

ELECTRO-MECHANICAL STUDIES OF SKELETAL TISSUES AT HIGH RESOLUTION

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ABSTRACT

Measurements of the electro-mechanical properties of bone tissues could prove important in the development of models to explain bone remodeling. Two high resolution scanning techniques have been used to obtain acoustic properties and microwave resistivity respectively for specimens of both human cortical and trabecular bone. An Olympus UH3 Scanning Acoustic Microscope (SAM) has been used for the former studies; a newly developed, innovative non-destructive, non-contact Scanning Evanescent Microwave Probe (SEMP) at Case has been used for the latter studies on the same specimens. SAM has been performed at 400 and 600 MHz, with nominal resolutions of 2.5 and 1.7 μm respectively. This portion of the studies showed that the lamellar organization of trabecular bone exhibits the same alternation of acoustic impedance in adjacent lamellae as has been observed within the adjacent concentric lamellar structures of secondary osteons (haversian systems). The SEMP generates evanescent microwaves at the tip of a quarter wavelength resonator at 1 GHz and has a resolution of 10 μm . The frequency is now being increased to 10 GHz, yielding a resolution approaching 1 μm .

INTRODUCTION

SAM is a powerful tool for studying the acoustic properties of materials at high resolution. An RF signal is applied to a piezoelectric transducer which generates an ultrasonic wave of an appropriate frequency. The wave travels through a high quality synthetic sapphire single crystal lens with a hemispherical indent at the far end. This lens provides the focusing function for the acoustic wave onto the specimen surface using a fluid, in this case water, as the coupling medium. After the incident wave interacts with the material a portion is reflected from the fluid-

specimen interface and a portion is transmitted into the specimen. The reflected amplitude, r , given by $r = (Z_2 - Z_1)/(Z_2 + Z_1)$ where the Z s are the acoustic impedances of the fluid and the specimen, is captured by the same lens and transducer now acting as the receiver. SAM stores the value of r as a voltage and then converts it to a shade of gray exhibited on a TV screen. The lens and/or the specimen moves point by point scanning over a given area resulting in a map related to the specimen's acoustic impedance. Backscatter scanning electron microscopy (SEM) has also been used with several of the specimens to obtain a measure of the density distribution in the same specimens studied with SAM.

The SEMP developed at Case consists of a quarter wavelength resonator, fabricated on a Duroid substrate of permittivity $10\epsilon_0$ coupled to a feedline by a three fingered interdigitated capacitor. In order to increase the probe's resolution, its tip is tapered to restrict the fields; additional confinement of the fields is obtained by using a small aperture in front of the tapered tip. Resonant frequency of the probe shifts with the impedance it is observing. The impedance is determined by the conductivity and permittivity of the specimen. Any variations in these values due to defects, impurities, grain boundaries, stresses, crystallite orientation will affect the probe output and thus can be mapped; mechanical, physical and chemical properties which affect the permittivity and conductivity of a material will be detected by changes in the reflection coefficient of the specimen. The square of the reflection coefficient R is given by: $1 - (n \delta e^2 \tau / m)(\mu / \epsilon)^{0.5}$, where n is the number of electrons/unit volume, δ is the skin depth, τ is the mean free scattering time, e is the electron charge, m is the electron mass, μ is the permeability and ϵ the permittivity of the

specimen. A more complete description will be found in Pathak et al.¹.

METHODS

Specimens of cortical bone were obtained from the mid-shaft of a human cadaveric femur. Specimens of trabecular bone were obtained from the middle of the femoral condyle of another human cadaveric femur. In both cases, the specimens were cut slowly with a diamond knife under running water. They were then polished with successive finer and finer paper down to 600 grit. The specimens were maintained in fluid in a freezer until thawed for use with either the SAM or SEMP.

The SAM studies were done using the 400 and 600 MHz burst mode lenses, nominal resolutions of 2.5 and 1.7 μm respectively. Since the focal length of the lens in each case is much less than 1mm, a drop of water is sufficient to couple the lens to the bone specimen by surface tension. The specimen is leveled, via the software resident with the UH3, so that the acoustic beam is everywhere perpendicular to the specimen's surface. Scan lengths range from 100 μm up to 2mm. Three controls determine the range of shades of gray; attenuation, intensity and contrast. It is imperative that these parameters be carefully controlled to avoid saturation of the signals.

The UH3 does not provide an internal calibration to determine the value of acoustic impedance from the reflection coefficient. A calibration system was developed based on correlating the voltage recorded in the receiving mode and the reflection coefficient measured for well-defined materials whose acoustic impedance's and elastic moduli were measured independently by ultrasonic wave propagation. Thus it is possible to determine the value of Z of any desired point on the specimen. $Z = \rho v$ and elastic modulus $E = \rho v^2$ are closely related. Using the calibration curves, it was then possible to obtain values of E for selected areas of both the cortical and trabecular bones.

The same specimens of both cortical and trabecular bone were then scanned over the same areas as with SAM using the SEMP at 1 GHz (nominal resolution 10 μm).

RESULTS

Figure 1A is a 400 MHz (120° aperture, burst mode) SAM micrograph of a portion of human cortical bone cut transverse to the bone axis. The same three key observations reported by Katz and Meunier² in their SAM study on related specimens at 600 MHz (120° aperture, burst mode) are observed here. This includes: the outermost lamella of each osteon having a darker shade of gray (lower density and elastic modulus); adjacent moduli alternate in their shades of gray - dark, light, dark, etc. - implying-compliant, stiffer, compliant, etc. - lamellar properties; and the outermost lamellae of adjacent abutting osteons appear to have their shades of gray interdigitate, indicating similar elastic properties, although their structures are quite distinct as confirmed by SEM studies. The specific area mapped on Figure 1A has an interesting feature which is why this specimen was chosen for the initial study using the SEMP as well. The two central haversian systems (osteons) exhibit much darker gray levels than the neighboring systems. This is presumed to be associated with lower densities and/or elastic moduli of both the organic and inorganic constituents of bone and/or alterations in their orientations, the types of variations in material properties that will affect the reflectivity recorded by SEMP. Figure 1B is the SEMP scan over an area close to that of Figure 1A on the same specimen. The voltage varies by 197 mV across the image. The circular-like regions indicated by the arrows corresponds to the very dark osteons observed on Figure 1A.

DISCUSSION

Since both the SAM and SEMP studies are done with the specimen maintained in a fluid environment, the studies can be repeated with little or no changes in the specimen structure and material properties. Thus, the scans are mapping the specimen's respective intrinsic properties, elasticity and microwave resistivity.

In order to achieve still higher resolution with the SEMP, its frequency is being increased to 10 GHz which should increase the resolution to approximately 1 μm . Since the deformation of bone is important in its resorption and remodeling, a miniature mechanical testing system is being designed which will fit on both the SAM and the

SEMP. This will permit studies of changes in both the elastic and the electrical properties during the various forms of applied deformation.

As support for these property studies, structure of the same specimens is observed both by optical microscopy and SEM. Since these require the bone specimens to be dry, they must be performed after the SAM and SEMP studies are completed.

The combination of SAM and SEMP is also being applied to other biological tissues such as skin, cartilage, etc. As the SEMP properties of different tissues are compiled it is possible that the probe could be made into a useful imaging tool, complementing x-ray and NMR imaging .



Fig. 1A 400 MHz (120 aperture, burst mode) SAM micrograph (resolution approximately 2.5 μm) of a portion of human femoral cortical bone cut transverse to the bone axis. The two center osteons show darker gray levels than does the surrounding bony tissue.

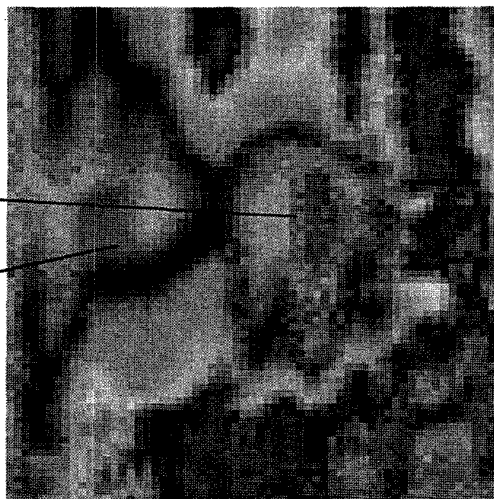


Fig. 1B 1 GHz SEMP scan over an area similar to that shown in Fig. 4 on the same specimen. The voltage across the image varies by 197 mV. The circular regions indicated by the arrows corresponds to the darkest osteon seen on Fig. 1A.

CONCLUSIONS

For the first time it has been possible to measure both the elastic and the electrical properties of skeletal tissues at the micrometer level of resolution. Data from these measurements could be useful for testing the validity of bone remodeling theories. Data from the use of even higher frequencies for both methods - the Kramer Scientific SAM operates at up to 2 GHz, 1/2 μm resolution; and at 10 GHz and higher for the SEMP, also sub-micrometer resolution - and a mechanical testing stage for deforming specimens during scans, could be the driving force for refining such remodeling theories.

REFERENCES

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2. J.L. Katz and A. Meunier, "Scanning acoustic microscopy studies of the elastic properties of osteons and osteon lamellae" J. Biomech. Eng. (1993) 115, 543-548.