Measuring the anisotropy of the midbrain in the linear regime

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Abstract

The anisotropy is measured in porcine tissue of the midbrain with a dynamic frequency sweep in the linear regime. The sample is measured on an ARES II rotational rheometer in a plate-plate configuration. The oscillatory experiment is a dynamic frequency sweep with a range from 1 to 100 rad/s. The sample is placed eccentric because this gives the opportunity to measure anisotropy, to have a better homogeneous strain field and the measured signal is increased allowing the measurement of a smaller sample than with a conventional centered configuration. Due to the microstructure of the midbrain two directions are measured. The midbrain is slightly anisotropic in the sagittal plane and in the transverse plane it is almost isotropic.
List of symbols

<table>
<thead>
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<th>Symbol</th>
<th>Description</th>
<th>Unit</th>
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<tr>
<td>$G$</td>
<td>Relaxation modulus</td>
<td>[Pa]</td>
</tr>
<tr>
<td>$G'$</td>
<td>Storage modulus</td>
<td>[Pa]</td>
</tr>
<tr>
<td>$G''$</td>
<td>Loss modulus</td>
<td>[Pa]</td>
</tr>
<tr>
<td>$G^*$</td>
<td>Frequency dependent dynamic modulus</td>
<td>[Pa]</td>
</tr>
<tr>
<td>$M$</td>
<td>Torque</td>
<td>[Nm]</td>
</tr>
<tr>
<td>$R$</td>
<td>Radius</td>
<td>[m]</td>
</tr>
<tr>
<td>$t$</td>
<td>Time</td>
<td>[s]</td>
</tr>
<tr>
<td>$\delta$</td>
<td>Phase shift</td>
<td>[rad]</td>
</tr>
<tr>
<td>$\gamma$</td>
<td>Shear strain</td>
<td>[-]</td>
</tr>
<tr>
<td>$\dot{\gamma}$</td>
<td>Shear strain rate</td>
<td>[s$^{-1}$]</td>
</tr>
<tr>
<td>$\theta$</td>
<td>Angle</td>
<td>[°]</td>
</tr>
<tr>
<td>$\sigma$</td>
<td>Shear stress</td>
<td>[Pa]</td>
</tr>
<tr>
<td>$\omega$</td>
<td>Angular frequency</td>
<td>[rad/s]</td>
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Chapter 1

Introduction

The human brain is one of the most important and difficult organs of the human body. During car accidents people can suffer injury or even die because the brain is damaged. The social costs of these accidents are immense. For instance these were estimated to be 160 billion Euro per year in the European Union [5].

To decrease the injury it is necessary to study the response of the brain during an impact. In the past researchers did study the brain and its material properties. In combination with finite element models (figure 1.1) which are developed nowadays, these data can be used to predict the behaviour of the brain during impact which can be used to create devices to decrease the injuries. There are a lot of differences between the studies causing differences in order of magnitudes between the measured data of experiments of different universities. The post-mortem time, the time between death and measurement, are different for various experiments. Not only the post-mortem time but also the different regions of the brain that are being measured leads to different data. Furthermore the researchers use different donors as some are using human tissue [10] (often high post-mortem times) where others are using bovine [11], porcine [3] or other animals [12] for their measurement. In this report the midbrain of

![Figure 1.1: Finite element model of the brain](image-url)
porcine brain is studied and a characterisation of anisotropy due to the preferential directions in the microstructure is done. The midbrain is chosen as it is assumed that this tissue will be the most anisotropic part of the brain.
Chapter 2

Linear viscoelastic material behaviour

2.1 Dynamic Excitation

To measure the material properties in the linear regime a dynamic excitation is put on the material. A sinusoidally strain is applied to the tissue in a range from 1 to 100 rad/s for all the tests. The stress will also respond sinusoidally like the strain. This can be expressed mathematically as follows:

\[ \gamma = \gamma_0 \sin(\omega t) \quad (2.1) \]

\[ \tau = G\gamma_0 \sin(\omega t + \delta) \quad (2.2) \]

2.2 Viscoelastic material

Brain tissue is a biological material with a viscoelastic material behaviour. This is a combination of elastic and viscous material behaviour. The shear stress in this material is not shifted with a \( \delta \) of 0 or \( \frac{\pi}{2} \) corresponding to purely elastic or viscous behaviour respectively but it is in between these values. Viscoelastic material can be analysed by decomposing the shear stress into two waves of the same frequency (\( \tau'_0 \) and \( \tau''_0 \))[8]. This is represented mathematically in (2.3) and graphically in figure 2.1.

\[ \tau = \tau' + \tau'' = \tau'_0 \sin \omega t + \tau''_0 \cos \omega t \quad (2.3) \]

Equation (2.3) can now be re-arranged to (2.4) which introduces two moduli: \( G' \) and \( G'' \), known as the storage modulus and the loss modulus, which are frequency dependent.

\[ \tau = G'\gamma_0 \sin(\omega t) + G''\gamma_0 \cos(\omega t) \quad (2.4) \]
Figure 2.1: Graphical representation of a dynamic excitation and the response versus time
Chapter 3

Experiment

3.1 Brain samples

Although the essence from the research is to predict the mechanical response of the human brain during impact human brain samples aren’t used during the experiments. This has two main reasons: the post-mortem time from a sample from a human being is too long and the availability is low. Porcine tissue however can be obtained a few hours after sacrifice. The tissue used in this study is obtained from approximately six months old pigs from a local slaughterhouse.

During the transportation the halves were put into a jar with Phosphate Buffered Saline solution (PBS) placed in a box with ice. The material was kept in PBS and ice to prevent dehydration and to slow down the degradation of the tissue. In the laboratory the midbrain was cut out. With the Vibrating-blade Microtome Leica VT1000S slices were cut at a height of 1.5 mm. A reason is that the recommended gap setting for parallel plates in the ARES is between 0.5 and 2 mm [1]. The cutting blade was placed under an angle of approximately 30 degrees, which is probably causing the difference in time dependency with the measured data of Benders [2]. With a cork bore samples were then cut out of the slices. It should be noticed that all the time the tissue was kept in the PBS and if possible cooled in ice. All the samples made have a diameter ranging from 10 to 13 mm.

The samples are made from slices parallel to the planes as shown in figure 3.1. Due to the microstructure it is expected that in the sagittal plane the material will be anisotropic and isotropic in the transversal plane.
3.2 ARES II Rheometer

With dynamic shear experiments researchers measure how brain tissue behaves in the linear viscoelastic regime. From the experiments the frequency dependent dynamic modulus $G$ and the phase angle $\delta$ can be determined, which describes the linear visco-elastic behaviour of the material.

A possibility for doing a dynamic shear strain experiment is with a sinusoidal oscillation. The machine that is used to do this is the ARES II rotational rheometer with a 10GM FRT transducer in a plate-plate configuration (figure 3.2). It consists of two plates which are parallel to each other. To prevent slip during the measurements sandpaper is glued on both of these plates (with grain size of 0.18 mm [10]). The sample is placed on the turntable. The upper plate is then lowered down onto the sample until the configuration is like in figure 3.2 (also used to determine the height of the sample). A compressive force of about 0.01 Newton is applied on the sample with the upper plate. The sample is placed to the edge of the lower plate [12]. This configuration is used because this gives the opportunity to measure anisotropy with a shear field that is approximately homogeneous and the measured signal is increased allowing the measurement of a smaller sample than with a conventional centered configuration. The plates are heated to a temperature of 37 degrees Celsius. With a pipet with PBS the sample is made wet during the experiment to prevent dehydration. From the measured torque $M$ and angle $\theta$ the shear stress and shear strain can be calculated by [12]:

$$\tau = \frac{MR}{2\pi R_1^2 \left( \frac{(R-R_1)^2}{2} + \frac{R_1^2}{8} \right)}$$  \hspace{1cm} (3.1)

$$\gamma = \frac{\theta R}{h}$$  \hspace{1cm} (3.2)

where $R$ is the radius of the plate, $R_1$ is the radius of the sample and $h$ is height of the sample.
3.2.1 Dynamic frequency sweep

The goal of dynamic frequency sweeps is to obtain the frequency dependent dynamic modulus $G$ in the linear regime of the material. The experiment is done with the ARES II Rheometer as described in paragraph 3.2. Brands et al. [4] found that a strain of 1% is the boundary of linear and non-linear viscoelastic behaviour for a frequency range of 0.1 to 16 Hz (Nicolle et al. [9] measured the same).

The dynamic frequency sweeps in this report are done with a frequency range of 1 to 100 rad/s. The first applied wave was ignored to get more accurate results. The waves had a minimum of 3 waves and the maximum time for a frequency to be applied is 5 seconds. Thus the waves have a range of 4 until 80 waves in a dynamic frequency sweep. The strain amplitude was 1%.

There are two different tests done in this report. In the first series of tests, the upper plate is raised and then lowered again between two tests, without changing the sample orientation. In the second series of tests, the upper plate is raised and then lowered again between two tests but now the sample orientation is changed to measure anisotropy.
Chapter 4

Results

In the previous studies on brain tissue, data was measured with the sequence: preconditioning, DFS and then stress relaxation. One of the goals of the project is to gain a better reproducibility of the experiments. Previous experiments were done with a preconditioning, dynamic frequency sweep and stress relaxation. The stress relaxation test is a large strain test. To see if this has any effect on the reproducibility, the sequences are done without stress relaxation. All samples are measured at 37 degrees Celsius during the experiment.

4.1 Influence of the vertical movement of the upper shaft

The goal of the first experiments is to investigate the effect of the shaft raising and lowering on the sample. First the sample is being tested for 90 minutes (every 10 minutes a DFS with a range from 1 to 100 rad/s) with the upper shaft constantly down (shown in figure 4.1). In this plot the line with the lightest color represents the lowest frequency (every line is a different frequency). To investigate the influence of the shaft going up and down a second test is done where the shaft is put up and down between the DFS tests with the same range of the previous experiment for again 90 minutes (shown in figure 4.2). The samples used in these two tests are from two different brains (exact properties are presented appendix A). The standard deviation from the first test (upper shaft constantly down) is about 3 percent for $G'$ and about 4 percent for $G''$. The upper shaft does have an influence on the measurement as the standard deviation is about 10 percent for $G'$ and 8 percent for $G''$. Although there is a difference it is still acceptable to use this procedure to measure anisotropy.
Figure 4.1: $G'$ and $G''$ versus frequency and time (Appendix A, sample 1)

Figure 4.2: $G'$ and $G''$ versus frequency and time (Appendix A, sample 2)
4.2 Anisotropy

The second goal is to determine the anisotropy in the midbrain. To achieve this the sample is rotated with the turntable and then measured with a dynamic frequency sweep. This is done for every 30 degrees in the sagittal plane. The angle is picked randomly during the measurements to prevent dependency of the time. There is still some dependency of the time which is corrected (Appendix B). The standard deviation of this test (after correction) is about 12 percent for $G'$ and 10 percent for $G''$. The results are shown in figures 4.3 and 4.4.

In the transverse plane the tissue is measured every 45 degrees. The standard deviation is about 10 percent for $G'$ and about 7 percent for $G''$. These are plotted the same way as the previous test in figures 4.5 and 4.6. Another sample from a different brain is presented in appendix C. The samples from the sagittal plane are slightly anisotropic with peaks at 120 and 240 degrees as shown in figure 4.4 and figure C.2. The last test is as expected (due to the microstructure) more isotropic. This can also be concluded from the standard deviations as the results from measurements in the sagittal plane are higher than in the transverse plane.

Figure 4.3: $G'$ and $G''$ versus frequency and time (Appendix A, sample 3)
Figure 4.4: Polar plot of $G'$ and $G''$ versus the angle without time dependency (Appendix A, sample 3)

Figure 4.5: $G'$ and $G''$ versus frequency and time (Appendix A, sample 5)
Figure 4.6: Polar plot of $G'$ and $G''$ versus the angle without time dependency (Appendix A, sample 5)
Chapter 5

Discussion

The experiments presented are influenced by several errors which makes the experiments harder to reproduce. The preparation of the samples are possibilities to create errors as brain is a very soft material and difficult to handle. For instance if the material is cut too quick when the slices are made, the material may already have suffered a large strain before the experiment is done. Furthermore it is hard to create a sample that is perfectly flat because the material is soft. This can cause an error in the measuring as the sample is placed between the parallel plate geometry.

After preparation the samples are kept in PBS and are still cooled. However due to the microstructure in the material or when the material of the sample is not purely from the midbrain it is possible that the geometry of the sample changes from a circle into an oval form. So when the material is turned into other directions when it is tested for anisotropy it is not sheared the same in every direction.

Another error is that the sample is placed on the turntable of the ARES by hand. It is not guaranteed that the sample is placed exactly in the middle. If it is then turned the distance to the center of the plate is then variable (see also figure 3.2). Other than the placement by hand displacement by the machine can occur during the measurement. If the sample is too dry it is possible that it sticks slightly to the upper plate resulting in a small displacement.

Although the brain is cooled during the preparation time it is measured at 37 degrees Celsius during the experiment. If the sample was heated too short it is possible that the material behaviour is different.
Chapter 6

Conclusion

To study the material behaviour of the potentially most anisotropic part of the brain (the midbrain) dynamic frequency sweeps were done. Porcine brain samples were prepared in two directions to measure the influence of the microstructure. The influence of a repeated removal and replacement of the upper plate during the experiment is also investigated and was found to be acceptable for further measurements for the detection of anisotropy. There is only a small anisotropy in the sagittal plane and almost isotropic in the transverse plane.
Appendix A

Properties of the samples

<table>
<thead>
<tr>
<th>Sample Description</th>
<th>Diameter [mm]</th>
<th>Height [mm]</th>
<th>Post-mortem time [h]</th>
<th>Standard Deviation [%]</th>
<th>$G'$</th>
<th>$G''$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shaft constant down (sample 1)</td>
<td>10.0</td>
<td>1.40</td>
<td>7.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Constant vertical movement of the upper shaft (sample 2)</td>
<td>11.15</td>
<td>1.45</td>
<td>7.5</td>
<td>$G' = 10, G'' = 8$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Measuring anisotropy in the sagittal plane (sample 3)</td>
<td>11.7</td>
<td>1.23</td>
<td>6.25</td>
<td>$G' = 12, G'' = 10$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Measuring anisotropy in the sagittal plane (sample 4)</td>
<td>11.6</td>
<td>1.56</td>
<td>4.0</td>
<td>$G' = 15, G'' = 10$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Measuring anisotropy in the transverse plane (sample 5)</td>
<td>12.3</td>
<td>1.56</td>
<td>9.17</td>
<td>$G' = 10, G'' = 7$</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Appendix B

Correcting the time dependency

A set of measurements can be expressed as a function $G$ depending on the time $(t)$ and other dependencies $(\theta)$: $G(t, \theta) = G_t(t)G_{\theta}(\theta)$

Where the function for the time dependency $G_t(t)$ can be written as:

$G_t(t) = At + B$ where $A$ is the slope and $B$ is the starting point of the line and is chosen to be 1.

A line can be fitted through the data with $G_t(t)G_{\theta}(\theta) = A^*t + B^*$ where $A^*$ is the slope of the measured data and $B^*$ is the value where the function begins.

The correction can be calculated with:

$(At + 1)G = A^*t + B^*$

Thus:

$A = \frac{A^*}{A^*}$, $G = B^*$

$A = \frac{A^*}{A^*}$

![Figure B.1: Data before and after the correction](image-url)
Appendix C

Other measurements

Figure C.1: $G'$ and $G''$ versus frequency and time (Appendix A, sample 4)
Figure C.2: Polar plot of $G'$ and $G''$ versus the angle without time dependency (Appendix A, sample 4)
References


