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# **REAL-TIME OBSERVATION OF TRABECULAR BONE MICROSTRUCTURE DURING MICROMECHANICAL TESTING**

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**Summary:** The paper deals with investigation of trabecular bone microstructure using real-time microtomography. Recorded  $\mu$ CT scans are used to record spatial mechanical behaviour of bone under loading and provides detailed information about inner architecture and hierarchical structure of bone tissue. Micro-FE models developed on basis of the  $\mu$ CT scans of the undeformed microstructure are used to investigate different material models suitable to describe bone tissue at the trabecular level. Overall, real-time microtomography enables microdamage detection in real-time during mechanical testing and provides a correlation to recorded stress strain curves.

### 1. Introduction

Mechanical properties of trabecular bone are greatly influenced by the microstructural arrangement of individual trabeculae (Wagner (1992)). The inner structure of the bone is very complex and for investigation of its influence on pre– and post–yield behaviour a combination of mechanical testing and numerical modelling is advantageous. Since the inner structure is very important in the development and design process of wide range of porous materials used in biomedical engineering, three-dimensional visualization of the inner structure is essential.

For assessment of the mechanical performance of implants used in reconstructive surgery not only the overall mechanical properties play an important role, but the inner structure which has a great impact on mechanical and transport properties of the tissue plays a key role (Knackstedt (2006)). In recent years 3-D imaging techniques were established which enables direct measurement of structural properties of materials used for the scaffold (Mathieu (2006) and Jones (2004)). These techniques can be used not only to measure the morphological properties of the material, but they can be successfully applied to measure its mechanical performance. To capture the 3-D deformation it is possible to use real time microfocus Computed Tomography. Trabecular bone sample is fixed in a special loading device enabling load application in increments. The deformed inner structure of the sample can be reconstructed during the whole deformation process. This enables to capture the strains in the individual trabeculae and to measure the highly localized strains within each trabeculae. Results obtained from the

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real-time microtomography can be used to compare post-yield behaviour of different samples of trabecular bone or to validate micro–FE models created from the micro–CT scans of the undeformed sample. Real-time tomography can also provide useful information in the design of tissue scaffolds where maximization of mechanical strength with optimization of flow and diffusion properties of the scaffold is essential for sufficient cell ingrowth.

### 2. Materials and Methods

### 2.1. Samples

Two samples of porcine trabecular bone were extracted from proximal femur. The specimens are of cylindrical shape with height of 10 mm and diameter of 10 mm and were extracted in the direction respecting the trabecular structure. Samples are placed in a small environmental chamber filled with physiological solution. Samples are fixed in a special loading device enabling compression and tension to be applied on the sample. The load–bearing frame of the device is made using materials with very low absorption of X-rays.

## 2.2. Image acquisition

High resolution tomographic images of the sample are obtained using X-ray tungsten microfocus tube (Hamamatsu photonics) with 5  $\mu$ m spot size (divergent cone beam) and flat-panel X-ray sensor ((Hamamatsu photonics) with active area 120 mm x 120 mm. The loading device with the mounted sample is placed on a rotating table controlled by stepper motors. The load is applied gradually in 1% strain increments and complete tomography of the sample is taken in 180 projections per increment. The overall strain applied to the sample was 10 %.



Figure 1: Experimental setup and detail of the loading device

### 2.3. Visualization of the deformation

From the projections obtained at different strain levels (0.01, 0.02, ..., 0.1) cross-sectional slices were reconstructed using cone-beam Radon transformation. The volumetric images can be resliced in any direction enabling to calculate strains in any cross-section using image correlation techniques. The deformed structure of trabecular bone in three selected strain levels is shown in Fig. 2



Figure 2: Deformation of the trabecular structure under applied load: (a) 0% strain (b) 2% strain, (c) 10% strain

#### 2.4. Image segmentation and FE model development

From the captured projections of the sample it is possible to reconstruct the trabecular structure and visualize its deformation in time. Using image correlation techniques it is even possible to calculate the strains within each trabecula (Verhulp (2004)), but this is computationally very expensive. Finite element models of trabecular microstructure were constructed based on the micro-CT images. A fully-automated segmentation algorithm was developed in order to ensure the same procedure for every slice. FE models of the trabecular bone microstructure were developed using either hexahedral (voxel) elements or tetrahedral elements. At this level of structural detail the trabecular bone is isotropic and homogeneous and therefore the material properties in the FE models are same for all the elements in the entire mesh.

#### 2.5. Finite element simulation

The reconstructed images of the undeformed state of the sample were used to derive the FE model of the bone inner structure. Material properties were identified by means of nanoin-dentation, performed using Berkovich-type diamond indenter. A small specimen of trabecular bone was mounted in the nanotester chamber after thorough grinding, polishing and ultrasonic cleaning. Material constants (E,  $\nu$ ) were obtained from the indentation curve by Oliver-Pharr (Oliver-Pharr (1992) method. The most common criterion used to study failure behavior of trabecular bone is the von Mises criterion (Nagaraja (2005) and Niebur (2000)). However, it has been shown, that von Mises criterion cannot capture the post-yield behavior of trabecular bone, since it does not incorporate the pressure dependency. Material constants for Drucker-Prager plasticity material model were found by fitting the nanoindentation curve with FE simulation of the experiment. Two materials constants – Young's modulus of elasticity (*E*) and Poisson's ratio ( $\mu$ ) were obtained directly from the experiment while cohesion (*d*), dilation angle ( $\beta$ ) and friction angle ( $\psi$ ) were identified in a plane contact analysis. Values of all material parameters obtained from the best fit (by method of least squares) are *E*=9.97 MPa,  $\nu$ =0.2, *d*=26 MPa,  $\beta$ =30  $\psi$ =5.

After identification of the material properties from the nanoindentation test, the calculated material properties were prescribed to all the elements in the model. The FE model was subjected to the 10% compressive loading and the response of the sample is calculated. Stress–strain



Figure 3: (a) Indentation curve fitted by the FE model, (b) Deformation field in the FE model with Drucker-Prager plasticity

diagram obtained from the numerical analysis was compared to experimentally determined one. Apparent stress is given by the sum of nodal forces at the fixed end of the specimen (reactions) divided by the cross–sectional area of the sample. Apparent strain is defined by the applied displacement divided by the total height of the sample. These values are calculated at each iteration time which enables for comparison with the experimentally determined stress–strain diagram.



Figure 4: Displacement field in the FE models of the trabecular bone sample: (a) smooth tetrahedral model (b) voxel model (only part of the model is shown)

#### 3. Conclusions

Real time micro–CT of trabecular bone under loading is a promising technique to study its pre and post-yielding behaviour. Projections obtained during the scanning can be used to develop FE models of the inner structure.

Response of the FE model developed from the  $\mu$ CT slices was compared to apparent material properties obtained experimentally using a special loading device.

Using FE models with advanced material properties obtained from fitting the nanoindentation test to its FE simulation seems to be a robust and precise method to predict the material properties of trabecular bone; provided the trabecular bone structure is segmented properly from the tomographic images. Moreover, the FE models can be easily used to calculate the response of the bone in complex loading or to assess effect of changes in the inner structure on mechanical performance of the bone (e.g. osteoporotic changes).

Described procedures are promising for detail analysis of deformation behavior of materials with complex inner structure, e.g. trabecular bone. Results obtained by FE modeling indicate a great importance of the quality of tetrahedral elements, need for second-order elements and proper surface detection when converting the  $\mu$ CT data into surface mesh. In both voxel and tetrahedral meshes the strain distribution is very localized imposing special requirements on the mesh quality. The proper plasticity criteria (here Drucker–Prager fitted from the nanoindentation) is essential to capture the deformation of trabecualae in compression.

Combined with material properties determined based on nanoindentation of single trabecula these models can be used to study mechanical behavior of bone under complex loading or to study effects of microstructural changes (e.g. in case of osteoporosis) on the overall mechanical performance.

#### 4. Acknowledgment

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