Characterization of Baboon Cortical Bone Microstructural Changes by Low-Field NMR and Correlation of Bone Mechanical Properties

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Abstract— A NMR spin-spin (T_2) relaxation technique has been described for determining the porosity, mobile and the bound water distribution in baboon cortical bone and correlate to their mechanical properties. The technique of low-field proton NMR involves spin-spin relaxation and free induction decay (FID) measurements, and the computational inversion methods for decay data analysis. The advantages of using NMR T_2 relaxation techniques for bone water distribution are illustrated. The CPMG T_2 relaxation data can be inverted to T_2 relaxation distribution and this distribution then can be transformed to a pore size distribution with the longer relaxation times corresponding to larger pores. The FID T_2 relaxation data can be inverted to T_2 relaxation distribution and this distribution then can be transformed to bound and mobile water distribution with the longest relaxation time corresponding to mobile water and the middle relaxation time corresponding to bound water. The technique is applied to quantify apparent changes in porosity, bound and mobile water in cortical bone. Overall bone porosity is determined using the calibrated NMR fluid volume from the proton relaxation data divided by overall bone volume. The NMR porosity, bound and mobile water components are determined from cortical bone specimens obtained from baboon donors of different ages, and the results are correlated to bone mechanical properties.

Keywords— Baboon bone, NMR, Spin-spin relaxation.

I. INTRODUCTION

Bone quality in terms of water distribution, porosity, and pore size distributions in cortical bone and related these measures can be used to predict bone mechanical properties. In this study it is to determine the porosity, mobile and the bound water distribution in baboon cortical bone then correlate with bone mechanical properties.

Water is not only present in the microscopic pores, which increase in number and size with age, water also exists within the extracellular matrix of bone tissue. Some studies have investigated the relationships between the mechanical properties and water distribution in cortical bone.

The results suggest that the removal of water from the extracellular matrix, in addition to that removed from the void spaces within bone, affects the mechanical properties of bone. Robinson [1] reported that water could exist as two fractions, one driven off at 50 °C associated with marrow-vascular-osteoid and one driven at 100 °C associated with the calcified matrix. This implies to us that water distribution in bone not only exists as mobile water in pores but also has some other forms because the amount of water loss in bone varies with the heating temperature (i.e., thermal energy level).

Bone is a two component composite material in which the mineral phase (mainly hydroxyapatite) confers the strength [2] and stiffness [3], and the organic matrix (mainly Type I collagen) primarily influences the toughness of bone [2, 4, 5]. While mineral and collagen each contribute to bone's competency, as do micro-architecture (e.g., porosity and osteonal morphology), macro-structure (e.g., curvature of diaphysis and thickness of cortical shell), and in vivo microdamage (e.g., microcracks and diffuse cracks), their interaction with water is equally important to the mechanical behavior of bone. Early studies demonstrated that bone stiffness, tensile strength, and hardness increase, whereas strain at fracture and energy to fracture decrease, following the dehydration of bone tissue [6-11].

The total fluid contained within bone tissue includes fluid explicitly within the intrinsic microstructural porosity [12-15], fluid contained within microcracks and diffuse damage [16], and fluid within the extracellular matrix. The distribution of water within bone tissue appears to change throughout life. It has been reported that water in bone tissues changes with skeletal growth [17] and with progressive mineralization [18,19]. The observation that mineral content increases with age, tapering at elder years [20, 21], implies that the amount of water in the tissue would likely be reduced in the elderly skeleton. Currently, the influence of water removal on the strength and toughness of cortical bone has studied by Nyman et al [22].

The results have shown that loss of water in the collagen phase decreases the toughness of bone, whereas loss of water associated with the mineral phase decreases both bone strength and toughness. However, in his study, the loss of water was used the dehydration method for such mechanism studies. Here, we hypothesize that NMR relaxation technique can non-destructive and non-invasive assess the bound water (water bound to collagen and mineral phases) and mobile water (water within the pores) in baboon cortical bone. The obtained information of water distribution within baboon bone tissue will be used for further bone mechanical property studies, and to more accurately assess bone quality.

II. BACKGROUND

A. Time domain NMR

To make an NMR measurement, the specimen to be inspected must be located in a magnetic field (B_0), and must be exposed to bursts (or pulses) of a radio frequency (RF) field (B_1). Following the RF pulse (or pulses) the resonating nuclei process in the field induces small transient signal voltages in an adjacent sensor coil. These NMR signals are the magnetization decay signals. This method is generally called time domain NMR. The signal is called the free induction decay (FID) signal and the peak amplitude of the magnetization (signal) is proportional to the concentration of selected atomic specie (e.g. hydrogen) in the measured volume of the material.

When nuclei absorb energy, thermal equilibrium is disturbed and the absorbed energy is exchanged exponentially with the surroundings. These exchanges are characterized by two primary time constants: the spin-lattice (T_1) and the spin-spin or transverse (T_2) relaxation times. Both T_1 and T_2 may have multiple values in a multiphase material and these parameters can be used in analysis of the properties of the material. The NMR instrument built at the Southwest Research Institute (SwRI) laboratory set up at 27 MHz proton resonance can produce better uniformly filed, and can obtain three components (protons in solid, bound and liquid-like or mobile phases) from the equilibrium nuclear magnetization of bone, i.e., M_{s+b+l} due to the different T_2 -Fid relaxation time constants.

B. Determination of bone porosity and bound water

It is known the total intensity of magnetization (M_{s+b+l}) from the NMR FID signal is due to the water present inside the pores (mobile water), the water that has undergone hydration with the bone (bound water), and the protons in the collagen and mineral matter (solid-like protons).

The total amplitude of T_2 relaxation envelopes, measured by the NMR spin echo train (CPMG) [23, 24], is a representation of the liquid phase inside the pores. The NMR CPMG magnetization amplitude of liquid inside the pores can be transferred to the volume of water using the known volume of water to obtain the NMR CPMG magnetization amplitude for the reference. Then the obtained bone NMR CPMG signal amplitude compares with the reference water signal to calibrate the volume of the liquid (water) inside the bone. The volume of bone is determined by water displaced method. The detailed calibration was described previously [25]. Therefore, after known the volume of the bone sample, the porosity of the bone can be estimated as the ratio of water volume inside the bone to the bone volume.

Since T_2 obtained from the water protons within the different sizes of pores, therefore, the inversion CPMG spectrum i.e., the distribution of T_2 spectrum corresponds to the pore-size distribution with the longer relaxation times corresponding to larger pores. Using the FID method, the distribution of T_2 -Fid spectrum determines the protons in solid, bound and mobile water phases.

C. Loosely (and Tightly) Bound Water Determination

To determine the loosely bound water within bone, the bone will be freely dried in air at room temperature by weighing the sample before and after drying to obtain the total water loss. Then, NMR CPMG measurement will be carried out (before and after free dry) to determine the total mobile water loss. Thus, the total weight of water loss subtracted from the total mobile water loss will be the loosely bound water loss. We will do these measurements for every half hour period. The samples will be free dried in air for one half hour, after recording the sample weight, then all the samples will be sealed within the test tubes and measured using the NMR. After all sample measurements are completed, the samples are allowed to free dry for another half hour and sealed in the test tube for another round of NMR measurements. These processing steps are performed approximately eight times, i.e., for a total of 4 hours of drying time in air. Since FID (T_2 -FID) measurement can be used to determine the total bound water and mobile water within the bone; therefore, known the loosely bound water, then tightly bound water can be determined [26, 27].

D. Theory (technique for inversion of relaxation decay time)

When the diffusion effect can be negligible on the low field NMR for CPMG method, that the relaxation rate $1/T_2$ is proportional to the surface-to-volume (S/V) ratio of the pore [28]:

$$1/T_2 = \rho (S/V)_{pore} \quad (1)$$

Where ρ is the surface relaxivity, which is a measure of the effects of the pore surface enhancing the relaxation rate. Equation (1) indicates that the NMR relaxation time is proportional to pore size.

For both CPMG and FID measurements, the observed NMR magnetization will depend upon the T_2 distributions of all pores or protons in different phases of the material. This implies that the NMR transverse relaxation (T_2) data can be expressed as a sum of exponential functions:

$$M(t_i) = \sum_{j=1}^m f(T_{2,j}) \exp(-t_i/T_{2,j}) \quad (2)$$

Where $f(T_{2,j})$ is proportional to the number of spins, which relax with a time constant, $T_{2,j}$. $M(t_i)$ is the NMR magnetization decay from fluid saturated cortical bone in the CPMG measurement or from protons in the different phases in the FID measurement. Equation (2) can be inverted into a T_2 relaxation time distribution with the longer relaxation times corresponding to larger pores. Using the FID method, the distribution of T_2 determines the protons in solid, bound and mobile water phases [29, 25].

III. MATERIALS AND METHODS

A. Baboon Bone Sample preparations

A total of 10 female baboon cortical bone samples including 5 in younger group (6 to 8-year-old, with ID numbers of 14373, 14435, 14696, 15287, and 16033) and 5 in elder group (26 to 29-year-old with ID numbers of 1557, 2000, 2002, 2594, and 2617) were collected and used in this project. The sample size was about 8 mm x 3mm x 40 mm. All the specimens were stored in phosphate-buffered-saline-soaked gauze at -20°C ; and prior to NMR measurements, the samples were completely thawed at room temperature.

B. NMR Experimental Studies

An SwRI built 0.5 to 40 MHz broad line NMR system was set up at a proton frequency of 27 MHz for these measurements.

^1H spin-spin (T_2) relaxation profiles were obtained by using NMR CPMG $\{90^\circ [-\tau - 180^\circ - \tau (\text{echo})]_n - \text{TR}\}$ spin echo method with a $6.5 \mu\text{s}$ wide RF- 90° pulse, τ of $500 \mu\text{s}$, and TR (sequences repetition rate) of 15 s. The FID signal was sampled and recorded at $2 \mu\text{s}$ intervals using a $6.5 \mu\text{s}$ wide 90° RF-pulse for the bone mobile water and bound water measurements. An inversion relaxation technique was used to invert both CPMG and FID data to T_2 relaxation distribution spectrum.

C. Mechanical Testing

After NMR measurements, specimens were monotonically loaded to failure to determine their mechanical properties. Specimens were loaded to failure under displacement control using a closed-loop elector hydraulic test machine (MTS, Minneapolis, MN) using a strain rate of 0.01%/sec. strain as monitored using a clip gauge extensometer and the applied load was recorded from a calibrated load cell. All specimens were tested at room temperature. The mechanical properties of bone stress, strain, toughness, and fracture of work energy were determined

IV. RESULTS AND DISCUSSION

Figure 1a and 1b show an example of NMR CPMG relaxation decay data for baboon cortical bone samples from different porosities plotted in a linear scale, respectively, indicating multiexponential relaxation behavior. The higher amplitude signal is for the bone from the higher porosity (6.95%) while the lower amplitude signal is for the lower porosity (3.52%). The intensity differences between them are proportional to the total liquid-like (mobile water) proton magnetization differences.

Figure 1a. NMR CPMG spin relaxation decay signal from 6.95% porosity (weight at 0.5984 g) in female baboon cortical bone sample.

Figure 1b. NMR CPMG spin relaxation decay signal from 3.52% porosity (weight at 0.8241 g) in female baboon cortical bone sample.

Using the T_2 relaxation decay data shown in Figures 1a and 1b, the inversion T_2 relaxation distribution patterns for these two specimens are shown in Figure 2a and 2b, respectively.

Figure 2a. Inversion T_2 relaxation time spectrum for female baboon cortical bone with porosity at 6.95%, and weight at 0.5984 g.

Figure 2b. Inversion T_2 relaxation time spectrum for female baboon cortical bone with porosity at 3.93%, and weight at 0.8241 g.

Figures 3a and 3b show an example of an NMR FID signal from the same baboon cortical bone as in Figures 1a and 1b, plotted in a linear scale.

Figure 3a. NMR FID spin relaxation decay signal from 6.95% porosity (weight at 0.5984 g) baboon cortical bone sample.

Figure 3b. NMR FID spin relaxation decay signal from 3.52% porosity (weight at 0.8241 g) baboon cortical bone sample.

The inversion T_{2-Fid} relaxation distribution patterns for these two samples in Figure 3a and 3b are shown in Figure 4a and 4b. The three peaks in the spectrum, from left to right, result from protons in the solid component (peak near 30 μ s), protons in bound water (peak near 300 μ s), and protons in mobile water (peak near 2-3 ms), respectively.

Figure 4a. Inversion FID T_2 relaxation time spectrum for 6.95% porosity (weight at 0.5984 g) baboon cortical bone sample.

Figure 4b. Inversion FID T_2 relaxation time spectrum for 3.52% porosity (weight at 0.8241 g) baboon cortical bone sample.

We can assume that the third peak (from the left to right) in the inversion T_{2-FID} relaxation spectrum is for mobile water component since the signal intensity obtained from CPMG measurement is in a good correlation with the intensity obtained from the intensity of the third peak of the FID inversion spectrum as determined in our previous measurement [27].

It is known that the T_2 relaxation distributions for liquid-like phase are from milliseconds to seconds (Figure 2), while the T_{2-FID} relaxation time distributions are only from microseconds to milliseconds even for three different phase components (Figure 4). The major difference between the T_2 relaxation times obtained using the NMR FID and CPMG methods is that the T_2 relaxation time constant is significantly shorter from FID technique than in CPMG technique. This is because that in FID measurement the signal decays in a time T_{2-FID} is often determined by field inhomogeneity while an ingenious CPMG measurement is a practical method for overcoming the inhomogeneity problem [30]. Therefore, it is better using CPMG obtained mobile water component for bound water calibrations. Therefore, from CPMG measurement the mobile water within bone can be calculated, and from FID measurement the ration of bound water to mobile water can be calculated, i.e., the bound water within bone can be calculated. Table 1 shows the porosity, bound and mobile water components determined from female baboon by NMR. Table 2 shows the mechanical property changes with the porosity and bound water change within the bone.

Table 1.
Porosity, bound and mobile water determined from female baboon bones by NMR measurements.

Baboon (ID # (26-29 y))	Sample Weight (g)	Sample Volume (cm ³)	Porosity (%)	Bound Water to Mobile Water Ratio	Baboon (ID # (6-8 y))	Sample Weight (g)	Sample Volume (cm ³)	Porosity (%)	Bound Water to Mobile Water Ratio
14373	.8241	.3928	3.52	4.378	1557	.6851	.3408	3.71	4.323
14435	.7822	.3755	3.46	3.209	2000	.6513	.3356	5.81	1.876
14696	.5984	.3022	6.95	2.193	2002	.6886	.3496	4.07	3.596
15287	.7617	.3748	4.82	2.001	2594	.7661	.3897	4.92	2.296
16033	.8556	.4320	6.10	1.759	2617	.5762	.2960	4.62	2.512
Mean			4.97 ± 1.54	2.708 ± 1.085	Mean			4.63 ± 0.80	2.921 ± 1.009

In table 2, the average of porosity is slightly increased, while the ratio of bound water to mobile water is slightly decreased compared between elder to younger groups. In this study, bound water is associated with both strength and work to fracture of bone, while mobile water is correlated with modulus of elasticity and appeared to quantify the level of microscopic pores with bone. It is found that the modulus of elasticity is significantly higher in the older bones compared to the younger bones. Furthermore, the increased elastic modulus is associated with a significant increase in loosely bound water compared to the young age group.

Since, in these two groups, the average porosity, and the average ratio of bound water to mobile water are not significantly different, it appears that the loosely bound water is an important factor for bone elastic properties. Also the volume fraction of loosely bound water is significantly higher in the old group compared to the young group. There are also significant differences in the yield strain, and toughness, between the young and the old groups suggesting the amount of bound water, and loosely bound water within the bone tissue influence the post-yield and failure behavior of cortical bones.

Table 2.
NMR baboon cortical bone water distributions are correlated to bone mechanical properties

Baboon (ID #)	Porosity (fraction)	Bound Water to Mobile Water Ratio	Loosely bounded water/Volume	Elasticity (Gpa)	Yield Strain (%)	Toughness (Mpa)
1557	0.0352	4.378	0.0182	25.0171	0.773	2.135
2000	0.0346	3.209	0.0282	25.448	0.794	1.337
2002	0.0695	2.193	0.0463	25.529	0.774	0.325
2594	0.0482	2.001	0.0277	20.147	0.778	1.140
2617	0.0610	1.759	0.0453	18.477	0.768	1.382
Average	0.0497 ± 0.015	2.708 ±1.085	0.0331 ± 0.012	22.924 ± 3.354	0.779 ± 0.010	1.264 ± 0.647
14373	0.0371	4.323	0.0201	20.695	0.964	2.623
14435	0.0581	1.876	0.0228	15.715	0.944	0.358
14696	0.0407	3.596	0.0145	16.990	0.931	1.715
15287	0.0492	2.296	0.0315	17.471	0.819	1.163
16033	0.0462	2.512	0.0252	13.176	0.952	2.0193
Average	0.0462 ±0.0081	2.921 ±1.009	0.0228 ± 0.0062	16.809 ± 2.736	0.922 ± 0.059	1.575 ± 0.861

V. CONCLUSION

This is the first attempt that the porosity, water distributions of mobile and bound water has been measured non-invasively and non-destructively in baboon cortical bone. The porosity, mobile and bound water pattern differences between baboon cortical bones are observed. These differences are correlated with the bone mechanical properties such as elasticity, yield strain and toughness for bone quality studies.

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