Use of Ultrasonic Parameters as Adjuvant Tool for Diagnosis and Monitoring of Bone Lesions

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Abstract. Ultrasound parameters were proposed to characterize femur *in vitro* from rats. Six quantitative parameters (Apparent Integrated Ultrasonic Backscatter - AIB, Frequency Slope of Apparent Backscatter – FSAB, Temporal Slope Apparent Backscatter - TSAB, Integrated Reflection Coefficient - IRC, Slope and Frequency Integrated Reflection - FSIR and Temporal Slope Reflection Coefficient - TSRC) were applied to the echo from the cortical and trabecular bone in twelve femur diaphyses *in vitro* from Wistar rats. The US signal is acquired from 5 previous chosen positions along the femur. The results showed that their values statistically belong to the same group. This is an indication that the proposed method (from acquisition protocol to parameter estimation) has potential to characterize bone tissue in animal models.

1 Introduction

Bone is composed of specialized and complex connective tissue [1]. Even with high strength and hardness, the tissue suffers numerous injuries that may cause a negative impact on patient condition [2,3] as well as in public health policies [3]. It is, thus desirable to have diagnostic tools preferably of low cost and non-ionizing radiation [4, 5] to assess bone quality and monitor bone condition.

In addition to commonly used methods to characterize bone [6,7] Quantitative Ultrasound - QUS has proved to be promising for the diagnosis of diseases on bone structure [5,6,7,8,9] and to follow up bone healing. QUS provides quantitative parametric information on bone tissue integrity [6]. Therefore, diagnosis by QUS can become an auxiliary tool that minimizes the subjectivity of image diagnostics and providing a more accurate diagnosis.

In literature, there are numerous parameters proposed to characterize bone. The most studied parameters for biological tissues characterization are: speed of sound [7], backscattering [10], reflected and attenuation coefficient [6]. Backscattering is the portion of the ultrasonic energy that is reradiated for particles with dimensions of wavelength order [11]. Beam reflection makes the wave return to the same medium, with the same speed, frequency and wavelength as the incident wave [12].

Wear et al. [8] correlated the attenuation coefficient, speed of sound and bone densitometry (X-rays) in humans with risk factors for osteoporosis and concluded that there are strong correlations between them.

Hoffmeister et al. [13] showed that the Apparent Integrated Ultrasonic Backscatter (AIB), proposed to assess soft tissues, is also effective for characterizing bone tissue.

Pereira et al., in rats, showed that parameters calculated from backscattering [14] and reflection [15] are promising to characterize bone tissue.

Although there are several studies that demonstrate a strong correlation between ultrasound parameters with gold standard exams – e.g. bone densitometry [8] - there is no standard method for characterizing bone and there are few studies using protocols in bones rats [14,15]. The use of rats in these studies will contribute to consolidating the technique of use of QUS for diagnosis, since the bones of rats are more similar to human's.

In this study, we aim to characterize femurs of rats *in vitro*, based on six QUS parameters: Apparent Integrated Ultrasonic Backscatter - AIB, Frequency Slope of Apparent Backscatter – FSAB, Temporal Slope Apparent Backscatter - TSAB, Integrated Reflection Coefficient - IRC, Slope and Frequency Integrated Reflection - FSIR and Temporal Slope Reflection Coefficient - TSRC.

2 Materials

2.1 Ethical Norms

The research was approved by the Ethical Committee for the Use of Laboratory Animals in Research of the Faculty of Medicine of the Federal University of Rio de Janeiro (UFRJ), followed the Guidelines for Care and Use of Animals in Research.

2.2 Samples Preparation

The samples consisted of 12 intact femurs *in vitro* from female rats (*Rattus Norvergicus Albinus*) weighting 230 ± 15 g. For sample preparation, followed the protocol: (*i*) The femurs were disarticulated at the hip, keeping the tibia and fibula articulated with the femur to minimize any mechanical stress, avoiding microtraumas, and changes in the bone structure; (*ii*) Soft tissues were dissected; (*iii*) Maintaining on average for 30 days in the presence of beetles larvae (*Dermestes Maculatus*) to remove completely the soft tissue.

2.3 Signal acquisition protocol

Signals were acquired in two periods, with a 10-day interval, subjected to the same environment (23 ± 1.5ºC), according to the protocol below:

• Femurs were positioned upon a reflector steel plate (1-cm thick).

• Transducer of 5-MHz frequency (model V326, Olympus® NDT Inc., Waltham, MA, EUA), diameter of 9.5 mm and 69.3 mm focal length, excited by pulse generator (model SR9000, Matec® Inc., Hopkinton, MA, USA), and the echoes displayed on an oscilloscope (model TDS 2024B, Tektronix® Inc., Beaverton, OR, USA).

• Glass cylinder with distilled water to couple and maintain the sample in the transducer focal area with its lower hole sealed with a PVC film (10.5-mm thick).

• A soluble gel was used for coupling the cylinder and femurs.

• The transducer placed at the midpoint of the middle lateral third of the femur diaphysis (Region of Interest - ROI).

• Five signals acquired along the femur, in 1-mm steps controlled by a stereotactic holder of 2-μ resolution.

• Reference signals collected from steel plate at the same distance of femurs.

For characterization of femur diaphysis the reflection echo from the bone surface and internal bone scattering were used.

2.4 Ultrasonic parameters measurement

The echoes from the bone surface and from the inside bone structure were used to characterize femur. (Fig. 1)

To identify the reflection echo (bone surface) it was first determined the length of the reference echo by selecting de position of the extreme limits corresponding to 10% of its maximum amplitude. Then a rectangular window was established around the reference echo. After that this window was used to define the limits of the reflection echo from the bone surface. The backscattered echo was taken as the RF signal beginning just after the reflection echo and with 4-μs duration. This time duration was chosen to ensure that the backscattered signal is derived from the internal region of bone, which has estimated average diameter of 3.16mm ± 0.2mm.

An algorithm was developed in Matlab® (MathWorks Inc., Natick, MA, USA) to estimate the ultrasonic parameters from femurs and reference signals. The parameters AIB, FSAB, and TSAB are estimated based on the Apparent Backscatter Transfer Function - ABTF [17] defined as:

$$ABTF = 10\log_{10} P_{specimen}(f) - 10\log_{10} \mathrm{P}_{reference}(f) \quad (1)$$

where $P_{reference}$ and $P_{specimen}$ are the power spectra of the signals from sample and from reference plate, respectively.

The AIB parameter expresses the average value of the apparent backscatter in a studied frequency range. AIB is obtained by integration of the ABTF curve, according to equation 2:

$$AIB = \frac{\int_{flow}^{fhigh} [ABTF]}{f_{high} - f_{low}} df \qquad (2)$$

The FSAB represents the fraction of the apparent backscatter related to each frequency and is obtained as the slope value resulting from a linear regression of the ABTF *versus* frequency plot. The TSAB represents the backscatter variation as the wave propagates through the tissue. It is estimated as the slope value of a linear regression of the AIB values *versus* time, calculated by dividing the backscattered signal into five rectangular windows with the same interval.

The parameters IRC, FSIR and TSRC are based on the Reflection Transfer Function - RTF (Equation 3), which has a definition similar to ABTF [7]. The integration of RTF over frequency gives the Integrated Reflection Coefficient - IRC.

$$RTF = 10\log_{10} P_{specimen}(f) - 10\log_{10} P_{reference}(f) \qquad (3)$$

Parameter FSIR is the slope value of the linear regression of the curve RTF *versus* frequency. FSIR is calculated similarly to FSAB, which is a parameter found in the literature [17]. Thus, FSIR is the fraction of apparent reflection corresponding to each frequency. The TSRC represents the reflection variation of the wave as it propagates through the femur. It is obtained as the slope value of a linear regression of the IRC values *versus* time, calculated by dividing the reflection signal into five rectangular windows with the same interval (thus, similarly to TSAB).

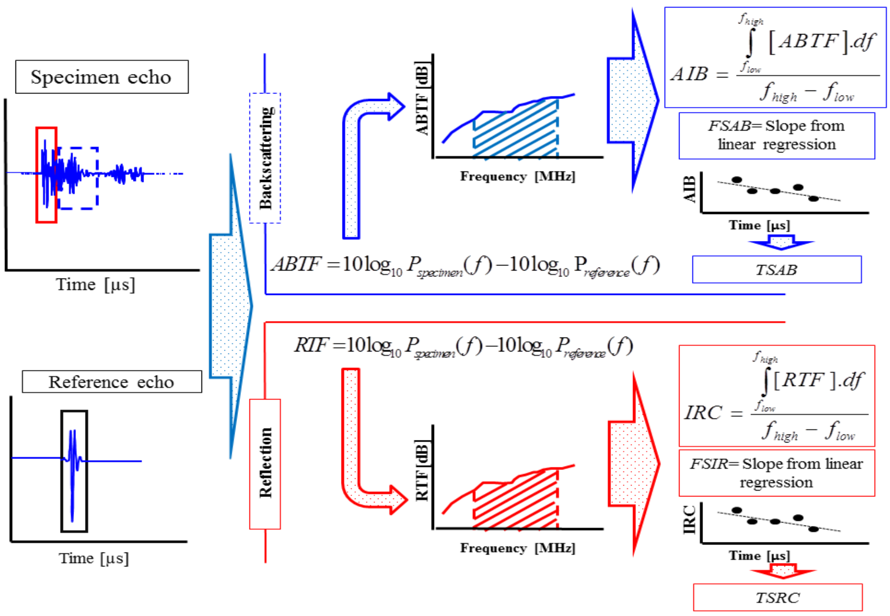

Fig. 1. Schematic diagram of the steps to obtain each of the parameters.

2.5 Statistical Analysis

Normality was testes by the Kolmogorov-Smirnov test and equal variance test. The statistical analysis using paired t-test (α = 0.05% level of significance) was applied to test the null hypothesis (all parameters came from the same population), as well as assess the repeatability of parameters and the method used for signal acquisition.

3 Results

The values of the ultrasonic backscattering parameters (AIB, FSAB and TSAB) from experiments 1 and 2 for each femur are shown in Table 1.

Table 1. Parameter values from backscattering in two experiments

Experiment	AIB [dB]	FSAB [dB.MHz^{-1}]	TSAB [dB.µs^{-1}]
1	-40.22	-1.31	-4.28
2	-41.46	-1.18	-2.58
3	-46.35	-2.43	-4.22
4	-42.02	-1.09	-3.69
5	-41.27	-1.21	-3.84
6	-43.17	-1.57	-2.62
7	-43.97	-1.80	-3.63
8	-41.52	-1.21	-3.88
9	-40.01	-0.97	-3.71
10	-43.52	-1.22	-2.47
11	-39.15	-1.35	-2.62
12	-46.88	-1.19	-3.41
1	-41.99	-1.68	-3.83
2	-37.22	-1.43	-2.96
3	-47.75	-2.29	-3.71
4	-43.23	-1.02	-3.83
5	-43.66	-1.48	-3.44
6	-49.54	-1.99	-2.82
7	-42.12	-1.39	-3.33
8	-45.89	-1.25	-3.28
9	-38.16	-0.99	-3.35
10	-43.08	-1.35	-2.91
11	-40.55	-1.38	-2.87
12	-41.25	-1.31	-3.12

The mean values and standard deviations of the ultrasonic parameters (IRC, FSIR and TSRC), in experiments 1 and 2, are shown in Table 2.

Table 2. Average values and standard deviation of each parameter in two experiments

Experiment	IRC [dB]	FSIR [dB.MHz^{-1}]	TSRC [dB.μs^{-1}]
1	-8.32 ± 1.21	-1.07 ± 0.19	-5.02 ± 1.86
2	-8.95 ± 1.53	-1.22 ± 0.28	-4.81 ± 2.23

The paired t-test (α = 0.05% level of significance) was used for the data of AIB and FSAB, which showed that AIB [T(11) = 0.42; p = 0.69] and FSAB [T(11) = 1.29; p = 0.23] belong to the same population. The normality test for TSAB failed, therefore we used the Wilcoxon Signed Rank test (α = 0.05% level of significance), which showed that TSAB [Z = 1.334; p = 0.196] belonged to the same population. Thus backscattering parameters were statistically repetitive [p > 0.05].

The paired t-test (α = 0.05% level of significance) was used for the data of reflection parameters (IRC, FSIR and TSRC), which showed that they [p > 0.05] also belonged to same population. Then, our method was statistically repetitive in all parameters.

4 Discussion

This study represents a contribution to the use of ultrasound as an adjuvant tool in diagnosis and monitoring of bone diseases and metabolic bone trauma. Ultrasound is a non-ionizing radiation requiring low cost instrumentation and suitable for *in vivo* bone structure characterization. Wistar rats femurs were adopted because they present bone structures closer to humans and so extrapolation of results is potentially easier. Six ultrasonic parameters were employed to characterize bone, based on scattering (AIB, FSAB, TSAB) and reflection (IRC, FSIR and TSRC).

Two experiments were conducted for each femur to check the repeatability of the proposed method. A set of five RF signals was acquired along the middle third of the femur lateral position by a stereotactic holder. Care was taken in minimizing anatomical variations, which would compromise the signal consistency of acquisition and parameter values.

Hoffmeister et al. [10] used the parameters AIB (-40.9 ± 2.0 dB), FSAB (-1.7 ± 0.5 dB.MHz^{-1}) and TSAB (-4.2 ± 0.6 dB.μs^{-1}) to characterize human trabecular bone *in vitro* and concluded that parameters are promising, which is in agreement with our results.

AIB values are slightly below the ones from literature [10], this can be attributed to the presence of cortical bone from femur rats (other researchers used only trabecular bone) [5,10,16] so energy loss by reflection may have occurred. On the other hand, FSAB e TSAB value present differences with respect to the literature [10] possibly because of differences in rats bone structure.

IRC parameter indicates the degree of reflection of bone tissue [6], thus a denser bone implies higher IRC value. In our study, mean values were consistent with the findings of Hakulinen et al. [16] (-10.0 ± 3.8 dB) in human trabecular bone and Pereira et al. [15] (-18.65 dB and -20.85 dB) for *in vivo* bone rats.

In our research, TSRC parameter showed a good correlation with bone cortex density. Thus TSRC parameter can be promising to monitor the progress of consolidation or to diagnose bone disease.
The next steps will be to increase the number of experiments and to apply the method in bone lesions *in vivo*.

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