	Experiment title: Bone material characteristics during fracture healing	**Experiment number**: SC3438
Beamline: ID 13	**Date of experiment**: from: 10 July 2012 to: 14 July 2012	**Date of report**: 10. April 2014
Shifts: 12	**Local contact(s)**: MONTERO Cedric	*Received at ESRF:*

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Report:

Summary:
We investigated the nanostructure of bone samples during the fracture healing process in mice. This approach was the continuation of a very successful experiment SC-3197 at ESRF (beamline ID 13) earlier in 2011, where we found gradients in bone material properties (mineral size and orientation) around osteocytes and blood vessels with submicron resolution.
We applied this high-resolution x-ray scattering techniques to a highly relevant model system in bone research: We investigated the hypothesis that fracture healing occurs in two phases, where bone tissues with different structural and mechanical qualities are formed successively. Using small and wide angle x-ray scattering (SAXS, WAXS) with a one-micron resolution we characterized the bone material and determined the mineral particle size and orientation as well as crystallographic parameters as a function of the position. By this approach we obtained important structural information, especially on the mineral phase.
During that beamtime we could not measure enough samples to get a clinically relevant statistics.

Scientific background:
Bone healing is a complex process where the mechanical and functional properties of bone tissue are restored through various cellular activities that lead to formation and resorption of fracture callus tissue. While mature bone is known to be a highly organized tissue with hierarchical structure, questions remain concerning the composition and properties of the fracture callus in different animal models. The spatial and temporal

distribution of various tissue types comprising the callus has been described earlier mostly at the histological level [1]. Recently the knowledge of the callus tissue structure at the submicron level and the material properties was improved, mainly by investigating samples from a sheep model [2, 3].

Experimental techniques, set-up, measurement strategy, sample details:

We used a combination of high-resolution SAXS and WAXS to map structural information on hydroxyapatite particles in thin bone sections with a one-micron beam. A similar methodology was used in previous bone research studies [4, 5, 6]. We used a monochromatic beam (13 keV) and an x-ray optic providing a one-micron beam size. The q-range covered approximately 0.1 to 3 nm^{-1} (SAXS) and 15 to 25 nm^{-1} (WAXS).

SAXS enabled the determination of mineral particle thickness and orientation while WAXS was used to analyze crystallographic parameters, such as lattice parameters, and texture information from the carbonated hydroxyapatite particles. For the performed experiments thin sections of PMMA embedded osteotomized mouase tibiae, cut in dimensions of 10 mm x 5 mm x 6 µm, were measured. The thin bone sections were mounted on silicon wavers and then mounted onto sample holders. Precharacterization with backscattered electron images allowed an exact correlation of the measurement positions with the previously defined areas. In order to map the thin sections we used a scanning stage (xy). To compare the nanostructure of the mineral phase and the matrix in a mouse osteotomy model, we tested 9 samples representing 3 different points of time (3 individual animals at 1, 2, and 3 weeks post osteotomy). We measured several scanning areas per sample in the cortex and callus regions (compare Figure 1) with side lengths from 30 µm up to 100 µm.

Results:

We investigated changes of mineral properties in the tissue (callus and cortex) during the fracture healing process in a spatially and temporally resolved way. Specifically, we found gradients in the parameters describing the mineral particle thickness and orientation depending on the position in the bone samples. See figure 1.

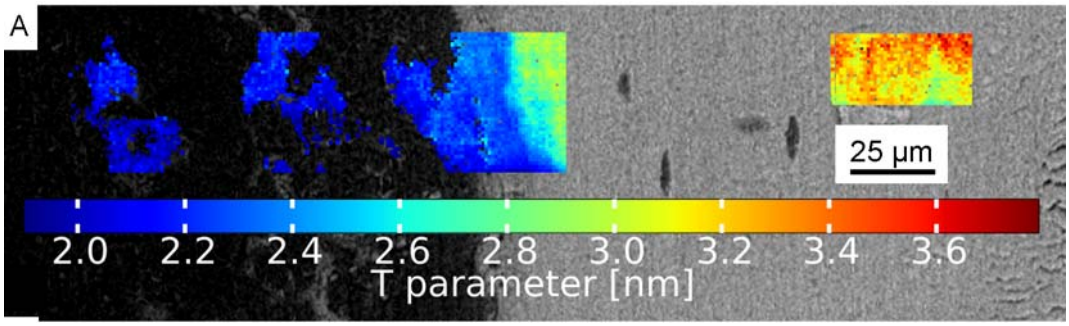

Figure 1: Bone healing in a 0.5 mm mouse osteotomy model 1 week post surgery: mouse tibia with periosteal healing callus. Panels show BSE (backscattered electron) images overlaid with maps of A) mean mineral thickness (T parameter) and. Left measurement area is part of the periosteal callus, right area is the cortex reference area.

To get clinically relevant conclusions we needed to get further beamtime and to measure more samples.

References

1. F. Shapiro, Bone development and its relation to fracture repair. The role of mesenchymal osteoblasts and surface osteoblasts. European Cells & Materials, 2008. 15: p. 53-76.
2. Y. Liu, I. Manjubala, H. Schell, et al., Size and Habit of Mineral Particles in Bone and Mineralized Callus During Bone Healing in Sheep. Journal of Bone and Mineral Research, 2010. 25(9): p. 2029-2038.
3. M. Kerschnitzki, W. Wagermaier, Y. Liu, et al., Poorly Ordered Bone as an Endogenous Scaffold for the Deposition of Highly Oriented Lamellar Tissue in Rapidly Growing Ovine Bone, Cells Tissues Organs 324467-T3, DOI: 10.1159/000324467
4. M. Kerschnitzki, W. Wagermaier, P. Roschger, et al., The organization of the osteocyte network mirrors the extracellular matrix orientation in bone. Journal of Structural Biology, 2011. 173: 303–311.
5. C. Li, O. Paris, S. Siegel, et al., Strontium is incorporated into mineral crystals only in newly formed bone during strontium ranelate treatment. Journal of Bone and Mineral Research, 2010. 25: 968-975.
6. C. Lange, C. Li, I. Manjubala, et al. Fetal and postnatal mouse bone tissue contains more calcium than is present in hydroxyapatite. Journal of Structural Biology, 2011.