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Assessment of trabecular bone tissue elasticity with resonant ultrasound spectroscopy

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ABSTRACT

The material properties of the trabeculae (tissue-level properties), together with the trabecular architecture and the bone volume fraction determine the apparent millimetre-scale bone mechanical properties. We present a novel method to measure trabecular tissue elastic modulus E_t using resonant ultrasound spectroscopy (RUS). The first mechanical resonance frequency f^e of a freestanding cuboid specimen is measured and used to back-calculate E_t. The steps of the back-calculation are (1) the apparent stiffness tensors $\mathscr{C}(\widetilde{E_t})$ is computed using micro-finite elements for a set of trial values of tissue Young's modulus $\widetilde{E_t}$ based on the computed tomography image of the specimen; (2) the modeled free-vibration resonance frequencies $f^m(\widetilde{E_t})$ of the specimen is calculated with the Rayleigh-Ritz method using $\mathscr{C}(\widetilde{E_t})$; (3) finally, E_t is obtained by interpolation using f^e and $f^m(\widetilde{E_t})$. Four bovine bone specimens were tested (nominal size $5\times 6\ \times 6\ \mathrm{mm}^3$). Average (standard deviation) of E_t was 13.12 (1.06) GPa. The measurement of a single resonance frequency enabled an estimation of tissue elasticity in line with available data. RUS is a non destructive technique relatively easy to implement compared to traditional mechanical testing. The novel method could contribute to a better documentation of bone tissue elasticity which is an important parameter of micro-finite element analyses for the clinical assessment of bone strength.

1. Introduction

The material properties of the trabeculae (tissue-level properties), together with the trabecular architecture and the bone volume fraction determine the apparent mechanical properties of trabecular bone at the scale of several millimetres (Cowin, 2001). These properties change during growth (Boskey and Coleman, 2010) and possibly in adult life in response to changes in loading conditions, or due to pathologies (Brennan et al., 2009; Oftadeh et al., 2015). Also, tissue-level properties are required as input for numerical bone models based on high resolution imaging (van Rietbergen and Ito, 2015).

Although many studies have been devoted to measure the elastic properties of trabeculae, it remains technically challenging and demanding to quantify these properties. Nanoindentation (Zysset et al., 1999) and acoustic microscopy (Raum, 2008) require fine surface preparation (polishing) and give only local values of elasticity which may not be representative of the specimen and depend on the probing direction. Similarly, elasticity values obtained from a bending test (Choi et al., 1990; Szabó et al., 2011) or an ultrasonic test (Rho, 1998) of an individual trabeculae may not be representative of larger volumes of the tissue. An alternative approach is to back-calculate the tissue-level elastic properties using experimental data of a mechanical test of a specimen of several millimetres (van Rietbergen et al., 1995; Ladd et al., 1998). That is, the value of trabecular tissue Young's modulus E_t is obtained by fitting the response of a micro-finite element (μ-FE) model to the apparent macroscopic modulus E of a specimen. As opposed to previously mentioned approaches, tissue modulus E_t obtained this way is representative of the entire specimen's volume.

Simulations using μ-FE methods has become a standard procedure to obtain in silico apparent trabecular bone properties (Niebur et al., 2000; Verhulp et al., 2008). However, the accuracy and precision of mechanical testing to obtain E is limited and may critically depend on experimental boundary conditions and specimen size (Keaveny et al., 1997). Also, mechanical testing may be destructive because rather larger strains are induced to increase precision.

The steps of the method are (1) the first free-vibration resonance frequency f^e of a cuboid specimen is measured; (2) apparent stiffness tensor $\mathscr{C}(\widetilde{E_t})$ are computed using μ-FE for a set of trial values of tissue Young's modulus $\widetilde{E_t}$; (3) the free-vibration resonance frequencies $f^m(\widetilde{E_t})$ of a model of the vibrating specimen are calculated using the set of tensors $\mathscr{C}(\widetilde{E_t})$; (4) finally, the actual tissue modulus E_t is obtained by interpolation using f^e and $f^m(\widetilde{E_t})$. In order to demonstrate the feasibility

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of the technique, we measured four bovine specimens taken from a single femoral head.

2. Method

2.1. Specimens

Four rectangular parallelepipeds (RP) specimens of bovine bone were prepared from the femoral head of a bovine obtained from a local butcher. The bovine was about two years old. In between preparation steps, specimens were stored at − 20°C. The femoral head was excised from the femur and the soft tissues were removed manually. RP specimens were prepared using a linear saw (Isomet 4000, Buehler GmbH, Dusseldorf, Germany) under continuous irrigation. During cutting, care was taken to obtain, for each specimen, pairs of parallel faces in three orthogonal directions. The specimens were pure trabecular bone and did not contain cortical bone parts as could be verified on micro-CT images. The orientation of the specimen with respect to anatomical axes was not controlled as the method introduced in this paper does not require any assumption on trabecuae orientation. Specimens were defatted for 12 h in a chemical bath of diethylether and methanol (1:1) and washed with saline water. This protocol does not modify bone elasticity (Cai et al., 2017). After deffating, specimens were stored at ambient temperature in phosphate-buffered saline solution (Gibco, cat. no. 70011044).

Specimen dimensions were determined using an electronic calliper (± 0.02 mm precision). The smallest specimen dimensions were $(4.93 \times 5.58 \times 5.83)$ mm^3 and the largest were $(4.90 \times 6.04 \times 6.10)$ mm^3. Mass was measured with a scale (precision ± 0.1 mg). Table 1 gives the dimension and mass of all specimens.

2.2. Image acquisition and processing

The specimens were imaged using a desktop micro-CT system (Skyscan 1172, Bruker, Kontich, Belgium), with a field of view of 2000×2000 pixels, a source voltage 80 kV, current 100 μA, and rotation step 0.3° over a 360° rotation. An isotropic voxel size of 9 μm was used. Images were reconstructed using a filtered back-projection algorithm (NRecon software, V 1.6.9, Skyscan NV, Kontich, Belgium).

After reconstruction, images were processed using ImageJ (Schneider et al., 2012): the pixel size was doubled to reduce image size; images were segmented with Otsu method, rotated and cropped in order to obtain specimen's faces aligned with the image frame.

2.3. RUS measurements

Specimens were taken out of the saline solution five to six hours before the RUS measurement. Any water between the trabeculae was removed with an air jet and absorbent paper. RUS measurements were conducted following the method and apparatus introduced by (Bernard et al., 2014). Briefly, the specimen was placed between a pair of shear ultrasonic transducers (V154RM, Panametrics, Waltham, MA), one acting as an emitter and the second one as a receiver (Fig. 1). Quasi stress-free boundaries conditions are achieved by placing the specimen on the transducers on two opposite corners and minimizing the force acting on the specimen. The force applied was not monitored but a single trained operator conducted all the measurements; accordingly we assume that the specimens were measured with comparable boundary conditions very close to free standing. A vectorial network analyser (Bode 100, Omicron electronics GmbH, Klaus, Austria) was used to record the frequency spectrum (phase and magnitude) of the specimen between 20 and 140 kHz with 50 Hz resolution. The receiver output signal was preconditioned before recording by a broadband charge amplifier (HQA-15M-10T, Femto Messtechnik GmbH, Berlin, Germany).

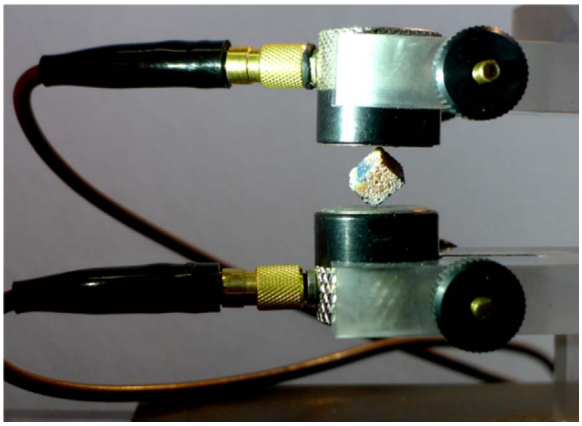

Fig. 1. RUS measurement configuration showing the mounting of a bovine specimen between ultrasonic transducers. Measurements are repeated with successive mounting on the different pairs of opposite corners.

RUS measurement of one specimen consisted in a series of 8 spectra acquisitions: the specimen was successively maintained by each of the four pairs of opposite corners and two spectra were acquired for each case, with a rotation of 90° of the specimen in between. The purpose of this procedure is to maximize the chance to excite the first vibration mode and to account for the possible small variability of the resonance frequency according to the specimen position.

For each specimen, the frequency f^e of the first peak was estimated from the 8 spectra acquisitions of a series. A typical acquisition of a series of eight spectra is shown in Fig. 2. In preliminary measurements, we found that for the different specimens, the first resonant frequency could not be identified for all spectra of an acquisition series. Typically in one to three acquisitions in a total of eight, the peak due to the first resonance could not be observed, likely because the corresponding eigenmode was weakly excited. To account for this, f^e was calculated as the average of the frequency of the three peaks of larger amplitude.

2.4. μ-FE and model frequency calculation

Specimens' CT scans were converted into FE models using direct voxel to element conversion in FAIM software (FAIM version 7.1, Numerics88 Solutions, Calgary, Canada). The size of the hexahedral elements was 18μ m. This operation includes removing non connected voxels. In our model, isotropic tissue elastic properties were assumed. All bone voxels were given a Young's modulus $\widetilde{E_t}$. Trial values of $\widetilde{E_t}$ for μ-FE models were between 4 and 20 GPa. Preliminary tests showed that the influence of the value of Poisson's ratio on the calculated first resonant frequency was negligible. Accordingly, a unique Poisson's ratio value of 0.3 was used for all calculations. Six different uniaxial strains were successively applied to each specimen following the Direct Mechanics method (van Rietbergen et al., 1996) as implemented in FAIM. This resulted in apparent stiffness tensors $\mathscr{C}(\widetilde{E_t})$. In 6×6 matrix

Table 1
Dimensions and mass of specimens.

Specimen	1	2	3	4
mass (mg)	115.48	117.82	135.18	123.48
dimensions (mm^3)	$4.92\times5.59\times6.05$	$4.90\times5.83\times6.83$	$4.90\times6.04\times6.85$	$4.93\times5.58\times5.83$