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Diagnosis of osteoporosis using the low frequency acoustic response of mice femoral bones irradiated by a high frequency acoustic radiation pulse.

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Abstract—

Quantitative characterization of the mechanical properties of bones is of great importance to diagnostic pathological processes. For example, osteoporotic bones have some differences in the mechanical properties if compared with the healthy ones. In this study a new technique based on acoustic radiation force has been used as an alternative to evaluate the differences in the mechanical properties of bones, that can lead to a new method to the diagnosis of bone diseases. The technique uses a single high-frequency ultrasound pulse (MHz) to excite the medium. Non-linear interactions of this acoustic wave in the tissue produces a lower frequency signal (kHz). Femoral bones where excised from 10 healthy mice and also from 10 mice where osteoporosis had been induced. Using µCT, the porosity, trabecular number, trabecular spacing, connectivity and the connectivity density of those bones where obtained. The following step was to irradiate those samples with a short focused acoustic radiation pulse (f=3.1 MHz, t=15 µs) and acquire the low frequency acoustic response using a dedicated hydrophone (ITC 6050) with acquisition band going from 1kHz to 70 kHz. A spectral analysis of the acquired signal has been done and the results compared with the µCT data in order to see if there where correlation between them. Also, a hypothesis test has been done to see if the technique can differentiate the samples coming from the healthy group and the osteoporotic. A strong correlation was obtained between the values from the spectral analysis of the low frequency acoustic response and from the trabecular number parameter µCT (spearman correlation coefficient of 0.72 and p-value of 0.02), also a moderate correlation has been found with the connectivity parameter (spearman correlation coefficient of 0.69 and p-value of 0.03) showing that the technique is sensible to the mechanical parameters .

Keywords—ultrasound, osteoporosis, Acustic Radiation Force.

I. INTRODUCTION

Quantitative characterization of the mechanical properties of bones is of great importance to diagnostic pathological processes. For example, osteoporotic bones have some differences in the mechanical properties if compared with the healthy ones. Ultrasound evaluation of bone tissue, by conventional techniques such as B-Mode, has limitations because it has a high acoustic impedance, preventing ultrasound waves to propagate [1], [2]. The main difference between the healthy and the osteoporotic bone is the insufficient mineral intake of calcium and phosphorous which leads to osteopenia [3]. Todays standards for the bone healthy characterization are X-ray absorptiometry (DEXA), and quantitative ultrasound (QUS). Dexa worksrealy well when trying to detect changes in mineral density but the radiation exposure is a drawback, specialy when trying to mke those measurements in newborns of infants or when trying to monitor trough time. In QUS the speed of sound and the atenuation of the wave is measured by transmiting and receiving transducers (placed in the surface of the bone) and these parameters are used for the determination of the properties of the bones.[4]–[6]

In this study a new technique based on acoustic radiation force has been developed as an alternative to evaluate the differences in the mechanical properties of bones, that can lead to a new method to the diagnosis of bone diseases. The new method uses a single high-frequency ultrasound pulse (MHz) to excite the medium. Non-linear interactions of this acoustic wave in the tissue produces a lower frequency signal (kHz). This signal carries information of mechanical properties of the studied region, therefore, can be processed into values that are weighted in those characteristics.

II. Metodology

A. *Mice Femura used as sample*

The study included two groups of mice femora a) Control Group (CG) with ix specimens, b) group induced osteoporosis (GO) also with six specimens submitted to CCl4 treatment. Five-week-old mice weighting aproximatelo 18 g were used. To induce HOD, the mice were treated with CCl4 (1 mL/kg body weight) dissolved in olive oil 1:4 (v:v) administered by intraperitoneal injection twice per week. The femoral bone of the right leg of each individual animal was removed to be used as a sample.

B. *Acoustic Measurements*

The experiments used a custom builtset-up. The Set-up consists of a focused ultrasonic excitation system that emits short pulses in the megahertz range and a acquisition system tha receive the response in the kilohertz range. The excitation was made using a focused ultrasound transducer which consisted of a semi-spherical piezoelectric ceramic with a diameter of 20 mm and resonance frequency at 3.25 MHz, with a focal distance of 50 mm. The transducer was resonantly driven by a wave from a function generator (Agilent 33220A Santa Clara California USA) configured in burst mode, with the help of a dedicated power amplifier to deliver up to 100 Vpp excitation.

The acoustic response resulting from the interaction of the exciting wave and the samples has a wide range of frequencies going from a few Hz to kHz. The acquisition of the signal was done using a Hidrophone (ITC 6050C Santa Barbara, CA USA; response band 0.300 kHz to 100 kHz; average sensitivity of 157 dB/V/µPa). Ad processed using an MATLAB algorithm.

C. *Micro-CT*

Each bone was scanned by a micro-CT instrument (1172; SkyScan, Kontisch, Belgium). Mechanical parameters of the bone were measured. The bones were scanned at low resolution, with an energy level of 55 kVp and intensity of 145 mA. The results are expressed according to standardized nomenclature [7].

II. Results

A. *Group Differentiation.*

The the average of ach value found for each group is shown in table 1 and the figure 1 is a boxplot of this data. ANOVA demonstrated that the Acoustic technique was able to differentiate the two groups ($p<0.001$).

Table 1: acoustic response value obtained for each group and the standard deviation.

Group	Mean (u.a)	Std.Dev (u.a)
CG	4.23	0.5
GO	2.71	0.9

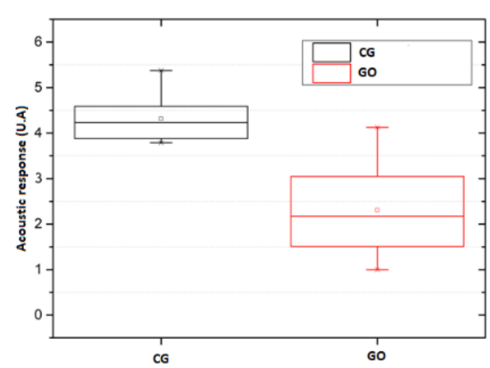

Figure 1: Boxplot of each group. in red the osteoporotic group (GO) and in Black the Control group (GO)

B. *Correlation between the Acoustics measurements and MicroCT parameters.*

The parameters obtained from the micro-CT are in the table 2. And the correlation between the acoustic signal and the micro-CT parameters are listed in the table 3. Results show a strong correlation of PEA AR with the trabecular number and moderate correlation with trabecular spacing(Tb.Sp), connectivity (Conn) e connectivity density(Conn.D).

Table 2: MicroCT parameters. BV/TV(%) is Bone volume fraction; Tb. Th (mm) is trabecular thickness; Tb. N is trabecular number; Tb. Sp is trabecular separation; Conn. Is connectivity and Conn.D is connectivity density.

	CG		GO	
	Mean	**Std.Dev**	**Mean**	**Std.De**
BV/TV (%)	11.961	3.47	10.398	3.53
Tb. Th (mm)	0.054	0.009	0.052	0.006
Tb. N(1/mm)	2.287	0.28	1.679	0.14
Tb. Sp (mm)	0.174	0.02	0.222	0.03
Conn.	598.00	175.11	358.00	136.54
Conn.D	264.84	79.13	172.66	51.50

Table 3: correlation between the acoustic signal and the micro-CT parameters.

micro-CT vs AS	Spearman Correlation (ρ)	*p-Value*
BV/TV (%)	0.60	0.069
Tb.Th(mm)	-0.08	0.819
Tb.N(1/mm)	0.72	0.020
Tb.Sp(mm)	-0.51	0.136
Conn	0.69	0.026
Conn. D	0.64	0.044

III. Conclusions

A strong correlation was obtained between the values coming from the spectral analysis of the low frequency acoustic response and from the trabecular number parameter coming µCT (spearman correlation coefficient of 0.72 and p-value of 0.02), also a moderate correlation has been found with the connectivity parameter (spearman correlation coefficient of 0.69 and p-value of 0.03) showing that the technique is sensible to the mechanical parameters that are changing between the samples. Also, when applied an analysis of variance (anova) in the data coming from each of the two groups a p-value smaller than 0.01 has been found, suggesting that the technique has potential to can be applied in the diagnostic of osteoporosis.

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