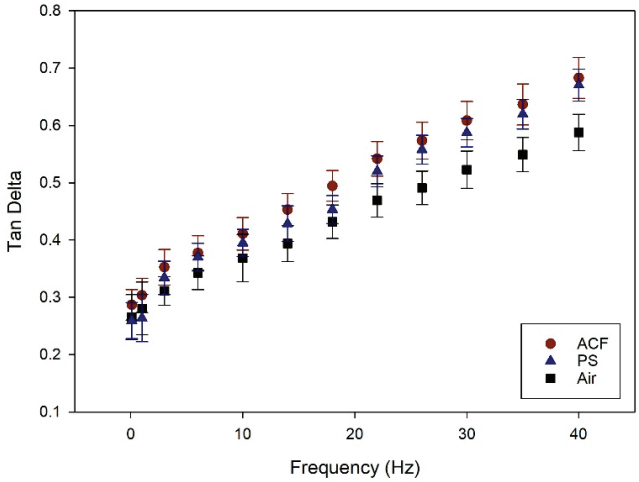

Fig. 6. Variation of tan delta with frequency for brain tissue tested under three different testing environments of artificial cerebrospinal fluid (ACF), physiological saline (PS) and air. Error bars represent 95% confidence intervals

4. Discussion

This study has demonstrated the impact of solution environments on the mechanical behaviour of brain tissue under both quasi-static and dynamic loading conditions. Given the substantial role of compressive loading in head trauma [38] and the potential exposure of the brain to compressive waves during head impacts [24], it becomes imperative to explore the compressive behaviour of brain tissue within diverse environments, encompassing various loading conditions and solution environments. In quasi-static testing, the stress–strain response varied depending on the solution environment, with the highest stress level observed in artificial cerebrospinal fluid, followed by physiological saline, and significantly lower stress levels in an air environment. In dynamic testing, both storage and loss moduli displayed significant differences across all tested frequencies among the solution environments. Additionally, the tan delta values from the three solution environments exhibited varying significant differences across different frequency ranges.

In the quasi-static compression testing, the stress level of brain tissue increased with strain, and the stress–strain curves displayed non-linear mechanical proper-

ties. Moreover, the stress–strain relations of brain tissue exhibited a dependency on the solution environment, consistent with a previous study on porcine brain tissue [36]. Although there were noticeable differences between the solution environments, significant disparities were only found between the ACF and air groups.

In dynamic testing, the storage and loss modulus of porcine brain tissue exhibited a consistent trend across all tested frequencies, regardless of the solution environment. However, the loss modulus displayed a more pronounced increasing trend as the environmental conditions transitioned from Air to ACF, indicating that the tissue's viscous response was more affected by the solution environment. For tan delta, significant differences were observed among the three solution environments across different frequency ranges. At frequencies above 18 Hz, the disparity between air and other solution environments became significant, indicating the mutual influence of frequency and solution environment on the mechanical response of brain tissue. In contrast to a previous study where the relative difference between solution environments decreased as the strain rate increased [37], the dynamic tan delta observed in this paper exhibited a different trend. However, it's important to note that direct comparisons were constrained by potential discrepancies in the types of loading protocols.

The mechanical experiments in this study were conducted using isolated porcine brain tissue. The variations in results may be attributed to differences between *in vivo* and *ex vivo* properties of the tissue. Previous research has indicated correlations between the mechanical properties of brain tissue *in vivo* and *ex vivo*, though these correlations are not always straightforward due to the absence of physiological conditions in *ex vivo* studies. An underestimation of the initial and long-term shear modulus was found due to preconditioning of the tissue when tested *in vitro* [12]. Additionally, the indentation response of porcine brain tissue was observed to be significantly stiffer *in situ* than *in vivo* [28]. Comparisons between *in vitro* and *in vivo* tests reveal that the shear modulus estimated from *ex vivo* is similar within the same range as *in vivo* MRE experimental results [22], [25]. However, further research is required to validate the correlation between *in vitro* and *in vivo* results.

This study investigated the mechanical properties of brain tissue, combining both white and grey matter. There is potential for future research to explore regional variations in brain tissue response under different solution environments. Previous studies have noted differences between white and grey matter [18], [27]. These variations could influence how different solution environments affect the mechanical response of brain tissue. However, our study provides valuable insights into the macroscopic mechanical behaviour of brain tissue. Moreover, the average material properties of brain tissue have often been focused under various loading conditions [29], [32].

During the sample preparation, the process of cutting brain tissue samples may introduce cell structure damage and swelling, potentially affecting the accuracy of mechanical property measurements. To minimize these artifacts, careful cutting procedure were employed. Future research will focus on alternative methods, such as non-invasive imaging and in situ testing, to improve the accuracy and reliability of mechanical characterization studies of brain tissue.

The mechanical properties of the brain play a crucial role in computational brain modelling [23]. Finite element (FE) models of the brain offer a non-invasive method to analyse its response to head impacts [14]. Achieving accurate brain models necessitates precise experimental results under multifactorial loading conditions. This study highlights the impact of solution environments on the mechanical behaviour of brain tissue. By fitting material parameters obtained from testing in artificial cerebrospinal fluid environments, the biofidelity of simulations could be enhanced. Brain injury to different types of soaking solutions could be linked by demonstrating how these solutions influence the mechanical properties of brain tissue. This understanding is crucial for accurate simulation of brain injuries and developing effective preventative and therapeutic measures. The analysis in this study primarily focused on the differences in mechanical properties under varying environmental conditions. Future research will delve into the mechanisms driving these differences, including changes in water content, environmental chemical potential and structural alterations within the brain tissue. Understanding these underlying mechanisms is crucial for a comprehensive interpretation of the observed mechanical behaviour.

The limitation of this study is that the mechanical properties of brain tissue were determined based on the mixed white and grey matter. Although both grey and white matter were present in these samples, the parietal lobe was chosen which helped maintain consistency across samples. Future research opportunities include examining the regional variations of brain tissue in various solution environments. Although the heterogeneity of brain tissue and the variation between white and grey matter have been previously studied [5], [8], our results offer valuable insights into the macroscopic mechanical response of brain tissue under various solution environments. Additionally, the average mechanical

properties, combining white and grey matter, have typically been analysed in compression and indentation tests [30], [32].

A small amount of geometrical change occurred during sample preparation presents limitations due to the extremely soft nature of brain tissue, which caused some deformation under its own weight [5]. However, the diameter of the samples was measured with callipers before testing, ensuring that shape alterations during preparation were accounted for in the mechanical results.

5. Conclusions

Summarizing, the mechanical behaviour of porcine brain tissue was investigated in compression using quasi-static nonlinear testing and Dynamic Mechanical Analysis under various solution environments. The stress–strain response of brain tissue in quasi-static testing varied depending on the solution environment. The dynamic storage and loss moduli showed significant differences across all tested frequencies among the solution environments, while significant differences in the tan delta were observed across different frequency ranges. Understanding the mechanical behaviour of brain tissue in complex environments is valuable for improving head simulations and brain material models.

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Competing interests

The authors declare that there is no conflict of interest.

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