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IN VITRO ULTRASOUND CHARACTERIZATION OF HUMAN PROXIMAL FEMUR MICROSTRUCTURE AND COMPARISON WITH MICRO-CT DATA

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Abstract: An *in vitro* investigation based on Quantitative Ultrasound (QUS) has been carried out in this paper on a sample of human femoral head, aiming at microstructure characterization and bone quality assessment. Two QUS parameters, Integrated Reflection Coefficient (IRC) and Apparent Integrated Backscatter (AIB) were measured by accurately analyzing ultrasonic signals backscattered from the target bone when immersed in water and insonified at 2.25 MHz. In particular, US data were acquired from 22 different positions along the maximum section perpendicular to the axial direction (head-neck axis) of the target sample. The obtained data were compared with local structural properties gathered at each considered position from a high-resolution micro-computed tomography (microCT) scan of the same sample. A linear regression analysis showed an appreciable correlation between QUS parameters and some of the micro-structural parameters. As expected, IRC correlated better with cortical bone volume fraction ($r = -0.53$), and AIB with trabecular bone volume fraction ($r = -0.60$) and trabecular spacing ($r = 0.47$). These results are particularly encouraging in view of a possible clinical translation of the proposed approach for early osteoporosis diagnosis.

Keywords: image processing; signal processing; ultrasound; computed tomography; bone characterization.

1. INTRODUCTION

Osteoporosis is a bone disease that involves the progressive decrease of both mass density and micro-architectural quality of skeletal structures, leading to an increased fracture risk. Osteoporotic patients are considered to have reduced quality of life and an increased mortality rate due to fractures and fracture-related complications [1]. Several works have demonstrated that significant mechanical variations in the structure of trabecular bone occur in case of Bone Mineral Density (BMD) reduction [1,2]. In addition, recent studies have proved that the evaluation of the trabecular bone micro-architecture can provide interesting information in terms of fracture prediction [3]. Finite element models have also been implemented to assess bone biomechanical behaviour and

their clinical potentiality in terms of fracture risk prediction has been evaluated [4]. Nowadays, Dual Energy X-ray Absorptiometry (DEXA) is considered to be the gold standard to measure BMD; however, it cannot provide information on the bone mechanical properties [5]. In order to gain knowledge on these properties, QUS parameters have been proposed in literature by considering the interaction between an ultrasonic wave and the trabecular bone [6-8]. Some QUS parameters, such as the apparent integrated backscatter (AIB), the frequency slope of apparent backscatter (FSAB) and the time slope of apparent backscatter (TSAB) have been correlated with measured physical characteristics of specimens (mass density, X-ray BMD, Young's modulus, yield strength and ultimate strength) [9]. Other *in vitro* studies were conducted to investigate the capability of Integrated Reflection Coefficient (IRC) and Broadband US Backscatter (BUB) to predict density and mechanical properties of bovine trabecular bones [10]. Ultrasound-based approaches represent an innovative solution and they are becoming more and more reliable [11].

In the present paper, the authors show the results of QUS methods applied to the signals backscattered from a human femoral head sample. Using an *ad hoc* designed experimental set-up, US acquisitions were performed on different portions of the bone sample, each time distinguishing between the cortical and trabecular signal contributions coming from the same portion correlating the obtained results with microstructure parameters provided by micro-computed tomography (microCT). To the best of our knowledge, this study represents the first example of a QUS characterization applied on a whole human femoral head, simultaneously including both the trabecular region and the cortical layer in their physiologic morphological configuration.

2. MATERIALS AND METHODS

A. Ultrasonic acquisitions

The aim of collecting the US signals backscattered from a sample of human femoral head *in vitro* was achieved by means of a suitable custom-developed experimental set-up.

In order to keep the bone specimen in a fixed and reproducible position, the specimen itself was drilled along the axial direction and a screw was carefully threaded into the realized hole. The drilling process was a crucial step that included an accurate analysis of the considered bone properties, in order to avoid damages to the trabecular structure. The screw was in turn linked to a mechanical system that allowed the sample rotation around the axial direction by means of a crank handle. In this way, it was possible to rotate step-by-step the femoral head and to assure that reflected US signals came from a specific target slice at enough distance from the screw hole. The bone was immersed in distilled water at room temperature (about 20°C) and the ultrasonic transducer was placed in such a way as to insonify the slice corresponding to the largest transversal section of the femoral head, as shown in Fig. 1.

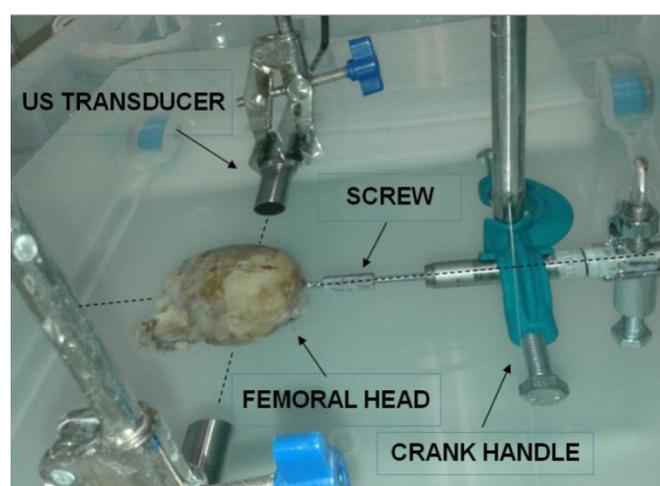


Figure 1. The adopted experimental set-up: the target femur bone rotated by means of a crank handle and the ultrasonic transducer.

The employed ultrasound transducer, acting both as a transmitter and as a receiver, was a single-element unfocused one with a capsule diameter of 12.7 mm (V-306-SU, Panametrics, Waltham, MA, USA). Sample insonifications were carried out considering 22 different rotation angles (Fig. 2). Measurement process started only when the target bone was in state of rest. Basic rotation steps were equal to 24° each, but in the region that could be explored also during *in vivo* clinical investigations the rotation steps were decreased from 24° to 12° each, as shown in Fig. 2. The transducer was connected to an ultrasonic pulser-receiver (Panametrics PR5077), which was able to operate at a pulse repetition frequency of 200 Hz (i.e., 200 pulses per second) and to excite the piezoelectric element by means of 400-V pulses centered at 2.25 MHz. The same pulser-receiver performed a 10-dB amplification and a 1-kHz high-pass filtering of the reflected signals, which were then digitized (100 MHz, 14 bits) by an acquisition board (PCI-5122, National Instruments, Austin, Texas, USA).

For each rotation step, 50 records, corresponding to the backscattering of 50 incident US pulses, were acquired and then averaged. This was useful to prove the acquired signal repeatability and to improve the signal-to-noise ratio (SNR).

The gathered average waveforms were therefore characterized by a high SNR, which facilitated the subsequent identification in each of them of the cartilage (Ca), cortical (Ct) and trabecular (Tb) portions. The average US signals are superimposed on the corresponding microCT slice in Fig. 2.

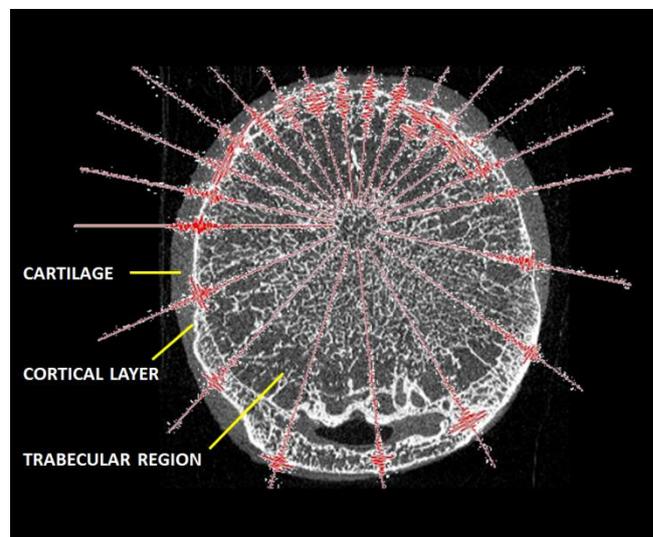


Figure 2. MicroCT image of the considered femoral head section with superimposition of the acquired US signals along the 22 evaluated directions.

One can notice that, as expected, the maximum signal value was found in correspondence of the interface between the Ca and the Ct layer. The first layer is depicted as the external region of dark grey voxels, whereas the second layer is represented by white voxels. We can also note that the US signal received from the internal Tb bone tissue is weaker because of the strong reflections generated by the cortical layer, due to the significant variation of acoustic impedance.

B. Quantitative ultrasound measurements

IRC and AIB values were calculated from the average US signal acquired for each considered rotation angle. A reference signal was also acquired replacing the target femur bone with a perfect reflector consisting of a steel plate. US signals were partitioned following the method proposed in [10]: considering the envelope of the reference signal, two time windows $\Delta T_1 = t_M - t_1$ and $\Delta T_2 = t_2 - t_M$ were defined starting from the time position of the maximum (t_M) and selecting the points (t_1, t_2) where the envelope signal reaches a fixed threshold (in this case 10% of the maximum). Therefore, in each average US signal, the region of interest ROI_{cort} , corresponding to the cortical region, was selected by considering the union of the time windows ΔT_1 and ΔT_2 around the maximum position. Signal region before ROI_{cort} was attributed to the cartilaginous layer. The region of interest ROI_{trab} , corresponding to the trabecular region, was defined by considering a time window of 13 μs after ROI_{cort} . Using the relationships described in [12], IRC and AIB were computed as follows:

$$IRC = \frac{1}{\Delta f} \int_{\Delta f} 20 \log_{10} \left(\frac{A_{cort}(f)}{A_{ref}(f)} \right) df \quad (1)$$

$$AIB = \frac{1}{\Delta f} \int_{\Delta f} 20 \log_{10} \left(\frac{A_{trab}(f)}{A_{ref}(f)} \right) df \quad (2)$$

where A_{cort} and A_{trab} denote the amplitude spectrum of the signal in ROI_{cort} and ROI_{trab} respectively, A_{ref} the amplitude spectrum of the reference signal, Δf the considered frequency bandwidth, determined as the -6 dB portion of the reference spectrum.

C. Micro-CT scan and data analysis

The bone sample was also scanned by a high-resolution microCT scanning system (Raytest IRIS micro-PET/CT, Raytest, Straubenhardt, Germany). The acquisition was performed as a low-dose (80 kV, 1 mA), isotropic voxel (60 μ m) scan.

The analysis included 50 slices (16-bit grey level, 886 \times 943 voxels) that were cropped from the original scan and corresponded to a 3-mm thickness of the sample centred on its maximum diameter along the head-neck femur axis. Twenty-two parallelepiped samples (3 \times 3 \times 19 mm³) were extracted from this analysis dataset at the angles shown in Fig. 2, each parallelepiped containing cartilaginous, cortical and trabecular volume, as illustrated in Fig. 3. The section of the parallelepiped boxes was chosen considering the beam diameter of the US transducer (3 mm), whereas height was empirically estimated from the US measures.

The custom-developed automatic algorithm used to segment Ca, Ct and Tb volumes was inspired by the dual threshold technique presented by Buie *et al* (2007) [13]. Volumes were segmented in three regions (referring to cartilaginous, cortical and trabecular ones), then structural parameters were calculated for cortical and trabecular regions using the software BoneJ [14].

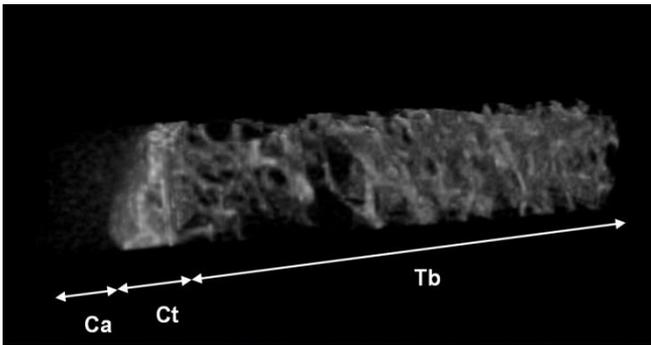


Figure 3. Three-dimensional microstructure of the bone sample at 0°. It is shown the cartilage thickness (Ca), the cortical thickness (Ct) and the trabecular thickness (Tb).

Considering the trabecular layer, we measured the following quantities: the voxel-based bone volume fraction (BV/TV), which is the volume of mineralized bone per unit volume of the sample; the mean trabecular bone thickness (Tb.Th, in mm) and spacing (Tb.Sp, in mm), both calculated

as presented by Dougherty *et al* (2007) [15]; the structural model index (SMI), which is a method for determining the plate-like or rod-like geometry of trabecular structures (based on the Hildebrand and Rüegsegger method [16]); the index was designed to be 0 for perfect plates, 3 for perfect rods and 4 for perfect spheres; the bone surface density (BS/TV in mm⁻¹); the fractal dimension, which uses the box counting method [17]; the connectivity density (Conn.D), which is a measure of the degree of connectivity of trabeculae normalized by TV [18]. Moreover, for the cortical layer, we measured the previously defined voxel-based BV/TV.

3. RESULTS

AIB, IRC and microCT parameter values were computed for all the 22 considered angular positions. The obtained mean values and the corresponding standard deviations are indicated in Table I.

Table I. Mean and standard deviation values for each considered parameter.

	Mean values	Standard deviation
Trabecular BV/TV	0.21	0.06
Tb.Th Mean (mm)	0.29	0.06
Tb.Sp Mean (mm)	1.06	0.36
SMI	4.19	0.40
BS/TV (mm ⁻¹)	0.71	0.24
Fractal Dimension	1.88	0.08
Conn.D (mm ⁻³)	0.04	0.02
Cortical BV/TV	0.04	0.01
AIB (dB)	-51.44	3.26
IRC (dB)	-46.24	3.73

The Pearson's linear correlation coefficients between the ultrasound parameters and the microCT ones were determined as well: the obtained values are shown in Table II for the trabecular layer and in Table III for the cortical one.

For the trabecular layer, AIB showed the strongest correlation value with respect to the structural parameters. In particular, the linear correlation values with BV/TV, Tb.Sp and BS/TV were -0.60, 0.47 and -0.46, respectively. These values have been remarked in bold font in Table II. The negative correlation value between AIB and BV/TV indicates that the greater the density of the trabecular layer, the smaller the intensity of the backscattered signal. This can be explained by the fact that when bone density becomes lower the ultrasound signal progressively detects more "discontinuities" in the trabecular structure. This was also confirmed by the positive correlation found between AIB and Tb.Sp, just indicating that trabecular backscattering increases proportionally to inter-trabeculae spacing. For the

cortical layer, it is interesting to observe that there is a positive correlation between IRC and BV/TV. The observed value is $r=0.53$, as remarked in bold font in Table III. This means that a greater bone cortical density is responsible for a greater echo signal intensity. As expected, AIB presented a better correlation with the trabecular parameters because it was computed from the backscattered component of the US signal due to trabeculae. On the contrary, IRC was better correlated with the cortical bone density because it was computed from the reflected component of the US signal due to the discontinuity between cortical and cartilaginous layer.

Table II. Linear correlation values between micro-CT and ultrasound parameters for the trabecular layer.

<i>Trabecular layer</i>	AIB	IRC
BV/TV	-0.60	0.21
Tb.Th Mean (mm)	-0.41	-0.01
Tb.Sp Mean (mm)	0.47	-0.20
SMI	-0.28	0.17
BS/TV (mm ⁻¹)	-0.46	0.03
Fractal Dimension	-0.32	0.07
Conn.D (mm ⁻³)	-0.20	0.03

Table III. Linear correlation values between micro-CT and ultrasound parameters for the cortical layer.

<i>Cortical layer</i>	AIB	IRC
BV/TV	-0.05	0.53

Since we made use of only one transducer with a 2.25 MHz frequency, we were not able to evaluate the effect of incident pulse frequency on QUS parameter values. In order to find the optimal frequency and to consequently improve the assessment of the bone status, further studies will be conducted involving the employment of the same approach using different US frequencies.

4. CONCLUSIONS

In the present work, the correlations between two QUS parameters (AIB and IRC) and structural data extracted by microCT were investigated on a human femoral head sample. Results showed an appreciable correlation between QUS parameters and some of the micro-structural parameters. In particular, IRC correlated better with cortical bone volume fraction, and AIB with trabecular bone volume fraction and trabecular spacing. The proposed approach was designed to give more importance to femoral head portions that may be explored in an *in vivo* analysis. This aspect could facilitate the extension of the described method to clinical applications aimed at early osteoporosis diagnosis.

5. ACKNOWLEDGEMENTS

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