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Dynamic mechanical characterization and viscoelastic modeling of bovine brain tissue

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Abstract

Brain tissue is vulnerable and sensitive, predisposed to potential damage under various conditions of mechanical loading. Although its material properties have been investigated extensively, the frequency-dependent viscoelastic characterization is currently limited. Computational models can provide a non-invasive method by which to analyze brain injuries and predict the mechanical response of the tissue. The brain injuries are expected to be induced by dynamic loading, mostly in compression and measurement of dynamic viscoelastic properties are essential to improve the accuracy and variety of finite element simulations on brain tissue. Thus, the aim of this study was to investigate the compressive frequency-dependent properties of brain tissue and present a mathematical model in the frequency domain to capture the tissue behavior based on experimental results. Bovine brain specimens, obtained from four locations of corona radiata, corpus callosum, basal ganglia and cortex, were tested under compression using dynamic mechanical analysis over a range of frequencies between 0.5 and 35 Hz to characterize the regional and directional response of the tissue. The compressive dynamic properties of bovine brain tissue were heterogenous for regions but not sensitive to orientation showing frequency dependent statistical results, with viscoelastic properties increasing with frequency. The mean storage and loss modulus were found to be 12.41 kPa and 5.54 kPa, respectively. The material parameters were obtained using the linear viscoelastic model in the frequency domain and the numeric simulation can capture the compressive mechanical behavior of bovine brain tissue across a range of frequencies. The frequency-dependent viscoelastic characterization of brain tissue will improve the fidelity of the computational models of the head and provide essential information to the prediction and analysis of brain injuries in clinical treatments.

Keywords:

Bovine brain; Dynamic mechanical analysis; Modulus; Viscoelastic modeling

1. Introduction

Computational simulations of human brain tissue have emerged with progress in brain mechanical investigations over the past decades[1]. Modeling is a promising platform that can be applied to predict brain mechanics[2] and develop brain injury criteria[3]. Brain tissue is considered as one of the most vulnerable organs and traumatic brain injury is predicted to be in the top three cause of death and disability in 2020[4]. The primary causes of brain injury are violence, sports, vehicle crashes and construction[5]. The direction and type of forces may contribute to brain injuries including angular, shear and translational forces[6]. Further, shaking motion of the brain within the skull often leads to brain diffuse injuries. Oscillations of the head with low acceleration can also produce mild brain trauma[7]. The mechanical properties of brain play an important role when head injuries are studied, such as traumatic brain injury simulations[8] and provide information in analysis of cortical folding[9] and tumor growth[10]. However, the accuracy and variety of these computational models requires quantitative data from experiments and are also dependent on the constitutive models used within simulations.

The mechanical characterization of brain tissue has been the focus of several studies. However, the obtained results are not always consistent across different studies due to the complexity of the soft tissue. Further, a wide range of loading conditions and protocols have been performed on brain tissue and there is lack of a standard testing protocol. Most protocols analyze brain tissue within the time domain and with "quasi-static" test conditions such as indentation[11], tensile[12] and shear[13]. Recently, the mechanical behavior of brain tissue has been investigated through the combination of compression, shear and tensile tests under multiple loading conditions[14][15]. In addition, dynamic sweep tests in the frequency domain have been previously performed to characterize the brain tissue, mostly conducted in shear[8][16][17]. In general, the potential differences are existed in the types of loading protocols and the dynamic compressive characterization of brain tissue has not been understood completely[18].

Brains are viscoelastic[19], and viscoelastic materials can be characterized by a storage and loss moduli[20][21][22]. The storage modulus characterizes the ability of the tissue to store energy in the elastic phase. The loss modulus characterizes the ability of the tissue to dissipate energy in the viscous phase, mostly lost as heat. Dynamic Mechanical Analysis (DMA) is a powerful method which can be used to characterize a material's viscoelastic properties over specific frequencies covering physiological and injury loading conditions. Unlike the conventional stress-strain tests, an oscillatory deformation is given to materials with a phase delay from the force.

The effect of microstructural heterogeneity of brain tissue has been recently focused[23][24] and it is necessary to investigate the connection between the macroscopic mechanical behavior and the regional microstructure for accurate prediction of injury across the brain structure. Brain tissue can be divided into gray and white matter. The gray matter is made of neurons with the function of data processing distributed around the surface of the cortex, and the white matter consists of myelinated nerve axons. Previous studies showed gray matter seems to be less stiff than white matter through indentation tests[25][23]. Recently, a study showed there is regionally microstructural variation even within the gray and white matter tissue[15]. Brain tissue was further differentiated into locations of corpus callosum, corona radiata, cortex and basal ganglia[26]. Corona radiata and corpus callosum are considered as white matter, while the latter has more oriented nerve fibers connecting the two hemispheres. Cortex and basal ganglia are considered as gray matter. The investigation of regionally

mechanical properties in brain tissue would be helpful for the clinic analysis as the degree of injury may vary with regions. While viscoelastic properties of brain tissue have been characterized, the regional effect on frequency-dependent properties have not been assessed by compressive DMA. In addition to the regional heterogeneity, white matter structure has been found to be transversely isotropic because of the highly aligned axonal fibers, while gray matter is simply isotropic[27][28]. Therefore, the mechanical response of white matter is potentially affected by the fiber direction[29].

To develop finite element (FE) models of brain tissue, the appropriate constitutive laws are essential. Most previous studies characterized viscoelastic behavior in the time domain and have used a Prony series in the single viscous solid phase[13][30]. Some studies applied a biphasic theory to describe the time-dependent response of brain tissue[31][32], which is based on Biot's theory of consolidation[33]. In addition, nonlinear material models are often adopted to predict the behavior of brain tissue for large strain, and the hyperelastic component has been determined following comparison of different constitutive models (e.g. neo-Hookean, Mooney-Rivlin, Gent or Ogden)[34][35]. The frequency-dependent properties of brain tissue are also vital in computational simulations when the dynamic mechanical response is analyzed. However, the application of constitutive laws describing the frequency-dependent viscoelastic behavior of brain tissue in compression is limited.

In this study, the frequency-dependent viscoelastic properties of brain tissue were characterized using Dynamic Mechanical Analysis. The white matter (corona radiata and corpus callosum) and gray matter (cortex and basal ganglia) were investigated under compression over a range of loading frequencies to characterize the regional and directional properties. The storage and loss moduli were analyzed, and a frequency-dependent constitutive model was calibrated using experimental data, so as to characterize the mechanical behavior of brains.

2. Materials and methods

2.1. Specimen preparation

Eight bovine brains under 12 months of age, were obtained from a supplier (Samples for Schools, UK). After delivery to the laboratory, the brain samples were wrapped in tissue paper and soaked in Ringer's solution (Oxoid Ltd, Basingstoke, UK). Samples were then stored at -40° C in a freezer in double heat-sealed plastic bags[36][37]. When the brains were required for testing, samples were taken out from the freezer and left in Ringer's solution for 12 h ahead of dissection. From previous studies, freeze-thaw treatment does not change the mechanical properties of biological tissue[38][39].

After the cerebellum and brainstem were removed, the cerebrum was cut in the coronal plane using a surgical scalpel (Swann-Morton Limited, Sheffield, UK). To investigate the regional properties of brain tissue, the specimens were collected from the four locations of cerebrum including corona radiata and corpus callosum, cortex and basal ganglia. This categorization is consistent with previous studies[23][14]. A circular trephine with the diameter of 8 mm was used through the anterior-posterior direction to extract homogeneous specimens into cylindrically shaped samples (Fig.1). Samples from the region of corpus callosum were extracted in the two orientations (i.e. (D1) orthogonal to nerve fiber bundles in the sagittal plane and (D2) aligned with the nerve fiber tracts in the coronal plane) to investigate the directional properties (Fig.2). The cerebral cortex is usually folded, and this circumvolution leads to a greater surface area for grey matter. The soft nature of brain tissue resulted in

some deformation of specimens under their own weight during the preparation, which may have increased the variability of the dimension measured. Brain samples were 5 ± 0.5 mm (mean ± deviation) in thickness and 8 ± 0.1 mm in diameter, measured prior to testing using a Vernier calliper (Draper Tools Ltd, Hampshire, UK).

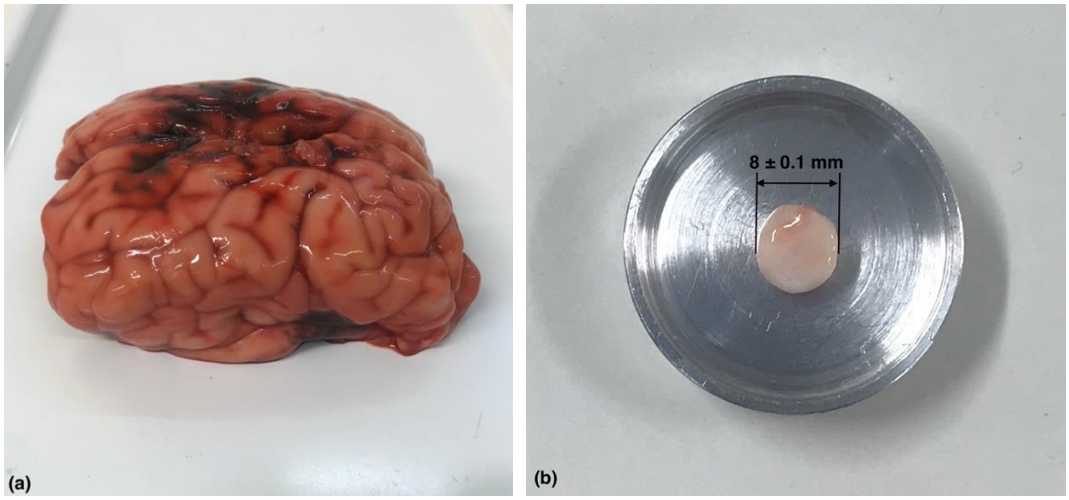


Figure 1: (a) A bovine brain obtained for mechanical testing. (b) Representative brain specimen in cylindrical shape of 8 ± 0.1 mm diameter.

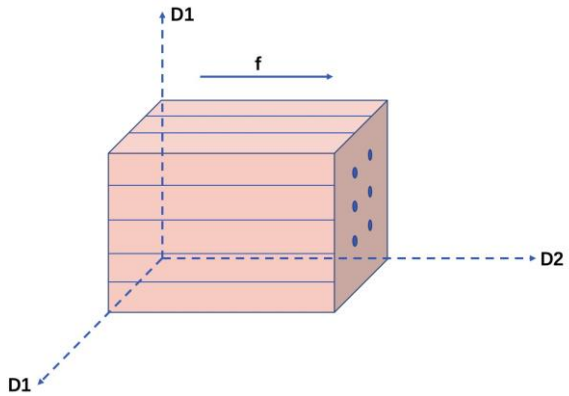


Figure 2: Schematic graphic of loading direction. Samples from the region of corpus callosum were tested orthogonal to nerve fiber direction D1 and aligned with the nerve fiber tracts D2. Vector f represents the nerve fiber direction.

2.2. Dynamic Mechanical Analysis frequency sweep

The viscoelastic properties of brain tissue specimens were characterized using a Bose ElectroForce 3200 (Bose Corporation, ElectroForce Systems Group, Minnesota, USA) testing machine operated with WinTest Dynamic Mechanical Analysis software (Bose Corporation, ElectroForce Systems Group, Minnesota, USA). Other biological and synthetic materials were previously tested using this approach[40][41][42]. The Bose testing machine was equipped with a 225 N load cell with a resolution of 0.002 N and a high accuracy displacement sensor with a resolution of 0.001 mm. A Fast Fourier Transform (FFT) was used to analyze the displacement sine wave input and load sine wave output. The

dynamic stiffness (k^*) was calculated as the ratio of the force amplitude and displacement amplitude. The phase angle (δ) was determined as the phase relationship between the force and displacement. The WinTest DMA software calculated storage (E') and loss (E'') modulus by converting the relevant stiffness and phase data through a shape factor (S) as shown in Eqs. (1) and (2). The shape factor for cylindrical specimens was calculated from Eq. (3). Further details on the characterization are available elsewhere[43][44]. To measure how energy dissipates in the tissue structure, the tan delta ($tan\delta$) as the ratio of loss to storage modulus (E''/E') was calculated for every frequency at different brain regions. The viscous response of a material increases with greater E''/E' ratio in a system.

$$E' = \frac{k^* \cos\delta}{S} \tag{1}$$

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

$$S = \frac{\pi d^2}{4h} \tag{3}$$

where d is the diameter and h is the thickness of a specimen.

2.3. Experimental setup and data analysis

The specimens were placed in the sample holder after dissection and preparation (Fig. 3). Amplitude sweep tests were performed at 1 Hz for about 680 cycles to determine the amplitude range within the linear viscoelastic region of the material, followed by the subsequent mechanical tests. All samples were compressed with a mean displacement of 1 mm (20% of a specimen height) using a circular flat indenter, followed by a sinusoidally varying displacement with an amplitude of 0.05 mm (i.e. between 0.95 and 1.05 mm), across a frequency sweep between 0.5 to 35 Hz in 12 steps for approximate 5000 cycles. The range of frequencies is relevant to the strain rates comparable with previous studies on bovine[31], porcine[26] and human brains[32] and also to which the brain might be exposed during physiological and injury conditions[7]. Prior to the data collection procedure, a preload of 10 mN was initially applied to specimens to ensure a zero configuration and a preconditioning cycle of 1 Hz with 0.05 mm amplitude was then performed to stabilize the samples[45][46][40]. All tests were performed at room temperature with the sampling rate of acquisition at 5 kHz for the highest frequency tested. A total of 96 brain samples were tested from four locations containing 23 corona radiata samples, 32 corpus callosum samples, 20 basal ganglia samples and 21 cortex samples. The specimens were hydrated with Ringer's solution before each test to minimize the friction between the compressed platen and the brain samples. During preliminary investigations the order of the tested frequencies did not alter the measured mechanical properties.

Figure 3: Experimental setup for the compressive DMA of brain tissue specimens.

To determine the effect of the regionally and directionally frequency-dependent behavior of brain tissue, Sigmaplot Version 14.0 (Systat Software Inc., London, UK) was used to perform the statistical comparisons. Storage and loss modulus and phase angle were compared at each frequency. A one-way analysis of variance (ANOVA) was performed to investigate significant differences. When ANOVA showed a statistically significant difference ($p < 0.05$), a Tukey HSD post-hoc analysis was used for all pairwise comparisons between various brain regions and directions in compressive DMA testing. The results for all analysis were considered statistically significant with a probability value of less than 0.05.

2.4. Constitutive modelling

For viscoelastic materials, the deviatoric stress is not linearly related to the deviatoric strain. To characterize the viscoelastic behavior of brain tissue, linear viscoelastic (LV) theory was applied to model the strain rates of brain injury[11] or combined with other constitutive laws[47][48]. The linear viscoelastic model can be effectively conducted in commercial Finite Element software with the experimental parameters. In a range of small deformations, the stress in this model was obtained by the following constitutive formulation based on the Bolzmann's superposition integral[49].

$$\sigma(t) = \int_{-\infty}^{t} u(t-\tau)\frac{d\varepsilon}{d\tau}d\tau \tag{4}$$

where σ is the deviatoric stress tensor, ε is the deviatoric strain tensor and $u(t)$ is the linear relaxation modulus. In this equation, the strain $\varepsilon(t)$ is considered to be zero for $t \leq 0$, and it could be transferred in the Laplace form by assuming the imaginary variable s to $j\omega$ as:

$$u^*(j\omega) = s\hat{u}(s) = \frac{\hat{\sigma}(s)}{\hat{\varepsilon}(s)} = \frac{\hat{\sigma}(j\omega)}{\hat{\varepsilon}(j\omega)} \tag{5}$$

where $\hat{u}, \hat{\sigma}$ and $\hat{\varepsilon}$ are relaxation modulus, stress and strain tensor in the Laplace domain, respectively. ω is a single angular frequency, the real part relevant to the actual force and $j = \sqrt{-1}$ standing for the imaginary number. $u^*(j\omega)$ is the complex modulus which can be given by the dynamic storage u'

and loss u'' modulus as:

$$u^* = u' + ju'' \tag{6}$$

In the physical models, a discrete relaxation function is considered with the expression as a discrete set of exponential decays defined in equation (7). Using this discrete function with Eqs. (4)-(6), the complex modulus can be defined from Eq. (8). Further, the dynamic storage and loss modulus of the generalized Maxwell model can be obtained as given by Eqs. (9) and (10) in the Prony series form:

$$u(t) = u_e + \sum_{i=1}^{N} g_i \exp\left(-t/\tau_i\right) \tag{7}$$

$$u^*(j\omega) = u_e + \sum_{i=1}^{N} g_i \frac{\tau_i j\omega}{1+\tau_i j\omega} \tag{8}$$

$$u'(\omega) = u_e + \sum_{i=1}^{N} g_i \frac{(\omega\tau_i)^2}{1+(\omega\tau_i)^2} \tag{9}$$

$$u''(\omega) = \sum_{i=1}^{N} g_i \frac{\omega\tau_i}{1+(\omega\tau_i)^2} \tag{10}$$

where the *N* relaxation modes are determined by the corresponding Prony constants g_i and relaxation times constants τ_i. u_e is the equilibrium modulus.

To estimate the parameters of a frequency dependent discrete relaxation function, a non-linear least squares algorithm was conducted, and the optimization constraint were iteratively updated until the coefficients converged with optimality tolerance of three decimal accuracy. The average experimental results for various regions or directions were used to calibrate the constitutive models based on the average square of deviation between the predicted dynamic modulus and the measured dynamic modulus from experiment through equation (11). The long-term modulus was obtained from the preliminary studies with low frequency testing conditions. The Prony coefficients g_i and relaxation times τ_i were initially assumed, based on the exponentially ascending order of the control variables. A minimum of three pairs of Prony constants was required to represent the viscoelastic behavior of the brain tissue[31] and from the preliminary studies, a four term Prony series was needed for these frequency-dependent linear viscoelastic models[50]:

$$\min(g,\tau) = \sum_{k=1}^{M}\left(\left(\frac{u'(\omega_k)}{\tilde{u}'_k} - 1\right)^2 + \left(\frac{u''(\omega_k)}{\tilde{u}''_k} - 1\right)^2\right) \tag{11}$$

where $u'(\omega_k)$, $u''(\omega_k)$ are the calculated values from equations (9) and (10), and $\tilde{u}'_k$, $\tilde{u}''_k$ are the measured data at M frequencies ω_k. The goodness of fit of data to the given model was assessed using the coefficient of determination R^2.

The mean frequency-dependent behavior of brain tissue for different regions and orientations was each simulated in COMSOL Multiphysics 5.5 (COMSOL, Stockholm, Sweden). The FE axisymmetric models were created using a cylindrical geometry with the average diameter and height of tested samples (8 mm in diameter, 5 mm in height), as shown in (Fig. 4). A mesh convergence analysis was conducted to validate the mesh density with the final mesh size of 0.2 mm. The top and bottom platens were modeled as rigid surfaces. The contact analysis between the platens and brain tissue specimens was performed in the simulation and the relevant friction coefficient of 0.1 in the lateral direction was adopted, which is within the range estimated for the soft tissue[34]. A Poisson's ratio of 0.49 for an incompressible material was selected to avoid any singularity conditions during the FE

implementation[51]. The brain specimens were compressed by the top platen, subsequently followed by a harmonic perturbation of 0.05 mm over a range of frequencies, 0.5-35 Hz, while the bottom platen was set as a fixed constraint.

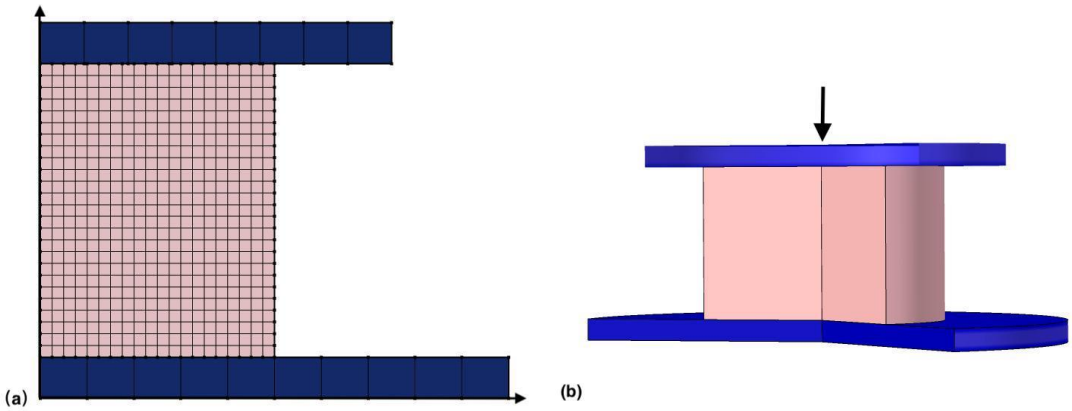


Figure 4: Numerical simulation of the brain specimens under dynamic compressive testing in (a) FE axisymmetric and (b) partial revolution 2D configurations to easily identify the cross-section.

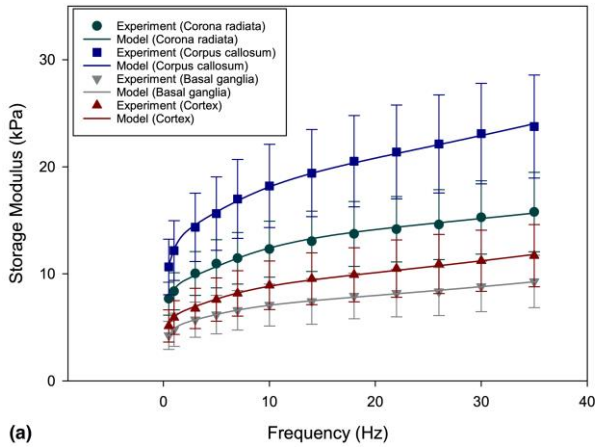
3. Results

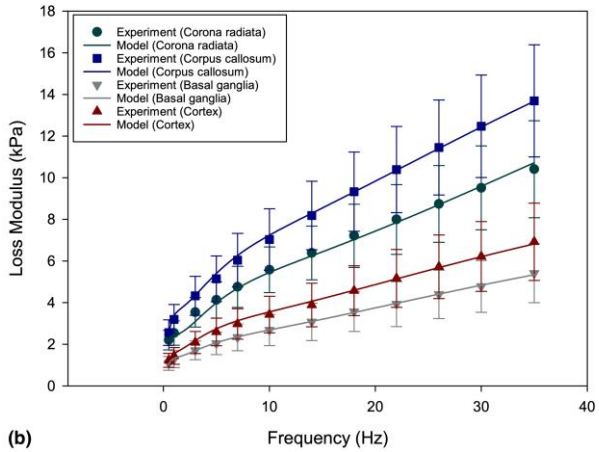
3.1. Regional dependency of viscoelasticity

The mean storage and loss modulus of brain specimens showed an increasing trend with increasing frequency (Figs. 5 (a) and (b)) for the different regions; the average loss modulus was lower than storage modulus for each tested frequency. The tested specimens from the corpus callosum showed the greatest mean storage and loss modulus (18.19 kPa and 7.82 kPa, respectively) over frequencies, followed by the specimens from the corona radiata (12.28 kPa and 6.08 kPa, respectively). The specimens tested from the cortex had marginally higher mean storage and loss modulus (8.86 kPa and 3.85 kPa, respectively) than from the basal ganglia with a lowest value of 7.05 kPa and 3.02 kPa, respectively.

The storage and loss modulus in the corpus callosum were significantly greater ($p < 0.05$) than in the basal ganglia and the cortex across all frequencies tested; the moduli in the basal ganglia and the cortex were not significantly different from each other. There was also no significant difference between the storage and loss modulus in the corona radiata and the corpus callosum ($p > 0.05$); however, from the frequency of 7 Hz a significant difference of storage modulus was considered between them (Figs. 6 (a) and (b)). For all increments of frequencies, the storage modulus in the corona radiata, the cortex and the basal ganglia was not significantly different while the loss modulus in the corona radiata was significantly larger than in the basal ganglia.

From the specimens tested, the average tan delta (i.e. ratio of E''/E') of brain tissue for each region showed an increasing trend with increasing frequency (Fig. 5 (c)). The corona radiata exhibited the greater viscous behavior with the $tan\delta$ ranging from 0.29 ± 0.03 (mean ± 95 % confidence intervals) to 0.67 ± 0.04; other regions showed a similar ability to dissipate energy with a mean value of around 0.41 across all frequencies. For the brain specimens tested below 10 Hz, the significant differences were only found between the tan delta in the corona radiata and the corpus callosum ($p < 0.05$) at 3 and 7 Hz, respectively. From the frequency of 10 Hz, the tan delta in the corona radiata was significantly greater than in other regions (Fig. 6 (c)).





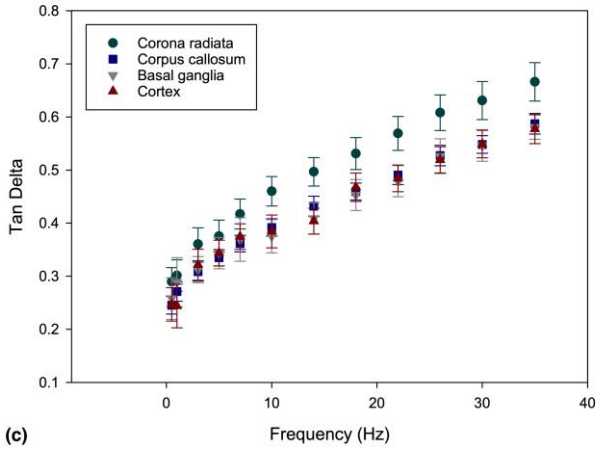
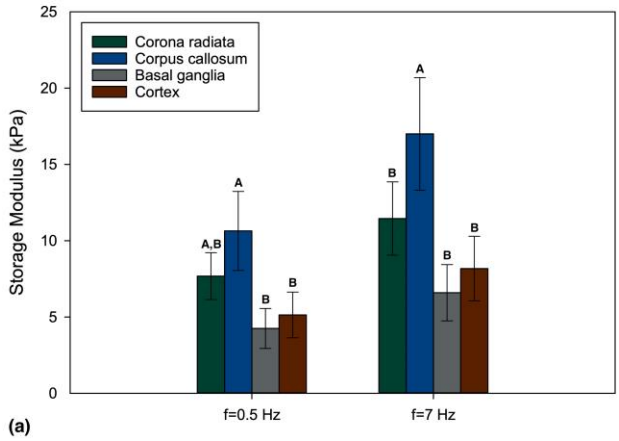
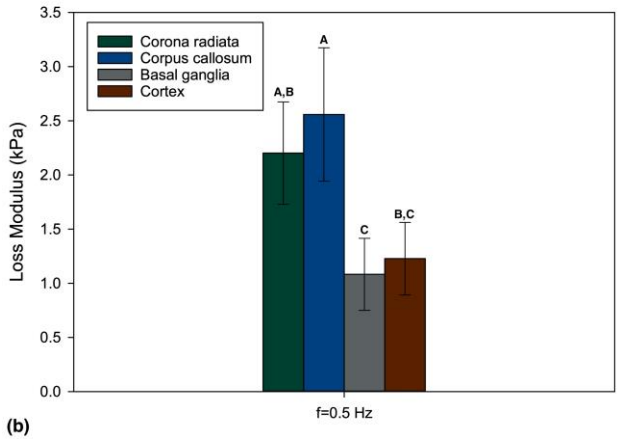


Figure 5: Frequency-dependent viscoelastic properties for brain tissue tested from the different regions of corona radiata, corpus callosum, basal ganglia and cortex. Mean (a) storage and (b) loss modulus, against frequency from experiments with relevant linear viscoelastic model predictions and the trendlines are data predicted following simulations which were solved at loading frequencies from 0.5 Hz up to 35 Hz in incremental steps of 0.1 Hz; (c) mean tan delta against frequency. Error bars represent 95% confidence intervals.





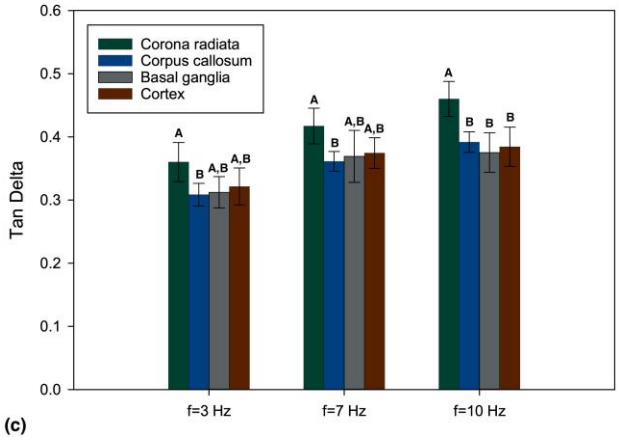
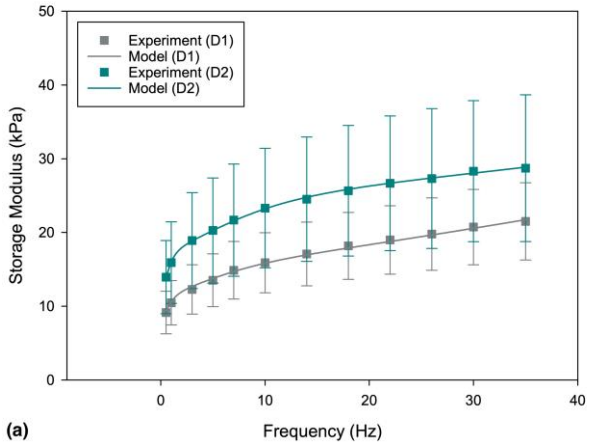
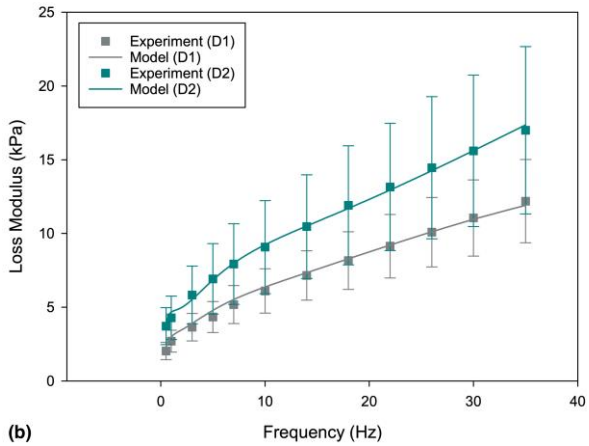


Figure 6: Grouped vertical bars of frequency-dependent viscoelastic properties (mean ± 95% confidence intervals) for brain tissue tested from the different regions of corona radiata, corpus callosum, basal ganglia and cortex. The statistical results (a) the storage modulus showed the two types of significant differences i.e. from 0.5 to 7 Hz and up to the end frequency sweep, (b) the loss modulus showed the same significant differences of regions over all frequencies tested; (c) the tan delta indicated significant differences were only found at 3 and 7 Hz; however, at the other frequency increments from 10 Hz there were the same significant differences of regions. In each regional group, viscoelastic properties not sharing a letter are considered to be significantly different (Tukey HSD).

3.2. Directional dependency of viscoelasticity

The effect of the nerve fiber direction on frequency-dependent viscoelastic properties was investigated. The viscoelastic storage and loss moduli exhibited an increasing trend with frequencies for different directions. The brain specimens from corpus callosum tested orthogonal to the fibers (D1) showed a slightly lower mean storage and loss modulus (Figs. 7 (a) and (b)). For the dynamic viscoelastic behavior of brain specimens, no significant directional dependency on the storage modulus was revealed over all frequencies tested while the loss modulus was found significantly larger at 1.8 to 1.6 times (Figs 8 (a) and (b)) for specimens tested aligned to the fiber tracts (D2) below the frequency of 7 Hz ($p < 0.05$). The trend of tan delta was consistent with the specimens tested in different nerve fiber orientations (Fig. 7 (c)) and no significant differences ($p > 0.05$) were found over the frequency range tested (Fig. 8 (c)).





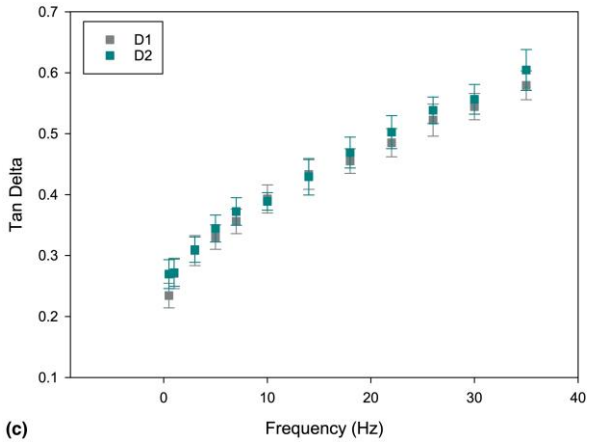
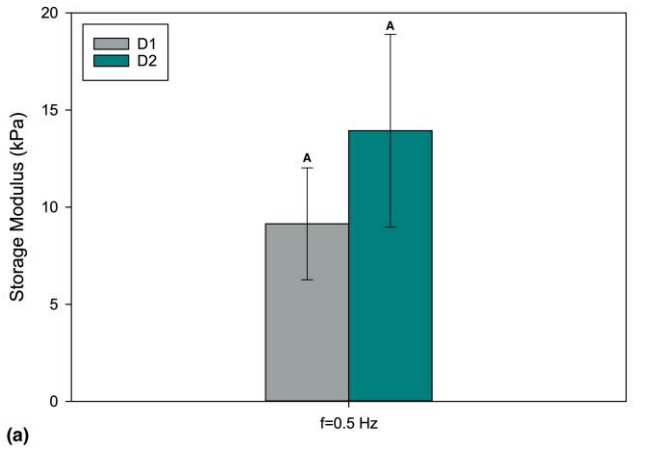
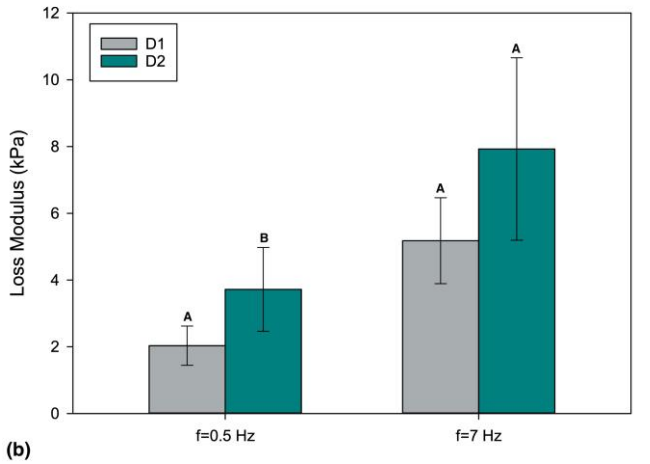


Figure 7: Frequency-dependent viscoelastic properties for brain tissue tested from the different directions: orthogonal to nerve fiber bundles (D1) and aligned with the nerve fiber tracts (D2). Mean (a) storage and (b) loss modulus, against frequency from experiments with relevant linear viscoelastic model predictions and the trendlines are data predicted following simulations which were solved at loading frequencies from 0.5 Hz up to 35 Hz in incremental steps of 0.1 Hz; (c) mean tan delta against frequency. Error bars represent 95% confidence intervals.





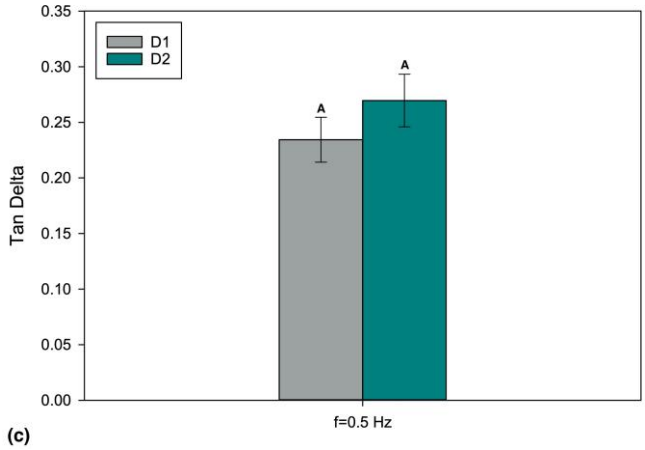
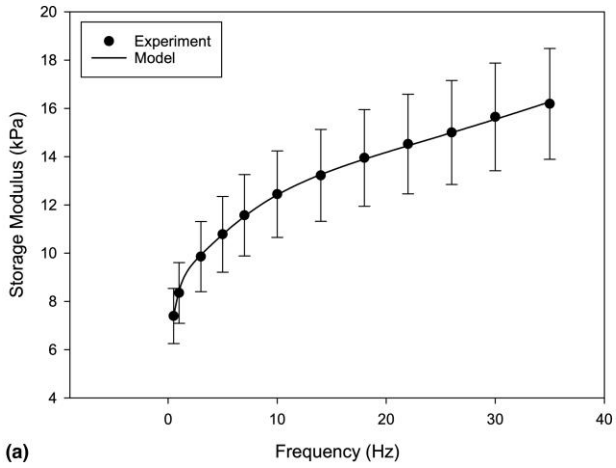
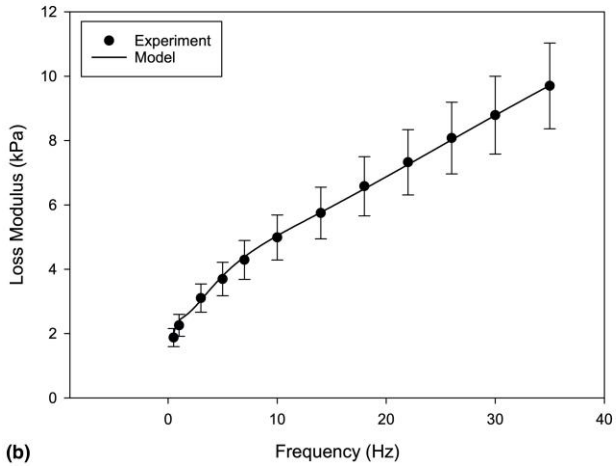


Figure 8: Grouped vertical bars of frequency-dependent viscoelastic properties (mean ± 95% confidence intervals) for brain tissue tested from the different directions: orthogonal to nerve fiber bundles (D1) and aligned with the nerve fiber tracts (D2). The statistical results (a) the storage modulus showed no significant differences of directions over all frequencies tested; (b) the loss modulus showed the significant difference only from 0.5 to 7 Hz; (c) the tan delta showed no significant differences were found across frequencies. In each directional group, viscoelastic properties not sharing a letter are considered to be significantly different (Tukey HSD).

3.3. General frequency-dependent characterization

The mean dynamic viscoelastic properties of brain tissue against frequency are shown in Fig. 9 for all tested samples. The storage modulus increased from 7.39 kPa to 16.19 kPa with a mean value of 12.41 kPa and the loss modulus ranged between 1.87 kPa and 9.70 kPa with a mean value of 5.54 kPa. The average tan delta of brain tissue exhibited an increasing trend with frequencies, ranging from 0.26 to 0.60 with an average value of 0.43.





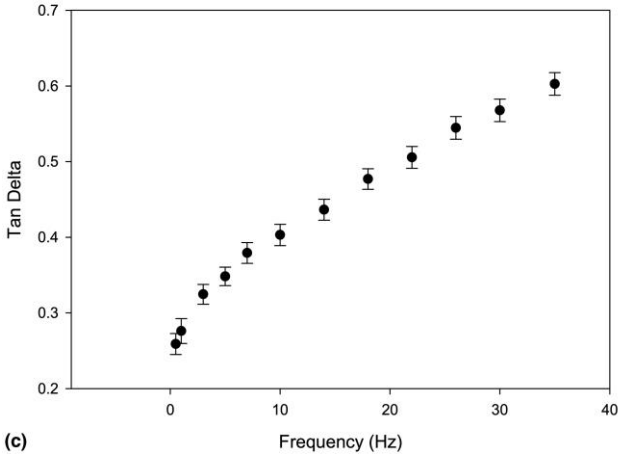


Figure 9: Frequency-dependent viscoelastic properties for brain tissue for all specimens tested. Mean (a) storage and (b) loss modulus, against frequency from experiments with relevant linear viscoelastic model predictions and the trendlines are data predicted following simulations which were solved at loading frequencies from 0.5 Hz up to 35 Hz in incremental steps of 0.1 Hz; (c) mean tan delta against frequency. Error bars represent 95% confidence intervals.

3.4. Viscoelastic model fitting

The mean results of the experimental storage and loss moduli of the brain tissue tested from various regions, directions and all brain specimens were used to obtain the optimized parameters of a four term Prony series in the frequency-dependent linear viscoelastic model (Table 1) with the corresponding coefficients of determination. The long-term modulus u_e was found to be 83.9 Pa. The number of four pairs of Prony constants were adequate to keep the simulation accuracy in the models. For the linear viscoelastic model in the frequency domain, the FE simulations were able to represent the mechanical behavior of brain tissue adequately from the mean storage and loss modulus.

Table 1: Constitutive parameters of frequency-dependent linear viscoelastic model derived from the mean dynamic viscoelastic properties over all frequencies for various regions, directions and general material characterization.

	Linear viscoelastic model parameter								
	Prony constant (kPa)				Relaxation time constant (s)				
	g_1	g_2	g_3	g_4	τ_1	τ_2	τ_3	τ_4	R^2
Corona radiata	5.80	3.54	4.93	58.75	6.59×10^{1}	2.57×10^{-1}	1.87×10^{-2}	7.58×10^{-4}	0.994
Corpus callosum	9.60	4.56	6.08	40.70	2.34×10^{1}	1.74×10^{-1}	1.89×10^{-2}	1.51×10^{-3}	0.998
Basal ganglia	3.59	1.76	2.14	15.44	5.00×10^{4}	2.09×10^{-1}	2.38×10^{-2}	1.63×10^{-3}	0.998
Cortex	4.57	1.88	3.05	18.85	5.00×10^{4}	2.15×10^{-1}	2.36×10^{-2}	1.71×10^{-3}	0.996
D1	8.53	3.77	4.91	29.36	1.12×10^{1}	1.60×10^{-1}	2.02×10^{-2}	1.98×10^{-3}	0.994
D2	11.94	6.42	8.60	97.49	1.12×10^{1}	1.95×10^{-1}	1.71×10^{-2}	7.21×10^{-4}	0.997
General	6.38	3.11	4.29	31.57	1.17×10^{1}	2.00×10^{-1}	2.01×10^{-2}	1.37×10^{-3}	0.998

4. Discussion

This study has demonstrated the effect of regions and directions on the frequency-dependent viscoelastic properties of brain tissue using Dynamic Mechanical Analysis. As the compressive loading plays a significant role in head trauma[52][53] and the brain could be exposed to compressive waves during the course of head impact[54], it is essential to determine the compressive behavior of the brain tissue over a range of frequencies. The dynamic storage modulus and tan delta from four regions showed different types of significant differences to various frequency ranges while the corresponding dynamic loss modulus exhibited the same significant difference of regions over all frequencies tested. No significant mechanical directional dependency was found in frequency-dependent viscoelastic properties except in loss modulus. For all specimens tested under the frequency sweep, the trends of

dynamic properties were similar, with properties increasing with frequency. Preconditioning tests were performed in this study which is a well-established process for the mechanical testing of biological tissue. Similar preconditioning behavior was conducted on human brain[15] and bovine brain[31] to ensure a repeatable mechanical response. The experimental results were used to establish viscoelastic constitutive models following the adaptation of storage and loss modulus against frequency to capture the compressive mechanical response of brain tissue.

The dynamic mechanical properties of bovine brain tissue showed regional dependency. Specimens tested from the corpus callosum exhibited a consistently significant difference with larger storage and loss modulus and tan delta than specimens tested from the basal ganglia, over all frequencies. In general, the dynamic storage and loss moduli of white matter (15.72 kPa and 7.09 kPa) in this study were greater than that of gray matter (7.97 kPa and 3.45 kPa). A similar trend for white and gray matter was found by some studies on human[55] and bovine brains[23] using indentation tests, but other studies reported the opposite trend on rat[56] and porcine brain tissue[26]; all of these experiments were performed in the time domain with stress-strain testing. These discrepancies may be induced by the potential differences in loading protocols and the extremely sensitive properties of brain tissue. Further, recent investigations on magnetic resonance elastography (MRE)[57][58] in the frequency domain showed the modulus of white matter was approximately 2 times greater than that of gray matter, which is comparable to the results from this study. Although MRE has been used to characterize brain tissue *in vivo*[59], current applications are limited to accurately quantify regional dependent properties within the small structure of the tissue specimens.

Significant directional dependency of the dynamic viscoelastic properties was only observed on the loss modulus from 0.5 to 7 Hz in the corpus callosum, considered as a highly anisotropic brain region, with larger values aligned to the nerve fiber tracts. Some studies reported a similar trend on lamb brain white matter through dynamic shear tests[25] and porcine brain tissue through tensile tests[12]. However, for the storage modulus and $tan\delta$, no significant differences were found over all tested frequencies and the bovine brain tissue is more likely to be isotropic, which is consistent to the previous studies on mouse brain tissue through indentation tests[60] and bovine brain through compression[24].

The mean dynamic viscoelastic properties of bovine brain tissue are frequency-dependency and the storage modulus was constantly higher than the loss modulus for every frequency. In comparison to a study[40] on the compressive viscoelastic properties of porcine brain tissue where the mean storage and loss moduli were 8.09 kPa and 4.85 kPa, respectively, the bovine brain in this study had higher dynamic moduli at comparable frequencies. In addition, animal brain tissues were tested using dynamic shear[61] and tensile methods[45] to analyze the oscillatory characterization. Despite comparisons being limited by the potential discrepancies in the types of loading and tested specimen species, the general trends of the dynamic storage and loss moduli against frequency were found to be similar.

Due to the ethical reasons and difficulties in obtaining human brain specimens, animal brains are often adopted for many mechanical experiments[62][63][64]. The discrepancies for the mechanical properties between human and animal brain tissue has been controversial. Human brain tissue was previously reported with stiffer mechanical properties than porcine brain tissue[65], while the similar dynamic moduli of human and porcine brain tissue were found through dynamic shear tests[8] and the dynamic mechanical behaviors in different animal brain tissues were measured to be close[66]. From the literature, the fresh human tissue exhibited relatively softer mechanical properties than the human autopsy results[67] which implied the animal brain tissue may generate closer data. Further, the

anatomical structure between animal and human brains has been analogous. Based on this similarity, the dynamic mechanical properties of bovine brains tested in this study may be used in the mechanical analysis and computational models of human brains.

To develop the brain FE models, dynamic viscoelastic properties of brain tissue are essential. Regarding head injuries, the brain could experience dynamic loading conditions such as shaken baby syndrome (SBS) where the violent shaking occurs with the head moving backwards and forwards[68]. However, due to the lack of experimental data for compressive frequency-dependent properties of brain tissue, most simulations were applied with the viscoelastic models to capture brain mechanical behavior in the time domain (i.e. stress-strain curves)[30][69]. The stress versus time behavior was studied previously from shear, compression and tensile tests[70][34][71] where the time dependent Prony parameters were estimated from the corresponding relaxation functions. The DMA tests in this study provide critical information on the frequency-dependent viscoelastic behaviors of brain tissue for different regions and directions, which has been manifested in the discrete relaxation mode of a Prony series with the exponentially ascending order of relaxation times. The linear viscoelastic model was previously adopted to replicate the mechanical response of biological material[11] with time domain experimental data and can also be applied to reproduce the dynamic response of viscoelastic material with adaptation of the storage and loss moduli. This constitutive model could improve the variety and accuracy in the brain computational models to develop the prediction of dynamic impact of brain injuries. Although the small dynamic deformation responses of brain tissue were the focus of this study, there is future opportunity to investigate large strain behavior based on the material parameters derived from dynamic experimental data; this would require further experimental work to investigate the large strain behavior of the tissue. A recent study showed a numerical approach to support the linear viscoelastic interconversion between the time and frequency dependent material properties of porcine brain tissue, based on the stress relaxation experimental data[72], even though the conversion of the frequency to the strain rate is inherently limited due to the continuously changing velocity during the oscillation. An optomechanical indentation method was performed with the same testing conditions through both the strain rate and frequency dependent approaches to indicate there is the great correlation between these data of different types[73]. In addition, inverse FFT is able to convert the frequency function to the time function and a comparison between the frequency and time dependent models becomes available.

The limitation of this study is that a freeze-thawed treatment was applied to prepare specimens. The variation between frozen and fresh tissue was previously studied and the results showed extensive overlap[74]. In addition, limited changes were shown for mechanical properties on porcine liver[39], aortic tissue[75], and ligaments[76].

For the sample preparation, the presence of the convolutions within the cerebral cortex, and the pia mater presents limitations during the extraction of specimens from the continuum of tissue from the cerebral cortex, additionally it is close to the sulci (i.e. the grooves which give a folded appearance to the brain). Although these factors may have an impact, much cortex tissue was also collected in the circumvoluted area mainly contributing to the testing results, with the magnitude of data measured and trends in this data being broadly in agreement across both these datasets.

5. Conclusion

In conclusion, bovine brain tissue is viscoelastic with frequency-dependent storage and loss modulus.

The dynamic mechanical tests were conducted to characterize the regional and directional properties of the bovine tissue throughout the range of frequencies tested. The viscoelastic storage and loss modulus showed an increasing trend against frequency with a mean value of 12.41 kPa and 5.54 kPa, respectively. In this study, the constitutive properties of bovine brain tissue for different regions of corona radiata, corpus callosum, basal ganglia and cortex were determined, and the frequency-dependent compressive behavior can be captured adequately through the linear viscoelastic model. Applications of the brain viscoelastic properties include the diagnosis of brain injury, complex head computational simulations and the development of protection equipment.

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