EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

ESRF

Experiment Report Form

The double page inside this form is to be filled in by all users or groups of users who have had access to beam time for measurements at the ESRF.

Once completed, the report should be submitted electronically to the User Office using the **Electronic Report Submission Application:**

http://193.49.43.2:8080/smis/servlet/UserUtils?start

Reports supporting requests for additional beam time

Reports can now be submitted independently of new proposals – it is necessary simply to indicate the number of the report(s) supporting a new proposal on the proposal form.

The Review Committees reserve the right to reject new proposals from groups who have not reported on the use of beam time allocated previously.

Reports on experiments relating to long term projects

Proposers awarded beam time for a long term project are required to submit an interim report at the end of each year, irrespective of the number of shifts of beam time they have used.

Published papers

All users must give proper credit to ESRF staff members and proper mention to ESRF facilities which were essential for the results described in any ensuing publication. Further, they are obliged to send to the Joint ESRF/ ILL library the complete reference and the abstract of all papers appearing in print, and resulting from the use of the ESRF.

Should you wish to make more general comments on the experiment, please note them on the User Evaluation Form, and send both the Report and the Evaluation Form to the User Office.

Deadlines for submission of Experimental Reports

- 1st March for experiments carried out up until June of the previous year;
- 1st September for experiments carried out up until January of the same year.

Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report, in English.
- include the reference number of the proposal to which the report refers.
- make sure that the text, tables and figures fit into the space available.
- if your work is published or is in press, you may prefer to paste in the abstract, and add full reference details. If the abstract is in a language other than English, please include an English translation.

ESRF	**Experiment title:** X-ray Analysis of Medullary Bone during Calcium Homeostasis	**Experiment number**: 3197
Beamline: ID 13	**Date of experiment**: from: 04.05.2013 to: 10.05.2013	**Date of report**: 07.07.2013
Shifts: 18	**Local contact(s)**: Manfred Burghammer, Emanuela Di Cola	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Michael Kerschnitzki * (Weizmann Institute of Science, Israel)

Wolfgang Wagermaier (MPI of Colloids and Interfaces, Germany)

Peter Fratzl (MPI of Colloids and Interfaces, Germany)

Anat Akiva* (Weizmann Institute of Science, Israel)

Britta Seidt* (MPI of Colloids and Interfaces, Germany)

Report:

Summary:

Using a submicrometer x-ray beam at ID13 we investigated the nanostructure of chicken medullary bone (MB) material, a special bone type unique to birds and dinosaurs. This spongy bone, present in the medullary cavity of the long bones, is intended as a labile calcium source for eggshell formation. Due to its rapid turnover rates it is used as a model system for extensive bone mineral storage and mobilization. By utilization of small and wide angle x-ray scattering with submicrometer resolution we characterized medullary bone mineral regarding its crystallographic structure, orientation and size (length and thickness of the mineral particles) as a function of the position.

Scientific background:

Bone is the most widespread mineralized tissue in vertebrates. It has a complex hierarchical structure based on a collagen matrix with embedded nanometer sized mineral particles[1]. During growth and maturation, bone is constantly undergoing processes of remodeling[2]. These are carried out by a complex interplay of the bone cells. During bone resorption, bone mineral is dissolved from the collagenous bone matrix[3]. However, to date it is not understood how the dissolved calcium and phosphate ions are stored and stabilized in the ambient serum without reaching local super-saturation, leading to spontaneous reprecipitation of mineral[4].

The most favorable circumstances to study the processes involved in calcium mobilization and transportation are those when these occur under extreme conditions. Such a situation can be found in egg-laying hens, in which calcium metabolism is incredibly intense. During eggshell production considerable amounts of the required Calcium (20-40 %) are derived from skeletal reserves[5]. To accommodate this, hens produce a spongy bone laid down within the medullary cavity of long bones. This Medullary bone builds up rapidly during the early stages of egg-laying. The rapid cycling (24 hours) in which medullary bone is resorbed and then rebuilt involving an extreme calcium metabolism[6], provides an opportunity to study the pathway of calcium storage, mobilization and transportation in its most intensive form.

Experimental technique and materials:

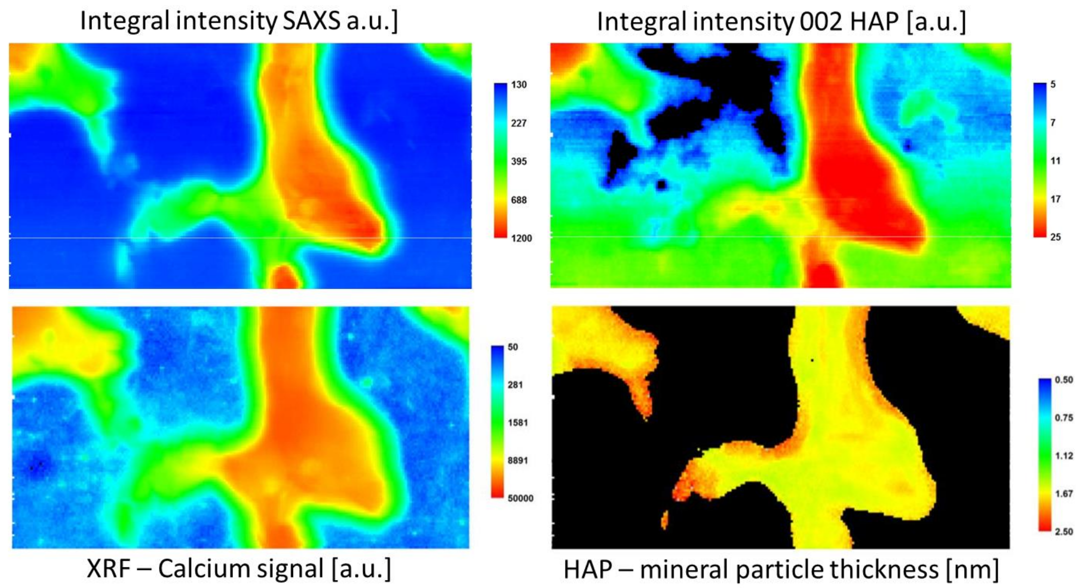
We used high-resolution small angle x-ray scattering (SAXS) and wide angle x-ray diffraction (WAXD) to map structural information from hydroxylapatite mineral particles in thin bone sections with a monochromatic (13keV) micrometer beam. The covered q-range was approximately 0.4 to 25 nm^{-1}. Additionally we used an XRF-detector to map the composition of the bone tissue in a position resolved way.

Thin sections of PMMA embedded chicken medullary bone samples were cut and grinded in dimensions of 1cm x 5mm x 30µm and measured at ID13. The thin bone sections with embedded position markers were glued onto sample holders which allow an exact correlation of the measurement positions with pre-characterized areas (back-scattered electron imaging). To compare the mineral and matrix nanostructure during calcium homeostasis we tested 3 medullary bone samples. To assure statistical significance, we measured several scanning areas per sample with side lengths from 50 µm up to 300 µm.

Results:

The results of this project will significantly help to improve the understanding of basic mechanisms involved in calcium mobilization from bones as well as calcium storage in the ambient bone serum. The novelty of this approach is clearly the particularity of the medullary bone model system, facilitating an intensive mineral turnover, which is only barely studied yet.

Specifically we succeeded to measure strong gradients in size of bone mineral particles as a function of the location inside the medullary bone structures (distance to the active areas of bone resorption). Furthermore we found calcium rich areas in the ambient bone marrow compartment which can not be related to the mature bone phase of the medullary bone material. Currently we try to relate these findings to different transient mineral structures which are known to be involved in the process of bone formation[7-10] and potentially as well in bone resorption.



References

1. Fratzl, P. & Weinkamer, R. Nature's hierarchical materials. *Prog. Mater. Sci.* 52, 1263-1334 (2007).
2. Currey, J.D. *Bones: Structure and Mechanics*, (Princton University Press, Oxford, UK, 2002).
3. Teitelbaum, S.L. Bone resorption by osteoclasts. *Science* 289, 1504-1508 (2000).
4. Omelon, S., *et al.* Control of Vertebrate Skeletal Mineralization by Polyphosphates. *PLoS One* 4(2009).
5. Edelstein, S., Harell, A., Bar, A. & Hurwitz, S. FUNCTIONAL METABOLISM OF VITAMIN-D IN CHICKS FED LOW-CALCIUM AND LOW-PHOSPHORUS DIETS. *Biochimica Et Biophysica Acta* 385, 438-442 (1975).
6. Bar, A. Calcium transport in strongly calcifying laying birds: Mechanisms and regulation. *Comp. Biochem. Physiol. A-Mol. Integr. Physiol.* 152, 447-469 (2009).
7. Mahamid, J., Addadi, L. & Weiner, S. Crystallization Pathways in Bone. *Cells Tissues Organs* 194, 92-97 (2011).
8. Mahamid, J., *et al.* Mapping amorphous calcium phosphate transformation into crystalline mineral from the cell to the bone in zebrafish fin rays. *Proc. Natl. Acad. Sci. U. S. A.* 107, 6316-6321 (2010).
9. Mahamid, J., Sharir, A., Addadi, L. & Weiner, S. Amorphous calcium phosphate is a major component of the forming fin bones of zebrafish: Indications for an amorphous precursor phase. *Proc. Natl. Acad. Sci. U. S. A.* 105, 12748-12753 (2008).
10. Mahamid, J., *et al.* Bone mineralization proceeds through intracellular calcium phosphate loaded vesicles: A cryo-electron microscopy study. *J. Struct. Biol.* 174, 527-535 (2011).