

Micro Computer Tomography (µCT) Set-Up for Long-Term Serial Measurements, Image Evaluation, and Subsequent Compressive Strength Tests on Frozen Sheep Vertebrae for Osteoporosis Research.

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Abstract. Injection of poly(methyl methacrylate) cements, one standard treatment for osteoporotic vertebral body fractures, may lead to critical loads and subsequent fractures in adjacent vertebral bodies. Biodegradable calcium phosphate cements (CPC) with bioinductive growth factors may be an alternative, since they have a Young's modulus comparable to that of cancellous bone.

Non-destructive tests with μ CT and quantitative image evaluation are used to assess new bone growth and material resorption following intravertebral injection of CPC. Immediate deep-freezing of excised bone prevents shrinkage or tissue disintegration and the samples have to be kept frozen for all following steps, including transport, μ CT measurements, and subsequent biomechanical tests. Here we will report on a set-up to preserve the frozen state of the material and allow stable long-term serial μ CT measurements. In addition, the image processing technique for the evaluation of bone growth and selected results on subsequently carried out compressive strength tests will be presented.

Introduction

In an older growing society the diseases related to osteoporosis or bone degradation will become a severe problem. Artificial bone replacements (with polimethyl methacrylate) cements, which is a standard treatment of today, have the disadvantage that the physical parameters, like the strength and hardness, are not in the same range as for natural bones. This will result in an overload of the neighbouring bones, which may fail subsequently. Biodegradable replacement material has the charm of vanishing with time and resulting in a bone as new. To emphasize the growing of new bones a bio-inductive growth factor is needed. These substances are active in stimulating the growth of cells. As they are mixed to the cement, they should directly alter the growing rate of bones. New ideas have to be tested first to animals. The large animal model the "Eisenberg" approach proposed [1] has the advantage to be directly comparable to the human case, as the invasive procedure is the



same. Popular science publications [2] still recommend the additional use of calcium and vitamin D to prevent osteoporosis. In these studies the success is linked to the number of femoral neck fractures, neglecting e. g. the effect of mussel strengthening by vitamin D. To get a quantitative comparison, the development of bone structures has to be controlled. Micro-CT is the first step to get a non-destructive insight, but in the next the image processing is needed to evaluate quantitative properties. The last step is the biomedical strength test.

Detailed medical results will be published elsewhere [6-8].

Micro-CT



Fig. 1. Dry ice cooled samples. Left total view, right insight to cool box.

Investigation of biological samples has the disadvantage that they rapidly degrade, shrink or even may crack while drying. In most cases such dissected body parts are stored either completely sealed in casted plastics (for further cutting and grinding) or in conserving liquids. Both variations have severe disadvantages, at least in respect to CT. At first voids will be filled with material and thus lowering the (density) contrast, secondly the added material may have a comparable attenuation coefficient to the tissue material and thus be no longer separable. And, third, the total attenuation will be higher resulting in a poorer photon statistics. From the medical science point of view the conserved material is no longer available for further physical tests, as the properties might have changed.

Thus immediate deep freezing comes into account. If you freeze fresh biological material covered in a thin air tight plastic envelop, it will stay in the same condition for quite a while. CT images will have the same density and spatial resolution as the original sample. There have been proposed several systems for gas flow cooling or cooling by Peltier elements. Both need additional cabling on the turning CT table and controlling. We used the very simple approach of dry ice in a Styropor (Fig. 1). The cooling chamber consists of several slices of Styropor, starting with a 50mm high slice at the bottom. Next slice contains four samples leaving a wall thickness of at least 50mm. next slice is 10mm in height but contains holes of 5-10mm in the sample area. Next again is a slice containing samples, covered by perforated but thicker slice. On top 50mm high container for the dry ice chamber is filled by crashed ice. Some small parts will drop down and immediately cool the samples. The ice particle may survive for a certain time during measurement, but will not be reconstructed nor lead to artefacts. The heavy lead stone on top keeps the assembled box closed and everything at the same position. As the cool gas is falling down and the change

from solid to gas produces a large volume, all parts of the box are slightly pressurised and continuously cooled.

In the case of serial measurements, which may be spread over month, there should be no shift in any of the measured quantities; the system has to be stable. What grants this? At first the climate in the room has to be controlled. We recommend a temperature change less than +-0.5 degree. During summer 2015, the outside temperature caused an unregulated excess of room temperature. During this period occasionally a concrete sample was measured several times. From the measured diameter the magnification of the shown CT system changed from 6.498 to 6.503 within three hours, but relaxed to the original value as the air cooling recovered [3]. This was a change of less than 0.1%, but on a 2k wide pixel detector this results in an additional pixel to the sample on both sides.

To prevent changes in X-ray spot position, both target and focussing coils have to be cooled and stabilized to a fixed temperature. In our set up we use a large flat panel detector of amorphous silicon. This must be temperature controlled to better than +-0.1 degree. If not the base line will shift and apparent attenuation will vary with temperature. The stability and the reproducibility of measured densities have been proven in a two year lasting project meanwhile more than five hundred undisturbed soil samples were measured and the roots could be segmented just reckoning the grey value range [4].

As a thumb rule, the micro-focus spot is assumed having a diameter in μ m as energy in W. Investigations have shown that X-ray spot size and form may vary with the used kV setting, tubes from different manufactures may have spot sizes not obeying this rule and the X-ray output measured in Sv (Sievert) may vary largely between tube systems [5]. The knowledge of these parameters is essential and will help in getting high quality and long-time stable images.



Technical details

Fig. 2. Choice of X-ray spectrum. https://w9.siemens.com/cms/oemproducts/Home/XrayToolbox/spektrum/Pages/radIn.aspx

Our standard μ CT (see fig. 1.) is assembled from the original mechanics by "Isotopentechnik Sauerwein" (today www.rayscan.eu), a "PerkinElmer" 1621 detector (www.perkinelmer.de), and a CT optimized 225kV "X-ray WorX" (www.x-ray-worx.com) μ focus X-ray tube. From the calculated spectra we choose the following parameters for this study: 100kV, 380 μ A, 0,5mmCu pre-filter. Sequences of 6 shadow image of one second duration were averaged. 3000 image during a stepwise rotation over 360 degree, each having a size of 2031 x 1801 pixels were taken. The source to detector distance was 1169.59mm while the source to object distance was 389.37mm. As we are using a

rectangular 400mm wide detector, we are not obeying the rule of Mr. Feldkamp (the opening angle must be less than "15degeree in total") in his algorithm, which we use for reconstruction. But this may be negligible, because we are using not the full height, but only 1801 lines. Fig. 3 gives an example of the resultant images. As μ CT on non-living objects are not dose limited an excellent spatial and density resolution results. The right



Fig. 3. Slice of four arbitrary samples. Image reduced to 0.25 actual size.

side of fig. 3 shows, that the labels printed by a laser printer can be read in the tomographic image.

Image processing

Quantitative analysis of the μ CT data was carried out using the 3-D software VGSTUDIO MAX 2.2 (Volume Graphics GmbH, Heidelberg, Germany) and applying half-cylinders with a radius of 2.5, 3, 3.5, 4, 4.5, and 5 mm (see Fig. 4 (a) and (b)). The longitudinal axis and length of the half cylinders for each individual vertebral body were defined on the basis of the respective parameters of the drill channel. The exact 3-D position of the half cylinders (e.g., rotation and depth) was chosen to exclude cortical bone. Total volume (TV), bone volume (BV) and cement volume (CV) were separately determined by global threshold determination in the drill channel (Fig. 5; radius of 2.5 mm) and the adjacent half cylinder segments (principle of onion shell). For this purpose, the respective maxima of the grey values for soft tissue, bone tissue, and cement were determined and the means between the maxima were used as a threshold for the volume determination of the individual components. Bone volume/total volume (BV/TV) and cement volume/total volume (CV/TV) were subsequently calculated.



Fig. 4. Schematic representation of the half-cylinders with a radius of 2.5, 3, 3.5, 4, 4.5, and 5 mm used for quantitative evaluation of the μ CT data.



Fig. 5. Determination of total volume (TV), bone volume (BV), and cement volume (CV) in the drill channel with a radius of 2.5 mm.

At 3 months, quantitative evaluation of the μ CT data revealed an increased bone volume/total volume as compared to untreated controls (L1) in vertebral bodies with an empty defect (L2) up to a distance of 1 mm from the original defect. In contrast, the implantation of pure CPC (L3) enhanced bone formation up to a distance of at least 2.5 mm.

Compressive strength testing

Lumbar spines were sawn into individual vertebrae (L1, L2, L3, and L4); the spinous and transverse processes, as well as the covering and base plates, were removed from all individual vertebrae (final height in each case 15 mm). Biomechanical compressive strength measurements were conducted using a universal material testing machine (FPG 7/20-010TM; Kögel, Leipzig, Germany) and the corresponding software (FRK Quicktest 2004.01TM). For biomechanical testing, frozen cancellous bone cylinders (10 mm diameter x 15 mm height) were obtained from the central part of the vertebral bodies (L1-L4) using a surgical diamond hollow milling cutter (diameter 10 mm). After a defined thawing time of

exactly 30 minutes, samples were semi-confined in 2 semilunar clamps (minimal inner diameter of 10.1 mm; length 9.8 mm) and then compressed along their longitudinal axis until fracturing. This axis was chosen because it represents the main loading axis in humans and is therefore of major interest for future clinical application.

At 3 months, no differences in the biomechanical compression strength of the differently treated vertebral bodies were observed.



Fig. 6. Bone volume (BV/TV) in differently treated vertebral bodies of female merino sheep 3 months after surgery.



Fig. 7. Left: Schematic illustration of the sample preparation steps for mechanical testing. Right: Biomechanical compressive strength of cancellous bone 3 months after surgery in differently treated lumbar vertebral bodies.

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