ESRF	**Experiment title:** High resolution SAXS and WAXS investigations of bone material properties as influenced by osteocytes	**Experiment number**: 3197
Beamline: ID 13	**Date of experiment**: from: 11.4.2011 to: 16.4.2011	**Date of report**: 26.8.2011
Shifts: 12	**Local contact(s)**: Manfred Burghammer	*Received at ESRF:*

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Report:

Summary:

Using a submicrometer x-ray beam at ID13 we investigated the nanostructure of different bone samples to test the hypothesis that osteocytes play a direct role in the mineral homeostasis and therefore directly influence bone material quality. By means of small angle x-ray scattering with submicrometer resolution we characterized the bone material, in order to investigate if the mineral is turned over more frequently in the vicinity of the cells and their processes than further away. We determined the mineral particle size and orientation as a function of the position around blood vessels, osteocytes and canaliculi. Currently we relate these findings to the role of osteocytes in basic bone formation mechanisms as well as to their influence on the bone material quality. Overall the experiment was very succesful and we were able to show that a beam with submicron size is necessary to obtain the local parameters around the osteocytes.

Scientific background:

Bone material has a complex hierarchical structure based on a collagen matrix with embedded nanometer sized mineral particles [1]. Bone remodelling is traditionally attributed to the action of osteoblasts and osteoclasts, which are bone cells and the primary target for osteoporosis treatment. Osteocytes, cells buried in the bone matrix, are thought to play a major role in directing the remodelling process [2] and have long been suspected to play a direct role in the mineral homeostasis [3] with some ability to deposit and resorb the bone mineral in their vicinity. Osteocytes constitute a complex network connected by cell processes situated in the so-called canaliculi. The total lacunar and canaliculi surface in an adult human is more than 100 times higher than the surface available to osteoblasts and osteoclasts. Thus it seems possible that osteocytes may act directly on the bone material they are facing. This implies that the mineral and the matrix in the neighborhood of osteocytes and of their cell processes should be more dynamic and, thus, may have a different structure compared to bone further away in the depth of the tissue.

With confocal laser scanning microscopy (CLSM) in combination with rhodamine staining the osteocyte network (figure 1) has been characterized in different bone types [4]. The typical size of the lacunae is 25 x 15 x 5 µm. The diameter of the canaliculi, connecting osteocytes mutually, is below 1 µm. Due to these submicron structures, high resolution techniques are mandatory to investigate gradients of mineral characteristics in the vicinity of osteocyte lacunae and canaliculi.

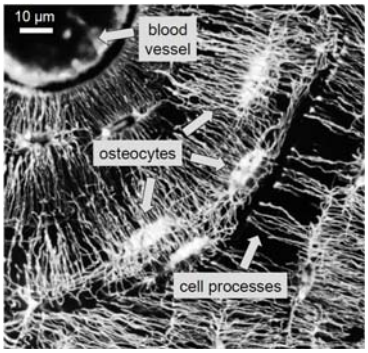

Figure 1: Osteocytic network in osteonal bone, measured by confocal laser scanning microscopy (CLSM). The diameter of the canaliculi is usually less than 1 µm.

Experimental technique and materials:
We used high-resolution small angle x-ray scattering (SAXS) to map structural information from hydroxylapatite mineral particles in thin bone sections with a submicrometer beam (0.5 µm). We used a monochromatic beam (13keV) and an x-ray optic providing a submicrometer beam size. The covered q-range was approximately 0.1 to 3 nm^{-1}. SAXS analysis allowed us to determine the mineral particle size and orientation.

Thin sections of PMMA embedded murine femora were cut and grinded in dimensions of 5mm x 5mm x 50µm and measured at ID13. To compare the mineral and matrix nanostructure in the vicinity and further away from osteocyte lacunae and canaliculi we tested 5 ovine bone samples. To assure statistical significance, we measured several scanning areas per sample with side lengths from 150 µm up to 300 µm.

Results:
The results of this project help clarifying whether osteocytes play a major role in mineral homeostasis and show the influence of this mechanism on bone material properties adjacent to the cells due to osteocytic bone resorption (osteocytic osteolysis). The novelty of our approach is the combination of the newly developed osteocyte network visualization tool (CLSM with rhodamine staining, see above) with the high resolution x-ray scattering experiments to correlate the geometry of the osteocyte network with material nanostructure.

The hypothesized mechanism, that mineral is turned over more frequently in the surrounding area of the cells and their processes than further away, leads to structural changes which we determined by SAXS-analysis (Figure 2). Specifically, we found gradients in size (T-parameter) and orientation parameter (rho-parameter) as a function of the measuring position on the bone samples and therefore relative to the osteocyte lacunae as well as the blood vessels. These graphs are not shown here, since these topical results are not yet published.

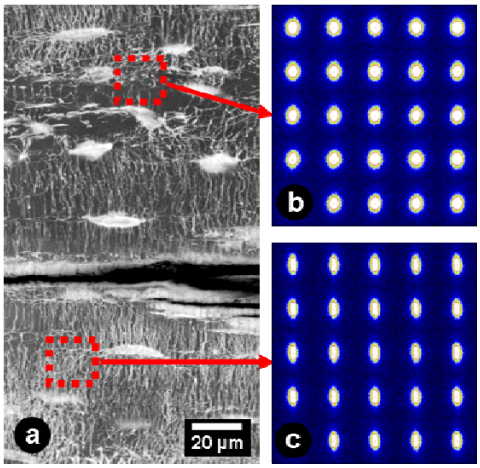

Figure 2: (a) Osteocytic network in ovine bone, measured by CLSM; (b) SAXS signal with low mineral particle orientation; (c) SAXS signal with highly ordered mineral particles.

From these SAXS patterns also the mineral particle size was determined and gradients around the osteocytes were found (not shown).

References
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