	Experiment title: High resolution SAXS and WAXS investigations of bone material properties at bone-implant interfaces	**Experiment number**: SC3564
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Report:

Summary:
Due to complications with surgeries of our cooperations partner we could not perform the originally planned experiment on implant integration in rat bone. But we could perfectly use the time for a continuation of a very successful experiment SC-3438 at ESRF (beamline ID 13) in 2012, where we analysed the bone material properties (mineral size and orientation) in healing mouse tibia osteotomies with submicron resolution (first results of the previous measurement, see Fig. 1). During the experiment SC-3438 we could not get enough data to draw clinically relevant conclusions.
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 and completed our earlier measurements with further samples. 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.

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.

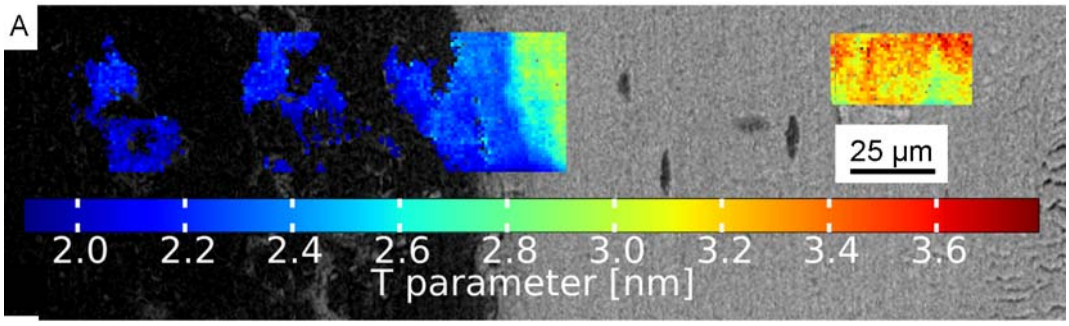

Figure 1: Bone healing in a 0.5 mm mouse osteotomy model 1 week post surgery: mouse tibia with periosteal healing callus. Panels show BSE (backscattered electron) images overlaid with maps of A) mean mineral thickness (T parameter) and. Left measurement area is part of the periosteal callus, right area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

We wrote a paper on these results which is currently under review.

References

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