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DIFFERENCES IN TRABECULAR BONE MECHANICAL STRENGTH MEASURED BY THE X-RAY MICROCOMPUTED TOMOGRAPHY AND COMPRESSIVE TEST

The article describes the concept of measurement of the mechanical strength of trabecular bone dissected from human femoral head. When the studies are performed based on the human trabecular bone, there is a risk that correlation can be not achieved due to high discrepancy in the distribution of the bone density and mechanical properties. The compressive tests and X-ray microcomputed tomography with associated density phantom were used to deliver quantitative data. There is a conclusion that the selection of the region in the femoral head has to be performed very carefully. The region where cylinder is dissected can influence the results and produce fake data, which could not be correlated in the density of bone.

1. INTRODUCTION

The X-ray microtomography (XMT) pioneered by Elliot and Dover in 1982 [1] until today there is developed and used in many studies [2, 3, 4, 5]. While XMT is becoming a common technique in the laboratory studies, the standard for quantitative determination of the absolute material density distribution has not been attained.

There is therefore a need to progress this concept in the development and validation of quantitative measurement of the bone density.

As the XMT has to be useful in the quantitative measurement of the density in the human bone biopsy samples, the precise protocol of the standard development had been developed. According to known literature the results from the accurate measurement of the bone mineral density done by Quantitative Computed Tomography (QCT) correlate to mechanical strength [6, 7]. Therefore correlation between results from XMT measurement and mechanical properties should be achieved to prove the usefulness of the method.

When the studies are performed based on the human trabecular bone, there is a risk that correlation can be not achieved due to high discrepancy in the distribution of the bone density and mechanical properties. They are strongly related to the site and region of the human skeleton. As a preparation to the accurate validation of the XMT standard the proper selection of the region of interest has to be achieved.

This paper describes the results of the measurement of the trabecular bone mechanical strength and stiffness in the different regions of the femoral head.

The main goal of the work was to test a method of selection, dissection and preparation of the trabecular bone specimens with associated protocol included XMT scanning and mechanical tests.

2. MATERIALS AND METHODS

The methods of sample preparation were based on the work described by Perilli at all [8]. However some steps were simplified or changed to improve the results and tests different operational options.

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2.1. BONE SAMPLE PREPARATION AND DISSECTION OF THE SPECIMENS.

The sample of bone was taken from the femoral head which was dissected during surgical procedure of total hip prosthesis replacement. The head was stored in the ethanol until the time of the specimens' preparation however it was not longer than two weeks after operation to avoid bone material modification.

The head was glued into the plastic cylinder to enable mounting the sample in the XMT scanner. Based on the projections and/or reconstructed images the mean trabecular direction (MTD) was identified. Next, sample was mounted on the table of milling machine in the position which enabled the extraction the cylinders in accurate angle aligned with the MTD. 9 cylinders were drilled with the same mount of the sample, while cylinder no. 1 was the one oriented just in the middle of the head in point where the MTD is cutting the surface of the head.

Each cylindrical specimen was fixed in the polymethylmethacrylate (PMMA) endcup, which was applied onto one end of the cylinder. The PMMA disc was 3mm high and had 12 mm in diameter with specific mark on site. Such a preparation enabled the repeatable vertical positioning of the specimens in the XMT scanner and compression machine.

2.2. QUANTITATIVE X-RAY MICROCOMPUTED TOMOGRAPHY

XMT scanning was performed for the cylinder no. 1 with application of the own physical phantom of the bone density. The scanner (Benchtop 160, X-tek Metris) settings are given in the table 1. The collected projections were then used to reconstruct the corresponding cross sectional images. The standard scanner manufacturer software was used to perform image reconstruction based on the filtered back projection algorithm. The images were stored as a one raw file in 16 bits unsigned format with size listed in the table 2.

The density phantom was designed as a box with width=12mm, height=3mm and length 40mm suitable for placing into XMT scanner chamber together with the bone specimen. The phantom box has five parallel rods to meet the range of levels of mineral content required for specific bone specimens.

The epoxy resin (polymethylmethacrylate PMMA) was selected as a water equivalent material [$\mu_{PMMA}(30keV)=0.36cm^{-1}$, $\mu_{water}(30keV)=0.38 cm^{-1}$] [9] while bone equivalent material was provided by differing proportions of hydroxyapatite (HA) mixed with epoxy resin.

The phantom was used together with new software, to establish a calibration curve and to calculate the absolute mineral density distributions of the bone microstructure. The calculation was performed using custom software designed by M. Binkowski to perform previous QCT studies [10, 11].

Table 1. XMT settings.

Detector size [pixels]	Voltage [kV]	Current [mA]	Filter [mm]
1900x 1500	65	113	no

Based on calibration curve the software calculated bone mineral density for bone segmented from the reconstructed images. Hydroxyapatite density (HAD) as a calibrated parameter has been calculated.

Table 2. Details of the reconstructed data.

Width	Height	No. of slices	Voxel size [μm]
543	731	1477	25x25x25

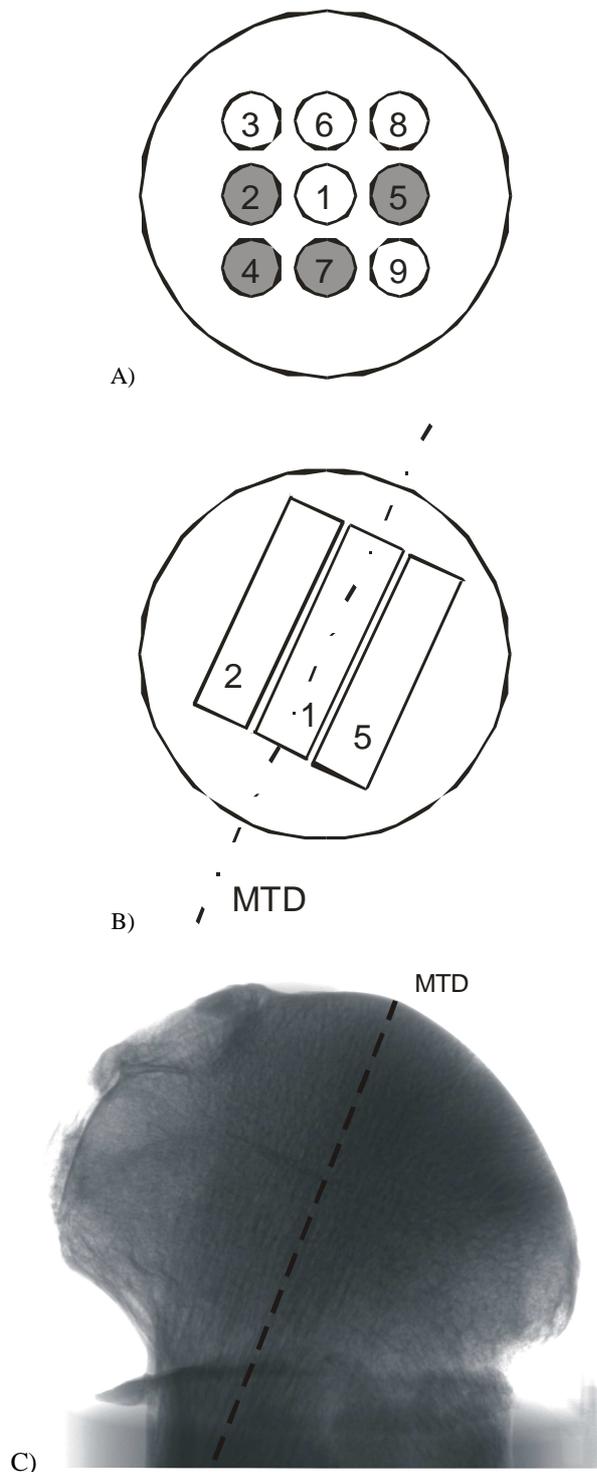


Fig. 1. The schematic view of the position of cylinders. A) top view, the gray circles show the specimens which were not tested due to not appropriate bone material B) left side view C) XMT projection with the mean trabecular direction (MTD).

2.3. MECHANICAL TEST

Some cylinders contained soft tissue like fat and biological material which was not trabecular bone. Therefore from 9 dissected specimens only 5 were in the condition appropriate for the mechanical tests.

The test were performed by servo hydraulic machine (Zwick Z050, MTS), which was controlled by PC and software provided by the manufacturer. The software enables operating with the initial load as well as the speed of increasing load and/or displacement. The experiment was monitored by video

extensometer (Messphysik) (see figure 4), while measurement of the deformation was performed by Digital Image Correlation System (Dantec).

Before test the diameter and length of the cylinders were measured. Those data and the standard force-standard way curve were used to estimate the stress-strain curve (see fig. 3). Based on linear part of that plot the module of stiffness was estimated by linear regression. The results of the measurements and calculations are listed in the table 3. For the whole specimen bone volume fraction (also called as a percent bone volume) was calculated as a function of Bone Volume (BV) and Tissue Volume (TV). The Hydroxyapatite Density (HAD) was calculated based on calibration curve given in the figure 2.

2.4. RESULTS

The plot of calibration curve and the results of the XMT measurement are shown in the figure 2 and table 3. The results of the mechanical tests are shown as a plot in figure 3 while the details of the mechanical measurement are shown in the table 3.

Mechanical parameters like Young modulus (E) and Maximum Force (Fmax) are well correlated ($R^2= 0,941$, $y=0,579x-159$). Those parameters were estimated for 5 different cylinders dissected from different region of the femoral head, while results from XTM were calculated only from specimen no. 1, (see table 4).

Table 3. Results from the XMT measurement.

	Mean \pm SD
BV/TV (%)	10.49 \pm 1.49
HAD [g/ccm]	0,773 \pm 0,011

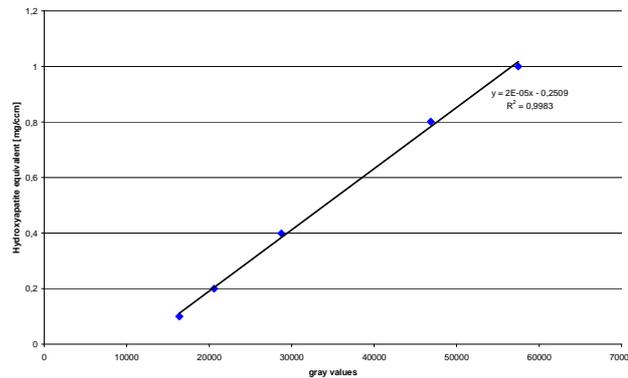


Fig. 2. Calibration curve estimated based on density phantom.

Table 4. Details of the tested cylinders, and results of mechanical tests⁴.

Num.	d [m]	lo [mm]	Fmax [N]	E [MPa]
1	9,35	33	1807	945
3	8,7	18	433	134
6	9,4	25	1000	307
8	9,3	19	651	155
9	8,9	19	419	160

⁴ Cylinders no. 2,4,5,7 were not sufficient for mechanical test .

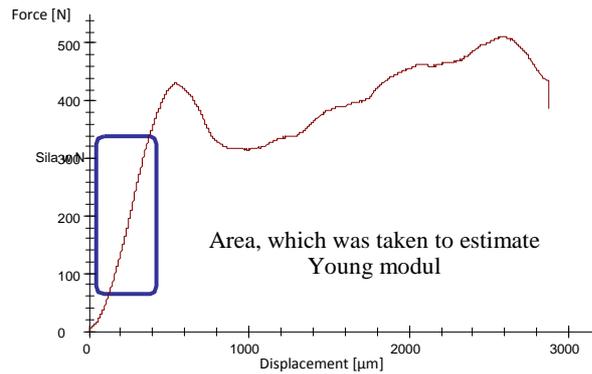


Fig. 3. Plot shows the example of the loading curve of the cylindrical trabecular bone specimen.



Fig. 4. The sample with the setup to compression test monitored by video extensometer.

3. CONCLUSIONS

In this studies mechanical strength of the trabecular bone has been measured in different location of the femoral head. The bone mineral density estimated by X-ray microcomputed tomography has been performed by applying the standard XMT and associated density phantom..

One particular case of mechanical test was illustrated by plot given in fig. 3. The linear part of the plot shown on that figure represents the state of the specimens where according to Hook's law sample is elastic. The maximum load can be observed further on that plot where load is equal about 420 N. The specimen collapse after that point and finally was broken and it was happen for displacement equal to almost 3000 microns.

The whole process of the dissection, gluing in the PMMA disc and XMT and mechanical tests were performed successfully.

We do conclude that the selection of the region in the femoral head has to be performed very carefully. The region where cylinder is dissected can influence the results and produce fake data, which could not be correlated in the density of bone.

The significance of this study could be improved if more sample of femoral head are tested using the all 9 cylindrical specimens scanned by XMT. However based on described case it can be concluded test a method of selection, dissection and preparation of the trabecular bone specimens with associated protocol included XMT scanning and mechanical tests has been successfully verified.

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