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Experimental Stress Analysis

Creating a Model of Cancellous Bone Tissue

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Abstract: The present paper deals with the creation of a model of cancellous bone tissue at the level of trabecular structure with focus on experimental determination of material characteristics for the given level of the model. An effective method of determining the material characteristics of cancellous bone is the use of imaging medical devices (a computer tomograph) i.e. on the basis of pixel intensity in the images. This enables us to determine the tissue density, which is consequently converted, using the existing correlation relations, to the modulus of elasticity. For more detailed description of the architecture, it is necessary to use the data from a micro CT or the images from the cut-off bone sample.

Keywords: Experimental, Image Processing, Computer Tomograph, micro CT

1. Introduction

A very effective tool to solve the problems of biomechanics is computational modelling that uses the method of finite elements [1, 2]. Particular models used to solve the problems of biomechanics of human can be divided into the models of geometry, material, bonds and loads. Currently it is not a problem to obtain a relatively accurate geometry of bones, teeth, arteries, veins and other intricate parts of human body, even in a three-dimensional form. It is possible to use computer tomography, and with the use of image processing methods [3, 4], or three-dimensional optical scanning to consequently process the obtained data in some of commonly available CAD software [5]. A frequently discussed problem of present time is, inter alia, determination of material characteristics of living tissues. When designing computational models of muscular and skeletal system, the most problematic area is a material model of cancellous bone tissue. Spongiosa is an internal component of the bone resembling a porous spongy structure that consists of trabeculae. Morphology and distribution of the individual trabeculae depends on mechanical load whereas the trabeculae are placed in the direction of the highest load [6]. Determination of mechanical quantities characterising the material of the bone is much more demanding than with common engineering materials. It was found out that material characteristics of spongiosa depend, inter alia, on its apparent density [7 - 9]. A correlation between the modulus of elasticity and apparent density was proved and described in the literature [8, 9]. There exists a number of methods of how to experimentally determine material characteristics, e.g. by destructive methods of tensile/compression test [10, 11] or by strain gauges [11] where the size of samples should be larger than 5 mm and they should contain minimum of 5 trabeculae. The results of measurements of various authors are very different because the cancellous bone is considerably porous. In addition, in vitro

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samples have different properties compared to in vivo samples (it is known that preservation of samples considerably changes mechanical properties [10]). Non-destructive tests are e.g. ultrasound measuring of material characteristics [12]. However, far more effective is the use of medical imaging devices, the so-called computer tomographs. For creation of even higher level of material model respecting an accurate architecture of cancellous bone, it is necessary to obtain the data from a micro CT or by experiment using images from cut-off layers of the bone sample.

2. Determination of mechanical properties of bone tissue from CT images

One of the commonly used examination methods in medicine is computer tomography - CT. By CT it is e.g. very easy to perform densitometric examinations of bone tissue [13] e.g. prior to insertion of dental implant. CT images enable us to measure the intensity of pixels, the so-called CT numbers, and these are converted to HU (Hounsfield Units) (1) [8], on the basis of which it is possible to identify the respective tissues.

$$HU = 1000 \cdot \frac{CT - CT_w}{CT_w - CT_a} \tag{1}$$

The conversion is based on the calibration of CT; the reference values are CT- number of water (CTw) and air (CTa). Reference values CTw = 1000 and CTa = 0 correspond to the values HU = 0, or HU = -1000. By the use of these reference values it is further possible to set a particular value of HU for the respective tissues.

Hounsfield units are used for quantification of bone tissue density because each tissue of the human body has its own characteristic value of HU. A basic division of HU spectrum is referred to in Fig.1 where the respective levels of HU are described by a given shade of grey. The highest values of HU are characteristic for tooth enamel (+3000). Within the HU scale, bone tissue ranges between ca. +150 and +2000.

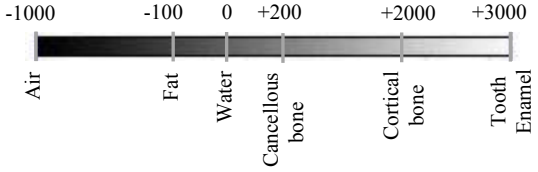

Fig. 1. Hounsfield units with gray scale

For experimental determination and conversion of pixel intensity to HU units, a team of authors from the Institute of Solid Mechanics, Mechatronics and Biomechanics (UMTMB) created the program *ROI Analysis* [14]. This program enables us to display CT images or their selected parts in the form of isosurfaces that express a constant level of HU and automatic determination of mean value of HU in the defined region (ROI region of interest) including a standard deviation that, due to the presence of air in the pores, obtains a relatively high value. This is necessary to take into account during the analysis. Fig. 2 illustrates the example of study of hip joint and pelvis. In the first region (ROI 1) the quality of bone is relatively high (296.2 ± 94 HU), because thee condyles and heads of long bones generally have sufficiently high density of cancellous bone tissue. In the pelvis bone (ROI 2) the density of bone is almost three times lower (113.1 ± 94 HU) than in the head of the hip. Fig. 3 describes the determination of bone quality in HU units in the transverse section of vertebra L4. In this

region the measured mean value was 117.9 ± 57 HU. Next, HU units are drawn for the entire vertebra that shows a clear difference between the cancellous and cortical bone. Moreover all images are accompanied by 3D graphs for better presentation of measured regions.

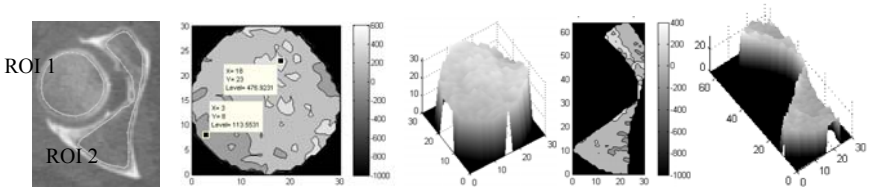

Fig. 2. Example of analysis of cancellous hip and pelvis bone

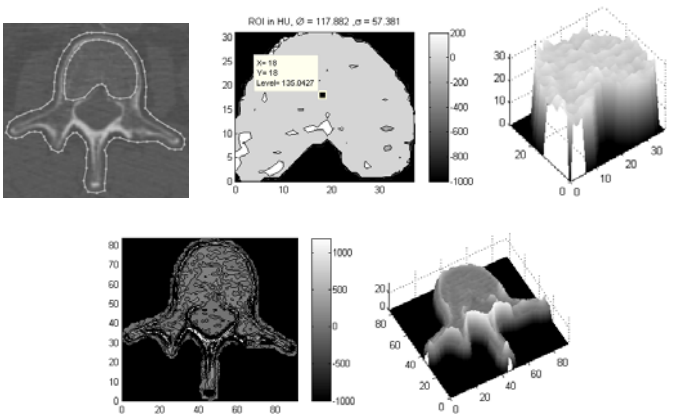

Fig. 3. Example of analysis of vertebra

A number of studies confirm a correlation between HU and Young modulus of elasticity, which is very important for the creation of the material model. For different bones there exist relations between apparent density ρ, modulus of elasticity E and Hounsfield units HU, due to which it is possible to convert the acquired HU matrix to the matrix of modulus of elasticity of bone tissue. E.g. the equation (2) provides relations for determination of density and modulus of elasticity of spongiosa of the mandible [8], [15]. For this purpose, UMTBM developed the software *CT Data Analysis* [16], which reads CT images and after having defined the respective regions it exports the matrixes with CT numbers or HU units for the respective pixels in the selected region. On this basis it is possible to create a material model respecting the density distribution of cancellous bone tissue in the respective pixels in a CT image (Fig. 3).

$$\rho = 1.205 \cdot HU + 139, \qquad \mathrm{E} = 2.349 \cdot \rho^{2.15} \tag{2}$$

Fig. 4 displays computational models of 2D segment of the mandible with non-homogenous isotropic material model of cancellous bone. The value of modulus of elasticity in the respective points can be identified according to the range of greyscale. The two dependences between a CT number and modulus of elasticity E were taken into account. 1. Linear - Fig. 4 on the left where, from the known value of CT number for the cortical bone, we can calculate the modulus of elasticity (the so-called pixel with the highest intensity has E = 13700 MPa [17] and Young modulus is linearly decreased in dependence with the pixel intensity), 2. Non-linear - Fig. 4 on the right where the dependence between the density and modulus of elasticity is expressed by relations (2).

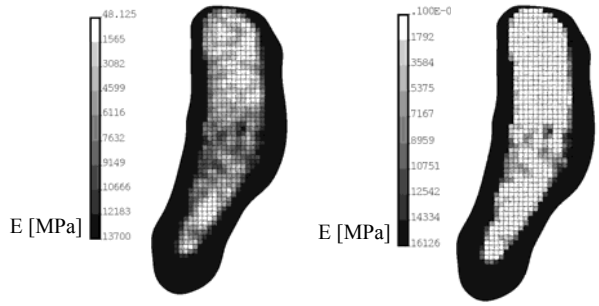

Fig. 4. Non-homogenous isotropic material model of cancellous bone in mandible

The advantage of determination of material model of cancellous bone tissue from CT is the possibility to solve specific problems of biomechanical character with a particular patient. Measuring of HU units and their conversion to bone density enables an effective, fast and easy performance of basic diagnostics of bone tissue.

3. Acquisition of experimental data of cancellous bone

A higher level of modelling of cancellous bone is represented by a structural model that respects the internal structure and distribution of the respective trabeculae. Therefore, as previously, we do not measure an apparent modulus of elasticity that is assigned to the given "non-trabecular" model of geometry. This does not respect the real architecture of spongiosa. Creation of trabecular model of cancellous bone tissue is, in our opinion, more important and is, without any doubt, more convenient especially for the analysis of dental implants. However, creation of such a model is much more demanding. It needs powerful computer technology, appropriate software and first of all input data. These can be obtained from micro CT equipment or experimentally by cutting off thin layers of bone embedded in a hardening substance and by gradual photographing. As a most appropriate embedding medium, which very well leaks into the tightest holes, we selected an epoxy resin (the authors used a similar procedure in their works [18], [19]). For better contrast of bone tissue, epoxy has to be coloured. Colouration must be sufficient so that after the removal of the bone layer we can only see its structure on the slide. In case the epoxy is not sufficiently coloured, the bone can shine through. As the most appropriate additive for colouration we selected (after completion of a series of testing experiments with black, aqueous colouring agent, crushed graphite and coal) a fine dry toner powder (see Fig. 5). This powder is industrially produced and achieves far greater fineness than the above-mentioned crushed additives that, when used, did not provide sufficient colouration so that the bone shone through also outside the slide. The use of additives for colouration often prevents the sample from stiffening.

3.1. Sample preparation

For removal of impurities and bleaching of bone, the bone segment is (see Fig. 5) first placed for the period of 48 hours into a 30 % hydrogen peroxide solution. The bone is consequently embedded in a mixture of epoxy resin (70%), toner powder (23.5%) and hardener (6.5%). The mixture is heated up to 70°C, because higher temperatures provide a substantially more pourable mixture. Next, the sample is placed into a vacuum pump from which the air is sucked off (see Fig. 5). Due to underpressure, the air is also sucked off the pores between the individual trabeculae, and the coloured epoxy leaks in. It is necessary to suck off carefully to avoid boiling of epoxy under the pressure nearing the pressure of vacuum.

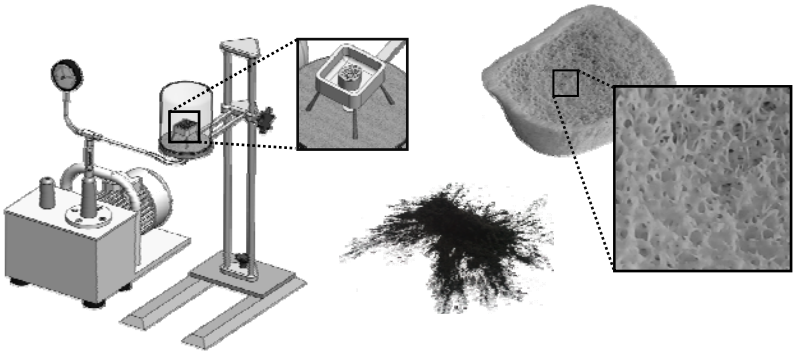

Fig. 5. Vacuum pump, toner powder and bone segment from the region of tibial condyle

3.2. Acquisition of images

The sample is gradually, layer by layer, milled and the individual layers are photographed. Prior to milling, the sample of bone with epoxy should be hardened for approximately 24 – 30 hours. In the work [20] our team conducted a sensitivity analysis of the layer thickness using the images from a micro CT. The results showed that after the removal of layers it is necessary to obtain the images of maximum thickness of 0.05 mm. Each of the milled-off layers is photographed with a camera fixed to the knee of the mill. To obtain the images, a digital camera Canon EOS 40D and an object lens PENTACON 1.8/50mm MC shutter f/5.6 were used, where the depth of image definition is at a given configuration of at least 4mm, which is sufficient for the required number of removed layers; in our case, the thickness of the segment was 3mm. The camera is equipped with CMOS sensor (22.2 x 14.8 mm, 3888 x 2592 px, pixel size u 5.7 μm) fitted with colour Bayer mask. Scanned data are stored in Canon RAW format CR2, and consequently, using the program UFRaw [21], all images are converted into the format PNG in greyscale.

To increase the contrast between the bone tissue and the embedding mixture, the following procedure was used. Embedded samples are illuminated by light from UV lamps. It is the light with maximum energy emitted in the region of the spectrum corresponding to UVA (λ = 315-400 nm, FWHM = 20 nm) with a certain overlap into the visible part of spectrum (violet-blue). Within this spectrum, the bone tissue behaves UV-actively. Hydroxyapatite contributes to this. Fluorescence occurs and UV light absorbed by bone tissue is then emitted in the visible region of the spectrum. The presence of light from the visible part of spectrum (violet-blue), which can be registered by CMOS sensor, however decreases the contrast. The increase in contrast is achieved by placing a filter in front of the camera. This filter must be able to retain as much light as possible in the violet-blue part of spectrum. Therefore it must have an additional colour that would fit violet-blue; the closest in this case is the yellow filter (Wartten #8, specifically used Carl-Zeiss G2 [22]). The impact of this filter on contrast is illustrated in Fig. 6.

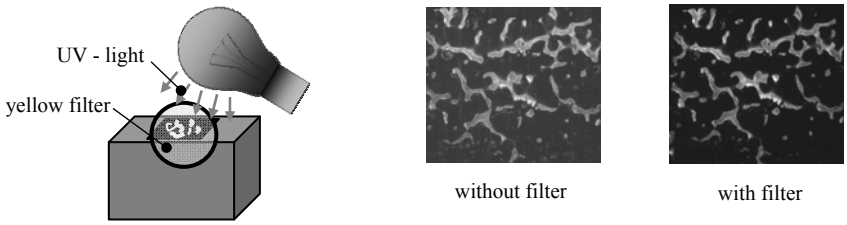

Fig. 6. Principle of taking images and using a yellow filter

This procedure enables us to sufficiently increase the contrast between the bone tissue and the embedding mixture and it also partially enables us to remove imperfections when embedding the samples (first of all "invisibility" of possible bubbles in embedding mixture). Placing an UV pass filter between the source of light (UVA+violet-blue) and the illuminated sample might further increase the contrast. The UV pass filter (Wratten #18A) would block the light in the visible part of spectrum and release only UVA radiation. The bone tissue would therefore repeatedly fluoresce but the embedding mixture would not be irradiated by the violet-blue light; therefore the yellow filter would not be necessary. The camera sensor is not able to register radiation within the UVA region; therefore the image would only display a fluorescent bone tissue.

3.3. Image segmentation and creation of polygonal network

To create a model of trabecular structure, software *STL Model Creator* was used [23]; it offers functions for hybrid segmentation, and from a series of images it can create a model of geometry in the format of polygonal network. Segmentation is an operation where we select the data that share common properties or belong to one tissue (see Fig. 7.). As the bone is due to the previous procedure sufficiently contrasting, a threshold filter would be sufficient for segmentation of the respective images. The interval of all pixels will be split on the basis of their intensity. Pixels with highest intensity correspond to the bone tissue while those with lowest intensity correspond to pores. An important parameter for development of the model of geometry itself is calibration of pixel size. This is performed on the basis of the attached scale. The size of pixel or voxel (a 3D pixel) is in our case 0.026x0.026x0.02 mm; altogether the development of the model of thickness of 3mm required 150 images (see Fig. 7).

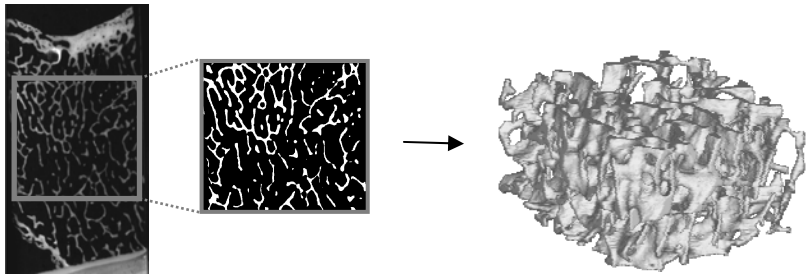

Fig. 7. Image segmentation and 3D model of geometry of cancellous bone.

4. Conclusion

The aim of this work was to show the possibilities of experimental determination of material characteristics of cancellous bone tissue. First, conventional CT images were used; these enable a very easy diagnostics of bone tissue using the software *ROI Analysis* a *CT Data Analysis* and also the development of computational models for FEM analyses. The following part of the work dealt with the development of the model respecting the distribution of trabeculae at micro level; the most frequently used are images from a micro CT. Domestic research institutions do not have access to this device; therefore it is necessary to acquire the images experimentally and in this way to replace a micro CT. Using these images enables us to conduct consequent FEM analyses on computational models representing a maximum possible level of computational modelling of cancellous bone (see Fig. 8 on the right).

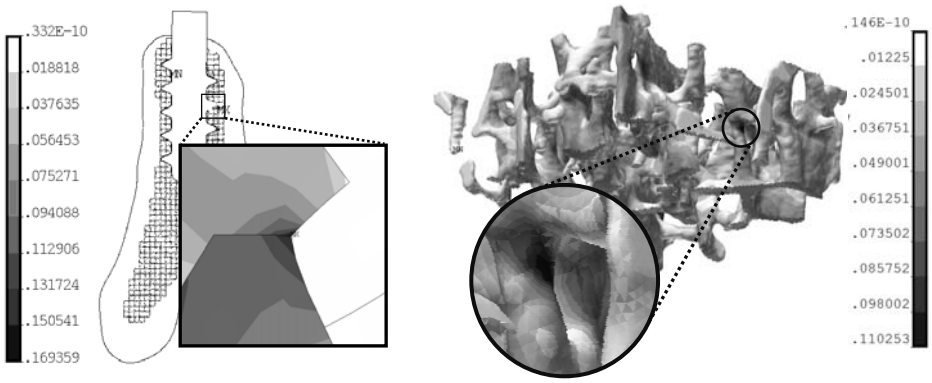

Fig. 8. Examples of FEM analysis (drawn intensities of strain)

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