ESRF	**Experiment title:** Impact of tissue microstructure and mineralization in the course of callus healing	**Experiment number**: **MD-583**
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Report:

Background:

The important role of mechanical stimuli at the cellular level for bone formation, adaption, and healing are well accepted. External loading leads to complex stress and strain fields inside the mineralized matrix. During fracture healing, the cells are exposed to rapid elastic and structural transformations of the involved tissues.

Experimental probing of stress and strain fields inside the fracture zone is not possible. Therefore, numerical (finite element) models have been proposed. Realistic modeling requires the incorporation of matrix mineralization, structure and anisotropic elasticity, which can be assessed by 3D Synchrotron Radiation (SR) μCT and Scanning Acoustic Microscopy (SAM). This combination provides the basis for numerical deformation analyses [1, 2].

The goal of this project is to develop models of the healing callus based on SRμCT and SAM data from rat osteotomy models. These models are the basis for a development of optimized mechanical stimulations schemes with respect to consolidation status.

Experiments and setup at ID19

10 rat femora were cut into blocks such that block II includes the osteotomy (Fig.1-left). The rats form groups characterized by a rigid or semiregid fixateur and by age (young (12 weeks) vs old (12 months)). For each animal, three blocks (I, II, III) were brought to ID 19 and images with synchrotron radiation micro-CT. Before scanning elastic maps (c33) were derived through acoustic microscopy using a 50 MHz transducer.

First, all 30 blocks were measured in absorption contrast ((5.1 μm)^3 voxelsize, E= 27 keV, delta(E)/E=1/10E-5). After that, out of the above mentioned samples 6 blocks (always Block II) were selected and scanned with holotomography by performing repeated scans at three different detector positions for each sample. The reconstruction algorithm is based on Filtered back Projection for the absorption image and includes a step of phase retrieval for holotomography [3].

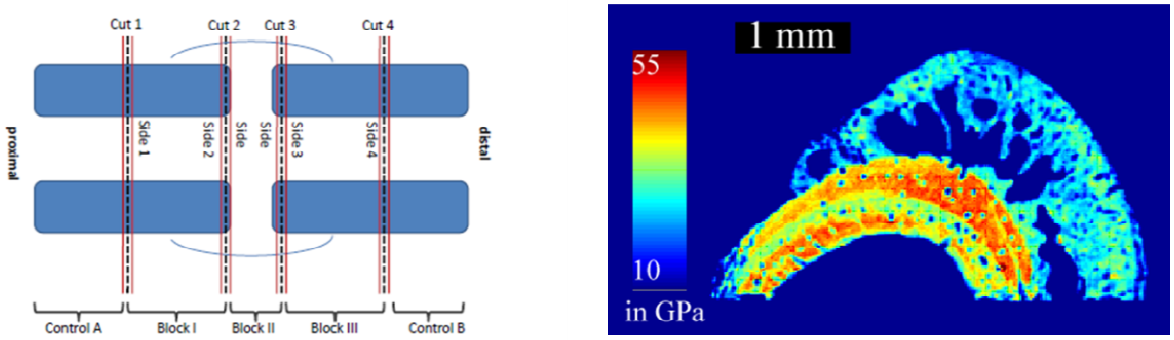

Fig. 1Left: Schematic setup of how the rat femora were cut. Scanning acoustic microscopy was carried out at side 1,2,3,4 plus at the sides of block II.
Right: Examplary c33 map obtained by 50 MHz SAM on side 3. The callus is less stiff than the cortex. The cortex appears to have regions with different stiffness.

Analysis and Results (preliminary)

SAM

After cuting the femora, the c33 surfaces of block I, II, III were polished and scanned using acoustic microscopy (50 MHz transducer) pixelsize equal to (16 µm)^2. The measured reflection amplitude maps were computed into elastic stiffness [4] maps. From those regions containing different types of bone (e.g. callus, cortical bone) were manually selected and its mean and standard deviation computed (Fig 1 - right). Stiffness values of callus in the young group is higher than those in the old group. Furthermore the transition from cortical to callus tissue will be investigated.

SR-CT

Analyis of the SR data is still in progress. Characteristical region within the callus as well as in the cortical bone are/ will be analyzed in terms of DMB (degree of mineralized bone) value and distribution (e.g. shape of the histogram).
E.g., the cortex of the sample shown in Figure 1 and 2 can be seperated into two regions with a significant difference of the DMB of 4%, porosity is found to be 3.6 % in the outer cortical region, 4.2 % in the inner cortical and 5.2 % for the callus. The average DMB of the callus of the shown sample is 4.6 % less stiff than the average cortex.

Major matter of interest is a deeper morphological description of the porosity side matched with SAM, seeing as we found very different structures of pores within the callus as well as in the cortex in terms of orientation, shape, size and connectivity, see Fig.2. A publication on this work entitled *"Impact of tissue microstructure and mineralization in the course of callus healing based on SRµCT and SAM data"* is in preparation.

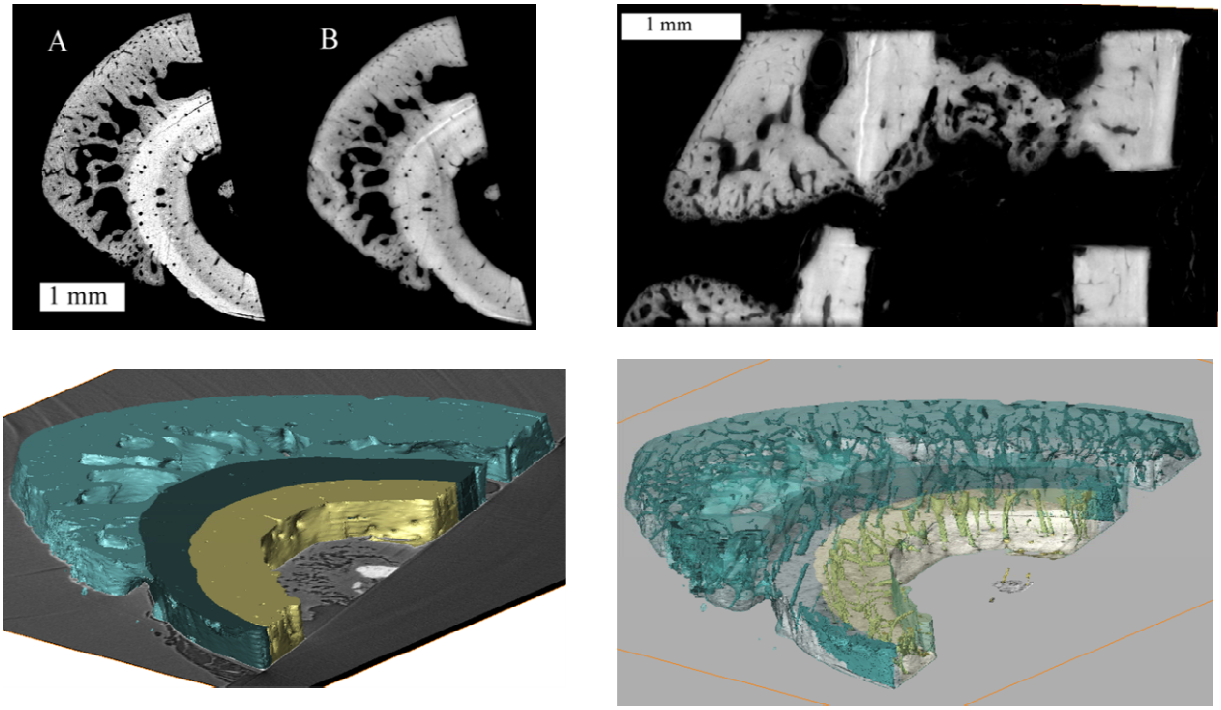

Fig. 2 Top left:Reconstructed slide of a side 2 (same as in Fig1-right)(A) attenuation contrast, (B) phase contrast. Top right: Reconstructed slide ot the osteotomy region (Block II), one sees that the callus front is different in terms of structure and DMB. Bottom: Volume rendering of the reigion already shown in Fig1-right. Left: segmentation based on DMB. Right: within the segmented regions the fine structure of the pores is shown.

References:

[1] Raum K.; Microelastic imaging of bone; Transaction On Ultrasonic Ferroelectrics And Frequency Control; 2008; IEEE 55: 1417-1431

[2] Grimal Q., Raum K., Gerisch A., & Laugier P.; Derivation of mesoscopic elasticity tensor of cortical bone from quantitative impedance images at the micron scale; 2008; Comput.Methods Biomech. Biomed Engin. 11: 147-157

[3] LANGER M., CLOETENS P., PEYRIN F., Regularization of Phase Retrieval with Phase-Attenuation Duality Prior for 3D Holotomography, IEEE Trans on IP, 2010 Sep, vol 19, n° 9, pp. 2428-2436

[4] Preininger B, Checa S, Molnar FL, Fratzl P, Duda GN & Raum K.; Spatial-temporal mapping of bone structural and elastic properties in a sheep model following osteotomy;2011; Ultrasound Med Biol.; 37(3):474-83