ESRF	**Experiment title:** The Effects of Strontium on Bone Mineral Properties Assessed by Synchrotron X-ray Radiation Small Angle Scattering.	**Experiment number**: MD-107
Beamline: ID-18F	**Date of experiment**: from: 29/10/2004 to: 02/11/2004	**Date of report**: 01/03/2005
Shifts: 12	**Local contact(s)**: Sylvain Bohic	*Received at ESRF:*

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Background: In the near future strontium (Sr), as the ranelate-compound will be used in doses up to 2g/day in the treatment of osteoporosis. As osteoporosis is mainly seen in elderly patients which already have a decreased renal function, they are at an increased risk for accumulation of the element. Recent findings from epidemiological studies in dialysis patients and experimental data obtained in a chronic renal failure (CRF) rat model established a dose-related multiphasic, deleterious effect of Sr on bone formation. In a previous project at the ESRF (LS-2137; ID18F 2002) we were able to localize the element in the mineralized parts of the bone of these rats. In the mentioned ESRF-project LS-2137 we were also able to demonstrate that Sr causes a crystal distortion in the hydroxyapatite lattice resulting in less crystalline mineral in both mineralizing osteoblast cell cultures and synthetic hydroxyapatite (Verberckmoes et al, 2004).
Moreover a recent electron microscopic study on synthetic Sr-containing hydroxyapatite performed at the University of Antwerp revealed the element to significantly reduce the crystal size. To which extent the contribution of such a physicochemical effect plays a role in the development of a mineralization defect is still unknown. Therefore the localization and measurement local apatite crystal distortions at the ultra-structural level in bone of experimental models during loading and withdrawal are needed.
Methods: CRF rats loaded with 0.2% SrCl2 in the drinking water for 2, 6 or 12 weeks followed by a washout period of 2 or 8 weeks were sacrificed at various time points during the course of the experiment. Undecalcified bone samples of control and Sr-loaded CRF rats were, after fixation in Burkhardt solution, embedded in methyl methacrylate prior to sectioning 5-10µ thin bone slices.
Rat tail tendons mineralized in vitro in the presence of various Sr-concentrations served as standard samples with an oriented collagen organization for SAXS measurements.
A monochromatic beam with a spot size of 2x10 µm at an energy of 17 keV was used in order to work on K-lines of Sr.

Results:

Small Angle X-ray Scattering

The results obtained obtained from the SAXS experiments did not yield the expected information.

1. After X-ray analysis the rat tail tendons seemed to be mineralized insufficiently to perform SAXS experiments. Although these samples showed a Von Kossa postive staining – Von Kossa stains Ca-containing precipitates in tissue sections – the mineral was not dense enough to be detected by X-rays. Currently new protocols are being developed in our laboratory to obtain mineralized rat tail tendons with an approriate degree of mineral deposition to allow X-ray analysis.
2. Although the sections of rat bone were of an adequate quality to perform SAXS, it was not possible to detect and hence standardize the orientation of the collagen fibrils in the mineralized bone layers with the current sample stage. When scanning the bone from strontium-poor to strontium-rich regions changing SAXS patterns were recorded. However it was was not possible to ascribe these effects to strontium since we were not able to distinguish the effects of collagen orientation changes from effects of strontium on the bone crystallites. A sample stage with 3-axle rotation possibilities would be necessary for future SAXS experiments on bone samples. Using such a sample stage it should be possible to detect the collagen arrangement of a certain region of interest and to orientate the sample so that the collagen fibers are arranged longitudinally to the incoming X-ray beam.

X-ray Fluorescence

Since (1) samples of interest were the same as in proposal 7894, which was not granted any beam time and (2) the beamline setting was suitable for the measurement of X-ray fluorescence of Sr in the bone samples, we decided to use the remaining beam time to investigate the strontium incorporation into and the efflux out of bone of CRF rats.

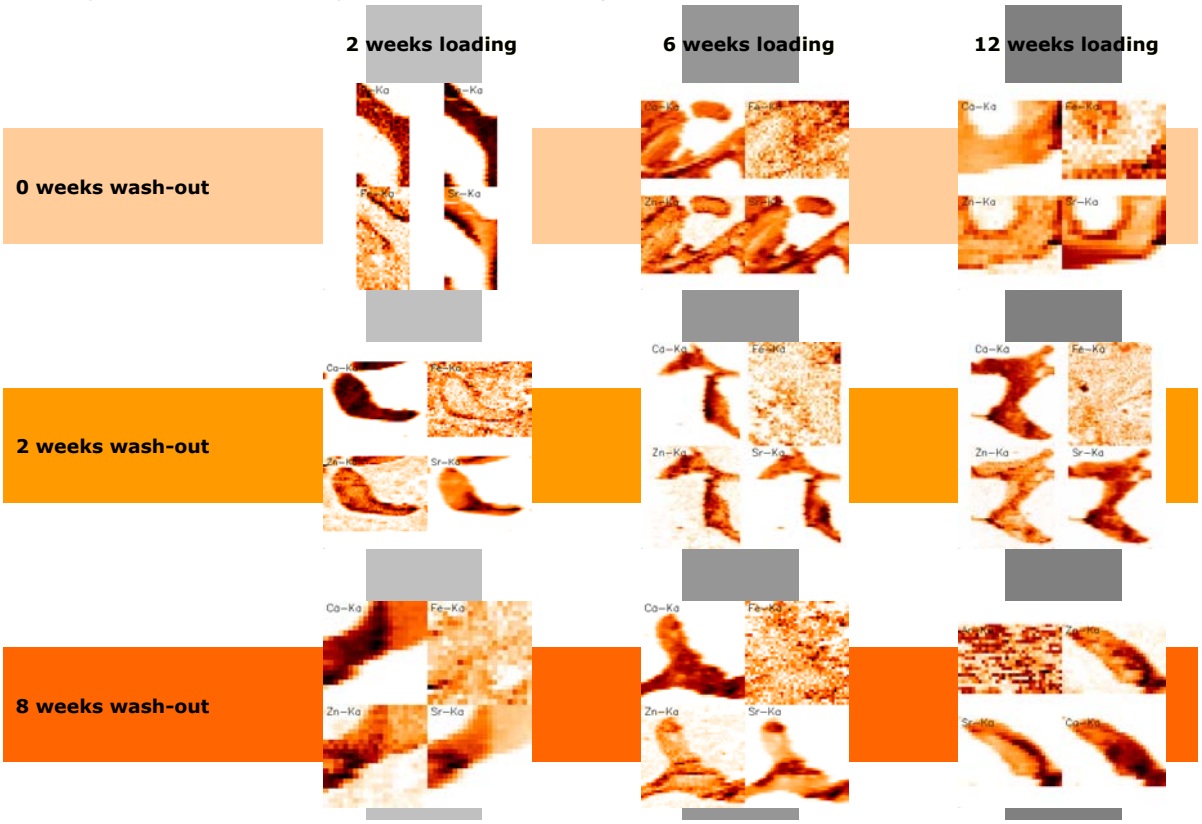

Figure 1. Strontium distribution in rat bone in function of time of Sr-loading (horizontal direction) and wash-out time (vertical direction). Strontium incorporation in the mineralized bone which was accompanied with the development of a mineralization defect (data not shown) already starts after 2 weeks of loading. This incorporation is extended during longer loading periods (6 and 12 weeks) as indicated by the thicker bands of strontium-rich mineral. The efflux of strontium out of the bone mineral is limited since the element is still detectable in the mineralized bone be it at a deeper layer in the bone. These results indicate that during the wash-out period the newly formed bone has a low strontium concentration and that it burries the strontium-rich bone layers. These findings together with the previous observations, where the mineralization defect induced during the loading period heals during the washout period (Oste et al, 2005), suggest that most likely the circulating strontium concentrations are responsible for the induction of the mineralization defect rahter than the strontium deposited in bone.

References

Oste L. et al., 2005 Time-evolution and reversibility of strontium-induced osteomalacia in chronic renal failure rats. Kidney int. 67: 920-30

Verberckmoes S.C., et al., 2004 Effects of Strontium on the Physicochemical Properties of Hydroxyapatite. Calcif Tiss Int, 75: 405-415.