EVALUATION OF SAMPLE PREPARATION PROCEDURES FOR MICRO-MECHANICAL TESTING OF TRABECULAR BONE

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1. Introduction

To improve the knowledge about the mechanical properties of trabecular bone it is necessary to assess the properties at micrometer scale. Testing of isolated human trabeculae is highly influenced by precision of the sample preparation. Article deals with description of the pre-testing procedure for the uniaxial testing, nanoindentation and finite element modelling of single trabeculae. Undesirable mechanical, biological and chemical influences have to be excluded during this procedure.

2. Tissue harvesting

The samples were cut out from caput femoris using diamond blade saw (Isomet 2100, Buehler GmbH.). First, the samples were degreased. The cleaning process was performed using ultrasonic bath with 1% solution of Alconox (Alconox, Inc.) anionic detergent. Process of delipidation consists of 15min phase of ultrasonic cleaning at a temperature not exceeding 40°C and rinsing with distilled water. After the removal of all bone marrow the samples were immersed in an ultrasonic bath with distilled water for 5min. The sample was then dried at room temperature. In purified bone structure suitable long straight trabeculae were identified and carefully extracted using a sharp-tip scalpel.

3. Nanoindentation pre-procedure

Nanoindentation is a variety of indentation hardness tests applied to small volumes. During the test, an indenter is pressed down into the specimen surface while load and penetration depth are measured [1]. In our tests, the depth of the indent was less than 1μm therefore it was necessary to reduce the surface roughness of the samples to minimal possible value. The trabeculae were fixed in longitudinal and transversal positions in low shrinkage epoxy resin (EpoxyCure, Buhler GmbH.).

Diamond grinding discs with grain size 35 and 15μm followed by monocrystalline diamond suspension with grain size 9, 3 and 1μm were used for grinding procedure regarding to the empirical grinding rule [2] with optimization described in detail in [3]. For the final polishing aluminium-oxide Al_2O_3 suspension with grain size 0.05μm on a soft cloth was used.

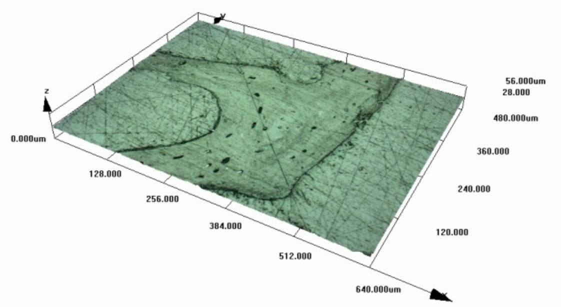

Fig. 1: 3D surface structure obtained by confocal microscope

Prior the mechanical testing the surface roughness of the sample was measured using a confocal laser scanning microscope (Lext OLS3000, Olympus Inc., Japan). Results of the scanning are represented by a 2D matrix of heights. The average roughness R_a (average distance from the profile to mean line) of the finished surface was 25nm.

4. Uniaxial test specimens

The specimens were prepared for displacement-controlled tension and fatigue tests in a custom-based uniaxial loading device. This device was specially designed for testing of microscopic specimens [4]. The specimen deformation was captured optically using a highresolution CCD camera (CCD-1300F, VDS Vosskuhler GmbH, Germany).

The ends of the trabeculae were dipped in a two-component glue (UHUplus Schnellfest 2-K-Epoxidharzkleber, UHU GmbH & Co. KG, Baden) and stored for 48h at room temperature. The drops of glue at the trabecula ends were used for manipulation with the sample using a pair of tweezers. The manipulation droplets of glue were used to attach the sample to the end-plates of the testing device. Fast-setting glue (Loctite Super Attak Ultra Plastik, Henkel Ireland Ltd.) was used for this purpose and the glue was allowed to set for 2 hours prior the experiment at room temperature.

5. FE model development

Finite element model was developed for simulation of the tension tests. Shape of the trabecula was obtained from two orthogonal projections using CCD camera. The geometry of the trabecula was approximated by elliptical cross-sections. The surface was defined by ellipse and orthogonal splines were drawn through the keypoints.

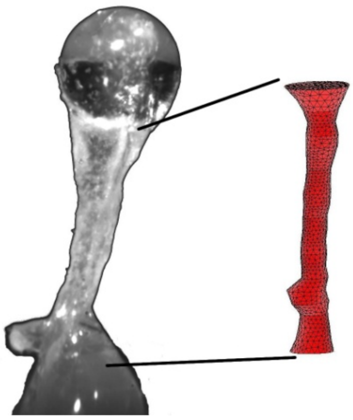

Fig. 2: FE model development

The volume was discretized by 10-node tetrahedral elements with quadratic shape functions. The discretized model is depicted in Fig. 2. Material properties required for the simulation were obtained from nanoindentation tests. The loading was controlled by displacement. Obtained relation between applied displacement and reaction force was compared with results of performed tension tests.

6. Experimental Results

Pre-testing procedure for the uniaxial testing, nanoindentation and finite element modelling of single trabeculae was described in this work. Presented procedures (precise preparation and fixation of micro scale biological specimens) allowed carrying out a unique set of micromechanical material tests of human trabecular bone. It enables to compare the behaviour of single trabecula under physical uniaxial loading with FE simulation of the test.

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