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Frequency dependent viscoelastic properties of porcine brain tissue

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Abstract

Brain tissue is vulnerable and sensitive, predisposed to potential damage under complicated loading conditions. Its material properties have been investigated, but the characterization of its viscoelastic properties is currently limited. The aim of this study was to investigate the viscoelastic properties of brain tissue. Porcine brain samples, dissected *ex vivo* in the coronal plane, were tested under compression using dynamic mechanical analysis over a range of frequencies between 0.1 and 35 Hz. Indenters with varying diameters of 8, 12 and 16 mm were used to study the effect on viscoelastic properties under a sinusoidally varying displacement with varying mean displacements (10%, 15% and 20% of a specimen height). As the indenter size increased, the storage and loss stiffness significantly increased ($p < 0.05$). The storage stiffness decreased significantly as the mean displacement decreased ($p < 0.05$). The average storage modulus was found to be 8.09 kPa and the average loss modulus was found being 4.85 kPa. Frequency dependent viscoelastic properties of brain are important to improve the accuracy in the computational modelling of the head to develop the prediction of brain injuries.

1. Introduction

The brain is considered as one of the most vulnerable organs; it is immersed in cerebrospinal fluid (CSF) and surrounded by the skull. Although the brain is well protected by the skull, traumatic brain injury (TBI) is still a major cause of death and disability induced by external forces[1]. Primary brain damage is caused by direct impact of a penetrating object at a rapid speed or under blast loading conditions[2]. Low-acceleration oscillations of the head can also induce mild brain trauma[3]. Additionally, diffuse axonal injury (DAI) is considered as one of the most common types of traumatic brain injury, and has been found to occur when the strain rates exceed 10 s^{-1} [4]. The mechanical properties of brain plays an important role when head injuries are analysed, such as brain swelling[5], cortical folding[6] and hydrocephalus[7]. The symptoms of brain injuries can appear immediately or several weeks later. Due to the existence of these uncertain cases, the predictive abilities of brain injuries are important and have been studied intensively leading to head simulation models[8, 9]. The accuracy and variety of the brain models, however, requires quantitative results from experiments under multi-factorial loading conditions.

To explore the mechanical properties of brain tissue, a wide range of loading conditions have been studied. Most tests have been performed in the time domain and with “quasi-static” loading conditions. Qian *et al.*[10] analysed the effect of strain rate on mechanical properties of porcine brain by indentation tests. Cheng *et al.*[11] performed unconfined compression experiments to investigate the porous properties of the bovine white matter using a poroviscoelastic model. Velardi *et al.*[12] studied the influence of heterogeneity on porcine brain through tensile tests. Budday *et al.*[13-15] characterized the mechanical response and regional behaviour of brain tissue through a combination of shear, compression and tension tests and identified constitutive models to describe brain behaviour under such loading conditions. In addition, the mechanical properties of brain tissue have been previously investigated in the frequency domain. The dynamic frequency sweep tests have been performed to characterise material properties of human brain[16], regional difference of white and grey matters on human and porcine brain[17] and nonlinear constitutive models for bovine brain[18], mostly conducted in shear. However, the effect of dynamic viscoelastic characterization of the brain tissue through dynamic mechanical analysis (DMA) under compression has not been quantified[19].

DMA can be used to determine the viscoelastic properties of materials over a wide frequency range covering physiological and injury loading conditions. Storage and loss stiffness are used to characterize viscoelastic properties of a structure[20]. The storage stiffness characterises the ability of the tissue structure to store energy in the elastic phase and the loss stiffness characterises the ability of the tissue structure to dissipate energy in the viscous phase. Unlike conventional indentation and stress relaxation tests, DMA enables a sinusoidally varying displacement control to analyse the viscoelastic properties of a structure with the resultant force response is out of phase[21].

Many previous studies have selected animal brains to conduct *in vitro* tests as a substitute for human brains because the former are more readily available. Prange and Margulies[22] through shear stress relaxation found the human brain samples were 29% stiffer than the porcine brain samples. Takhounts *et al.*[23] using the same testing method showed human samples to be 40% stiffer than bovine samples. These experiments were performed in the time domain with stress-strain methods while the results in the frequency domain were different. Galford and McElhaney[24] described from vibration experiments that the dynamic storage and loss moduli of monkey brain tissue were 1.4 and 2 times higher than that of human brain tissue, respectively. However, Nicolle *et al.*[17] through oscillatory shear tests found the moduli of human and porcine brain tissue were similar. The mechanical response and anatomical structure between human and animal brains are usually considered with no significant discrepancy. Therefore, there are similarities reported in previous studies on

mechanical response of brain tissue between human and porcine, implying that porcine brains may be used as models for human brains.

Testing conditions and protocols in the research of material properties have a significant influence on experimental results. Arbogast and Margulies[25] found the complex shear modulus of porcine brain tissue at 5% applied strain is lower than at 2.5% strain. A similar trend was identified by Darvish and Crandall[18], the variations of shear modulus on bovine brain tissue relevant to strain. Budday *et al.*[26] observed the contact stiffness of bovine samples had a linear relationship with the indenter size. In addition, the variations are also affected by the factors of sample species, tissue heterogeneity and even environmental temperature[22, 27]. While viscoelastic properties of brain tissue have been characterised, the effect of indenter size and mean indentation depth on storage and loss properties has not been assessed by compressive DMA. The severity of brain injuries is also relevant to the strain rate and the magnitude of the displacement of brain away from an initial centre line[28, 29]. Therefore, the understanding of frequency-dependent properties per external loading condition, such as indenter size or strain, is important.

The aim of this study was to characterise the frequency dependent viscoelastic properties of porcine brain tissue using DMA under compression. The variation of dynamic stiffness properties was assessed by comparing different indenter sizes and indentation depths. The storage and loss moduli were also analysed over a range of loading frequencies.

2. Material and methods

Four half porcine brains under 8 months old were obtained from a supplier (Dissect, Kings Heath, Birmingham, UK). Following arrival in the laboratory the samples were wrapped in tissue paper soaked in Ringer's solution (Oxoid Ltd, Basingstoke, UK) and then stored at -40°C in a freezer in double heat-sealed plastic bags[30, 31]. From previous studies, such freezing treatment does not change the mechanical properties of biological tissues[32]. Before each mechanical test, a half brain was taken out from the freezer and placed in a fridge, thawed in Ringer's solution overnight at around 4 °C ahead of dissection and preparation for mechanical testing[31].

Ten test specimens which comprised both white and grey matter were obtained from the four half porcine brains. After the cerebellum, spinal cord and medulla oblongata were removed, the cerebral hemispheres were mainly composed of the frontal, parietal and occipital lobes and cut in the coronal plane using a surgical scalpel (Swann-Morton Limited, Sheffield, UK). Specimens were dissected into cylindrical shaped samples (Fig. 1) through the anterior-posterior direction. The brain samples were 7 ± 1 mm (mean $\pm$ deviation) in thickness and 28 ± 1 mm in diameter measured using a Vernier calliper (Draper Tools Ltd, Hampshire, UK).

Figure 1: (a) A half porcine brain before dissection and (b) a representative image of a test specimen.

A Bose ElectroForce 3200 (Bose Corporation, ElectroForce Systems Group, Minnesota, USA) testing machine operated using WinTest Dynamic Mechanical Analysis software (Bose Corporation, ElectroForce Systems Group, Minnesota, USA) was used to determine the viscoelastic properties of the brain tissue samples. This approach has been previously used to test biological and synthetic materials[21, 33-35]. A 225 N load cell was used which controls force with a resolution of ± 0.002 N over a wide range of frequencies. The Wintest DMA Analysis software used fast Fourier transform to analyse the force and displacement waves. The dynamic stiffness (k^*) and the phase angle (δ) were characterized subsequently. Dynamic stiffness was determined by the ratio of force amplitude to displacement amplitude. The phase angle was calculated as the phase difference between the force and displacement. The ratio of loss to storage stiffness (k''/k') as the $\tan \delta$ is a measure of energy dissipating in a system. A material with greater k''/k' ratio displays the greater proportion of viscous behaviour in the system. The storage (k') and loss stiffness (k'') were calculated using Eqs. (1) and (2). Further details on the characterisation are provided elsewhere[31, 33, 36].

$$k' = k^* \cos \delta \quad (1)$$

$$k'' = k^* \sin \delta \quad (2)$$

Figure 2: Compressive DMA setup for the porcine brain specimens.

Specimens were placed in a 28 mm diameter custom-designed container and compressed using a circular flat indenter. Three different indenter diameters of 8, 12 and 16 mm were used during the dynamic testing (Fig. 2). To minimize the friction between the brain specimens and the compression indenter, samples were hydrated with Ringer's solution before each mechanical test. All samples were tested by applying a sinusoidally varying displacement with a mean displacement (MD) of 1.4 mm (20% of the specimen height) and an amplitude of 0.14 mm, i.e. between 1.26 and 1.54 mm, over the frequency range from 0.1 to 35 Hz in 14 steps. The acquisition frequency at 35 Hz is 5 kHz. An example of the force against displacement data is shown in Figure 3. This frequency range covers most of the loading frequencies to which the brain might be exposed during physiological and traumatic loading[3]. For the 16 mm diameter indenter two further mean displacements of 1.05 mm (15% of the specimen height, with an amplitude of 0.105 mm) and 0.7 mm (10% of the specimen height, with an amplitude of 0.07 mm) were investigated. The mean displacement range covered mechanical levels of brain tissue from spontaneous recovery to irreversible injury[37, 38]. Between each test, the brain specimens were irrigated with Ringer's solution to keep hydrated. Preliminary tests were used to confirm that the order of the testing did not alter the measured mechanical properties. Before the data collection procedure, a preconditioning cycle of 5 Hz was applied[39, 40] following the same displacement parameters detailed above.

Storage (E') and loss moduli (E'') were calculated by converting from the relevant stiffness using a shape factor (S) calculated using Eq. (5) and Eq. (6)[33, 41]. The shape factor for cylindrical samples can be calculated from Eq. (3).

$$S = \left(\frac{\pi}{h}\right) \left(\frac{d}{2}\right)^2 \quad (3)$$

$$E^* = \frac{k^*}{S} \quad (4)$$

$$E' = \frac{k'}{S} \quad (5)$$

$$E'' = \frac{k''}{S} \quad (6)$$

where h is the sample thickness, d is the diameter of a specimen. In this instance, the diameter d was considered to be equivalent to the diameter of the indenter.

Sigmaplot Version 13.0 (Systat Software Inc., London, UK) was used to perform regression analysis for the curve fit of stiffness and modulus against frequency. The relationship between brain specimens tested under different mean displacements and indenter diameters were analysed by a one-way analysis of variance method (ANOVA). A Kruskal-Wallis ANOVA on ranks was used if the normality test (Shapiro-Wilk) failed ($p < 0.05$). If ANOVA showed a statistically significant difference ($p < 0.05$), a Student-Newman-Keuls Method (SNK) was used for all pairwise comparisons of testing groups which would be significant when $p < 0.05$.

Figure 3: Representative load displacement experimental data, from multiple cycles, for a given sample at 22 Hz.

3. Results

Figure. 4(a) shows storage stiffness properties for three individual brain samples. The storage stiffness for all tested samples increased with frequency initially, then decreased at higher frequencies. The trend of storage stiffness can be characterized by a logarithmic curve fit (Eq. 7) from 0.1 to 18 Hz and a second order polynomial fit (Eq. 8) up to the end frequency sweep.

$$k' = A \ln(f) + B \quad \text{for } 0.1 < f < 18 \quad (7)$$

$$k' = C(f^2) + D(f) + F \quad \text{for } 18 \leq f \leq 35 \quad (8)$$

Here, k' is the storage stiffness, f is frequency and A , B , C , D and F are empirically derived storage constants

by the least-squares fit method, which are summarised in Table 1.

Figure. 4(b) shows loss stiffness properties for three individual brain samples. The loss stiffness increased with increasing frequency and was lower than storage stiffness for most frequencies tested while for the end testing frequency, the storage and loss stiffness was found to be similar. The trend of loss stiffness can be characterized by a second order polynomial fit across all frequencies tested (Eq. 9) which has the same equation to the second part of storage with different constants.

$$k'' = H(f^2) + I(f) + J \quad \text{for } 0.1 \leq f \leq 35 \quad (9)$$

Here, k'' is the loss stiffness, f is frequency and H , I and J are empirically derived loss constants by the least-squares fit method summarised in Table 1.

For specimens tested under various mean displacements, there was a statistically significant increase in storage stiffness ($p < 0.05$) of 1.6 (35 Hz) to 2.4 (0.1 Hz) times as the mean displacement increased from 10%h to 20%h (Fig. 5). The loss stiffness with 20%h MD was significantly higher than with 10%h MD ($p < 0.05$) while there was no significance difference ($p > 0.05$) for other two pairs (i.e. 15%h and 20%h MD; 20% and 30% MD). For the average storage and loss stiffness, the mean displacement of 20%h (229.7 N/m and 140.3 N/m) was greatest, followed by the mean displacement of 15%h (171.2 N/m and 111.0 N/m) and the mean displacement of 10%h (133.6 N/m and 87.4 N/m) had the lowest value.

For specimens tested using varying indenter sizes, the average stiffness (Fig. 6) against frequency follows the same trend of individual samples. The dynamic storage and loss stiffness response of brain tissue was dependent on the indenter size, increasing with indenter diameter ($p < 0.05$). The storage stiffness increased up to 258.4 N/m at a diameter of 16 mm, 144.2 N/m at a diameter of 12 mm and 67.9 N/m at a diameter of 8 mm and then experienced a decreasing trend at higher frequencies. The loss stiffness had an increasing trend across all tested frequencies from 39.6 N/m to 227.7 N/m at a diameter of 16 mm, 21.5 N/m to 123.0 N/m at a diameter of 12 mm and 10.2 N/m to 58.7 N/m at diameter of 8 mm. Increasing the indenter diameter led to an increase of stiffness (k' and k'') by 1.83 ± 0.05 (mean $\pm$ deviation) from $D = 12$ mm to $D = 16$ mm, 2.12 ± 0.04 from $D = 8$ mm to $D = 12$ mm and 3.88 ± 0.12 from $D = 8$ to $D = 16$ mm.

Figure 7 shows the mean storage and loss moduli against frequency for all tested samples. The storage modulus increased up to 9.14 kPa with an average value of 8.09 kPa and loss modulus ranged between 1.38 kPa and 7.91 kPa with an average value of 4.85 kPa. The converted results of the average stiffness from brain specimens tested under three indenter diameters of 16 mm, 12 mm and 8 mm were not significantly different ($p > 0.05$).

From the samples tested, the mean $\tan\delta$ (i.e. ratio of k''/k') of brain tissue showed an increasing trend with frequencies, ranging from 0.32 to 0.98 (Fig. 8). No significant differences in $\tan\delta$ were found for different diameters and mean indentations ($p > 0.05$).

Figure 4: Variation of stiffness with frequency for three individual samples from a single hemisphere tested using a 16 mm-diameter indenter and under a 20% specimen height mean displacement. (a) storage stiffness and (b) loss stiffness.

Figure 5: Variation of stiffness with frequency for brain tissue tested under three different mean displacements of 10%, 15% and 20% of the specimen height with the 16 mm diameter indenter. (a) mean storage and (b) mean loss stiffness (N/m). In (a), L1 represents a logarithmic curve fit (Eq. 7) from 0.1 to 18 Hz and L2 represents a second order polynomial fit (Eq. 8) up to the end frequency sweep. In (b), a logarithmic curve (Eq. 9) was fitted across all frequencies tested. Error bars represent standard deviation.

Figure 6: Variation of stiffness with frequency of brain tissue tested under three indenter diameters of 16 mm, 12 mm and 8 mm with the mean displacement of 1.4 mm (20% of the specimen height). (a) mean storage and (b) mean loss stiffness (N/m). In (a), L1 represents a logarithmic curve fit (Eq. 7) from 0.1 to 18 Hz and L2 represents a second order polynomial fit (Eq. 8) up to the end frequency sweep. In (b), a logarithmic curve (Eq. 9) was fitted across all frequencies tested. Error bars represent standard deviation.

Figure 7: Variation of modulus with frequency. (a) mean storage and (b) mean loss modulus (kPa) of brain tissue obtained from three indenter sizes with the mean displacement of 1.4 mm (20% of the specimen height). In (a), L1 represents a logarithmic curve fit (Eq. 7) from 0.1 to 18 Hz and L2 represents a second order polynomial fit (Eq. 8) up to the end frequency sweep. In (b), a logarithmic curve (Eq. 9) was fitted across all frequencies tested. Error bars represent standard deviation.

Figure 8: Variation of $\tan(\delta)$ with frequency for the all brain samples tested. Error bars represent standard deviation.

Table 1: Statistical details derived from mean stiffness against frequency plots of Fig. 5 for three mean displacements and Fig. 6 for three indenter diameters. Curve fit results also derived from mean storage and loss modulus against frequency plots of Fig. 7 ($p \leq 0.05$ for all trends). Coefficients are in N/m for stiffness

Constant	Storage/Loss regression equation	Stiffness coefficients (N/m)		Modulus coefficients (kPa)
		Mean displacement (mm)	Indenter diameter (mm)	

while are in kPa for modulus.

h, a specimen height

		10%h	15%h	20%h	8	12	16	
A	Storage	20.61	24.15	26.57	7.37	14.57	26.57	0.95
B	Storage	94.23	124.79	181.91	45.91	99.36	181.91	6.29
	R^2	0.98	0.98	0.99	0.99	0.99	0.99	0.99
C	Storage	-0.069	-0.096	-0.092	-0.011	-0.048	-0.092	-0.003
D	Storage	2.73	3.82	2.91	0.38	1.83	2.91	0.089
F	Storage	129.39	160.79	235.76	64.61	126.85	235.76	8.36
	R^2	0.99	0.99	0.99	0.99	0.99	0.99	0.99
H	Loss	-0.063	-0.08	-0.102	-0.026	-0.052	-0.102	-0.003
I	Loss	5.66	7.02	8.39	2.16	4.46	8.40	0.30
J	Loss	26.83	36.38	52.96	13.23	28.39	52.96	1.82
	R^2	0.99	0.99	0.99	0.99	0.99	0.99	0.99

Discussion

This study has used dynamic mechanical analysis under compression to demonstrate that brain tissue is viscoelastic with both storage and loss stiffness, and moduli, being frequency dependent. Further, the viscoelastic storage and loss stiffness are dependent on the indentation mean displacement and the indenter size, increasing with higher mean displacement and larger indenter diameters. The viscoelastic storage and loss moduli are independent on the indenter size. For all samples tested under different testing protocols, the trends for storage and loss stiffness were similar. Many other studies used the similar curve fit method to define viscoelastic material properties including heart chordae[40] and mitral valve[21]. In this study, the storage stiffness, and moduli of porcine brain showed an increasing trend at initial frequencies and then a decreasing trend at higher frequencies. This finding is consistent with previous results for porcine bladder[34], mitral valve leaflet[21], and human/porcine brain[42], for the latter magnetic resonance elastography (MRE) was performed to describe the material properties. Loss stiffness of porcine brain exhibited an increasing trend with frequency; a similar tendency has been found in brain tissue by dynamic testing in shear[17, 43] and MRE methods[44]. In spite of differences in testing devices and experimental conditions, the general trends were consistent with previous studies. The dynamic stiffness properties (Fig. 4) varied with frequencies for the three samples, dissected from the same cerebral hemisphere which implies sample variability.

The storage and loss stiffness of porcine brain tissue decreased with lower mean displacement of the indenter.

The size of indenter significantly altered the storage and loss stiffness of brain tissue, both of which decreased with decreasing indenter diameter which can be found in bovine brain under indentation testing[26]. Furthermore, the change of stiffness throughout the tested frequencies was significant. For brain samples tested under the three indenter diameters, the average minimum values of storage and loss stiffness were approximately 48% and 17% of the corresponding maximum values, which were consistent to three indenter diameters. For brain samples tested under three mean displacements, however, the change of stiffness varied from 48% to 34% and 17% to 11% with lower mean displacement for storage and loss stiffness, respectively. This indicates that the mean displacement has a greater effect on the frequency-dependent trend of stiffness as compared to the indenter size.

The storage and loss modulus were derived from the relevant stiffness. Compared to stiffer biomaterials such as articular cartilage[45] in compression, adhesion in soft biological materials plays a significant role in contact mechanics affecting the impacting area and inducing mechanical errors[46, 47]. To avoid this issue, a circular flat indenter was selected[26, 39] rather than a hemispherical indenter[48]. When the custom-designed flat indenter was performed on brain tissue, the impacting area remains constant over the whole sinusoidal displacement range[49, 50]. As the thickness of each sample was controlled, the modulus of the specimen is considered to be inversely proportional to the square of the indenter diameter and proportional to the specimen stiffness. Some previous studies[26, 51] have indicated that modulus is independent of the indentation depth where the contact stiffness assumed as the ratio of the linear stress-strain curve is constant. In DMA testing the strain-rate is continuously changing during oscillation at a frequency so the dynamic stiffness might vary with different indentation depth. A range of shape factors are available in literature for indentation studies[52]. Although different shape factors will alter the predicted values across studies, it is worth noting that as it is a constant any predicted trends are not affected within a study; instead, predictions between studies may be off-set.

Many studies have measured mechanical properties for porcine brain tissue. Rashid *et al.*[53] found the elastic secant modulus to be 19-65.2 kPa, Budday *et al.*[26] found the modulus of white matter tissue to be 1.604 kPa, while Miller *et al.*[54] found the instantaneous Young modulus was 3.24 kPa. The average dynamic modulus through the frequency tested for the cylindrical samples in this study was 9.4 kPa that is comparable to the range studied by Tamura *et al.*[55] where the initial elastic modulus was found to be between 5.7-23.8 kPa. However, all of these studies were performed in time domain (i.e. static stress-strain experiments) which are difficult to compare the results of this investigation because dynamic modulus obtained in frequency domain is different to Young's modulus.

Many soft biological tissues were tested under oscillatory shear methods to analyse the viscoelastic properties[56]. In comparison to a study by Fallenstein[16] where human brain tissue was tested *in vitro* at 9 to 10 Hz with shear storage modulus ranging between 0.6 to 1.1 kPa and loss modulus ranging between 0.35 to 0.6 kPa, the axial compressive moduli reported in this paper were higher at comparable frequencies. However, any comparison is limited by the potential differences in loading protocols between shear and compression tests.

This study was based on the viscoelastic properties of brain which combined white and grey matter. There is future opportunity to investigate the regional variation of brain tissue under dynamic mechanical analysis. Indeed, some studies have found variation between the white and grey matter[17, 22, 57] and the interregional heterogeneities of brain tissue[58]. However, the results obtained in our study provide a useful measure of the mechanical behaviour of brain tissue. Average material properties of brain tissue have often been studied, e.g. by Rashid *et al.*[53] and Qian *et al.*[10] through compression and indentation tests, respectively. Generally,

white matter with aligned fibre tracts is considered more anisotropic than grey matter.

Realistic viscoelastic properties of brain are important for the computational modelling of the brain. Finite element (FE) models of brain provide a non-invasive way to analyse the brain response to head impacts[59]. The viscoelastic parameters as material constants in simulations were mostly taken from indentation[2], stress-strain and stress relaxation tests[11, 60] where an N-term Prony series were used to fit the experimental data. The frequency-dependent properties of brain tissue are also important in FE simulations to analyse the mechanical response under dynamic loading. A comparison between time and frequency dependent properties of brain tissue in the previous literature is limited due to different experimental protocols[19], however, a recent study found it is possible to predict the frequency-dependent properties based on experiments in frequency domain[61]. A linear viscoelastic model was adopted to model biological tissues under cyclic loading, characterised by the storage and loss moduli. Further, methods to convert data from the frequency domain to time-domain are reported in literature[62]; further, the maximum strain rate in this study ranges approximately between 7-14 /s. Based on this approach, the frequency dependent values presented in this paper could be used to fit viscoelastic constitutive models such as through Prony series representations following adaptation of the storage and loss moduli obtained to represent the mechanical behaviour of brain tissue.

Limitations

A limitation of this study is that a small amount of geometric variability occurred when preparing samples. The extremely soft nature of brain tissue also leads to some deformation of specimens under their own weight during the preparation[15]. However, the diameter of samples was measured using callipers prior to testing, this ensured that the resultant moduli accounted for any variability in specimen shape during preparation. Further, the indenter sizes in this study are large enough to ensure the homogenous behaviour of tissue[63].

In this study, a freeze-thaw protocol has been used to store specimens. Although there are some limitations when comparing frozen to fresh soft tissue, the data from the results showed extensive overlap between the frozen and fresh samples[64]. Previous studies reporting tests on porcine liver[65], ligaments[66] and aortic specimens[67] revealed limited changes in mechanical properties. Further, the method of freezing preservation including freezing temperature may be more critical to mechanical properties[68, 69]. In this study, the storage protocol of tissue at -40°C, consistent with established procedures to maintain initial stress-strain response of soft tissues[21, 70].

For experimental data of brain tissue obtained from compression tests and stress relaxation tests in time domain, a whole range of material models exist to curve fit. Previous studies used constitutive models of hyperelastic materials on brain tissue including Gent, neo-Hookean, Mooney-Rivlin and Ogden models[15, 53]. And some studies used a Prony-Series in time domain to model the rate-dependent response of brain tissue[11, 60]. However, such models for viscoelastic materials within the frequency domain are more limited. Further studies are required for direct application of the data, however, one strategy is to characterise frequency-dependent results using a Prony-Series, validating the models against experimental data.

Conclusions

It can be concluded that porcine brain tissue shows frequency dependent-viscoelastic properties over the range of frequencies tested, 0.1-35 Hz. The storage stiffness exhibited a combination of a logarithmically increasing

trend and a quadratic decreasing trend against frequency while the loss stiffness exhibited a quadratic increasing trend against frequency. The dynamic stiffness properties were affected by different indenter sizes and indentation depths. The storage and loss stiffness decreased with lower mean displacement of indenter. The indenter with a larger diameter led to higher storage and loss stiffness while the storage and loss moduli were constant with a mean value of 8.09 kPa and 4.85 kPa, respectively. These findings could be used in diagnosis of traumatic brain injury and head simulations in frequency domain.

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