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Time-lapsed investigation of three-dimensional failure and damage accumulation in trabecular bone using synchrotron light

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Abstract

Synchrotron radiation micro-computed tomography (SRμCT) is a very useful technique when it comes to three-dimensional (3D) imaging of complex internal and external geometries. Being a fully non-destructive technique, SRμCT can be combined with other experiments in situ for functional imaging. We are especially interested in the combination of SRμCT with mechanical testing in order to gain new insights in the failure mechanism of trabecular bone. This interest is motivated by the immense costs in health care due to patients suffering from osteoporosis, a systemic skeletal disease resulting in decreased bone stability and increased fracture risk. To better investigate the different failure mechanisms on the microlevel, we have developed a novel in situ mechanical compression device, capable of exerting both static and dynamic displacements on experimental samples. The device was calibrated for mechanical testing using solid aluminum and bovine trabecular bone samples. To study different failure mechanisms in trabecular bone, we compared a fatigued and a non-fatigued bovine bone sample with respect to failure initiation and propagation. The fatigued sample failed in a burst-like fashion in contrast to the non-fatigued sample, which exhibited a distinct localized failure band. Moreover, microscopic cracks – microcracks and microfractures – were uncovered in a 3D fashion illustrating the failure process in great detail. The majority of these cracks were connected to a bone surface. The data also showed that the classification of microcracks and -fractures from 2D section can sometimes be ambiguous, which is also true for the distinction of diffuse and distinct microdamage. Detailed investigation of the failure mechanism in these samples illustrated that trabecular bone often fails in delamination, providing a mechanism for energy dissipation while conserving trabecular bone architecture. In the future, this will allow an even better understanding of bone mechanics related to its hierarchical structural organization.
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Keywords: Synchrotron light micro-computed tomography; Bone biomechanics; Image-guided failure assessment; Microfracture; Microdamage

Introduction

Micro-computed tomography (μCT) is an important and still relatively new technique in the fields of biomedical engineering and materials science to visualize internal and external geometrical structures directly in three dimensions (3D). Conventional laboratory equipment delivers high fidelity 3D datasets with nominal spatial resolutions of about 10 μm at acceptable measurement times [40,41]. For higher spatial resolution tomography experiments, synchrotron radiation sources can be used at dedicated beamlines around the world [4,45,50]. Synchrotron radiation sources offer a much higher brilliance compared to X-ray tubes, such that the investigation of samples at the micro- and even submicrometer level becomes feasible. Moreover, the high brilliance of the synchrotron light allows the use of monochromatic radiation, i.e., the selection of a single photon energy. Monochromaticity avoids beam hardening and therefore turns a reconstructed tomogram in a quantitative mapping of X-ray attenuations. With the standard setup of most experimental stations spatial resolution of about

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1 μm can be reached, where the limit is due to the finite thickness of the scintillating screen [21]. Special X-ray optics can be used to overcome also this limit and resolution down to 100 nm have been reported [43,44]. Besides the most commonly used absorption contrast also phase contrast can be applied for imaging using coherent synchrotron light [5,10], which is especially advantageous for imaging of specimens composed of light elements [6]. Being a fully non-destructive and non-invasive technique, μCT and synchrotron radiation μCT (SRμCT) can be combined with other experiments in situ for functional imaging [20,22]. SRμCT is the ideal instrument to investigate failure in complex opaque structures in a truly 3D manner with micrometer spatial resolution [2,12].

In this study, we are especially interested in the combination of SRμCT with mechanical compression testing to gain new insights in the failure mechanism of trabecular bone. We aim to improve our understanding of osteoporosis; a systemic skeletal disease characterized by low bone mass and architectural deterioration of the bone tissue resulting in decreased bone stability and an increase in fracture risk [18,28]. The disease occurs mainly in post-menopausal women and the aged but is also relevant in certain younger populations. Trabecular bone is responsible for force transduction from joint faces onto the compact bone surface and vice versa. Moreover, trabecular bone in the lumbar spine carries up to 90% of the exerted force. Therefore loss of trabecular bone and/or the impairment of its mechanical properties increase local stresses and strains and thus fracture risk. The World Health Organization (WHO) defines osteoporosis as a difference in bone mass of 2.5 standard deviations from a healthy young normal [51]. This definition implies that bone stability can be directly correlated to bone mineral density, which is true for large populations [19,27,39,52], but fails to be accurate for an individual patient. In fact bone density alone accounts for 10 to 90% of the variation in trabecular bone strength on an individual basis [9] leaving 90 to 10% of the variation unexplained. Where in the last decade mainly densitometry [14,25,26] was used for diagnosis of bone strength and osteoporosis, it becomes more and more clear nowadays, that also micro-architecture as well as local bone tissue properties should be taken into account for accurate diagnosis and understanding of the competence of bone. Until recently it was only possible to investigate bone failure in the post hoc analysis of two-dimensional bone sections, studying fracture patterns [8,11,29,36] or micro-damage [1,7,23,37,42]. These investigations uncover a wide range of features, however, they all are destructive and deliver only 2D data. In contrast image-guided failure assessment (IGFA) being a new tool for the non-destructive 3D imaging of bone fracture can be applied at different time points during the failure process [30,31,34]. Previous IGFA experiments on bone were conducted on desktop tomography systems were used and thus nominal spatial resolutions in the order of 10–50 μm were achieved.

Here, we present a novel in situ mechanical compression device (IMCD) developed for the purpose of combining mechanical tests with concomitant 3D synchrotron imaging, to assess spatial resolutions in the 1 to 10 μm regime. We describe the design of the IMCD and present data to validate it for mechanical testing. We also present first results from an exemplary IGFA study on trabecular bovine bone.

Materials and methods

IMCD design

The principle design of the IMCD was based on a previous development: the micro-compression (MCD) device [34], which was used in a desktop scanner for IGFA of trabecular bone specimens and other cellular materials [35]. The main similarity between the IMCD and the MCD is the fact that the core piece of the device is a hollow radiolucent tube, housing the sample and functioning as an abutment for the load imposed on it. The sample can be kept in a hydrated environment. The IMCD was developed to perform in situ loading experiments at the Materials Science (MS) beamline of the Swiss Light Source (SLS) [53]. 3D design drawings, as well as a photograph of the device in operation of the IMCD are shown in Fig. 1. The device was specifically designed to house cylindrical wet and dry bone samples of 4 mm height and 3.7 mm diameter, but can be modified to hold a variety of sample geometries. As can be seen in Fig. 1, the IMCD consists of three major parts: the sample chamber, the actuator housing, and the mechanical guides. The sample chamber, located in the lower part of the device, is made out of brass and a high-strength radiolucent poly-amid-imide tube (Torlon 4203, Solvay S. A., Brussels, Belgium). The whole chamber is directly screwed onto the tomography sample handler [53]. The actuator housing and mechanical guides are in the upper part of the device and are connected via a bearing. Once the sample chamber is screwed to the actuator housing they can both rotate as one unit around the fixed guides together with the turntable of the sample handler. Force is exerted on the sample using a piezoelectric actuator (PI P-246K020, Physics Instruments GmbH, Karlsruhe, Germany), which is capable of exerting 300 μm displacement with an accuracy of 0.1 μm. The temporal stability of the prescribed displacement is guaranteed by the internal closed loop displacement measurement system of the actuator. The actuator can be used to apply static and dynamic displacement on the specimen located in the sample chamber. The force sensor is directly mounted on top of the actuator pin. Two load cells are currently available for the device with ranges of 100 N (Burster 8413-100, Burster Präzisionsmesstechnik GmbH and Co. KG, Gernsbach, Germany) and 500 N (Burster 8413-500) and accuracies of about 0.5 N and 2.5 N, respectively.

Displacement of the load cells due to their own compliance was measured in calibration experiments, which was then used to correct the acquired displacement data. The piezoelectric actuator was controlled using a high voltage amplifier (Physics Instruments GmbH, Karlsruhe, Germany). The whole IMCD setup was controlled with a PC and control software programmed in LabView (National Instruments, Austin, Texas, USA).

Device validation

Specimen Preparation: To validate the IMCD as a mechanical testing device engineered and biological materials were used. The engineered samples were solid aluminum cylinders, 4 mm in height and 3.7 mm in diameter (alloy AA6082). Since the samples were all from the same batch they should exhibit very similar mechanical properties. The biological samples were trabecular bovine bone cylinders (age unknown). For mechanical testing, slabs were cut from a distal bovine femur using a FK23 band saw (Bizerba GmbH and Co. KG, Balingen, Germany), such that the slab surfaces were perpendicular to the principal loading axis. Using a diamond trephine (MedArtis AG., Munich, Germany) and a Promac 212 drill press (Tooltek Co. LTD., Taichung Hsien, R. O.C.) cylindrical bone specimens of 3.7 mm diameter were cored from the slabs. With an Isomet 5000 precision blade saw (Bühler LTD., Lake Bluff, Michigan, USA) the cores were cut to 4 mm long samples. During the whole preparation procedure the bone samples were constantly irrigated with phosphate buffered saline (PBS) (pH 7.4), except for the cutting with the precision saw where pure water was used to cool them and minimize alteration of bone properties. Subsequently, bone marrow was extracted from the cylinders using 0.2% handsoap (Johnson Diversey, Sturtevant, USA) in

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