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Local strain and damage mapping in single trabeculae during three-point bending tests

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Abstract

The use of bone mineral density as a surrogate to diagnose bone fracture risk in individuals is of limited value. However, there is growing evidence that information on trabecular microarchitecture can improve the assessment of fracture risk. One current strategy is to exploit finite element analysis (FEA) applied to 3D image data of several mm-sized trabecular bone structures obtained from non-invasive imaging modalities for the prediction of apparent mechanical properties. However, there is a lack of FE damage models, based on solid experimental facts, which are needed to validate such approaches and to provide criteria marking elastic–plastic deformation transitions as well as microdamage initiation and accumulation. In this communication, we present a strategy that could elegantly lead to future damage models for FEA: direct measurements of local strains involved in microdamage initiation and plastic deformation in single trabeculae. We use digital image correlation to link stress whitening in bone, reported to be correlated to microdamage, to quantitative local strain values. Our results show that the whitening zones, i.e. damage formation, in the presented loading case of a three-point bending test correlate best with areas of elevated tensile strains oriented parallel to the long axis of the samples. The average local strains along this axis were determined to be $(1.6 \pm 0.9)\%$ at whitening onset and $(12 \pm 4)\%$ just prior to failure. Overall, our data suggest that damage initiation in trabecular bone is asymmetric in tension and compression, with failure originating and propagating over a large range of tensile strains.

Keywords

Trabecular bone; Local strain detection; Damage model; Whitening; Microdamage

1. Introduction

Finite element (FE) analysis is a commonly used tool in biomechanics research for prediction of the mechanical competence of trabecular bone structures (Borah et al., 2001; van Rietbergen, 2001; van Rietbergen et al., 2002; Muller and van Lenthe, 2006). A major driver of this particular field is the need to complement current diagnostic measures solely based on areal bone mineral density (aBMD). It is by now commonly accepted that aBMD, i.e. bone mass, alone is only a modest predictor of fracture risk by itself and therefore needs to be complemented by additional parameters describing bone quality (Stone et al., 2003; Felsenberg and Boonen, 2005). Two very important parameters describing the quality of bone are trabecular microarchitecture, as well as the bone matrix material properties (Felsenberg and Boonen, 2005). While trabecular bone microarchitecture is to some extent clinically accessible in a non-invasive fashion by using X-ray computed tomography (Feldkamp et al., 1989; Ruegsegger et al., 1996) or magnetic resonance imaging (Wehrli et al., 2002), the bone matrix material properties can so far only be determined *in vitro* from mechanical testing experiments (Choi et al., 1990; Turner et al., 1999; Zysset et al., 1999; Nalla et al., 2003, 2005). This may change in the near future, however, as tools to probe bone matrix material properties *in vivo* are currently being developed (Diez-Perez et al., 2010; Hansma et al., 2006). In the meantime, the absence of tools that can assess the bone matrix properties in patients pose a large problem. Consequently the prediction of mechanical competence from FE models is generally not based on experimentally derived data and almost all studies rely on calibration strategies, which tune model parameters, such as yield strain (Niebur et al., 2000; Bayraktar et al., 2004), in order for the simulations to match apparent experimental data. Hence, the predictive power of most of these models is questionable. Yet to be fair, there are also very few experimental studies providing appropriate material parameters. While the mechanical properties of cortical bone have been studied on the sub-millimeter and microscale to a larger extent, there is only limited data available on the mechanical properties of individual trabeculae. However, such investigations are direly needed as with the advent of ever more powerful computing resources geometrical non-linear FE simulations can even model large deformations (Stolken and Kinney, 2003). This allows the investigation of local plasticity and failure in trabecular bone structures, which requires experimental data for further development.

In this communication we propose a strategy for the direct measurement of local strains involved in damage initiation and plastic deformation in single trabeculae concomitant with a mechanical testing experiment. For this purpose we are exploiting the stress whitening effect found in bone, which was recently linked to microscopic damage accumulation and microfractures in mm-sized samples of human vertebral trabecular bone (Thurner et al., 2007) and has been previously described in cortical bone (Zioupos and Currey, 1994; Currey et al., 1995; Zioupos and Currey, 1998). Using an externally applied texture and particle-tracking, we employ high-speed photography to optically detect displacement and compute local strain fields in single bovine trabeculae subjected to a three-point bending test. The local strain fields can be directly linked to the accumulated damage quantified in form of whitened pixels. From the experimental data we are able to deduct the local strain fields involved in damage formation and peak strain values experienced just prior to catastrophic failure. Our results suggest that microdamage forms predominantly in areas subjected to tensile deformation. Whether or not these conclusions are also true for human trabecular bone remains to be determined.

2. Materials and methods

2.1. Sample preparation

Proximal parts of bovine femora were obtained from a local grocery store (Gelson's Market, Santa Barbara, CA, USA). Single trabeculae were tested directly after preparation. Using a butcher's band saw, femoral bones were first cut in half along the frontal axis of the bone. The halves were then cut perpendicular to this axis into slabs of ≈ 5 cm in thickness. Bone marrow was subsequently extracted from the specimens using a water jet. Samples with rod-like shapes were cut out, which were mostly found in the regions closer to the diaphysis. A total of 10 single trabeculae were excised for mechanical testing. The average sample length was (2.52 ± 0.21) mm and the average diameter (0.56 ± 0.14) mm.

2.2. Mechanical testing with high-speed photography imaging

For three-point bending tests of single trabeculae and imaging with high-speed photography, we used a custom-made mechanical testing device previously described in detail (Thurner et al., 2007). The testing device (cf. Fig. 1(A)) was modified to accustom single trabeculae loaded in a three-point bending geometry. The two lower points of support, with a span length of approximately 2 mm, are provided by a u-shaped jig (Fig. 1(B)) in the sample chamber. The third point of support was provided by a triangulated plunger, which exerted the displacement on the sample in the middle between the two lower points of support. The signal from the load cell (LBC-100, Transducer Techniques Inc., Temecula, CA, USA), placed between plunger and piston, was amplified by a gain factor of 10 using an analog amplifier (LPF-100B, Warner Instruments Corp., Hamden, CT, USA) to improve the signal to noise ratio. All samples were tested immersed in a buffer solution containing 150 mM NaCl and 10 mM Hepes, at pH 7.4. In order to establish contact between the plunger and the sample without damaging the sample, the piston first was lowered slowly until a preloading force of 0.6 N was reached and then stopped. The preloading force was experimentally determined so that the sample did not exhibit any more rotation along the long axis (i.e. x -axis, inset in Fig. 1(B)). The preloading force was well below the plastic deformation regime of the samples (cf. Fig. 5).

In the second phase the sample was loaded up to 600 μm displacement, at a nominal speed of 600 $\mu\text{m/s}$, resulting in a strain rate of about 0.5 s^{-1} on the bottom fiber of the samples. For imaging we used a high-speed camera and two fiber lights (Thurner et al., 2007). All videos were recorded with a frame rate of 60 frames/s and a shutter speed of 1/12,000 s.

Compression testing of mm-sized bovine trabecular bone was done using a custom-made mechanical testing device, consisting of a load frame and a piston driven by a stepper motor. The piston impinges onto the partly transparent sample chamber, made from passivated aluminum frame and plunger as well as a PMMA window as described elsewhere (Thurner et al., 2007).

2.3. Mechanical data analysis

In order to calculate the elastic modulus and tensile strain at the tension side from a typical force–distance curve obtained experimentally (cf. Fig. 5), the trabecula is regarded as a Timoshenko beam (Gere and Timoshenko, 1991) using Cowper's values for the shear coefficient K (Cowper, 1966). For three-point bending tests of single trabeculae, the following equations are obtained for the highest tensile strain ϵ (Eq. (1)), the highest tensile stress σ (Eq. (2)) and the elastic modulus E (Eq. (3)), assuming a circular cross sectional area A of the specimen with radius R and hence $I = \pi R^4/4$:

$$\varepsilon = \frac{12w_{\max}R}{L^2 \left(1 + \frac{24(1+\nu)I}{AKL^2}\right)} \quad (1)$$

$$\sigma = F \cdot \frac{L}{\pi R^3} \quad (2)$$

$$w_{\max} = \frac{FL^3}{48EI} \left(1 + \frac{24(1+\nu)I}{AKL^2}\right) \Rightarrow E = \frac{FL^3}{48w_{\max}I} \left(1 + \frac{24(1+\nu)I}{AKL^2}\right). \quad (3)$$

Here $w_{\max}$ represents the maximum sample displacement perpendicular to the x -axis (cf. Fig. 1(B)), L is the distance between the lower supporting points and F is the load on the specimen. Using Eq. (3), the elastic modulus was retrieved by fitting a straight line to the most linear portion of the obtained force–distance curves. The yield point was calculated from obtained stress–strain curves (Eqs. (1) and (2)) by using the 0.2% offset criterion (Turner and Burr, 1993). The failure point was defined as the global maximum in the force–distance curve.

2.4. High-speed photography image processing

Whitening of bone was quantified using a simple thresholding algorithm as previously described in detail (Turner et al., 2007). For the synchronization of whitening values with recorded load–distance curves a standard digital image correlation (DIC) algorithm – programmed in LabVIEW (National Instruments, Austin, TX, USA) – was applied tracking a high contrast region on the plunger through consecutive frames.

2.5. Sample patterning and local strain assessment

A first deployment of a standard interrogation window based DIC did not produce satisfying results, because (a) the specimen surface per se does not exhibit sufficient contrast, and (b) the intensity values in the areas that whiten change dramatically during the test. Therefore we generated an artificial particle pattern on the samples. We imprinted the samples with a grid-like template using a modified inkjet printer (Deskjet 5650, Hewlett Packard, Palo Alto, CA, USA) to achieve a homogeneous pattern with sufficient contrast on the sample surfaces. Fig. 2(A) shows the digital pattern with 600 dots per inch resulting in $(25.4/1200)$ mm $\approx$ 20 μ m diameter of one grid point. For printing, each specimen was briefly dried on a paper towel and placed into the printer on a double-sided sticky tape to hold it in place standing upright on its narrow side. After printing, the ink was dried for 5 min in ambient air. The samples were subsequently rewetted in buffer solution for at least 5 h. Fig. 2(B) shows the obtained result on one specimen and Fig. 2(C) the underlying sticky tape. The real dot size on the printed bone in Fig. 2(B) was estimated to be 30 μ m and the initially designed periodical pattern resulted in a stochastic pattern on the bone surface. For local strain detection, Vic-2D software (Correlated Solutions, Inc., Columbia, SC, USA) was used as a correlation algorithm to provide displacement and strain data for our bone specimen. The software was validated using a typical image of an unstrained sample, which was strained to various levels and all data points analyzed. This showed that typical statistical errors for the chosen subset (21 pixels) and stepsize (1) were of the order of 0.006% and the relationship between imposed and measured strains yielded a perfect correlation ($R^2 = 1$) (data not shown). The software derives x - and y -displacement fields and uses these values to compute local strains as given by Eqs. (4)–(8) (Pilkey, 2004).

$$x\text{-strain: } \varepsilon_{xx} = \frac{du}{dx} + \frac{1}{2} \left[\left(\frac{\partial u}{\partial x} \right)^2 + \left(\frac{\partial v}{\partial x} \right)^2 \right] \quad (4)$$

$$y\text{-strain: } \varepsilon_{yy} = \frac{dv}{dy} + \frac{1}{2} \left[\left(\frac{\partial u}{\partial y} \right)^2 + \left(\frac{\partial v}{\partial y} \right)^2 \right] \quad (5)$$

$$\text{shear strain: } \varepsilon_{xy} = \frac{1}{2} \left(\frac{\partial u}{\partial y} + \frac{\partial v}{\partial x} + \frac{\partial u \partial u}{\partial x \partial y} + \frac{\partial v \partial v}{\partial x \partial y} \right) \quad (6)$$

$$\text{principal strains: } \varepsilon_{1,2} = \frac{\varepsilon_{xx} + \varepsilon_{yy}}{2} \pm \sqrt{\frac{\varepsilon_{xx} - \varepsilon_{yy}}{2}^2 + \varepsilon_{xy}^2} \quad (7)$$

$$\text{von Mises strain: } \varepsilon_{vMises} = \sqrt{\varepsilon_1^2 - \varepsilon_1 \varepsilon_2 + \varepsilon_2^2} \quad (8)$$

ε : strain, u : displacement in the x -direction, v : displacement in the y -direction.

Finally, the detected whitening zones were used as regions of interest to compute the average local strains at whitening onset in all samples as well as the maximum local strain just prior to failure using MATLAB (The MathWorks, Natick, MA, USA).

2.6. Finite element modeling

We used a computer-aided design and FEA software package (SolidWorks and CosmosWorks, SolidWorks Corp., Concord, MA, USA), to create a model of a single trabecula bone sample (cf. Fig. 6) subjected to a three-point bending test under similar conditions as in the experiment. The sample was modeled as a cylinder with 480 μm diameter and 2.5 mm length, in order to resemble to the sample depicted in Figs. 6–9. The plunger contact point on top of the modeled trabecula was displaced by 258 μm in the y -direction (down); this displacement corresponds to the experimental plunger displacement prior to the failure point of the specimen. The two supporting points at the bottom were modeled as hinges, which allow free rotation around the z -axis (out of the image plane) but define a non-slip fixed boundary condition in the x - and the y -direction. The FEA is based on a linear elastic material model (linear static analysis) and bone was assumed as an isotropic material with a density of 1.9 g/cm^3 , an elastic modulus of 2.0 GPa, and a Poisson's ratio of 0.3 (Farah et al., 1989).

2.7. Histological detection of microdamage

To compare microdamage detected using basic fuchsin staining and the stress whitening mm-sized bovine trabecular bone cubes with similar sizes as ones in Thurner et al. (2007) as well as additionally excised trabeculae were loaded to whiten but not to failure. Subsequently these samples were stained en bloc in 1% basic fuchsin in increasing concentration (70%, 80%, 90% and 100%) of ethanol in vacuum at room temperature for 5 days. The en bloc stained samples were then embedded in PMMA (poly-methyl-methacrylate), serially sectioned to 200 μm thickness, and ground to 100 μm thickness. By this technique only microdamage present in the sample at the end of mechanical testing is stained with basic fuchsin. All samples were then viewed under a laser confocal scanning

microscope (LCSM) (Zeiss, Thornwood, NY; Excitation 543 nm; Emission 560 nm) Images acquired under LCSM were then compared to optical images acquired during the test.

3. Results

Typical video footage of a three-point bending experiment is shown in Fig. 3; here, whitening starts at a displacement of about 60 μm . Generally, whitened areas in all samples have ellipsoidal shapes, are exclusively developing on the tensile side of the specimens (red contours, Fig. 3(B)–(F)), initiate prior to detected yield points, which is at 114 μm displacement for the sample shown in Fig. 3, and steadily increase in the region of plastic deformation (Fig. 3(D)–(F)). Upon formation of a macroscopic crack, i.e. at the failure point, the ellipsoidal whitening zone on the tension side fades (Fig. 3(E)) and a whitening zone appears situated in front of (and propagating with) the crack tip (Fig. 3(E) and (F)). A histological section of a similar sample subjected to whitening but not failure, shows excessive microdamage in the whitened region, confirming the link between whitening and damage processes in bone (cf. Fig. 4(A)). Additionally, also the investigation of larger trabecular samples loaded to white yields similar results; whitened regions in a frontal section of the sample were correlated to whitened areas as shown in Fig. 4(B).

The force–displacement and whitening–displacement curves for the sample shown in Fig. 3 are presented in Fig. 5(A). Using Eq. (3) the average elastic modulus for all samples was calculated to be $(2.0 \pm 1.0 \text{ GPa})$. We did not observe significant differences in the mechanical data of pristine and ink-imprinted trabeculae (data not shown). To determine the local strain fields involved in whitening and thus damage formation in a quantitative fashion, we analyzed captured video footages of 7 out of 10 samples using the Vic-2D software. From the obtained displacement fields, strains in the x - and the y -direction as well as the shear, 1st principal, and von Mises strain fields were computed (Eqs. (4)–(8)). The experimentally derived local strain fields for one typical specimen just prior to failure (258 μm displacement) are given in Fig. 6 (left column). The strain fields show good qualitative correlation with the FE simulations displayed in Fig. 6(A). Fig. 7 shows a quantitative comparison between local strains in the x -direction of the same specimen obtained from the experiment and FE simulation at whitening onset (66 μm displacement). The section analysis compares local strain in the x -direction within the fiber experiencing the highest tensile strains and shows significantly higher strains in the real sample (Fig. 7(A)), which is most likely due to heterogeneous sample properties.

The tensile strain along the long axis (x -axis) of the trabecula – ϵ_{xx} – predicts the whitening zones seen in the experiments best. This is shown in Fig. 8 where the development of ϵ_{xx} is given together with the corresponding frames from the high-speed video. Detected whitening zones in the same sample are highlighted by red contours in Fig. 9(A) at initial onset (66 μm displacement) and in Fig. 9(B) just prior to failure (258 μm displacement). An overview of the average local strain values for all investigated samples is given in Table 1. For the strain in the x -direction, on average the local strain in whitened zones at whitening onset is determined to be $(1.6 \pm 0.9)\%$ and the highest local strain just prior to the failure point is $(12 \pm 4)\%$.

4. Discussion

In this communication we use video footage of individual trabeculae subjected to a three-point bending test for the concomitant detection of microdamage and local strain fields. While microscopic damage accumulation in bone is directly accessible via the stress whitening effect, local strains are detected by tracking fiducial markers created through a printing process and by using digital image correlation software. In comparison to previous

experiments on mm-sized cuboidal bone samples (Turner et al., 2007) the used three-point bending geometry results in a well-defined loading case, allowing local strain detection, replication of the experiment using FE analysis as well as estimation of strains and stresses using Timoshenko beam theory. Additionally the sample geometry is also beneficial for the whitening detection; single trabeculae immersed in saline are semitransparent also whitening within a sample or on its opposite side are detected.

Whitening was previously correlated to microdamage in cuboid trabecular bone samples using scanning electron microscopy (Turner et al., 2007) and more recently also in single trabeculae (Jungmann et al., 2007). The correlation between whitening and microdamage is also shown in Fig. 4 using two different examples, a mm-sized bone sample and a single trabecula. In both cases, whitened areas coincide with detected microdamage. It is interesting to note that through the whitening effect we are detecting a phenomenon on a scale beyond the resolution of our imaging system. Since resolution and field of view are correlated the choice of resolution is important. The resolution needs to be adjusted to the investigated hypothesis and higher resolution is not always necessarily an improvement. In our case it may well be that whitening becomes invisible beyond a certain magnification. Similar optical effects, as presented here, have also recently been reported by Sun et al. (2010), who also noted rather large localized strains in human cortical bone (Sun et al., 2010). Importantly, our results show that the whitening zones, and hence damage formation, in the presented loading case, correlate best with areas of elevated tensile strain parallel to the long axis of the samples (cf. Figs. 3, 4, 6 and 8). In contrast no whitening is observed on the counterpart areas exhibiting compressive strain on the trabeculae. This is consistent with previous work by Zioupos et al. (1995), who showed in a rather elegant manner that microdamage initiation in cortical bone is localized in regions of tensile stress, and that only yield criteria that differentiate between compressive and tensile anisotropic properties can effectively predict the localization of microdamage.

The correlated local strain and stress whitening analysis yields an approximate initiation strain of $(1.6 \pm 0.9)\%$ for whitening, and hence, microdamage formation. However, the maximum tensile strains in the first frame where whitening/microdamage is detected are considerable higher as can be seen in Figs. 7 and 9. While linear elastic FE analysis and Timoshenko beam theory predict a strain of 3% and 4.5% in the sample shown in Fig. 7, respectively, the local strain detection yields strains of up to 8.5%. Hence, at the first point of detection the sample may have experienced plastic or “damage”-strains of possibly up to 4%. While using an average local strain of the whole whitened region reduces the strain value significantly it is likely overestimating the strain needed for the initiation of whitening and microdamage. Therefore it may well be that the actual strains needed for microdamage formation are rather at the lower end of our range, i.e. 0.7%. Interestingly, similar values have been previously identified by Bayraktar et al. who determined tissue yield strains at around 0.4% (Bayraktar et al., 2004). In contrast to this rather low threshold for damage accumulation our average strain value would suggest damage formation, at average tensile strains of about 1.6% with the top of the range being 2.5%, which is comparable to reported values of local strains of about 3% around crack tips in bone (Nicolella et al., 2001). In a sense it is not surprising that strains bridging out towards these values have been found, since the analysis is based within the damaged region of the sample. Despite the limitation of accurately determining a single onset strain value our study delivers a microdamage onset range (0.7%–2.5%), which is entirely based on experimental data.

In addition, experiments are subject to a few further limitations. The local strain maps exhibit some heterogeneity (Fig. 8), which could of course be due to the fact that trabecular bone tissue is neither an isotropic nor a homogeneous material. However, the heterogeneities could also be due to limited resolution of the fiducial marker pattern for digital image

correlation, so smaller size and higher number of particles would be beneficial. Lastly, we could only use a 2D digital image correlation approach which is not ideal for a curved surface and may lead to some systematic errors, however, the strain component along the long sample axis, in which we are most interested, should be the one least affected due to this. We expect, however, that further improvement in experimental procedure and data analysis will allow pinpointing a microdamage onset strain threshold more accurately.

Additionally to microdamage initiation strains, this study gives some insight into the asymmetry of microdamage accumulation. In the chosen loading case microdamage via whitening is exclusively detected on the side of the specimen subjected to tensile strains. This observation also holds when taking into account the histological analysis. Comparing our results to an earlier report by Stolken and Kinney (2003), who used a geometrical non-linear FE model to simulate the behavior of a single trabecula under compressive load, we can find some striking similarity. Although the trabecula in Stolken and Kinney's communication is subjected to compression parallel to its long axis, it ultimately fails in buckling producing a somewhat similar strain state as experienced in the three-point bending test presented in this communication. One of the two damage models proposed by the same authors, the brittle failure model, predicts a damage zone on the side of the samples subjected to tensile strains parallel to its long axis, similar as detected in our experiments. In contrast their plastic damage model based on a von Mises yield criterion predicted damage on both the compressive and tensile side of the specimen, which would be rather similar to our 1st principal strain and von Mises stress component shown in Fig. 6. Similar symmetric damage models were also employed in other studies (Niebur et al., 2000; Bayraktar et al., 2004; Chevalier et al., 2007). Our results however, suggest that a damage model, with predominant damage initiation in regions of tensile strains, and asymmetric in tension and compression is more appropriate. At least this is true for our loading case, whether this holds for larger volumes of trabecular bone subjected to apparent compressive strains needs yet to be proven. Importantly, this is in good agreement with the damage model proposed by Zioupos et al. who have elegantly shown that among several criteria tested only the Tsai–Wu criterion, which allows for different material properties in tension and compression, reliably predicts microdamage in regions subjected to tensile stress (Zioupos et al., 1995).

As mentioned the lower range of our microdamage initiation is close to values obtained by the calibration study by Bayraktar et al. as well as in other studies (Niebur et al., 2000; Bayraktar et al., 2004; Chevalier et al., 2007). However besides some exceptions, most damage models, applied in FE studies of mm-sized or larger trabecular bone samples, are usually empirical and adjusted through calibration with mechanical tests. Hence, a promising future step would be incorporation of damage criteria obtained from experiments, as presented here, into FE simulation to test their predictive power. Such an approach has been elegantly described for FE modeling of individual trabeculae in previous publications (McNamara et al., 2006a,b; Mulvihill and Prendergast, 2010); In both cases FE models were informed by tensile testing experiments of similar trabeculae (McNamara et al., 2006a,b). Another recent approach to reduce calibration of FE models utilised elastic modulus values obtained from nanoindentation (Chevalier et al., 2007). It is noteworthy, that the value for the elastic modulus used by Chevalier et al., but also in other studies (Niebur et al., 2000; Bayraktar et al., 2004) was usually between 10 and 20 GPa and much higher compared to the value of 2.0 GPa that we measured for our samples. Nevertheless, our result for the elastic modulus is in good agreement with ones from other micromechanical tests (Kuhn et al., 1989; Choi et al., 1990; Lucchinetti et al., 2000). It needs to be noted though that the experiments by Chevalier were all performed on dried samples, which likely increase tissue modulus. Similarly, a recent micromechanical study testing the bending properties of dry trabeculae by Lorenzetti (2006) likely yields higher values for elastic moduli (≈ 15 GPa) as reported here. On the other hand a recent study by Busse et al. also performed on dry

trabeculae from human vertebral bone reported similar elastic moduli in the range of 2 GPa and reported load displacement curves point to similarly high strains of $\approx 30\%$ just prior to failure (Busse et al., 2009). Also, a computational study by McNamara et al. predicted strains over 6% in individual trabeculae subjected to 0.3% apparent tensile strain (McNamara et al., 2006a,b). Similarly high local strain values, although in cortical bone, have also been reported in other recent studies (Sun et al., 2010; Nicolella et al., 2006). In case of the study by Chevalier et al. the question needs to be considered as to whether nanoindentation potentially overestimates the elastic modulus, due to probing a very small volume of usually $\sim 1 \mu\text{m}^3$ (Rho et al., 1999; Roy et al., 1999; Zysset et al., 1999; Tai et al., 2005; Mittra et al., 2006). This is certainly much smaller than the volume we tested and even smaller than typical element sizes used for FEA. However, structural features like bone lamellae or cement lines being above the $1 \mu\text{m}^3$ may also influence the mechanical properties of bone matrix. In contrast to the work by Chevalier et al., our values are much closer to the models for individual trabeculae put forward by McNamara et al. and Mulvihill et al. with lower elastic modulus values of about 2–3 GPa (McNamara et al., 2006a,b; Mulvihill and Prendergast, 2010). Put together, our work and experimental efforts of McNamara et al. and Chevalier et al. represent important steps to reduce calibration of FE models. Nevertheless, further work is needed to combine these developments and advance the generation of experimental data that can be made useful for FEA and help to minimize calibration. Especially, in the case of geometrical linear FE models a calibration approach for a damage model seems questionable since failure of trabecular bone is a very localized process involving only a few trabeculae, which are subjected to extremely high local strains (Bay et al., 1999; Arthur Moore and Gibson, 2002; Nazarian and Muller, 2004; Thurner et al., 2006, 2007). A geometrical linear model might simply homogenize high local strains to overall only slightly increased local strains and hence underestimate the strains needed to create microdamage.

In conclusion, we present a way to detect local strains during micromechanical three-point bending tests of bone. The simple rod-like geometry of the samples and the presented local strain analysis allowed us to correlate whitening (micro-damage), with tensile strains and to quantitatively determine the tensile strain needed for damage initiation. Our experiments complements efforts in FEA of trabecular bone structures (Williams and Lewis, 1982; Dagan et al., 2004; Nagaraja et al., 2005; Shefelbine et al., 2005; van Lenthe et al., 2006) by providing important data for formulation of a future damage model with solid experimental foundation. From our results we propose that damage in trabecular bone tissue forms asymmetric with microdamage originating at tensile strains of 0.7%–2.5%.

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Fig. 1.

(A) Custom-made mechanical testing device with fluid reservoir, fiber lights, and a Photron Ultima 512 high-speed camera equipped with KC lens and infinity lens 3 and 4 for trabecular cubus and single trabeculae mechanical testing, respectively. (B) Testing equipment for single trabeculae bone samples in a three-point bending test. A coordinate system for orientation is shown in the lower left corner: x -axis is the long axis of the trabecula, y -axis the short and z -axis points out of the image plane (not shown).

Fig. 2.
(A) Template pattern for microdot inkjet printing. (B) Resulting pattern on a single trabecula bone sample. (C) Recess in the printed grid pattern.

Fig. 3. Single trabecula bovine bone in a three-point bending test at different displacements. The red contour shows the detected whitened area. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Fig. 4.

Comparison of microdamage detection using basic fuchsin staining and stress whitening. (A), Single trabecula bovine bone in a three-point bending test to a maximum displacement of 140 μm — scale bar: 500 μm (I) and histological section of the same sample showing microdamage in the same area as the whitened zone (II). (B), Cuboid trabecular bone sample in a compression test to a maximum apparent strain of 9% with two regions of interest (ROI) highlighted in red and green along with a whitening vs. time development (I). Same ROIs are shown in the transmission micrograph (II). Zoom-in histological section of the two ROIs revealing microdamage at the whitened zones along with a zoom-out overview of the sample — scale bars: 20 μm (III). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Fig. 5.

Detected whitened pixel (gray) and force exerted on sample (black) versus bending distance of single trabeculae bone samples. The two circles on the force curve denote the yield point (first) and failure point (second), respectively. (A) Whitening and force development of the sample shown in Fig. 3. (B) Whitening and force development of the sample presented in Figs. 6–9.

Fig. 6.

Strain components for one single trabecula sample (A) and linear elastic finite element analysis results (B). Scalebar is 500 μm . The color bars represent linear scales with the following Min–Max values for experiment and simulation: $\epsilon_{xx} = -0.1$ – 0.17 , $\epsilon_{yy} = -0.26$ – 0.41 , $\epsilon_{xy} = -0.18$ – 0.1 , 1st principal = -0.02 – 0.42 , von Mises = 0 – 0.23 (in simulation von Mises stress is shown). (Experimental and simulation displacement: 258 μm). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Fig. 7.

Experimental (A) and simulated (B) results for the local strain components in the x -direction on a single trabecula at the onset of whitening (top) and section analysis of the fiber with the highest tensile strain at the bottom of the sample (bottom). Experimental and simulation displacement was 66 μm .

Fig. 8.

(Left) Frames from high-speed video and (right) corresponding strain fields in the x -direction (ϵ_{xx}) at given plunger displacements. Red areas represent a positive strain (tension) and purple areas negative strain (compression). Used colorbar for strain overlay (bottom). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Fig. 9.

(A) Detected zone at the initial whitening onset highlighted by a red contour line (66 μm displacement). The strain distribution in the region of interest (i.e. whitened zone) at whitening onset is shown below. (B) Maximal whitened area prior to the failure point (258 μm displacement). Again, the strain distribution at failure is shown. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1

Strain components, corresponding average values, and standard deviations at whitening onset and just prior to failure, in whitened regions, for 7 tested samples.

Strain component	Whitening onset (%)	Failure (%)
ϵ_{xx}	1.6 ± 0.9	12 ± 4
ϵ_{yy}	-7 ± 6	-15 ± 12
ϵ_{xy}	0.2 ± 0.7	0.8 ± 4
1st principal	2 ± 1	13 ± 6
Von Mises	1.0 ± 0.5	8 ± 5