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Mechanical Properties of Single Bovine Trabeculae are Unaffected by Strain Rate

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Abstract

For a better understanding of traumatic bone fractures, knowledge of the mechanical behaviour of bone at high strain rates is required. Importantly, it needs to be clarified how quasistatic mechanical testing experiments relate to real bone fracture. This merits investigating the mechanical behaviour of bone with increasing strain rate. Various studies examined how cortical and trabecular bone behave at varying strain rates, but no one has yet looked at this question using individual trabeculae. In this study, three-point bending tests were carried out on bovine single trabeculae excised from a proximal femur to test the trabecular material's strain rate sensitivity. An experimental setup was designed capable of measuring local strains at the surface of such small specimens, using digital image correlation. Microdamage was detected using the bone whitening effect. Samples were tested through two orders of magnitude, at strain rates varying between 0.01 and 3.39 1/s. No linear relationship was observed between the strain rate and the Young's modulus (1.13–16.46 GPa), the amount of microdamage, the maximum tensile strain at failure (14.22–61.65%) and at microdamage initiation (1.95–12.29%). The results obtained in this study conflict with previous studies reporting various trends for macroscopic cortical and trabecular bone samples with increasing strain rate. This discrepancy might be explained by the bone type, the small sample geometry, the limited range of strain rates tested here, the type of loading and the method of microdamage detection. Based on the results of this study, the strain rate can be ignored when modeling trabecular bone.

Keywords

strain rate; microdamage; strain; three-point bending; trabeculae

1 Introduction

Bone fracture is often caused by impact, involving high strain rates (Pithioux et al., 2004). To help prevent such fractures, an understanding of the mechanical behaviour of bone with increasing strain rates is needed (McElhaney, 1966). Guedes et al. (2006) stated that polymer matrix composites are characterized by a strain rate dependent mechanical behaviour. Hence, being a polymer/protein matrix composite material, bone is expected to be influenced by loading rate.

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Various past studies investigating the effect of strain rate on bone mechanical properties, yielded conflicting results. An increasing Young's modulus was generally reported with increasing strain rate (McElhaney, 1966; Crowninshield and Pope, 1974; Currey, 1975; Wright and Hayes, 1976; Hansen et al., 2008), however Ferreira et al. (2006) reported a decreasing trend and Pithioux et al. (2004) no trend. Two studies (Crowninshield and Pope, 1974; Currey, 1975) reported an increasing yield strain and stress with increasing strain rate, contradicting a recent study (Hansen et al., 2008) where a decreasing trend for the yield parameters was observed. Concerning failure strain, a decreasing trend with increasing strain rate was seen mostly in cortical bone (McElhaney, 1966; Crowninshield and Pope, 1974; Pithioux et al., 2004), however Guedes et al. (2006) found that failure strain increased with strain rate in trabecular bone. Two studies on macroscopic trabecular bone samples (Carter and Hayes, 1976; Guedes et al., 2006) found a weak power law relationship between strain rate and ultimate mechanical properties of compressed trabecular bone. The range of strain rates employed in each study varied, with McElhaney (1966) covering a large range of 0.001-1500 1/s, but Hansen et al. (2008) using a smaller range of 0.14-29 1/s. The large variation in the ranges chosen in each study might be due to the fact that the strain rate occurring during traumatic bone fracture is undefined (Hansen et al., 2008).

Despite the mentioned strain rate studies, the influence of strain rate on single trabeculae under bending has not been investigated so far. In this study, the strain rate sensitivity of trabecular tissue was examined. Single bovine trabeculae were subjected to three-point bending and the Young's modulus, local strains at failure and at microdamage initiation as well as the amount of microdamage were investigated.

2 Materials and Methods

2.1 Excising single trabeculae

The proximal end of a frozen bovine femur, acquired from a meat wholesaler, was separated from the rest of the femur. Then, the proximal femur was cut with a bandsaw (BG 200, Medoc, Logrono, Spain) along the longitudinal and transverse directions, resulting in several cm-sized pieces. These pieces were cleaned of marrow using a cold water jet, produced by connecting a plastic pipette tip to a water tap through a plastic tube. After marrow removal the individual trabeculae were revealed. The pieces cut were kept hydrated in tap water during cutting and subsequently stored in Hank's Buffered Salt Solution (HBSS) of pH=7.4.

Initially, thirteen individual trabeculae were excised from the bone pieces using a pair of scissors and a scalpel. Following application of the speckle pattern (see below), each trabecula was wrapped in gauze soaked with HBSS and stored at -20°C in sealed plastic bags until testing. The pieces from which the trabeculae were excised were stored in the same manner and later reused to harvest additional trabeculae. From the used pieces an additional thirty trabeculae were excised, which were similarly stored at -20°C until testing.

In total, forty-three individual trabeculae were used for this study. The group of thirty samples was subjected to a supplementary freeze-thaw cycle compared to the initial group of thirteen trabeculae. As no significant difference was found performing a two-tailed Student's t-test on the data (significance level 0.05), the data of both groups were merged.

Care was taken in choosing trabeculae to match the 2 mm span of the three-point-bending jig. The cross-sectional dimensions (0.1-1 mm) at midspan were measured in two approximately orthogonal directions, using a digital vernier caliper. Although damage creation by the caliper tips cannot be completely excluded, care was taken to minimize this risk during sample measurement.

2.2 Speckle pattern

To detect surface strains, a point grid pattern was printed on the sample surface eventually facing the camera using an inkjet printer (D2460 deskjet, Hewlett Packard, USA). First, printing of a grid, created using graphics software (Photo-Paint X4, Corel, Ottawa, Canada), at a resolution of 1200 dpi was started. As soon as the printer started printing on the paper, it was stopped and opened to access the location just about to be printed. Double-sided sticky tape was applied to this paper zone and single trabeculae, rinsed with distilled water, were placed on it. Then, the printer was set to continue creating an ink grid on the specimens (cf. Figure 1).

2.3 Mechanical testing

Each sample was placed on a three-point-bending jig (Appendix A, Figure 5) in a bath filled with 10 mM Phosphate Buffered Saline (PBS) solution of pH 7.4. The bath was mounted on the platform of the mechanical tester (ElectroForce3200, Bose, Eden Prairie, MN, USA). Load was applied via a rounded plunger fixed to the mechanical tester, controlled by the provided WinTest software. During testing, fiber optic lights (DC-950H, Dolan-Jenner, Boxborough, MA, USA) illuminated the specimen from two sides. A three-axis stage was used for positioning the high-speed camera (Ultima 512, Photron, San Diego, CA, USA), which recorded the bending tests using commercial software (Photron FASTCAM Viewer 2.4.5.1, Motion Engineering, Indianapolis, IN, USA).

To test the effect of strain rate, the three-point bending tests on individual trabeculae were to be run in displacement control. This proved impossible due to technical problems; due to small displacements involved in these experiments, the proportional integral derivative (PID) feedback parameters could not be adjusted to ensure stable control of the testing machine. To prevent damage to the jig and to the load cell (5 lb, model 31, Honeywell Sensotec, Columbus, OH, USA), tests were carried out without PID control, in so-called direct command mode. This meant that the displacement rate could only be controlled indirectly by varying the voltage rate.

The resulting testing procedure consisted of two stages. First, the plunger was manually lowered until it touched the top part of the trabecula being tested. Contact was defined as a preload of 0.01 N. While the actuator was touching the sample but not moving, the camera was triggered. Then, a little time later, the specimen was loaded past the failure stage, with a voltage rate varying between 0.03 and 6 V/s (corresponding to strain rates ranging between 0.01 and 3.39 1/s, see below). The displacement was measured using a built-in linear variable differential transformer (LVDT). The actuator/sensor system has a maximum travel of 12.5mm. The displacement sensor has a resolution of 1 μm . To avoid collision between the upper and lower parts of the jig, the experiment was set up such that the plunger, pulled to its ultimate downward position, did not touch the rollers.

The high-speed camera recorded the mechanical test with frame rates varying from 60 to 500 frames/s, matching the respective data acquisition rates used. Tracking of a white ink mark on the plunger provided synchronization between video footage and mechanical data (Jungmann, 2006); the time lag between starting the mechanical test and the camera resulted in a first stationary and then moving ink mark in the video footage.

2.4 Data analysis

Twenty-nine out of the forty-three single trabeculae prepared were analyzed. Fourteen samples were excluded due to various problems. Five specimens were lost and another five specimens were damaged, possibly during sample preparation. Although care was taken not to bend or locally damage the individual trabeculae, some damage might still have occurred

during excision or when attaching the samples to and separating from the sticky tape, when printing the samples. Specimens were judged to be damaged, if they whitened and failed outside the middle third of the sample, along the longitudinal direction, or, if parts of the outer layer of the trabecula were missing. These signs indicating overt damage were easily detectable in the video footage of each test.

From the remaining datasets, the longitudinal tensile strain at failure and at whitening onset, the Young's modulus and the maximum amount of whitening until failure were derived. Seven calculated values exceeding the mean by more than four times the standard deviation were excluded from further analysis, where the mean and standard deviation were taken after exclusion.

Displacement rates were determined from recorded mechanical data. The displacement rates were converted into strain rates experienced by the extreme tensile fibre (i.e. the one furthest away from the neutral axis) at midspan of the simply supported beam with a point load using the following formula:

$$\dot{\epsilon} = \frac{6dh}{l^2} \quad (1)$$

where $\dot{\epsilon}$ denotes the strain rate estimates, d the displacement rate of the plunger and h the height of the specimen. This formula is derived by combining Hooke's law, the Euler-Bernoulli formula for the deflection of a beam at midspan, the expression for stress in a bending beam and the formula for the bending moment at midspan of a simply supported beam with a point load at midspan. This formula assumes small displacements, therefore the strain rates derived here can be compared to other studies varying strain rate. Strain rates used here varied between 0.01 1/s and 3.39 1/s. To better visualize the orders of magnitude of the strain rates tested, graphs were plotted using a semi-logarithmic scale. A straight line was fitted to the data points using the method of least squares (Figure 2) and the coefficient of determination (R^2) was calculated. Also, for each parameter, a linear regression t-test was carried out (under the null hypothesis the slope of the fitted line equals 0, significance threshold 0.05). The R^2 and P-values are shown in Table 1.

Young's modulus—Force-displacement data was obtained from the mechanical tester. The linearly elastic region of the force-displacement curves was manually selected, and a linear curve was fitted to these data points, giving the slope in the elastic region. An elliptical shape was assumed for determining the cross-sectional area, second moment of area and the shear coefficient. To test the elliptical shape assumption, six single trabeculae were excised from a second bovine femur. These were embedded in resin and cross-sections inspected under a microscope. Four cross-sections could be compared to an ellipse, one to a circle and one to the letter D. Therefore, assuming an ellipse for the calculations was justified.

A shear coefficient varying between 0.828 and 0.907, assuming a Poisson's ratio value of 0.3, was used (Cowper, 1966), depending on the sample geometry. The slope, the shear coefficient and the Poisson's ratio along with the variables derived from geometrical measurements of the single trabeculae (i.e.: cross-sectional area, second moment of area and length) were incorporated in the Timoshenko formula (2) (Gere and Timoshenko, 1991; Wang, 1995), to obtain the Young's modulus of the single trabeculae examined.

$$v_{\max} = \frac{Pl^3}{48EI} + \frac{24(1+\nu)I}{AKl^2} \quad (2)$$

where $v_{\max}$ denotes the deflection at midspan, P the applied force, l the length of the beam, E the Young's modulus, I the second moment of area, the Poisson's ratio, A the cross-sectional area of the beam and K the shear coefficient. For each specimen, the Young's modulus was calculated and plotted against the strain rate (Figure 2a).

Microdamage—The whitening effect (Currey et al., 1995) was used to detect microdamage (Turner et al., 2006a; Turner et al., 2006b; Turner et al., 2008). To quantify whitening, the images recorded by the high-speed camera were processed, as previously described (Turner et al. 2006a) using a custom LabVIEW program (Jungmann, 2006). Briefly, a threshold was defined, based on the brightest pixel found on the bone surface in the first few frames of each video. All pixels above the threshold were counted as whitened pixels in subsequent frames resulting in a whitening curve. A whitening curve of a single trabecula is shown on Figure 3, as an example, along with a force-displacement curve. The whitening effect itself is depicted on Figure 4.

The amount of whitening just prior to failure, used as an indicator of the amount of microdamage, was normalized by dividing the number of whitened pixels by the cross-sectional area of the corresponding specimen. As we assume whitening to scale with volume, normalization should reduce the effect of the sample geometry. Resulting whitening values were plotted against strain rate (Figure 2c).

Strain—Strain maps were generated from recorded images using commercial digital image correlation software (Vic-2D 2009, Correlated Solutions, Columbia, SC, USA). To obtain strain maps covering the area of interest, the subset size was set between 21 and 33 pixels (depending on the sample) and the step size to 1. A decay filter (90% center-weighted Gaussian filter) of size 15 was applied to smooth the calculated strains. The ink pattern on the surface of the samples and the chosen parameters (i.e. step size and filter size) were validated for two-dimensional (2D) strain mapping using Vic-2D (Appendix A, Table 2).

The software provided real-time strain measurements and therefore, strain maps matched with the different phases of whitening (Figure 4). For each sample, the longitudinal Lagrangian strain maps corresponding to whitening initiation and failure were retained for further analysis. The bottom extreme fiber was selected at midspan, and the maximum tensile strain value noted at whitening initiation and failure, respectively. These values are shown on Figure 2b and Figure 2d as a function of strain rate.

3 Results

No linear relationship was found between the tested parameters and the strain rate based on the calculated R^2 and P-values. Young's modulus values ranged from 1.13 to 16.46 GPa, and no linear correlation ($R^2=0.026$, P-value=0.423) was found between Young's modulus and strain rate, as shown in Figure 2a and Table 1. The amount of microdamage (normalized whitening) ranged from 5.6 to 38.7 10^3 pix/mm², and no linear correlation ($R^2=0.007$, P-value=0.732) was observed between the amount of microdamage and the strain rate, as shown in Figure 2c and Table 1. The maximum tensile strain at failure ranged from 14.22 to 61.65 %, and from 1.95 to 12.29 % at whitening onset. No linear relationship could be established between the strain rate and the maximum tensile strain at failure ($R^2=0.000$, P-

value=0.960) and at whitening onset ($R^2=0.051$, P-value=0.280), as shown in Figure 2b, Figure 2d and Table 1.

4 Discussion

Our experiments suggest that the mechanical properties of individual bovine trabeculae are unaffected by changes in strain rate.

Testing of individual trabeculae is challenging, which is reflected in the considerable number of samples excluded. Numerous samples flipped over once on the jig and thus, only showed an unprinted or partially printed side to the camera. In addition, the displacement rate could not be directly controlled due to problems with the PID control. This meant that the displacement rates tested were an outcome of the voltage rate applied and the sample geometry, rather than an imposed variable. For this reason tests could not be repeated at the same displacement rate. Finally, the geometry of the samples was not well defined as the exact final position of the sample being bent was only defined once placed on the jig. This way, sample height could easily be deduced from the images, whereas no additional information could be gained regarding sample width. However, the sample's rough dimensions were known by making two orthogonal measurements at the centre of the sample before positioning it on the rollers. These values combined with the known height were used to define the geometry and used in the analysis.

2D strain calculations were carried out assuming a flat surface, whereas in reality the surface of an individual trabecula is curved. Most likely this has little influence on the results of this study since only the longitudinal strains were used in the analysis. However, it must be noted that out of plane strains could not be accounted for in the 2D strain analysis. Also, the maximum strain most probably occurs at the very bottom of each sample, an area not captured by the camera in this study.

The reported strain values were compared to two studies testing human single trabeculae (Yeh et al., 2001; Busse et al., 2009). The failure strain values are consistent with the findings of Yeh et al., measuring trabecular tissue strain at the point of fracture exceeding 20% ($26.6 \pm 2.8\%$) for hydrated samples in cantilever bending. Recently, Busse et al. (2009) reported displacement values, which theoretically (based on equation 1) correspond to even higher strains, i.e. sample average maximum tensile strains of 36% at failure and 18% at yield for dry samples in three-point bending. The strains measured here at whitening onset in single trabeculae are higher than the strain values measured at the emergence of the dark zone in cortical bone (Sun et al., 2010), reporting an average strain of $1.1 \pm 0.65\%$. Sun et al. measured five points on the edge of the dark zone in each of their samples and retrieved a range of values from 0-3% for damage initiation. In this study the highest measured value for strain was used, which can explain the higher strains obtained for damage initiation.

Comparison of previous studies (McElhaney, 1966; Crowninshield and Pope, 1974; Saha and Hayes, 1974; Currey, 1975; Wright and Hayes, 1976; Saha and Hayes, 1976; Carter and Hayes, 1976; Pithioux et al., 2004; Guedes et al., 2006; Ferreira et al., 2006; Hansen et al., 2008) shows that there is no consensus on the effect of strain rate on the bone mechanical properties. These studies were carried out in compression and tension using cortical as well as trabecular bone samples over different strain rates and sizes. They are to some extent contradictory and show various effects of strain rate on the Young's modulus, yield stress and strain as well as on the ultimate stress and strain. A recent study (Ziopoulos et al., 2008) showed that the amount of microdamage in cortical bone loaded in tension was affected by strain rate.

Two studies (Carter and Hayes, 1976; Guedes et al., 2006) found a weak power law relationship between the strain rate and the ultimate mechanical properties of compressed trabecular bone. Carter and Hayes (1976) found that the compressive strength of human trabecular bone was proportional to the strain rate raised to the 0.06 power when testing through six orders of magnitude (0.0001-100 1/s). Guedes et al. (2006), by testing bovine trabecular bone through three orders of magnitude (0.00015-0.15 1/s), observed that the collapse strain was proportional to the strain rate raised to the 0.03 power. Therefore, it is possible that a similarly weak effect would occur at the single trabecula level.

The results of this study showed high scatter, similarly to other studies (Guedes et al., 2006; Pithioux et al., 2004). Pithioux et al. (2004) experienced great variability within each group of tests and suggested the potential importance of the defects, i.e. pores and heterogeneous tissue properties, on the results. This could also explain the scatter in our results, which might have been reduced by taking the average value of measurements at each strain rate, as done by Guedes and coworkers (Guedes et al., 2006). There the average values followed well the fitted model. However, as explained above, this was not possible given the limitations of the testing machine. None of the papers mentioned are directly comparable to this study, therefore one can only make assumptions regarding the nature of the difference in results.

The very weak correlations in the results presented here might also be due to the relatively small sample geometry, type of loading, the relatively small range of strain rates tested here and bone type. The structure of individual trabeculae differs from that of cortical bone. Cortical bone has an organized lamellar structure, whereas trabeculae are made up of packets (Roschger et al., 2003) with different degree of mineralization, resulting in an irregular structure.

To the best of our knowledge, there are no reports on the dependence of mechanical properties of individual trabeculae on strain rate. However, our reported values for Young's moduli of single trabeculae agree well with values previously published (Ryan and Williams, 1989; Mente and Lewis, 1989; Kuhn et al., 1989; Choi et al., 1990; Bini et al., 2002; Busse et al., 2009). Importantly, the data presented here show that microdamage initiation within single trabeculae is independent of strain rate, as is the large magnitude of local strain that can be sustained prior to failure. Based on the parameters studied, the strain rate can be ignored when characterising trabecular bone tissue.

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Appendix A. Validation of the Strain Calculation

The validation of the ink pattern on the surface of the samples and the chosen parameters for 2D strain mapping using the Vic2D software was carried out on the selected single bovine trabecula sample from Figure 3 and Figure 4. The reference image, i.e. the image taken at the start of the experiment containing the undeflected sample, was stretched along the longitudinal axis of the sample in approximately 1% increments up to 15% elongation, the latter approaching the failure strain of the selected sample. When stretching the image, only the width of the image was increased using a free graphics editor software (GIMP). Lagrangian longitudinal normal strains were calculated using equation (3) and knowing the

change in length of the image due to stretching. These strains are referred to here as theoretical strains.

$$\varepsilon_{xx} = \frac{du}{dx} + \frac{1}{2} \frac{du^2}{dx} \quad (3)$$

The digital image correlation in Vic2D was carried out using a step size of 1, i.e. computing strains for each pixel inside the region of interest. Lagrangian longitudinal normal strain values were calculated and smoothed (using a decay filter of size 15) with the Vic2D software. The values calculated at the upper edge of the sample were disregarded as on this sample this region contained no speckle pattern. The Vic2D strains values were then compared to the theoretical Lagrangian strain values (Appendix A, Table 2). The relative difference between the theoretical and the Vic2D strains was less than 1% and therefore was considered negligible. Fitting a straight line between these values resulted in a perfect correlation ($R^2=1$). The standard deviations were less than 4% which showed that the step size used produced strain results within an acceptable error.

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Figure 1.
Example of a speckle pattern printed onto a single trabecula sample.

Figure 2.

Extracted parameters plotted against strain rate. A linear model fitted to the data points by linear regression (note the logarithmic scale on the x-axes) with R^2 values are shown (P-values in Table 1). (a) Young's modulus (b) Maximum tensile strain at whitening onset (c) Maximum amount of normalized whitening just prior to failure (d) Maximum tensile strain at failure.

Figure 3.
Evolution of whitening synchronized with the force-displacement data of a selected single bovine trabecula (Figure 4) during three-point bending.

Figure 4. Recorded images demonstrating whitening initiation and progression (left) with corresponding longitudinal local strain maps (right) at various stages (deflections with corresponding times noted) of the three-point bending test of the same single trabecula sample as in Figure 3. Whitening onset was detected at 54 μm deflection.

Figure 5.

(a) Setup used for the experiments showing the translation stage (for centering the plunger between the lower two rollers), the extension piece (for ease of sample exchange), the submersible load cell and the bath. (b) Close-up view of the three-point bending jig showing the plunger and the lower two rollers as well as a bone sample. The white marker at the bottom of the plunger is for motion tracking.

Table 1

R^2 and P-values describing the linear relationship between strain rate and maximum tensile strain at failure, maximum tensile strain at whitening, maximum amount of whitening until failure and Young's modulus.

Strain rate versus	Linear R^2	P-value
Young's modulus	0.026	0.423
Maximum normalized whitening at failure	0.007	0.732
Maximum tensile strain at failure	0.000	0.960
Maximum tensile strain at whitening onset	0.051	0.280

Table 2

Comparison between the theoretical strains and strains computed by Vic2D of an image artificially strained, including the standard deviation, at different strain levels.

Theoretical ε_{xx} [-]	Vic2D mean $\varepsilon_{xx} \pm$ std. dev. [-]	Relative difference* [%]
0.00000	0.00000 $\pm$ 0.00000	-
0.01034	0.01040 $\pm$ 0.00041	0.62
0.02078	0.02072 $\pm$ 0.00058	-0.27
0.03132	0.03134 $\pm$ 0.00054	0.05
0.04198	0.04198 $\pm$ 0.00039	0.01
0.05004	0.05006 $\pm$ 0.00025	0.05
0.06087	0.06090 $\pm$ 0.00028	0.04
0.07182	0.07179 $\pm$ 0.00030	-0.04
0.08287	0.08287 $\pm$ 0.00030	0.00
0.09402	0.09400 $\pm$ 0.00029	-0.02
0.10528	0.10528 $\pm$ 0.00027	0.00
0.11665	0.11663 $\pm$ 0.00032	-0.02
0.12812	0.12812 $\pm$ 0.00032	0.00
0.13970	0.13972 $\pm$ 0.00039	0.01
0.14845	0.14846 $\pm$ 0.00038	0.00
0.16022	0.16021 $\pm$ 0.00032	0.00

* $((\text{Vic2D mean } \varepsilon_{xx} - \text{theoretical } \varepsilon_{xx}) / \text{theoretical } \varepsilon_{xx}) * 100$