TRABECULAR BONE PROPERTIES EVALUATED BY SCANNING ACOUSTIC MICROSCOPY

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Abstract

A reflection scanning acoustic microscope (SAM), operating at 50 MHz, was used to evaluate changes in properties of trabecular bone. Samples obtained from human cadaver calcaneus were explored to reconstruct acoustic impedance images and estimate variations of acoustic impedance of bone. For each sample, the acoustic impedance (Z) was estimated over the entire sample section. The mean degree of mineralization of bone (MDMB) was measured over the entire section of bone samples by quantitative microradiography. A moderate but significant correlation was found between Z and MDMB indicating that mean degree of mineralization of bone contributes to changes in acoustic impedance.

Introduction

Bone mechanical properties are determined by different characteristics, such as microstructure (organization of collagen fibers and hydroxyapatite crystals, integrity of collagen-mineral interface), degree of mineralization, micro-architecture, and tissue elasticity. These characteristics strongly contribute by varying degrees to bone strength and resistance to fracture. Imaging techniques, such as dual-energy X-ray absorptiometry (DXA) and quantitative ultrasound imaging in the MHz range, enable evaluation of skeletal status at a macroscopic scale (organ level), and are used for fracture risk prediction [1, 2]. The impact of bone pathologies and their therapeutic treatments on bone tissue material properties and micro-architecture, and a better understanding of bone physiopathology require the use of precise and reliable investigation techniques that provide information about bone characteristics at the level of bone structural unit. The degree of mineralization of bone can be measured by quantitative microradiography [3] and also quantified in three dimensions with spatial resolution of about one micrometer using 3D-synchrotron radiation microtomography [4]. Bone mechanical properties (elasticity and hardness) can be measured by nanoindentation with depths of between 100 and 600 nanometers [5]. Scanning acoustic microscopy in the GigaHertz frequency range allows measurements of material micromechanical properties at spatial resolution approximating that of optical microscopy [6, 7]. Previous studies reported the use of SAM to image heterogeneities of bone surface properties [8]. Acoustic impedance maps, which display variations in both bone density and elasticity, have been obtained for cortical bone at 20 MHz [9] and at the level of individual lamellae at 900 MHz [10, 11]. Bone elastic moduli was measured by SAM at 400 MHz, using a calibration curve, based on a correlation between grey levels of SAM images and elastic moduli of reference materials [12]. The purpose of the current study is to assess acoustic impedance of trabecular bone tissues using SAM in order to investigate the relationship between the mean degree of mineralization of bone obtained by microradiography and impedance values at 50 MHz.

Material and methods

Bone samples

The study was performed on 19 trabecular bone samples obtained from human cadaver calcaneus, with no history of bone disease. Thirteen specimens were obtained from men between 61 and 86 years of age (mean ± std: 76.5 ± 7.6 years) and six specimens from women aged between 65 to 91 years (mean ± std: 82.5 ± 9.0 years).

After excision, all the bone samples were fixed in 70% ethanol, then dehydrated in absolute alcohol and finally embedded in methylmethacrylate (MMA). The bone samples were then ground and polished to obtain plane parallel surfaces and avoid surface roughness.

Data acquisition

The samples of human calcaneus were explored using a scanning acoustic microscope (SAM100EX, Q-BAM, Halle, Germany) operating with a 50 MHz focused transducer (60° aperture angle; 30 µm lateral resolution). The acquisitions were made with the bone samples immersed in distilled and degassed water, maintained at 25°C. The sample was positioned at the transducer focus and an image of reflectivity (C-scan) of the entire surface (approximately 1cm^2) of the sample was acquired.

Estimation of acoustic impedance

Under the condition of normal incidence, the reflection coefficient (R) is proportional to the measured voltage (V) on SAM. The relation between the reflection coefficient and the voltage was obtained by plotting R as a function of V (calibration curve) for a variety of homogeneous reference materials of predetermined impedance (using pulse echo method for speed of sound measurements and Archimedes' principle for density estimation). For each scanned point of bone images, the reflection coefficient was calculated using the calibration curve, estimated under the same experimental conditions, electronics settings and temperature. The acoustic impedance ($Z=\rho c$; ρ is the density of the material under study expressed in kg/m^3, c is the acoustic wave velocity expressed in m/s) was obtained from the equation, $R= (Z_b - Z_w)/(Z_b + Z_w)$, were Z_b and Z_w are the acoustic impedances of bone and water, respectively. Our procedure allows evaluation of quantitative impedance values that are independent of the acquisition system electronics, sample topography and inclination [12, 13].

For each bone sample, the mean impedance value was estimated from the entire surface, by averaging impedance values of each scanned point.

Evaluation of the mean degree of mineralization of bone

The evaluation of the mean degree of mineralization of calcaneus bone (MDMB) was performed by computerized quantitative contact micro-radiography. The measurements were made on sections of uniform thickness of 100 ± 1 µm adjacent to the sections explored by scanning acoustic microscopy. The degree of mineralization of bone expressed in g mineral / cm^3 of bone corresponds to the mass of the mineral content per unit volume of bone [3].

Results

Figure 1 displays a typical reflectivity image obtained at 50 MHz from the surface of a calcaneal sample, positioned at the focus, with the corresponding distribution of the impedance parameter within this region.

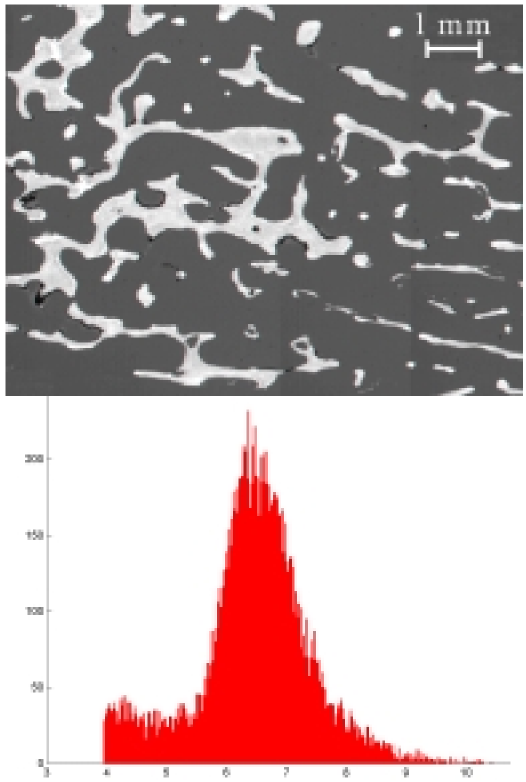

Figure 1: Reflectivity image of a calcaneal sample (top), taken from a man (73 years of age) obtained at 50 MHz. The histogram (bottom) showed the distribution of impedance values within the entire surface.

Individual mean impedance values estimated from the entire sample of human calcanei varied between 5.36 ± 0.27 MRayl and 7.19 ± 0.43 MRayl. The mean acoustic impedance and standard deviation of trabecular bone was obtained by averaging the mean Z-values over all the bone samples and was 6.23 ± 0.55 MRayl. The average mean Z-value was 6.34 ± 0.48 MRayl for the male group and 6.00 ± 0.67 MRayl for the female group. The mean degree of mineralization estimated over the entire section of each individual calcanei samples varied between 0.90 ± 0.05 and 1.43 ± 0.14 g mineral / cm^3 bone. A least square linear fit between Z and MDMB of calcaneus bones showed a moderate but significant correlation ($R=0.56$, $p<0.05$) between mean acoustic impedance and mean degree of mineralization of bone (Figure 2).

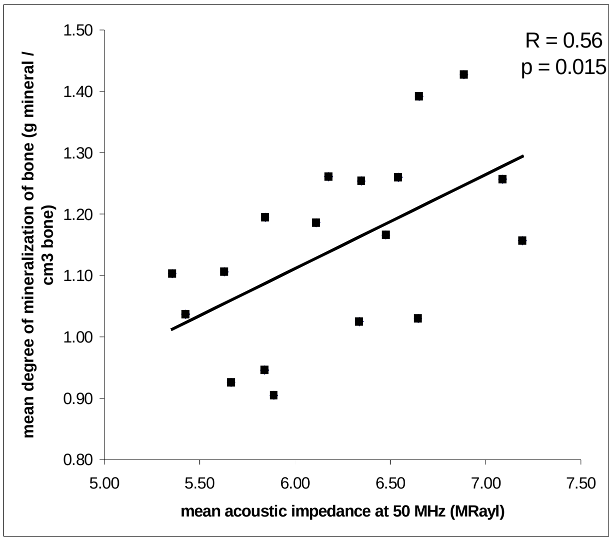

Figure 2: Correlation between mean Z-values at 50 MHz and the mean degree of mineralization. The two parameters were averaged over the entire section of trabecular samples.

Discussion

This preliminary study aimed at measuring acoustic impedance of bone in order to assess changes in the tissue properties, and ultimately, investigate the relationship between the mean degree of mineralization of bone and acoustic impedance. The mapping of acoustic impedance of bone sections was achieved with spatial resolution of about 30 µm, using a scanning acoustic microscope. The study was performed on trabecular bone taken from human calcaneus, with predetermined mean degree of mineralization.

Mean acoustic impedance values, obtained on trabecular bone taken from cadavers between 61 and 91 years of age, were in the same range of values as those reported on human cortical bone [9]. To our knowledge, no acoustic impedance values of trabecular bone has been reported. The low standard deviation values indicate that the acoustic impedance was uniform within the bone sections as well as within the women and men groups. The average group mean values of acoustic impedance of men were not significantly higher than that of women group. This result is consistent with the average group of mean degree of mineralization values, which did not significantly vary between men and women groups [3]. Concerning all the trabecular bone samples, the mean acoustic impedance obtained at 50 MHz appeared to increase with the mean degree of mineralization of bone. Our finding emphasizes that acoustic impedance likely reflects changes in the degree of bone mineral density at the tissue level. The moderate correlation found between mean acoustic impedance values at 50 MHz and mean degree of mineralization of bone indicate that only 30% of the variance of the acoustic impedance is accounted for variations in mean degree of mineralization. Part of the unexplained variance may be due to independent variation in elastic properties. Further work is required to elucidate the contribution of elastic properties on acoustic impedance. Measurement errors in acoustic microscopy and/or X-ray microradiography might also be responsible for the limited correlation coefficient. The lack of precision of impedance measurements may result from different error sources that may be dependent upon the acquisition system and the sample preparation (non uniform inclusion material, effect of inclusion on bone properties, etc..).

Bone strength and resistance to fracture are dependent on bone mineral density and micro-architecture, but also on bone tissue quality (organization of hydroxyapatite crystals, collagen cross-links, micro-cracks). Quantitative scanning acoustic microscopy has a great potential for the assessment of bone properties at the tissue level. The current work indicate that bone mineralization contributes to changes in acoustic impedance. Further analysis and complementary techniques are necessary to establish a model of the influences of bone micromechanical properties on acoustic impedance. Yet, quantitative acoustic microscopy is a promising relevant tool for the evaluation of bone quality. This technique is suitable for bone assessment in transgenic mice and follow-up of therapeutics effects.

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