FINITE SHEAR BEHAVIOUR OF BRAIN TISSUE
UNDER IMPACT LOADING

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ABSTRACT
A method is presented for determining the dynamic shear behaviour of brain tissue using a rotational plate-plate rheometer in a strain and frequency range representative for impact conditions. The non-linear strain behaviour was characterised using shear stress relaxation experiments and expressed in terms of a damping function. Results were corrected for the non-homogeneous strain field applied. Brain tissue was found to exhibit shear softening (45% at 20% strain) that could be fitted with a second order Mooney-Rivlin model. The time dependent behaviour of brain tissue seems to be independent of the strain level. It was characterised up to 1000 Hz by small strain oscillatory shear experiments and application of the Time Temperature Superpositioning principle. The data presented can be used to fit a non-linear material model for brain tissue under shear loading for frequencies up to 1000 Hz and strains up to 20%.

INTRODUCTION
The head is identified as being most frequently involved in life-threatening injury in crash situations [1]. During an accident, the head is subjected to an external mechanical load. This load is transferred to the brain tissue. If the mechanical response of the brain tissue exceeds a certain limit, brain damage will be caused via an injury mechanism. Insight into brain injury mechanisms is needed to improve prevention measures and diagnosis and treatment of brain injury.

To obtain insight into injury mechanisms, the internal mechanical response of the head under impact conditions has to be known. Since it is impossible to determine this response in-vivo, i.e. in the real living human, numerical modeling of the head is often applied [2, 3, 4].
One of the shortcomings of these numerical models is the simplified constitutive modeling of the material behaviour of brain tissue, due to the lack of data on material behaviour valid for impact conditions. Typical frequencies during such impact are on the order of 1000 Hz, whereas studies on isolated axons indicate strains of about 20% to be responsible for injury [5].

In a previous study [6] we characterised the small strain dynamic shear behaviour of porcine brain tissue. In this study, oscillatory shear experiments and stress relaxation experiments have been performed on porcine brain tissue at finite strain levels, thus completing the material characterisation in the full relevant dynamic range.

MATERIALS AND METHODS

Sample information
Porcine brain tissue was harvested from six-months-old pigs, obtained from a local slaughterhouse, immediately following sacrifice by electrical shock. During transportation, complete hemispheres were submerged in a physiological saline solution to prevent dehydration. Sagittal slices were cut using a standard butchers meat slicing device with a rotating knife blade (manufacturer: ‘Bizerba’). From these slices, 24-mm diameter cylindrical samples were cut using a cork bore. Samples were prepared from tissue located near the center of the brain and contained a mixture of white and grey matter. To eliminate scattering of measurement data by potential anisotropy, all samples were cut in the same direction with their cylinder axis perpendicular to the sagittal plane. The sample heights varied between 2 and 3 mm. Testing was completed within approximately four hours after sacrifice.

Experimental setup
Simple shear experiments have been performed on a rotational plate-plate viscometer (Rheometrics ARES [7]). Samples were placed between 25-mm diameter flat parallel disks. A prescribed rotation was applied on the one plate, while the torque was measured on the other plate that was fixed. The strain applied is defined as the plate displacement at the edge of the sample divided by the sample height. For determining the sample height, the distance between the plates was lowered until the normal force measured reached $10^{-4}$N. The plate distance was then measured with an accuracy of 1 µm using a build-in measurement device of the viscometer. Correct temperature conditions were obtained by controlled air temperature. Dehydration of the brain tissue samples was prevented by controlling humidity using a custom build moist chamber. To prevent slip between the sample and the plates two methods of sample fixation were investigated. Firstly an adhesive (standard Cyano-acrylate glue [8]) has been used to fixate the samples to the plates and secondly the coefficient of friction of the plates has been increased by fixing waterproof sandpaper (P220) to the plates using two-sided adhesive tape.

Data acquisition
Since the experiments were conducted on a rotational viscometer, a non-homogeneous strain field was present between the plates. Inertial effects and viscous heating in the sample can be neglected and it is assumed that there is no material displacement in z-direction (i.e. perpendicular to the plate surfaces). The shear strain, $\gamma(r,t)$, then can be written as

$$\gamma(r,t) = \frac{\theta(t)r}{H} = \frac{\gamma_0(t)r}{R}, \quad 0 < r < R$$  (1)
were $\theta$ represents plate rotation, $H$ the sample (and gap) height, $\gamma_0(t)$ the strain at the outer edge of the plate and $R$ the disk radius. The total transient torque is,

$$T(t, \gamma_R) = 2\pi \int_0^R \tau(r,t) r^2 dr$$  \hspace{1cm} (2)

By introduction of the shear modulus $G(\gamma,t)$ as,

$$\tau(r,t) = G(\gamma,t) \gamma(r,t)$$  \hspace{1cm} (3)

and using Equation (1), the torque can be rewritten as,

$$T(t, \gamma_R) = \frac{2\pi R^3}{4\gamma_0} \int_0^R G(t, \gamma) \gamma^3 d\gamma$$  \hspace{1cm} (4)

In the data processing software within the viscometer used, the shear modulus is assumed to be independent of the strain level. This apparent modulus, $G_a(t)$, is obtained directly from the viscometer and can be obtained by solving the integral of equation (4), yielding,

$$G_a(t) = \frac{2T(t, \gamma_0(t))}{\pi R^3 \gamma_0(t)}$$  \hspace{1cm} (5)

It represents the true material parameter $G(t)$ for strains within the linear regime of the material only. For strains outside the linear range $G_a(t)$ represents some average modulus over the strain range in the material.

**Experimental protocol**

Two types of loading conditions have been used, oscillatory strain for characterising the linear behaviour of the materials and step strain with subsequent stress relaxation for the non-linear behaviour.

**Oscillatory strain.** During the oscillatory experiments a sinusoidal strain was imposed on the sample,

$$\gamma(t) = \gamma_0 \sin(\omega t)$$  \hspace{1cm} (6)

When steady state is reached and strain amplitude $\gamma_0$ is sufficiently small, the shear stress $\tau$ will also be sinusoidal, but with a phase shift $\delta$ due to the viscous behaviour,

$$\tau(t) = G_d \gamma_0 \sin(\omega t + \delta)$$  \hspace{1cm} (7)
Both viscoelastic characteristics, phase shift $\delta(\omega, T)$ and dynamic modulus $G_d(\omega, T)$, are functions of the angular frequency and temperature $T$. To obtain information on the material behaviour at higher frequencies than the maximum frequency of the viscometer, 16 Hz, the Time Temperature Superposition principle (TTS) was employed. A set of isothermal characteristics, such as the phase angle $\delta$ and dynamic modulus $G_d$, was determined within the, viscometer-limited, frequency range at different temperatures, $\omega_T$. Next, the phase angle characteristic was shifted along the logarithmic frequency axis to an arbitrarily chosen reference characteristic of this set, to form one smooth curve: the master curve. This master curve is valid for the temperature at which the reference characteristic is measured (i.e., the reference temperature $T_{ref}$). The amount of horizontal shift per isothermal characteristic was quantified by the horizontal shift factor, $a_T$, and the effective frequency for which a shifted result is valid, $\omega_{eff}$, was obtained by,

$$\omega_{eff} = a_T \omega_T$$  \hspace{1cm} (8)

Next, the horizontal shift factors obtained in this manner, were applied to the dynamic modulus, $G_d$. A small vertical shift, $b_T$ was then applied to the modulus to obtain a smooth dynamic modulus master curve. This vertical shift factor is commonly associated with density changes due to temperature changes and is usually on the order of 1 [9]. In this manner, frequencies higher than the maximum allowed test frequency can be assessed by lowering the temperature below the reference temperature. The TTS principle is valid when indeed a smooth master curve can be obtained from the isothermal characteristics [10] and has already successfully been applied to brain tissue before [6, 11].

Oscillatory strain experiments were performed at a constant strain amplitude of 1% and increasing frequencies up to 16 Hz at several temperatures between 4 and 38°C. The 1% strain value been chosen since it lies within the linear range of the material [6]. Characteristics were shifted to the 38°C characteristic to form a master curve and shift-factors were determined.

The small strain results were used to study the effect of the different adhesion methods on brain tissue by comparing them with results obtained without adhesive or sandpaper. An overview of all experiments is provided in Table (1).

### Stress Relaxation.

For investigating the non-linear strain behaviour of a material, oscillatory experiments can only be used when a material model is assumed beforehand. To investigate the non-linear strain behaviour of the material without making model assumptions,

#### Table 1. Overview of experimental conditions: n: number of samples, $\Delta T$: temperature range, MC: Moist chamber (yes/no), d: sample diameter, $\Delta \gamma$: strain range, DFS+TTS: Dynamic Frequency Sweeps and application of Time Temperature Superpositioning, SR: Stress Relaxation.

<table>
<thead>
<tr>
<th>Measurement type</th>
<th>n</th>
<th>$\Delta T$</th>
<th>$T_{ref}$ [°C]</th>
<th>MC</th>
<th>d [mm]</th>
<th>$\Delta \gamma$ [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>DFS+TTS</td>
<td>5*</td>
<td>4-38</td>
<td>38</td>
<td>y</td>
<td>24</td>
<td>1</td>
</tr>
<tr>
<td>SR</td>
<td>4*</td>
<td>38</td>
<td>38</td>
<td>y</td>
<td>24</td>
<td>5-20</td>
</tr>
</tbody>
</table>

* 2 adhesive fixed samples, 2 sandpaper fixed samples
stress-relaxation experiments were performed. Since the application of a step strain is not possible in reality, stress relaxation was achieved by imposing a strain, $\gamma_R$, on the sample within 0.1 s and keeping it constant thereafter (see Figure(1)). Strain values, $\gamma_R$, ranging from 5 to 20% percent were applied on 4 samples of brain tissue and the apparent modulus, $G_a(\gamma_R)$, was measured during 100 s. It was observed that the relaxation curves for different strain values have a tendency to be parallel. This suggests that, the stress relaxation modulus can be separated into a strain and a time dependent part,

$$G_a(t, \gamma_R) = h_{a}(\gamma_R) G(t)$$

(9)

In this $G(t)$ represents the linear viscoelastic modulus (which does not depend on strain) and $h_{a}(\gamma_R)$ the damping function determined from the apparent modulus[12]. The validity of time strain factorisation for brain tissue has been investigated by applying equation(9) on the experimental data. For each sample, a scaling function $H(t, \gamma_R, \gamma_{ref}^{\gamma_R})$ was defined as,

$$H(t, \gamma_R, \gamma_{ref}^{\gamma_R}) = \frac{G_a(t, \gamma_R)}{G_a(t, \gamma_{ref}^{\gamma_R})}$$

(10)

If factorisation is valid, the scaling function $H(t, \gamma_R, \gamma_{ref}^{\gamma_R})$ will be independent of time and correspond to the damping function $h_{a}(\gamma_R)$ when $\gamma_{ref}^{\gamma_R}$ is chosen in the linear range of the material. The scaling function, $H(t, \gamma_R, \gamma_{ref}^{\gamma_R})$, was time-averaged to $\bar{H}(\gamma_R, \gamma_{ref}^{\gamma_R})$ and the large strain relaxation moduli, $G_a(t, \gamma_R)$ then were normalised to $\gamma_{ref}^{\gamma_R}$ using:

$$G_{norm}(t, \gamma_{ref}^{\gamma_R}) = \frac{G_a(t, \gamma_R)}{\bar{H}(\gamma_R, \gamma_{ref}^{\gamma_R})}$$

(11)
Since the experiments were conducted on a rotational plate-plate viscometer, a non-homogeneous strain field was present between the plates. For non linear material behaviour the relaxation modulus will depend on time and strain applied. For this reason, the apparent modulus needs to be corrected for the non-homogeneous strain field to obtain the relaxation modulus $G(t, \gamma_R)$.

**Correction for non-homogeneous strain field**

**Method.** Soskey and Winter presented a correction method valid for stress relaxation experiments in a rotational plate-plate viscometer [13]. First equation(4) was differentiated with respect to $\gamma_R$,

$$G(t, \gamma_R) = \frac{2T(t, \gamma_R)}{\pi R^3 \gamma_R} \left( \frac{3}{4} + \frac{1}{4} \frac{\partial \ln \left( \frac{T(t, \gamma_R)}{\partial \ln \gamma_R} \right)}{\partial \ln \gamma_R} \right)$$

(12)

Substitution of the apparent relaxation modulus, equation(5), in Equation(12) gives,

$$G(t, \gamma_R) = G_a(t, \gamma_R) \left( 1 + \frac{\partial \ln G_a(t, \gamma_R)}{4 \partial \ln \gamma_R} \right)$$

(13)

Until now, no specific type of constitutive relation is assumed in this derivation. However, if strain time factorisation according to equation(9) is valid, equation(13) can be rewritten as,

$$G(t, \gamma_R) = h_a(\gamma_R) \left( 1 + \frac{\partial \ln h_a(\gamma_R)}{4 \partial \ln \gamma_R} \right) G_a(t)$$

(14)

It can be concluded that, when time strain separation is valid, only the damping function needs to be corrected to obtain the real relaxation modulus from the apparent modulus measured.

To actually apply the correction, four damping functions were selected from literature and fitted to the time-averaged scaling functions, $\overline{H}(\gamma_R, \gamma_{R}^{ref})$ using a Nelder-Mead simplex (direct search) method as implemented in Matlab 5.3 [14]. The quality of each fitted damping function was determined by evaluating the average error of the fit at each experimental data point and the standard deviation of the error. The experimental results, $\overline{H}(\gamma_R, \gamma_{R}^{ref})$, then were corrected by applying the best fit in equation(14).

**Damping functions.** The first damping function used, was originally intended for fitting the damping function of a polymer melt (melt I: a rheological well defined Low Density Poly Ethylene melt [15] ). It is a double exponential function proposed by Osaki [16],

$$h_{Osaki}(\gamma) = f_1 \exp(-n_1 \gamma) + f_2 \exp(-n_2 \gamma)$$

(15)

In this function, $n_1, n_2, f_1$ and $f_2$ are material parameters.
Based on their constitutive modelling, Khan and Larson [17] proposed another damping function for fitting the same melt,

\[ h_{\text{Larson}}(\gamma) = \frac{1}{1 + \frac{1}{\alpha} \gamma} \]  

(16)

Now only one material parameter, \( \alpha \) needs to be determined.

The next two damping functions are determined from hyper elastic functions already used in literature for fitting material data on brain tissue. For simple shear the damping function of a hyper elastic material can be written as,

\[
\begin{align*}
\tau &= \frac{\gamma_0}{B_3} + \left( \frac{\partial W(\gamma)}{\partial I_1} + \frac{\partial W(\gamma)}{\partial I_2} \right) \frac{\gamma_0}{B_3} 
\end{align*}
\]  

(17)

with \( \gamma_0 = \lim_{\gamma \to 0} \tau(\gamma) \), \( \tau(\gamma) \) the shear stress, \( I_1 \) and \( I_2 \) the first and second invariant of the Finger tensor \( B \) (\( B = F \cdot F^\top \) with \( F = (\nabla \mathbf{u})^\top \), the deformation tensor) and \( W(\gamma) \) the hyper-elastic strain energy density function (SED). The first SED based damping function is derived from the Mooney-Rivlin SED original proposed for rubbers by Mooney [18]. It was used for fitting free compression experiment results with brain tissue by [19, 20, 21] and has the form,

\[
\begin{align*}
W &= \sum_{i=1, j=1}^{N} C_{ij}(I_1 - 3)^i(I_2 - 3)^j 
\end{align*}
\]  

(18)

were \( N = 1 \) was used in [19, 20] and \( N = 2 \) in [21]. Application of \( N = 2 \) and \( i \neq j \) in equation(17) and using that for simple shear \( I_1 = I_2 = \gamma^2 + 3 \) gives,

\[
\begin{align*}
h_{\text{MR2}}(\gamma) &= 1 + 2\gamma^2 \frac{C_{02} + C_{20}}{C_{01} + C_{10}} 
\end{align*}
\]  

(19)

The last damping function is based on the Ogden SED. It was fitted to simple shear results with brain tissue by Prange et al. [22]. It can be written in terms of principal strains, \( \lambda_i \), as,

\[
\begin{align*}
W &= \frac{2\mu}{\alpha^2}((\lambda_1^\alpha + \lambda_2^\alpha + \lambda_3^\alpha) - 3) 
\end{align*}
\]  

(20)

with \( \alpha \) and \( \mu \) material parameters and \( \lambda_1, \lambda_2, \lambda_3 \) the principal strains in 1,2, and 3 direction. During simple shear the principal strains can be written as \( \lambda_1 = \lambda_2^{-1} = \lambda \) and \( \lambda_3 = 1 \). The shear stress now can be written as,

\[
\tau = \frac{2\mu \lambda^{\alpha} - \lambda^{-\alpha}}{\alpha \lambda + \lambda^{-1}} 
\]  

(21)

For simple shear, \( \lambda \) can be expressed in terms of shear strain, \( \gamma \), and equation(21) can be applied in equation(17) to obtain the damping function \( h_{\text{Ogden}}(\gamma) \).
RESULTS

Oscillatory strain experiments

Master curves of phase angle and dynamic modulus have been constructed from small strain oscillatory results. Results obtained with two different fixation methods (adhesive and sandpaper) are compared with those obtained without fixation in Figure(2). Results obtained without adhesive presented in [6] are presented as reference.

Both dynamic modulus and phase angle obtained using fixed samples, are within the range of the results without fixation. Furthermore, it can be seen that the spread of the dynamic modulus is less when sample is fixed to the plates. The phase angle results show a larger spread. Nevertheless it seems that the small strain results without fixation as previously presented in [6] did not suffer from bad sample adhesion.

Stress relaxation experiments

Stress-relaxation experiments were performed on four brain tissue samples. Strain values applied vary between 5 and 20%. In Figure(3) apparent stress relaxation moduli of brain samples are shown. All brain tissue samples show shear strain softening for strains up to 20%, i.e. the relaxation modulus decreases as a function of strain applied. While shear strain softening seems consistent per sample, the associated change of relaxation modulus (approximately 35%) is less than the spread between relaxation moduli of different samples (approximately 90%). Also it can be observed that the relaxation modulus does not reach a plateau value after 100 s.

Time strain factorisation. The scaling function $H(t; \gamma_R; \gamma_R^{ref})$ was determined from the results using equation(10), and choosing $\gamma_R^{ref} = 5\%$ (samples G1, G2 and S1) and 6% (sample S2) as reference strains. The value of $H$ was evaluated for $t = 0.1$ to 9.6 s with time intervals of 0.5 s. The results are shown in figure(4).

The scaling functions of sample G1 do not seem to depend on time. The same can be said for the 10% results of G2 and the 20% results of S1. The 20% result of sample G2 and the 16% and 20% results of the S2 sample show a decreasing trend in time. The largest decrease in scaling function takes place in between 0.1 and 0.6 s. No final conclusions will be drawn on the applicability of strain time separability here since the number of samples is too low.

Figure 2. Effect of fixation method (adhesive and sandpaper) on small strain master curves ($\gamma_0 = 1\%$). *Results presented in [6] printed as reference.
The time averaged damping function values, $\bar{H}(\gamma_t, \gamma^p_t)$, as well as normalised relaxation moduli, $G_{norm}(t)$ (equation(11)), are shown in Figure(5).

The results for sample G1 deviate considerably from those of G2, S1 and S2. For this reason results of sample G1 are excluded from further investigation. For the latter three samples the average shear softening at 20% strain is 30% ± 6.8% (mean ± standard deviation). The mean relaxation modulus varies from 367 ± 130 Pa at 0.1 s to 173 ± 47 Pa at 10 s for these samples. The coefficient of variation (standard deviation normalised with mean value) remains within 35% for all times to 50 s.

**Correction for strain field.** To obtain realistic fits for the damping function, we assumed the damping function value at 5% strain to be valid for lower strains also and copied the value to 1% strain. Figure(6) shows the four damping functions from literature, fitted on the combined experimental data of the samples G2, S1 and S2. The fitted material parameters as well as the fit errors are shown in Table(2). The Osaki damping function shows the lowest error but gave some convergence problems in the fit procedure. The second best fit was the second order Mooney-Rivlin fit, while the Larson fit was only slightly less good. The Ogden fit gives a
Figure 4. Time dependency of scaling function \( H(t; \gamma_R, \gamma_{ref}^R, t) \). Results of four brain samples shown in separate figures. Measurements marked (2) indicate repeated measurements.

Table 2. Values of material parameters in damping functions fitted to brain data.

<table>
<thead>
<tr>
<th>Damping function</th>
<th>Fit parameter</th>
<th>Mean error ± standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Osaki fit</td>
<td>( f_1=15.4, f_2=-14.4, n_1=-3.0, n_2=-3.2 )</td>
<td>( -4.0 \times 10^{-6} \pm 3.0 \times 10^{-2} )</td>
</tr>
<tr>
<td>Larson fit</td>
<td>( \alpha=29.0 )</td>
<td>( 8.0 \times 10^{-3} \pm 3.4 \times 10^{-2} )</td>
</tr>
<tr>
<td>2nd order Mooney-Rivlin</td>
<td>( C_{02}/C_{01} )</td>
<td>( 5.7 \times 10^{-3} \pm 3.0 \times 10^{-2} )</td>
</tr>
<tr>
<td>Ogden fit</td>
<td>( \mu = 420.2^*, \alpha = 4.8 \times 10^{-7} )</td>
<td>( -1.0 \times 10^{-1} \pm 1.3 \times 10^{-1} )</td>
</tr>
</tbody>
</table>

\*Value cannot be determined uniquely from fit on damping function only.

poor prediction of the shear softening in the damping function, furthermore the result was very insensitive for changes in material parameters \( \alpha \) in equation(21). For this reason the second order Mooney-Rivlin fit was used to determine the correction factor in equation(13). This yielded the corrected results in Figure(6), showing that the average shear softening at 20% strain equals 44.7%±7.0%. Fitting the 2nd order Mooney-Rivlin model to the corrected data provides the
Figure 5. Damping functions of the four brain tissue samples tested (left), relaxation moduli normalised to 5% result (G1,G2,S1) and 6% result (S2) (right) and . Double damping function values of S1 at 16% and S2 at 5%, indicate repeated measurements.

Figure 6. Correction for non-homogeneous strain field. Fit results of four material model functions (left). Corrected data using 2nd order Mooney-Rivlin model (MR2 corrected) (right). 'Experimental' data point at 1% strain copied from 5% strain.

following fit parameter $C_0^2/C_0^2 = -5.4$. The mean error equals $6.1 \cdot 10^{-2} \pm 6.5 \cdot 10^{-2}$.

**DISCUSSION**

**Experimental results**

Brain tissue exhibits shear softening when loaded in the non-linear strain range using stress relaxation experiments. It was also observed that during 100 s the relaxation modulus does not reach a plateau value$^1$. Moreover, in the constant strain part (i.e. after t=0.1 s) of the log-log relaxation plot, the curves for different strain values have a tendency to be parallel.

$^1$Fung investigated the creep behaviour of muscle tissue under tension and did not find a plateau value after one day [23]. Although although muscle tissue is rather different from brain tissue, this illustrates that the absence of a plateau value is not uncommon for biological tissues.
This indicates that strain dependent behaviour is independent of time dependent (relaxation) behaviour. This corresponds with findings by Prange et al. [22], who also concluded that factorisation was allowed. As a result of this factorisation, the time dependent behaviour of brain tissue can be derived from small strain oscillatory experiment results for frequencies up to 1000 Hz by application of TTS [6, 11], while the large strain behaviour can be characterised by a damping function obtained from, relatively slow, stress relaxation experiments. Fitting several damping functions known from literature, on the measurement data, revealed that a second order Mooney-Rivlin model fits the raw material data best. The first order Mooney-Rivlin model and the Ogden model, used previously in literature to describe the strain dependent part of the brain tissue behaviour were not able to predict the shear softening observed. The first order Mooney-Rivlin cannot predict shear softening at all, while the Ogden model could not predict the amount of shear softening present in brain tissue.

Methods

When large strains were applied to the brain tissue, sample slip occurred when smooth, clean metal plates were used. For this reason two approaches have been applied to eliminate sample slip in the large strain experiments; fixing the sample to the plate using glue, and increasing friction using sandpaper on the plates. It could be seen that the small strain results were within range of the results without slip precautions. The large strain stress relaxation results did not reveal consistent differences between the fixation methods results. Both time relaxation behaviour as well as strain softening showed same tendencies. A drawback of using the adhesive, is the unknown adhesive thickness which could not be accounted for in the data processing. This might have been the reason for the deviation of the sample G1 results in Figure(5). For this sample a relatively thick layer of glue was applied. Since the glue behaves as a rigid solid when compared to the very compliant brain tissue, the real brain sample thickness will be lower than the measured plate distance. As a result the reported relaxation modulus and shear softening will be overestimated [24] as is apparent the results. When sandpaper was used, the thickness of paper and tape can easily be accounted for by zeroing the plate height with paper and tape attached to them. A drawback of sandpaper is that sample slipping might still be possible.

Measurement repeatability was investigated for the sandpaper fixed samples, S1 and S2 in Figure(5). When the 5% measurement of sample S1, was repeated, a 1.8% difference of the damping function was found. For sample S2 the 16% measurement was repeated after the 20% strain measurement and a 5.0% difference in damping function was found.

Limitations of current research

In determining the damping functions of brain tissue, a strain value of 5% was chosen as reference strain for determining the scaling function, $H(t, \dot{\gamma}_R, \dot{\gamma}_R^{ef})$, in equation(10). To obtain the true $h(\dot{\gamma}_R, \dot{\gamma}_R^{ef})$ should be chosen in the linear range of the material. For brain tissue this means below 1% strain [6]. Unfortunately, this was not possible in our relaxation experiments, since the torque signal displayed too much noise for strains below 5%, attributed to the fact that the signal was close to the lower measurement limit of the viscometer.

The results of the large strain measurements had to be corrected for the non-homogeneous strain field between the plates of the rotational rheometer. Direct application of this method, according to equation(13), requires measurements at small strain intervals to determine the derivative of $G_0$ with respect to $\dot{\gamma}_R$. This was not feasible with brain tissue, since the time a
sample could be used was limited by sample degeneration. Instead, several literature known
damping functions have been fitted to the measurement results and the correction has been
applied on these.

Finally the number of samples is too low to derive statistically valid conclusions from the
results presented. Nevertheless it is believed that the trends shown are realistic.

CONCLUSIONS
In this study a method is presented to determine the large strain behaviour of porcine brain
tissue on a rotational plate-plate rheometer. The non-linear behaviour was characterised using
stress relaxation experiments. It was shown that brain tissue exhibits shear softening (45% at
20% strain) and it seems that the time dependent behaviour of brain tissue does not depend
on the strain level. The time dependent behaviour was determined for frequencies up to 1000
Hz by application of Time Temperature Superpositioning Principle on small strain oscillatory
experiment results. The strain dependent behaviour could be fitted well using a second order
Mooney-Rivlin material model. As a result the material behaviour of brain tissue has been
derived from the experimental results for frequencies up to 1000 Hz and strains up to 20%.

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