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Viscoelastic properties of the nucleus pulposus of the intervertebral disk in compression

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The viscoelastic properties of the nucleus pulposus were measured in compression for 35 specimens dissected from 9 sheep. Measurements on 19 specimens were made on the day of slaughter; the remaining 16 specimens were stored frozen and thawed before testing. A preload of 0.2 N was applied to each specimen and a cyclic compression of 10 μm applied at eight frequencies in the range 0.1–10 Hz at a temperature of 37 °C. Freezing appeared to increase the storage modulus, E', but not the loss modulus, E'', or $\tan\delta = E''/E'$. These parameters, E', E'' and $\tan\delta$, had values of 64 ± 28 kPa, 24 ± 11 and 0.33 kPa ± 0.07, respectively. The value of $\tan\delta$ passed through a minimum at a loading frequency of 0.9 ± 0.2 Hz. The water content of the specimens was 80 ± 2%.

1. Introduction

The measurements reported in this paper were intended to characterize the viscoelastic properties of the nucleus pulposus of the intervertebral disk in compression. These properties are fully characterized by the dependence of a storage modulus, E', and a loss modulus, E'', [1, 2] on the frequency, f, of a sinusoidal applied stress. Alternatively, the viscoelastic properties may be expressed by a complex Young's modulus $E^* = E' + iE''$, where i is the square root of -1 [1, 2]. Then E^* has a magnitude of $(E'^2 + E''^2)^{1/2}$ and an associated phase angle, δ, given by $\tan\delta = E''/E'$. When $\delta = 0$, there is no phase difference between a time-dependent applied stress and the strain which it induces, i.e. the material is then purely elastic and exhibits no viscous properties.

The nucleus pulposus forms the inner part of the intervertebral disk. A knowledge of its properties is of value for understanding the mechanical behavior of the intervertebral disk [3] and, hence, of the intact spine [4]. The nucleus pulposus is sometimes removed surgically, in the treatment of back pain, and information on the viscoelastic properties of the healthy tissue may be useful for developing suitable replacement materials [5]. The viscoelastic properties of the nucleus pulposus have been measured in shear [6, 7] and values of E^* have been estimated from indentation tests [8]. These experiments were performed on human tissue. However, human disks obtained from post mortem examination usually show signs of degeneration which affects the properties of the nucleus pulposus [7, 9]. Sheep nucleus pulposus was investigated here because it has a similar appearance and water content to the human tissue [10] and because sheep disks are considered to be a reasonable model for human disks [11].

2. Methods

2.1. Specimens

Spines were obtained from sheep aged under 1 year. They were removed when the sheep were slaughtered, at a local abattoir, and sealed in a polythene bag surrounded by muscle to keep the intervertebral disks moist. They were dissected, to obtain specimens of nucleus pulposus from the lumbar spine, within 4 h of slaughter. Thirty-five specimens were obtained from the spines of nine different sheep. The sheep has six lumbar intervertebral disks but it was not always possible to dissect out six specimens of adequate size for this study, from each spine. Each nucleus was divided into two parts; each part was closely sealed in Clingfilm and kept in a small glass container with a tightly fitting plastic lid. One half of each specimen was used for mechanical testing; the other half was used to determine the water content of the specimen.

Nineteen specimens, from six sheep slaughtered in the morning, were used for mechanical testing in the same afternoon. Sixteen specimens for mechanical testing, from three sheep, were kept frozen at −40 °C, for a period of 5–14 days, and thawed before use.

2.2. Mechanical testing

Specimens were tested in compression in a DMTA3 (Rheometric Scientific GmbH, Munich, Germany) testing machine at a temperature of 37 °C. A preload of 0.2 N was applied and sinusoidal cyclic compression applied at eight different frequencies in the range 0.1–10 Hz. The amplitude of the sinusoidal displacement was 10 μm. Each test was repeated immediately under identical conditions.

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2.3. Water content

The water content was determined by weighing before and after drying in an oven at 60 °C for 48 h. Oven drying at 67 °C has been used previously for determining the water content of intervertebral disk tissue [12].

2.4. Statistics

To determine whether there was evidence for a minimum in a plot of $\tan\delta$ against $\log_{10}f$, a straight line and a second-order polynomial were both fitted through the experimental results. The F-test [13] was then used to determine whether the polynomial gave a significantly better fit to the data points than the straight line, i.e. whether a curve which passed through a minimum provided a significantly better fit than a straight line. These calculations were performed using a spreadsheet (Excel, Microsoft Corporation, Redmond WA).

Data sets were checked to determine whether the values were normally distributed by the Anderson–Darling test. They were then compared using a t-test; differences were judged to be significant at the $p = 0.05$ level. These calculations were performed using specialist statistics software (Minitab, Minitab Inc., State College PA).

3. Results

3.1. Frequency dependence

Overall, there was no clear dependence of E' and E'' values on frequency. There was an apparent increase in their values with increasing frequency (in the range 0.1–10 Hz) for most specimens. This increase was not always linear and sometimes appeared to pass through a minimum. However, when the tests were repeated, the values of E' and E'' consistently increased, indicating that their values were changing as a result of the test. Fig. 1(a) and (b) shows results from a typical specimen.

Values of $\tan\delta = E''/E'$ showed a clear dependence on frequency, f, passing through a minimum value at 0.9 ± 0.2 Hz (mean ± SD); values of $\tan\delta$ obtained from initial and repeat tests were closely similar. Fig. 1(c) shows a result from a typical specimen. The subjective impression that there was a minimum in plots of $\tan\delta$ against $\log_{10}f$ was confirmed by a second order polynomial giving a significantly ($p < 0.05$) better fit than a straight line for 15 out of 18 fresh specimens and 14 out of 16 which had been frozen. For each specimen, the lines were plotted through the data from the first test. The second order polynomials were used to calculate the value of f at which $\tan\delta$ was a minimum for each specimen. The results from both fresh specimens and those which had been frozen were normally distributed and were not significantly different from each other. They were, therefore, pooled to obtain the best estimate of the value of $\log_{10}f$ and, hence, f at which $\tan\delta$ was a minimum.

3.2. Viscoelastic moduli

Storage and loss moduli, E' and E'', respectively, are summarized in Table I. These results are those measured in the first test, for each specimen, at $f = 1$ Hz. A single value is presented because there was no clear dependence of measured values on frequency (see 3.1). Values at 1 Hz were chosen because these measurements were closest to the frequency at which $\tan\delta$ had its minimum value (see 3.1).

Some values of the elastic moduli were not included in the summary data of Table I because of their high values. One fresh specimen had abnormally large values for the storage modulus ($E' = 203$ kPa) and for the loss modulus ($E'' = 85$ kPa); both were more than 3 standard deviations from the mean. According to Chauvenet's criterion [14] any value with a probability of occurrence of less than $1/2N = 1/(2 \times 19) = 0.026$, where N is the number of samples, may be considered inconsistent. For normally distributed data, any sample which deviates from the mean by more than 2.58 standard deviations has a probability of occurrence of less than 0.01. Thus the abnormally high values for E', E'' and $\tan\delta$, that had been calculated from them, were omitted from the data set before the summary data of Table I were compiled. One of the samples which had been frozen also had large values of E' (170 kPa; 2.8 standard deviations from the mean) and E'' (72 kPa; over 3 standard deviations from the mean); results from this specimen were also rejected.

Data from the fresh specimens and those which had been frozen are pooled in Table I when this is statistically valid. Results for E', E'' and $\tan\delta$ were normally distributed, for both fresh specimens and those which had been frozen. According to the t-test, there was a significant difference ($p = 0.03$) between the two sets of data for E'. However, no significant difference was observed for E'' or $\tan\delta$ so their values were pooled to obtain better estimates for their means, standard deviations and ranges of values.

3.3. Water content

The water content from the same specimens is summarized in Table I. Three fresh specimens which gave abnormally high values (90%, 91% and 95%) have been omitted following Chauvenet's criterion (see 3.2); these values are so high that it is likely that a mistake was made in the experimental procedure but this could not be checked because the procedure destroys the specimen. Both specimens which had abnormal values for E' and E'' had normal water contents. These were 80%, by mass, for the fresh specimen, and 79% for the specimen which had been frozen. These results were not omitted from the summary data of Table I because they were obtained from different experiments to those which yielded the anomalous E' and E'' values.

There was a significant difference ($p < 0.01$) between the water content of the fresh specimens and those which had been frozen. Both sets of results were normally distributed and so could be compared using a t-test. Because of the difference between the two sets, pooled data are not presented in Table I.

There appears to be no significant correlation between E', E'', $\tan\delta$ and water content. The only exception is that E'' for specimens which had been frozen appears to be weakly correlated ($r = -0.5$, $p = 0.05$) with water content. However, this result is not conclusive because no significant correlation was observed for the fresh specimens.

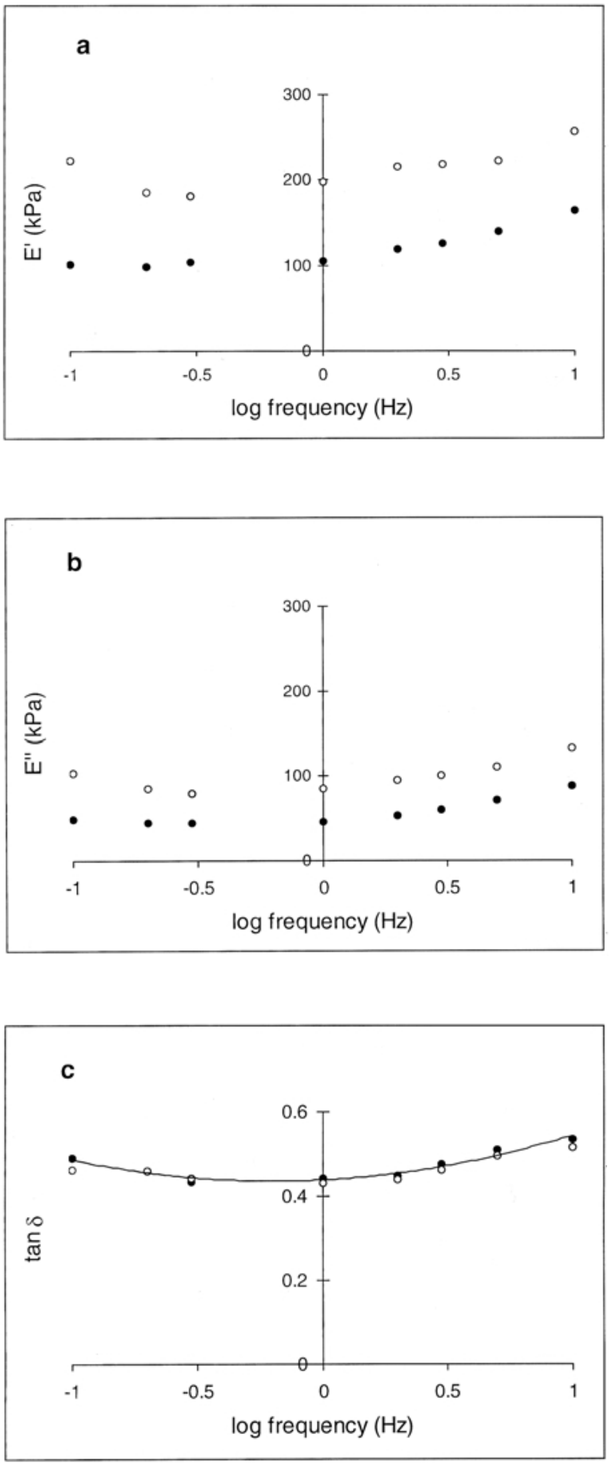

Figure 1 Viscoelastic properties of the nucleus pulposus plotted against the logarithm (to the base 10) of frequency, f (in Hz): (a) storage modulus, E' (in kPa), (b) loss modulus, E'' (in kPa) and (c) $\tan\delta = E''/E'$. These results correspond to a single sample, which had not been frozen, but are typical of those obtained for other samples. In each case, a filled circle represents the results of the first test and the open circle represents the results from the second test. In (c) the curve is the second order polynomial which gives the best fit to the results of the first test.

4. Discussion

It is likely that the viscoelastic properties of the nucleus pulposus are very close to being linear (for further details of linear viscoelasticity see [15]). The reason is that the intact intervertebral disk exhibits linear viscoelasticity during compression [16]. For a linearly viscoelastic system, $E'' = 2\pi fM$, where M is a measure of its viscosity [1, 2]. Thus, in principle, E'' increases with f but the increase was not sufficiently marked to be apparent here.

It was noted that the values of E' and E'' were increased when a repeat test was performed. This increase could be a result of consolidation or gradual water loss by evaporation. Any barrier, to prevent water loss, would have constrained the specimen and so affected the results. Attempts to control the relative humidity around the specimen could have led to uptake of water which would also have affected the results of the tests. This increase in E' and E'' is likely to have been occurring during the first test, leading to their values being somewhat overestimated and, perhaps, masking any dependence of E'' on f. However, the changes in E' and E'' values in the first test were sufficiently small (see Fig. 1(a) and (b)) that they were unlikely to effect the order of magnitude of the results (see below).

The appearance of a minimum in the plot of $\tan\delta$ against $\log_{10}f$ is unlikely to be an experimental artefact. Consolidation of the specimen or loss of water would be expected to result in a gradual increase or decrease in $\tan\delta$ (depending which of E' and E'' changed value more rapidly). In any case, $\tan\delta$ values were closely similar in the initial and repeat tests. Thus, a statistically significant minimum is likely to be evidence of a real effect. This result indicates that viscous effects in the nucleus pulposus are minimized at a frequency close to 1 Hz, i.e. of the order of the fundamental frequency associated with normal locomotion.

The results presented here indicate that E' and E'' have values of 64 ± 28 and 24 ± 11 kPa (mean ± SD), respectively. The values of the standard deviations, which are expected to reflect biological variability as well as experimental error, are large compared with the changes in E' and E'' observed in a single test (see Fig. 1(a) and (b)). Thus there is no justification for quantifying the frequency dependence of E' and E'' for the sheep population. The ranges of values for E' and E'' correspond to a range of E^* values of 20–130 kPa. Iatridis *et al.* [6] reported that the shear modulus G^* for human nucleus pulposus was 7–20 kPa. Young's modulus is related to shear modulus by $E = 2G(1 + \nu)$, for isotropic materials subjected to low strains [1, 17]. This relationship implies that E^* lies in the range 20–60 kPa for human nucleus pulposus which is in agreement with the results obtained here for sheep. Umehara *et al.* [8] obtained values of 5–98 kPa from indentation tests.

The values of $\tan\delta$ listed in Table I indicate that the strain induced in the nucleus pulposus lags behind the stress with a phase angle in the range 9–24° with a mean value of 18°. This result is comparable to the range of values, obtained in shear, for human nucleus pulposus, of 23–30° [6].

The water content of 81 ± 2%, by mass, obtained from fresh samples of sheep nucleus pulposus is identical to that of 81 ± 2% obtained in a separate study [18] and is very similar to values of 80 ± 4% for human nucleus pulposus [19]. It is the water content of the nucleus pulposus which is largely responsible for its mechanical properties [20]. Thus the water content of sheep nucleus pulposus indicates that it is likely to have similar mechanical properties to the human tissue.

TABLE I Values of storage modulus, E', loss modulus, E'', $\tan\delta = E''/E'$ and percentage water content by mass. Each range of values is represented by the mean, standard deviation (SD), the minimum value and the maximum value. In each case, outliers are omitted as described in the text. Fresh and frozen data sets are pooled only when there is no significant difference between them

	E' (kPa)	E'' (kPa)	$\tan\delta$	%water
Fresh				
Mean	64	23	0.34	81
SD	28	13	0.08	2
Min	20	3	0.16	79
Max	119	50	0.44	84
Frozen				
Mean	83	26	0.33	79
SD	20	8	0.05	1
Min	43	12	0.22	77
Max	114	42	0.40	80
Pooled				
Mean	—	24	0.33	—
SD	—	11	0.07	—
Min	—	3	0.16	—
Max	—	50	0.44	—

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