

ASSESSMENT OF PHYSICAL PROPERTIES OF TISSUES AND BONE SUBSTITUTES MATERIALS IN HISTOLOGIC SECTIONS BY SCANNING ACOUSTIC MICROSCOPY (SAM)

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INTRODUCTION: Large bone loss is mostly treated by autologous bone transplantation. Bone substitutes such as calcium phosphates (Ca-P) have been developed to enhance the healing process in cases where the amount of bone available is not sufficient to reconstruct the injured part. The assessment of mechanical property changes of these *in vivo* degrading materials and their gradual replacement by bone is of great practical importance. Scanning Acoustic Microscopy (SAM) is a non-destructive method, which works on the principle of propagation and reflection of acoustic waves at interfaces and is able to provide information about local density and stiffness with a lateral resolution of a few tenths of microns.¹ The aim of this study is to set up a SAM measurement system that permits characterization of histologic specimens with an additional focus on their mechanical properties. Aspects like sample preparation and long-term stability of the measurements are discussed since high resolution scans are of extremely long duration.

METHODS: The Scanning Acoustic Microscope used in this study works in reflection mode and is operated at 50 MHz in a pulse mode (Fig. 1, left). Using the time difference between the upper and lower surface reflection signal (Fig. 1, right), ultrasonic wave velocity can be calculated. This parameter together with acoustic impedance is used to calculate density and modulus. Experiments were performed on histologic samples (embedding medium poly (methyl methacrylate) with different tissues (bone, Ca-P cement, dentine, enamel, soft tissues). The effect of sample preparation on SAM measurements was investigated. Milled and additionally polished samples were used.

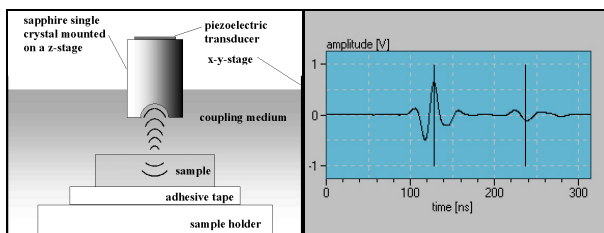


Fig. 1: left, the SAM setup; right, measurement of ultrasonic wave velocity: the first signal stems from the upper, the second from the lower surface reflection.

Samples were measured in different coupling media for 48 hours with SAM. Among the screened media distilled water and a 0.1 M phosphate buffer solution (PBS) at pH 7.4 are currently under detailed investigation. Laser profilometry has been performed to characterize the surface roughness (R_a , $R_{z(DIN)}$).

RESULTS: The reflected signal in SAM measurements is 5-15 % stronger for the polished sample and surface roughness decreases with additional polishing. A correlation between surface roughness and reflection behavior of the sample can be presumed. Concerning coupling media, water based solutions permit a good signal transmission whereas paraffin oil and glycerin did not allow good wave propagation. So far, different tissues like cement, bone, connective tissue, dentine and enamel could be distinguished unambiguously with respect to impedance, velocity, density and modulus. In the long-term measurements a decrease in impedance of 2-12 % has been measured after 48 hours for both systems (H_2O , PBS). Surface roughness showed a significant increase during this time so that dissolution or deposition processes on the sample surface could be supposed.

DISCUSSION & CONCLUSIONS: Scanning Acoustic Microscopy seems to be a helpful tool to analyze histologic sections in addition to fluorescence microscopy, polarized light microscopy and surface staining for cellular details. Information about mechanical properties can be precisely associated with a topographic location. The operating frequency of 50 MHz allows investigations with a resolution of 30 μm of both surface and bulk properties, which is a particular feature of this microscope. However, the absolute values measured must be interpreted with caution, since sample preparation affects the reflection behavior of the sample. Therefore, the method appears to be useful for comparative analysis of samples with the same preparation history. The results have to be calibrated with other established methods.

REFERENCE: ¹ A. Briggs (1992) *Acoustic Microscopy*, Clarendon Press, Oxford.