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Cortical bone elasticity measured by resonant ultrasound spectroscopy is not altered by defatting and synchrotron X-ray imaging

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ABSTRACT

In the study of mechanical properties of human bone, specimens may be defatted before experiments to prevent contamination and the risk of infections. High energy synchrotron radiation micro-computed tomography (SR-μCT) is a popular technique to study bone microstructure. However, little is known about the effects of defatting or irradiation during SR-μCT imaging on different elastic coefficients including shear and longitudinal moduli in different anatomical directions. In this work, these effects are evaluated on a set of 24 samples using resonant ultrasound spectroscopy (RUS), which allows one to accurately measure the complete set of elastic coefficients of cortical bone non destructively. The results show that defatting with diethylether and methanol and irradiation up to 2.5 kGy has no detectable effect on any of the elastic coefficients of human cortical bone.

1. Introduction

Structure-function relationships of bone have been intensely studied in the past decades, particularly with the availability of X-ray-based micro-computed tomography (μCT) techniques allowing a detailed quantification of bone microstructure. Among these, μCT imaging techniques using X-ray synchrotron sources (SR) can deliver high resolution images of bone with increased signal-to-noise ratio and less beam hardening effects and less beam artifacts compared to laboratory-based μCT imaging devices (Peter and Peyrin, 2011). In that vein, coupling mechanical tests with SR-μCT is a powerful method to investigate the relationships between microstructure and mechanical properties in bone (Akhtar et al., 2008; Granke et al., 2011). Common limitations of such studies are the artifacts potentially introduced by the experimental protocol used, including sample preparation steps and measurements. These procedures may alter to a greater or lesser extent the tissue structure and, therefore, have impact on the final outcome of the studies.

To experimentally measure mechanical properties of human bone, specimens must be preserved and may be defatted to prevent contamination and the risk of infections. Generally, defatting is done by chemical fixation, using different products such as ethanol and formalin (Wieding et al., 2015; Stefan et al., 2010). Many studies have investigated the influence of these preservation methods on some particular bone mechanical properties such as the Young's modulus (Haimi et al., 2008; Linde and Sørensen, 1993) and fracture toughness (Smith et al., 2011). Formalin affects post-yield behaviour, increases hardness and reduces toughness, while controversial effects on elasticity have been reported when ethanol was used (Sedin, 1965; Linde and Sørensen, 1993; Stefan et al., 2010). Nevertheless, in all these studies, different chemical composition was used and the properties were measured by destructive mechanical tests (Smith et al., 2011; Stefan et al., 2010).

The effect of irradiation is also a concern. Previous studies have reported that gamma irradiation doses of 25 kGy, i.e., the standard dose for sterilization, significantly reduce the high-cycle fatigue life of allograft bone tissues (Islam et al., 2016) and the strength of human cortical bone, while Young's modulus measured in three-point bending does not seem to be significantly affected (Currey et al., 1997; Nguyen et al., 2007). Similar results have also been reported using X-ray irradiation (Barth et al., 2011), being used in bone imaging techniques.

Most of the studies are limited to the investigation of the effect of irradiation or specimen preparation to the Young's modulus in the longitudinal direction (Stefan et al., 2010; Kaminski et al., 2012; Haimi et al., 2008; Currey et al., 1997; Barth et al., 2011). However, since cortical bone is a typical anisotropic material, quantifying a single modulus is not sufficient to fully characterize the elastic behavior. This is the aim of the present study to quantify the possible effects of typical

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defatting and SR-μCT imaging conditions on human cortical bone elasticity. To this goal, resonant ultrasound spectroscopy (RUS) is used. RUS is a non destructive technique that allows one to characterize the same samples in native state (before any chemical or irradiation alteration), defatted state and after irradiation. Furthermore, RUS provides the elastic tensor of cortical bone (Bernard et al., 2013), that is, the five elastic coefficients of the transversely isotropic material, and not only Young's modulus as in previous studies.

2. Materials and methods

2.1. Specimens

Bone specimens were harvested from the left femur of 12 human cadavers. The femurs were provided by the Département Universitaire d'Anatomie Rockefeller (Lyon, France) through the French program on voluntary corpse donation to science. The tissue donors or their legal guardians provided informed written consent to give their tissue for investigations, in accord with legal clauses stated in the French Code of Public Health. Among the 12 donors, 8 were females and 4 were males ($50-91$ years old, 72.6 ± 13.0, mean $\pm$ SD). The fresh material was frozen and stored at -20 °C.

The samples were slowly thawed and then, for each femur, approximately a 10 mm thick cross section was cut perpendicular to the bone axis from the mid-diaphysis. Then, using a water-cooled low-speed diamond wire saw (Model 3241, Well, Lyon, France), two rectangular parallelepiped shaped specimens were prepared in the lateral and medial anatomical quadrants of each cross section, which led to a set of 24 specimens. The nominal specimen size was $3 \times 4 \times 5$ mm^3 in radial (axis 1), circumferential (axis 2) and axial direction (axis 3), respectively, defined by the anatomic shape of the femoral diaphysis. All specimens were kept hydrated during sample preparation.

2.2. Elasticity measurements by resonant ultrasound spectroscopy

The elastic tensor, assuming a transversely isotropic symmetry was assessed by RUS. Here, the indices of the elastic tensor are written with the Voigt notation,

$$C_{ij} = \begin{pmatrix} C_{11} & C_{12} & C_{13} & 0 & 0 & 0 \\ C_{12} & C_{11} & C_{13} & 0 & 0 & 0 \\ C_{13} & C_{13} & C_{33} & 0 & 0 & 0 \\ 0 & 0 & 0 & C_{44} & 0 & 0 \\ 0 & 0 & 0 & 0 & C_{44} & 0 \\ 0 & 0 & 0 & 0 & 0 & C_{66} \end{pmatrix}, \quad (1)$$

where $C_{12} = C_{11} - 2C_{66}$ and $(1-2)$ is the isotropy plane; C_{11} and C_{33} are longitudinal elastic coefficients and C_{44} and C_{66} represent shear elastic coefficients.

RUS was conducted following (Bernard et al., 2014, 2015). Briefly, the elasticity measurements by RUS are described as follows. A bone specimen was held by two opposite corners between two ultrasonic transducers (V154RM, Panametrics, Waltham, MA), one for emission and one for reception, to achieve a free boundary condition for vibration. The frequency response of the vibration in the frequency range 100–500 kHz, tuned so as to measure the 30-40 first resonant frequencies, was recorded by a vector network analyzer (Bode 100, Omicron Electronics GmbH, Klaus, Austria) and a broadband charge amplifier (HQA-15M-10T, Femto Messtechnik GmbH, Berlin, Germany). Six consecutive measurements were performed on each specimen. Between each measurement, the specimen was turned with a slightly different orientation in order to maximize the number of detectable resonant frequencies. Then, the resonant frequencies of the specimen were extracted from the six measured responses using the method dedicated to high damping material (Lebedev, 2002). Finally, knowing the apparent mass and dimensions of each specimen, the elastic coefficients were automatically calculated by solving the inverse problem formulated in a Bayesian framework (Bernard et al., 2015). This RUS protocol usually yields a precision of 0.4% for C_{44} and C_{66}, 3% for C_{11} and C_{33}, 5% for C_{13} (Bernard et al., 2013).

2.3. Bone defatting

Bone specimens were defatted following a protocol that prevents the risk of infections and allows the sample conservation at room temperature. Briefly, the procedure consists of the following steps: (1) rinsing in saline at ambient temperature; (2) defatting for 18 h using a chemical bath of diethylether and methanol (1:1) at room temperature; (3) draining off the excess of chemical fluids on absorbent paper; and (4) rinsing by sonicating with distilled water in a temperature-controlled ultrasonic bath. After this procedure, defatted specimens were kept separately in a tube filled with saline and preserved in a fridge at 4 °C. This procedure was used previously in ex-vivo studies such as (Tang et al., 1996; Granke et al., 2011). In our experience, samples defatted using the above procedure can be measured in synchroton facilities as they comply with safety regulations.

2.4. Synchrotron radiation microtomography

SR-μCT imaging was performed on the beamline ID19 at the European Synchrotron Radiation Facility (ESRF, Grenoble, France). This SR-μCT setup is based on a 3D parallel beam geometry acquisition (Salomé et al., 1999; Weitkamp et al., 2010).

A full set of 2D radiographic images were recorded using a CDD detector (Gadox scintillator, optic lenses, 2048×2048 Frelon Camera) by rotating the sample in 1999 steps within a 360° range of rotation. The detector system was fixed to get a pixel size of 6.5 µm in the recorded images in which a region of interest of 1400×940 was selected to fit the sample. The aim was to assess bone mineral density and the vascular network of the microarchitecture of cortical bone. Then, the bone images were binarized by selecting a single threshold to assess bone porosity.

The beam energy was tuned to 26 keV. Due to the limited beamline time at the ESRF facilities, half of the specimens were scanned in monochromatic configuration by using a (Si111) double crystal monochromator (total scan time $\sim$55 min/specimen) and the rest were scanned in pink beam configuration (total scan time $\sim$15 min/specimen). The total dose received by the specimen was estimated to be 0.5 kGy for monochromatic scanning and 2.5 kGy for pink scanning.

2.5. Experimental protocol

The experimental protocol is depicted in Fig. 1 and includes: (1) RUS measurements of the elastic coefficients (C_{ij}^N) on the 24 native specimens right after sample preparation; (2) the same set of specimens

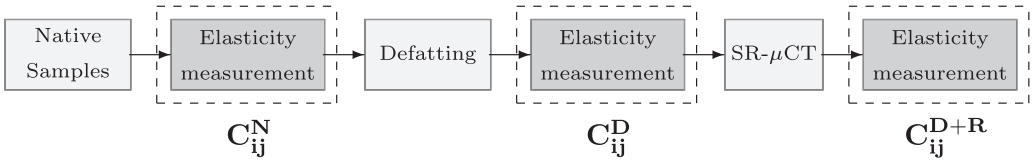

Fig. 1. Experimental protocol.