ESRF	**Experiment title:** A comprehensive study of the relationship between acoustic impedance and degree of mineralization of calcified cartilage and bone	**Experiment number**: MD-52
Beamline: ID19	**Date of experiment**: from:25/02/2004 to:29/02/2004	**Date of report**: 06/2005
Shifts:	**Local contact(s)**: Dr Elodie BOLLER	*Received at ESRF:*

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The evaluation of microstructural and intrinsic material properties of biological tissues requires the development of high resolution modalities to map the spatial distribution of these properties at the microstructural (or tissue) level. High Frequency Scanning Acoustic Microscopy (SAM) allows the evaluation of spatial changes in material properties at the microscopic scale. SAM has been used to determine, through local measurements of acoustic impedance, inhomogeneities in tissue acoustic properties of site specific mineralized tissues (calcified cartilage and bone). Synchrotron radiation microtomography (SR-µCT) is currently used for the determination of 3D microstructures and quantitative assessment of mineral density in mineralized tissue. Because the acoustic impedance Z is closely related to tissue density (ρ) and elasticity (stiffness) through the relationship: $stiffness = \rho Z^2$, the combined evaluation of tissue density (SR-µCT) and acoustical impedance (SAM) leads to the quantitative assessment of intrinsic tissue stiffness, which a relevant mechanical property of mineralized tissue. The current collaborative work (between the LIP UMR 7623, the Q-BAM group (Martin Luther University, Halle, Germany), the Laboratoire of Pharmacologie CNRS UMR 7561 and CREATIS CNRS UMR 5515) involved *in vitro* investigation of two types of mineralized tissues (calcified cartilage and subchondral bone from rat patellae and cortical bone from human radii) using SAM and SR-µCT modalities. The goals were threefold: (i) validation of SAM imaging to resolve tissue microstructure, (ii) determination of tissue stiffness (at a microscopic scale) and (iii) providing an insight into the interplay between tissue mineralization and stiffness.

The experiments were performed on beamline ID19. Since the aim of the experiment was to compare the SAM and SR-µCT images, we selected a pixel size on the detector of 4.9 µm (spatial resolution of 10 µm). Using the 2048 x 2048 CCD based 2D detector, this choice enables to get a field of view of 10 x 10 mm2 and thus to encompass the entire samples. The energy was set to 23.3 KeVfor bone and 20.5 KeV for rat patellae. For each sample 900 radiographic images under different angles of view were recorded. The 3D images were then obtained by applying an exact tomographic reconstruction algorithm, based on filtered backprojection . The size of the cubic voxel in the reconstructed images was 4.9 µm.

Analysis of Cortical bone: The study aimed at i) validating 200 MHz SAM for the assessment of cortical bone microstructure using reference data obtained with SR-µCT, ii) deriving the anisotropic stiffness coefficient c_{33} at the tissue level. Ten human specimens of cortical bone (radius) were explored using 200 MHz SAM (spatial resolution of 8 µm). Site-matched regions were analyzed on both the acoustic impedance

(Z) and degree of mineralization of bone (DMB) images (Fig. 1). Structural parameters, including diameter and number of haversian canals per cortical area (Ca.Dm, N.Ca/Ar) and porosity Po were assessed with both methods using a custom developed image fusion and analysis software. A model was used to relate the DMB to mass density and calculate local stiffness c_{33} from the combination of Z and DMB. A strong correlation was found for microstructural properties derived from both techniques (Table 1)

Table 1 : Microstructural features derived from SAM and SR-µCT measurements

	SR-µCT	SAM	R^2	RMSE
N.Ca (mm-1)	10.4 ± 4.8	10.8 ± 5.3	0.97	5.6
Ca.Dm (µm)	54.3 ± 9.3	54.9 ± 9.5	0.72	5.2
Po (%)	5.7 ± 2.4	6.5 ± 2.3	0.91	0.92

The derived stiffness c_{33} (35.9 ± 12.8 GPa) was highly correlated to the square of Z (R^2=0.99, $p<10^{-4}$) while the correlation coefficient of a power fit between DMB and c_{33} was only moderate (R^2 = 0.31, $p < 0.0001$). This indicates that measurements of DMB are unlikely to predict accurately bone elasticity. Factors such as collagen cross-linking, orientation, mineral cluster size which will not affect density measurements could influence both acoustic and elastic properties. Our findings suggest that SAM fulfills the requirement for simultaneous evaluation of cortical bone microstructure and elastic properties at the tissue level (3 publications in peer reviewed journals and 4 presentations at international conferences).

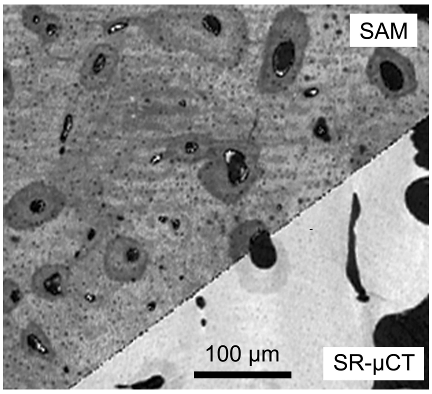

Fig 1. Detail of a fused acoustic impedance *Z* (SAM) and *DMB* (SR-µCT) image. For a better illustration the diagonal line separates regions, for which either the SAM or the SR-µCT image is in the foreground.

Analysis of Calcified cartilage: The cartilage/bone interface undergoes constant remodeling (cartilage and bone formation, mineralization and resorption) during skeletal development and degenerative diseases. The interaction between cartilage and bone and the role of the calcified cartilage layer are not well understood. Our major aim was to determine the relative contribution of tissue mineralization to acoustic impedance variation of cartilage/bone interface measured at 400 MHz (3 µm lateral resolution) during the growing process. Sixteen patellae taken from 8 immature (5-week-old) and 8 mature (15-week-old) Wistar rats were explored by sR-µCT. The 3D images were reconstructed and a software is under development to extract the image sections, taking into account the complex saddle shape of the rat patella, to extract the region of analysis (corresponding to acoustic region) and determine the local degree of mineralization of the samples. SR-µCT images displayed changes in bone structural organization and mineralization at a microscopic scale (Fig. 2). While no evident transition between hyalin cartilage and bone was observed in immature patella, two regions of different mineralization could be distinguished in mature bone. These preliminary observations are concordant with acoustic findings. SAM results indicated no Z variation in cartilage/bone zone for immature rats. In mature animals, a region just beneath hyalin cartilage where the lower Z (4.5 ± 0.6 Mrayl) may correspond to the less mineralized transitional zone between bone and cartilage. A region of higher reflection coefficient (Z = 5.2 ± 0.7 Mrayl) may, presumably, indicate higher mineralization in the deeper and earlier formed bone. The complete study of the relationships between Z and mineralization is under way.

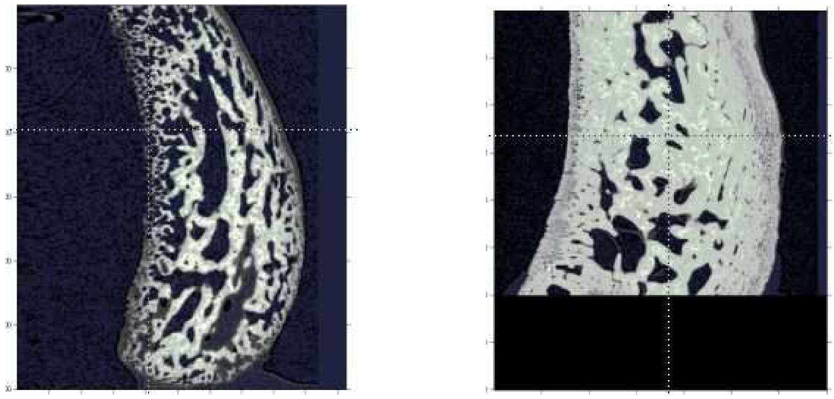

Fig. 2 : SR-μCT images of a longitudinal cross section of rat patellae immature (left) and mature (right).

Publications :

1. Raum K, Leguerney I, Chandelier F, Bossy E, Talmant M, Saïed A, Peyrin, F, Laugier P. Structural and elastic determinants of axial transmission ultrasonic velocity in the human radius. Ultrasound in Medicine and Biology 2005, (In press).

2.Raum K, Leguerney I, Chandelier F, Talmant M, Saïed A, Peyrin, F, Laugier P. Spatially resolved elastic stiffness mapping in bone Part I: Assessment of structural and tissue properties with SAM and SR-μCT. Submitted to J Biomechanics, June 2005.

3. Raum K, Cleveland R, Peyrin F, Laugier P. Spatially resolved elastic stiffness mapping in bone - Part II: Derivation of elastic stiffness from mineral density and acoustic impedance. Submitted to J Biomechanics, June 2005.

Communications :

Raum K, Leguerney I, Chandelier F, Talmant M, Saïed A, Peyrin F, Laugier P. Structural and elastic determinants of axial transmission ultrasonic velocity in the human radius. 148th Meeting of the Acoustical Society of America (ASA), November 15-19, 2004, San Diego, California, USA. JASA, 116 (4 Pt 2), 2478.

Chandelier F, Leguerney I, Raum K, Talmant M, , Saïed A, Peyrin F, Laugier P. Assessment of cortical bone microstructure and material properties using high resolution scanning acoustic microscopy. 148th Meeting of the Acoustical Society of America (ASA), November 15-19, 2004, San Diego, California, USA. JASA, 116 (4 Pt 2), 2491.

K Raum, F Chandelier, I Leguerney, M Talmant, A Saïed, F Peyrin, R Cleveland, P Laugier. Bone microstructure and elasticity assessed by high resolution scanning acoustic microscopy. World Congress on Ultrasonics - Ultrasonics International (WCU/UI 05), 28th August 28 - 1st September, 2005 Beijing, China

K Raum, F Chandelier, I Leguerney, M Talmant, A Saïed, F Peyrin, R Cleveland, P Laugier. Bone microstructure and elasticity assessed by high resolution scanning acoustic microscopy. Bone quality : what is it and can we measure it ? May 2-3, 2005 Bethesda, Maryland, USA