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Research Paper

The role of angular reflection in assessing elastic properties of bone by scanning acoustic microscopy

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ABSTRACT

For an assessment of the mechanical performance of bone, a quantitative description of its mechanical heterogeneity is necessary. Previously, scanning acoustic microscopy (SAM) was used as a non-destructive method to estimate bone stiffness on the micrometer scale. While up to now only the normal incidence of acoustic waves is taken into account, we extend in our study the evaluation procedure by considering the full opening of the acoustic lens. The importance of this technical aspect is demonstrated by determining the contrast in Young's modulus between newly formed osteons and the surrounding higher mineralized interstitial bone.

Several regions of human cortical bone of a femur in cross-section were imaged. For all the regions quantitative backscattered-electron imaging (qBEI) to estimate the local mass density was combined with SAM measurements. These measurements reveal a non-monotonic dependence between acoustic reflectivity and Young's modulus, which shows that it is actually necessary to consider the lens opening in a quantitative way. This problem was experimentally and theoretically approached by using lenses with two different opening angles operated at different frequencies (52° at 400 MHz and 80° at 820 MHz) to image the same specimen.

The mass density of bone in osteons was found to be 1930 kg/m^3 on average, while the higher mineral content in interstitial bone results in a 9% increase of the density. The contrast in the effective Young's modulus E, as determined through SAM, is more pronounced, with an average value of 14 GPa in osteons and a more than 60% increase in interstitial bone. Additionally, SAM maps show oscillations in E with a periodicity of the typical bone lamella thickness of approximately 7 μm in both osteons and interstitial bone. This mechanical heterogeneity can be explained by the varying orientation of the mineralized collagen fibers.

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1. Introduction

An assessment of the quality of bone (Seeman and Delmas, 2006) has to address the different hierarchical levels of its structure (Fratzl et al., 2004; Wagner and Weiner, 1992). Important progress has been made in the imaging of the bone architecture using micro-computed tomography (Muller, 2009), also employing synchrotron radiation (Peyrin, 2009; Raum et al., 2006b), and recently using dual beam electron microscopy (Reznikov et al., 2013). Much less structurally and functionally investigated is the bone material itself. Due to bone remodeling and mineralization the bone material is very heterogeneous on a length scale of roughly 100 μm. In cortical bone remodeling leads to the formation of new osteons, which consist of a central canal for the blood supply and is surrounded by concentric bone lamellae (Parfitt, 1994). Between the osteons the space is filled by interstitial bone, which remained from older osteons that were partially resorbed due to remodeling. The higher age of the interstitial bone results on average in a higher mineral content compared to the bone forming the osteons. This spatial heterogeneity of the mineral content is accessible, e.g. via scanning electron microscopy in the backscattering mode (Roschger et al., 1998). The distribution of the bone mineral density (BMDD) is an important parameter describing bone material quality (Lukas et al., 2011; Roschger et al., 2008; Ruffoni et al., 2007). Closer linked to the mechanical performance and therefore even more important is to understand the mechanical heterogeneity of the bone material. Young's and/or reduced modulus are the most easily accessible mechanical property. It can be estimated via nanoindentation (Bushby et al., 2004; Faingold et al., 2012; Fratzl-Zelman et al., 2009; Gupta et al., 2006a; Hengsberger et al., 2002; Ziskind et al., 2011). However, acquiring the local elastic constants of large representative areas with nanoindentation is very time-consuming (Fischer-Cripps, 2006, 2007). Low resolution and, typically, dry measurement conditions are further disadvantages of this technique. There is a need for an "efficient" method of characterization of bone on the micrometer scale.

Scanning acoustic microscopy (SAM) has seen several developments since the creation of the first fast transmission setup by Lemons and Quate (1974). The scanning procedure has become faster and the signal-to-noise ratio improved. The resolution of modern acoustic microscopes reaches lengths below 1 μm. SAM has been used for investigations on soft (skin, eye) and hard (bone, tooth) biological tissues (Chappard et al., 2011; Katz et al., 2001; Meunier et al., 1988; Walter and Briggs, 1996). One operation mode of SAM is reflective acoustic microscopy, where the transmitting lens functions also as the receiving lens (Blouin et al., 2011; Raum, 2008). In most cases the lens is spherical with its acoustical axis perpendicular to the sample surface. The basic design of these microscopes is a pulse generator, which sends an electrical pulse to a piezo-transducer mounted on the acoustic lens. The wave travels through the lens and the coupling fluid, is reflected on the fluid/sample interface and returns to the transducer again through the fluid and the body of the lens. The returning signal is integrated over a time interval. The acoustic lens is attached to a scanning unit (*x*-, *y*- and *z*-direction), which makes it possible to acquire a planar intensity image in focus with resolutions down to approximately one micrometer, depending on the lens and frequency used. In general, the obtained voltage will depend on the mechanical properties of the material, its density and the lens geometry.

A simplifying assumption used in previous work is that, independent of the lens opening angle, the signal strength in focus is proportional to the acoustic reflection coefficient for normal incidence, i.e. at zenithal angle $\theta=0^\circ$ (Hirsekorn et al., 1995). Using this assumption of an opening angle of the acoustic lens of 0°, elastic properties of bone have been estimated from SAM measurements (Eckardt and Hein, 2001; Raum et al., 2006a, 2003, 2006b; Raum et al., 2003, 2006b; Rupin et al., 2009). For this the SAM images where combined with estimations of the density using synchrotron radiation micro-computed tomography (Rupin et al., 2009). However, the assumption of neglecting the angular reflectivity may not be valid in general, especially for bone tissue with a high mineral content. Therefore, we are revisiting this problem and assume that the whole opening angle of the lens is contributing to the detected signal (Briggs, 1992; Maev, 2009; Royer and Dieulesaint, 2000; Sheppard and Wilson, 1981). Several theoretical models describe these contributions according to the angular dependency of the acoustic reflectivity (Atalar, 1978; Atalar et al., 1977; Bertoni, 1984; Breazeale et al., 1977; Quate et al., 1979; Somekh et al., 1984; Wickramasinghe, 1978). Our approach is, firstly, to determine how much of the opening angle of the lens is really contributing in the measurement. For that purpose we use a simplified plane wave model adapted from Somekh et al. (1984). We, secondly, evaluate the acoustic reflectivity of a cross-section of cortical bone of a human femur. For our improved evaluation procedure it is essential to perform measurements using lenses of different opening angles to overcome difficulties which arise from the non-monotonic dependence between acoustic reflectivity and the Young's modulus (Fig. 1). The SAM imaging is complemented by a measurement of the local mass density using quantitative backscattered electron microscopy imaging, qBEI (Roschger et al., 1998, 2008). The information of three measurements on the same region of the sample (qBEI and SAM with two lenses) is then combined in the evaluation to obtain two-dimensional maps of the effective Young's modulus of bone with micrometer resolution. Our study concentrates on the distinction between osteons and interstitial bone in cortical bone of a human femur. Both regions can have similar reflectivity in the SAM measurement, when measured with the higher opening lens. However, the combined evaluation of the three measurements allows to detect the higher effective Young's modulus of interstitial bone compared to osteons. The mechanical contrast between lamellae remains preserved in both osteons and interstitial bone.

2. Theory

2.1. *Angular reflectivity*

The acoustic reflectivity of an interface is dependent on the angle of incidence θ and, generally, on the azimuthal angle φ.