MECHANICAL PROPERTIES OF INDIVIDUAL TRABECULAE IN A PHYSIOLOGICAL ENVIRONMENT

Martin Frank, Dorothee Marx, Dieter H. Pahr and Philipp J. Thurner
Institute of Lightweight Design and Structural Biomechanics
TU Wien
Getreidemarkt 9, BE02
1060 Vienna, Austria
e-mail: frankm@ilsb.tuwien.ac.at

ABSTRACT
Reliable mechanical properties of trabeculae are needed at the tissue-level for prediction of mechanical behavior of the overall trabecular structure using Finite element analysis (FEA). The aim of this study was to develop a set-up to test trabeculae in tension in a close to physiological environment, and to determine reliable tissue-level properties. Ten bovine trabeculae were tested until failure. Tissue-stress can only be indirectly determined, since it is based on a defined cross-sectional area. Different geometrical assumptions for the cross-section were compared. The mean tissue Young’s modulus, based on the assumption of an elliptical cross-sectional area, was 9.9 ± 3.4 GPa, the mean tissue ultimate tissue strain 9.8 ± 3.9 %. Back-calculation of the tissue Young’s modulus by means of FEA illustrated a significant reduction to 8.2 ± 2.4 GPa (p < 0.001). However, with simple geometric assumptions, it is possible to estimate a reasonable upper and lower boundary for the tissue Young’s modulus. Full-field strain measurements were done to detect localization of strain. It was shown that local strain peaks occur already early after yielding, with a local strain at fracture of 19.7 ± 6.6 %. These findings clearly show that individual trabeculae can withstand much higher tissue strains as previously reported.

KEY WORDS
tensile test, hydration, bone fracture risk assessment, post-yield behaviour, local strain, structural influence

1 Introduction
Risk assessment of bone fracture in the elderly is a critical task. Dual-energy X-ray absorptiometry (DXA) is used as a gold standard, despite a rather low sensitivity [1]. According to Zysset et al. the usage of finite element models (FEM) can significantly improve the prediction of bone strength [2]. In homogenized FE, cylindrical biopsies are tested in compression-tension experiments and the apparent modulus is determined [3]. Material properties can be back-calculated using FEM [4], which does not constitute an actual measurement. μFE simulations are able to predict local tissue behaviour at the scale of individual trabeculae. However, μFEA is dependent on reliable input parameters at the tissue level and thus, it is necessary to perform mechanical tests of individual trabeculae. Mechanical data at this scale is still rather limited due to technical challenges [5]. As explained in the study of Bini et al. samples are very likely to fail even before testing, resulting in a small amount of useable data [6]. The values determined for the Young’s modulus in tensile experiments differ over one order of magnitude [5], from 0.8 [7] to 16.2 GPa [8]. Although there is evidence that bone shows a different mechanical behaviour under wet and dry conditions at the lamellar level [9], this has not yet been quantified at the scale of individual trabeculae [8]. In fact, there are several studies available that have evaluated the elastic behaviour for tension and 3-point bending tests [12]. They showed that the mechanical parameters can be derived in a reproducible manner, but their sample preparation and testing was tedious and time-consuming. Given the high variability between current studies and the rather low amount of reproducible samples, there is the need to develop a reliable test set-up with a higher throughput. Further, it is necessary to quantify the structural influence of the curved shape of trabeculae on the mechanical behaviour. The goal of our study was to develop such a set-up to test individual trabeculae in tension close to physiological conditions and to evaluate the structural influence.

2 Methods

Ten samples were obtained from the proximal head of the second phalanx of a 16 month old bull, sourced immedi-
ately after death from a local butcher. Individual trabeculae were dissected with a hand-held miller (Dremel 400, USA) in hank’s balanced salt solution (HBSS) at room temperature. Sample geometry was acquired with a μCT100 (SCANCO Medical AG, Switzerland) at a nominal resolution of 3.3 μm (voltage 55 kV, current 200 μA, integration time 200 μs, average data 3). Geometrical measurements were obtained manually in ImageJ (1.45s, National Institutes of Health, USA). The mean trabecular length was 0.59 ± 0.12 mm, the mean major axis 0.18 ± 0.05 mm, the mean minor axis 0.13 ± 0.03 mm resulting in a mean aspect ratio of 3.6 ± 1.4.

A speckle pattern was applied indirectly with a black spray paint (see figure 1-C). For alignment, samples were placed in a custom-made silicon chamber. Two component epoxy glue (UHU Plus Endfest 300, Germany) was added to both ends of the sample for sample fixation in the test set-up (see figure 1-B). Epoxy glue was dried over night (> 12 h) at room temperature. Then, samples were rehydrated in HBSS for 2 h. Samples were tested in a custom made sample holder in a water-bath filled with HBSS as illustrated in figure 1-A. The set-up was mounted in a servo-electric load-frame (SELmini-001, Thelkin AG, Switzerland), and tests were performed displacement controlled. The force was measured with a 10 N load cell (HBM-S2M, Germany, relative error 0.02%). All experiments started at a pre-load of 0.05 N to align the sample and testing was done until failure.

Video recording was performed with a camera (UI-3250CP-M-GL, IDS GmbH, Germany), at 10 Hz for the frontal plane. Additionally, a second video camera (UI-3243CP-M-GL MP, IDS GmbH, Germany) was mounted at 45° to record the sample from the lateral plane via a mirror. Direct tissue strain measurements were done with a point tracking algorithm [14]. This algorithm allows sub-pixel resolution, since the centroid of each point is used for tracking. Individual points were selected at the top and the bottom of each trabeculae (see figure 2). The change in length between the bottom and the top points was monitored and the average was calculated to determine the tissue strain. Additionally, a digital image correlation software (GOM Correlate, Germany) was used to determine full field strain maps.

2.1 Determination of the trabecular cross-sectional area

Since tissue-stress can only be determined indirectly, a cross-sectional area has to be defined. This cross-sectional area was determined in three different ways. A: The cross-section was assumed elliptical and the major and minor axis were measured at the median slice resulting in a mean cross-sectional area \( A_{ell} \) of 0.0189 ± 0.0086 mm² (see figure 3-B). Mechanical parameters presented in table 1, column Elliptical, are based on this area. B: The cross-sectional area is calculated in the median slice in the \( \mu \)CT scan (the horizontal slice loacted at the middle position in

![Figure 1. A: Overview of the tensile test set-up. B: Individual trabecula embedded in epoxy. C: Zoomed area of trabecula with applied speckle pattern.](image)

![Figure 2. Optical tissue strain determination, with selected points at top and bottom region of the trabeculae. Lines represent the mean position of the corresponding points. A: initial frame at pre-load. B: last frame before fracture.](image)
resulting in a mean cross-sectional area of $A_{\text{mid}} = 0.0197 \pm 0.0083 \text{mm}^2$). C: The cross-sectional area is calculated by dividing the volume of one trabecula by its length (see figure 3-A) resulting in a mean cross-sectional area of $A_{\text{mean}} = 0.0236 \pm 0.0091 \text{mm}^2$.

Mechanical parameters presented in table 1, column Mean, are based on this area.

The following mechanical parameters were determined: The Young’s modulus $E$ is calculated as the slope of a linear regression as described by [15]. Basically, a plot of the $R^2$ value dependent on the window length is used to obtain the largest $R^2$ value. Further, the end point of the linear regression is used to determine the point of yield ($\epsilon_y$ and $\sigma_y$). The same regression line is used to get the 0.2% point of yield ($\epsilon_{0.2}$ and $\sigma_{0.2}$). Further, tissue stress and strain at failure are evaluated ($\sigma_u$ and $\epsilon_u$). Hardening stress $\sigma_h$ is calculated as the difference between ultimate stress $\sigma_u$ and yield stress $\sigma_y$, as described by Caretta et al. [12]. For each curve, the hardening coefficient $B$ is calculated based on the following formula,

$$
\sigma = \sigma_y + \sigma_h \cdot (1 - e^{-B \cdot \epsilon_{pl}}),
$$

with an exponential fit in SciPy (V 0.18.0). Elastic work $W_{el}$ and post yield work $PYW$ are calculated by integration of the stress-strain curve until and after the point of yield. The mean tissue-stress-strain in figure 4 is calculated with the mean mechanical parameters and the formulas described in figure 4.

2.2 Linear-elastic finite element analysis

As already mentioned, trabeculae are not regularly shaped elliptical rods. We performed a linear-elastic FE analysis to back-calculate the most accurate elastic behaviour of individual trabeculae. Image processing and a direct voxel to element conversion was done in medtool (Dr.Pahr Ingenieurs eU, Austria). All nodes of the bottom plane were connected with rigid elements to a single reference node, where all degrees of freedom (DOFs) were constrained. The same procedure was done with the top plane, except that the DOF in the axial direction was not constrained. In the FE analysis (Abaqus V6.14) the top reference node was displaced by the same value as obtained from the experiment and the reaction force was calculated. The back-calculated Young’s modulus was then determined by the relative error in the reaction force.

2.3 Statistics

Statistical analysis was done with R (Version 3.3.2) [16]. Significance levels were determined with ANOVA and Tukey honest significant difference HSD post hoc test. Values below a significance level of 0.05 were considered as significant.

3 Results

Figure 4 illustrates the corresponding tissue-stress-strain curves for uni-axial testing. Using the video feeds from the frontal and lateral plane we are able to observe trabecular alignment during the whole test. Out of ten tested samples, one was disregarded, since it showed a significant misalignment. All determined mechanical parameters are summarized in table 1. A tissue-stress at failure of 9.8% on average was determined and a mean tissue-stress at failure of 169 MPa. Tissue-stress values always have to be treated with caution, if the sample geometry is not regular. Therefore, we verified the structural influence of the geometry on the tissue elastic behaviour.

$$
\sigma_h = \sigma_u - \sigma_y
$$

$$
\epsilon_{pl} = \epsilon - \epsilon_y
$$

$$
\text{if } \epsilon \leq \epsilon_y: \sigma = E \cdot \epsilon
$$

$$
\text{if } \epsilon > \epsilon_y: \sigma = \sigma_y + \sigma_h \cdot (1 - e^{-B \cdot \epsilon})
$$

Figure 4. Determined tissue-stress-strain curves of trabeculae tested in uni-axial tension, based on an elliptical cross-section. The dark line represents the mean tissue stress-strain curve (based on the formulas shown in the graph, calculated from the mean values indicated in table 1). Red crosses illustrate the point of yield, red circles the point of failure.
Table 1: Mean ± standard deviation of mechanical tissue parameters. a: significant difference between Elliptical and FE, b: significant difference between Mean and FE, c: significant difference between Elliptical and Mean

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Elliptical</th>
<th>Mean</th>
<th>FE</th>
</tr>
</thead>
<tbody>
<tr>
<td>$E$ [GPa]</td>
<td>9.9 ± 3.4$^a$</td>
<td>7.6 ± 2.4$^b$</td>
<td>8.2 ± 2.4$^{ab}$</td>
</tr>
<tr>
<td>$\sigma_y$ [MPa]</td>
<td>73 ± 29 $^a$</td>
<td>59 ± 25 $^c$</td>
<td>-</td>
</tr>
<tr>
<td>$\epsilon_y$ [%]</td>
<td>0.8 ± 0.4</td>
<td>0.8 ± 0.4</td>
<td>-</td>
</tr>
<tr>
<td>$\sigma_{y0,2}$ [MPa]</td>
<td>91 ± 28 $^a$</td>
<td>72 ± 25 $^c$</td>
<td>-</td>
</tr>
<tr>
<td>$\epsilon_{y0,2}$ [%]</td>
<td>1.2 ± 0.5</td>
<td>1.2 ± 0.5</td>
<td>-</td>
</tr>
<tr>
<td>$\sigma_u$ [MPa]</td>
<td>169 ± 25 $^a$</td>
<td>138 ± 24 $^c$</td>
<td>-</td>
</tr>
<tr>
<td>$\epsilon_u$ [%]</td>
<td>9.8 ± 3.9</td>
<td>9.8 ± 3.9</td>
<td>-</td>
</tr>
<tr>
<td>$B$</td>
<td>39 ± 13</td>
<td>39 ± 13</td>
<td>-</td>
</tr>
<tr>
<td>$\sigma_b$ [MPa]</td>
<td>96 ± 28</td>
<td>79 ± 22</td>
<td>-</td>
</tr>
<tr>
<td>$PYW$ [J m$^{-3}$]</td>
<td>12.8 ± 5.6$^a$</td>
<td>10.3 ± 5.9$^c$</td>
<td>-</td>
</tr>
<tr>
<td>$W_u$ [J m$^{-3}$]</td>
<td>0.3 ± 0.2</td>
<td>0.3 ± 0.2</td>
<td>-</td>
</tr>
</tbody>
</table>

3.1 Structural influence on the elastic behaviour

As already mentioned, stress can only be determined indirectly, for example with a force measurement, based on a defined cross-sectional area. Since an assumed elliptical cross-section is commonly used [7, 10] tissue stresses based on this assumption were compared to those of back-calculated ones from FEA. A comparison of the actual cross-sectional area at the median slice with the assumed elliptical shape shows that there is no significant difference ($p = 0.614$). Because of the curved shape of a trabecula, there might be still a structural influence on the measured or calculated mechanical behaviour. Thus, stresses based on the median slice will be overestimated, compared to the real parameters. In contrast, there is a significant difference between the mean area and the assumed elliptical one ($p < 0.0001$). Subsequently, stresses based on the mean cross-sectional area $A_{mean}$ will be underestimated. This allows us to determine an upper and lower boundary value for the real stresses, with simple geometric assumptions.

The determined Young’s modulus, based on an assumed elliptical cross-section is 9.9 ± 3.4 GPa. We further evaluated the Young’s modulus, based on the mean cross-sectional area $A_{mean}$. This results in a value of 7.6 ± 2.4 GPa, with a significant difference to the elliptical assumption ($p = 0.001$). Here, the Young’s modulus is clearly lower because of the increased cross-section, due to the curved shape at the ends of trabeculae. The Young’s modulus back-calculated from the linear elastic FE analysis is 8.2 ± 2.4 GPa. Compared to the modulus based on the elliptical assumption and to the one based on the mean area, there is a significant difference ($p = 0.001$ in both cases).

3.2 Local strain behaviour

Full-field strain determination showed that localization of strain occurs quite early (see figure 5-B-2, as indicated by a broadening of the histogram, compared to figure5-B-1). In the elastic region, the strain field is very homogeneous (as seen in figure5-B-1) and values for global and local strain are very similar. At point 2, the 0.2 % yield point, the strain field is already more distributed, but still in a narrow range. At point 3, the point of failure, we can detect local regions of extremely high strains (at the point of failure 19.7 ± 6.6 % on average between all samples). In comparison the tissue-strain at failure is 9.8 %. As a result, a trabecula exhibits multiple regions of very high strains in the last part of the post yield region.

4 Discussion

The determined ultimate tissue-strain in our study was 9.8 %, a much higher value than reported in other studies. For example, Jiroušek et al. determined a strain at failure of 2.9 % [11] and Carretta et al. 5.1 % [12]. Only, Hernandez et al. measured a comparable tissue strain of 8.8 % at failure [13], when they added HBSS onto their samples. From these findings, we can estimate that hydration indeed increases the ultimate tissue-strain by approximately a factor of 2. This finding is very important for fracture analysis, since trabeculae seem to be able to absorb a much higher energy in the post-yield region, when hydrated and being close to physiological conditions. Similarly, Hengsberger et al. found out that the Young’s modulus of individual lamellae was about 2 times larger in dry samples, compared to physiological ones [9].

The determined tissue Young’s modulus, based on an assumed elliptical cross-section was 9.9 ± 3.4 GPa. This value is comparable to previous studies, such as [12, 11, 10], although there is a large variation between available studies [5]. Carretta et al. mentioned that there is a structural influence on the mechanical behaviour of individual trabeculae [12], however, this has not been quantified yet. Thus, we evaluated the structural influence of the trabecular shape on the elastic material behaviour. The comparison of the different assumptions on the cross-sectional area of a trabeculae showed that there is a significant difference between the obtained elastic material parameters. The most accurate method is to back-calculate tissue mechanical parameters from FEA. Nevertheless, several studies that evaluated the Young’s modulus of individual trabeculae assumed a constant cross-section [6, 7, 10]. Since not all studies have the possibility to do μCT scans and perform a finite element analysis, we suggest to determine an upper and lower boundary for the determined parameters. We propose to additionally calculate the mean cross-sectional area $A_{mean}$, based on the volume. This results in a lower boundary for e.g. the Young’s modulus. Similarly, the determined Young’s modulus, based on the elliptical cross-section serves as an upper boundary. Back-calculation of the real Young’s modulus from FEA showed that this value is indeed close to the average of the other two values. So far, we tested the structural influence on the elastic behaviour. In the future we will also investigate the structural influence on the post-yield behaviour.

The back-calculation of the Young’s modulus is
based on a linear elastic μ-FE model. Bone is assumed to be isotropic, which is a common assumption for μ-FE modelling [17]. However, the FE-model should be adapted to non-linear material behaviour to further back-calculate all mechanical parameters.

The local strain field determination illustrated that trabeculae show locally a homogenous pure elastic behaviour before yield (see figure 5-B-1). Only, after the yield point, locally elevated strains start to occur (see figure 5-B-2). Subsequently, the full field strain becomes more heterogenous. Very high local strains, close before fracture, indicate the generation of diffuse damage and micro cracks and local material failure (see figure 5-B-3). The pattern is very intermittently shaped and thus, an indication for multiple cracks. This behaviour seems to be important to allow an individual trabecula to deform at almost 10% before failure. Further research will be done to verify the local damage behavior of trabeculae, for example using the whitening effect [18, 19]. This approach was already successfully used to quantify the local tensile strain needed for damage initiation in 3-point bending tests [20]. In a prospective study, we will also quantify directly the influence of the test environment (wet vs. dry) on the mechanical behaviour in our new tensile test set-up.

5 Conclusion

Individual trabeculae are capable to sustain very high tissue strains until failure, locally up to 20%, close to physiological conditions. Thus, it is important to use mechanical parameters, derived close to physiological conditions, to use FEA for fracture risk assessment. Further, trabeculae may be assumed to have an elliptical cross-section for approximating mechanical parameters with an error of about 18%.

Acknowledgement

We gratefully acknowledge the provision of the bovine bone samples from Fleischerei Leopold Hoedl (1230 Wien, Loogsgasse 1)

References


