EUROPEAN SYNCHROTRON RADIATION FACILITY

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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 via the User Portal:
https://wwws.esrf.fr/misapps/SMISWebClient/protected/welcome.do

Deadlines for submission of Experimental Reports

Experimental reports must be submitted within the period of 3 months after the end of the experiment.

Experiment Report supporting a new proposal ("relevant report")

If you are submitting a proposal for a new project, or to continue a project for which you have previously been allocated beam time, you must submit a report on each of your previous measurement(s):

- even on those carried out close to the proposal submission deadline (it can be a *"preliminary report"),*
- even for experiments whose scientific area is different form the scientific area of the new proposal,
- carried out on CRG beamlines.

You must then register the report(s) as "relevant report(s)" in the new application form for beam time.

Deadlines for submitting a report supporting a new proposal

- 1st March Proposal Round - **5th March**
- 10th September Proposal Round - **13th September**

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.

Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report in English.
- include the experiment number 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:** Time- and space resolved mechanical behavior of regenrating bones	**Experiment number**: MD 1117
Beamline: ID02	**Date of experiment**: from: 01-02-2018 to: 05-02-2018	**Date of report**:
Shifts: 12	**Local contact(s)**: BOESECKE Peter	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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Prof. David Gloaguen, Nantes University

Dr. Fabienne Jordana, Nantes University

Ms. Ameni Zaouali, Nantes University

Report:

1. Abstract

Proposed experiments intend to highlight the distribution of, on the one hand, (hydroxyapatite - HA) crystal microstructural features (size distribution, spatial pattern), and, on the other hand, mechanical stresses induced by bone reconstruction treated with BCP (Biphasic Calcium Phosphate biomaterial), mainly supported by the mineral part of bone architecture. Such investigations are achieved through mechanical state mapping in various regeneration levels until complete reconstruction, considering samples harvested at different regeneration stage, and under incremental mechanical load. The evolution of the mechanical properties as regard to bone regeneration microstructure was investigated thanks to *in situ* tensile testing under synchrotron WAXS and SAXS (Wide- Small Angle X-ray Scattering). The size and orientation of bone mineral particles as well as the spatial distribution of particle agglomerates in rat calvaria defects were investigated at different healing stages. The resulting two dimensional maps of elastic strain, mean thickness and degree of orientation for the mineral phase, revealed the strong correlation between the bone mechanical properties and the crystalline phase organization.

2. Experiment details

Mechanical investigations have been carried out on parallelepiped-shaped samples ($10 \times 5 \times 0.6$ mm^3) harvested from rat calvarias at different regeneration stages (2, 4, 6, and 8 weeks after surgery). Dogbone-shaped samples have been managed by embedding 2 mm of the ends of their longest dimension (10 mm) in a non-invasive, low polymerization temperature, high stiffness resin. The samples were studied through *in situ* tensile testing in the elastic regime in a step-by step mode (initial mechanical stress state + 4 incremental loads) up to 50 N. For each loading step, SAXS and WAXS mapping were achieved across the bone defect, in transmission mode, thanks to a Pilatus detector with scanning steps of 60 µm in a continuous line-scan mode along and perpendicular to the tensile axis with 38×26 µm^2 spot size and 15 keV beam energy ($\approx$ 1.0 Å wavelength). The drawn maps ensure a thorough investigation of the strain distribution in HA nanocrystallites across the interface between natural and regenerated bone over a 2×2 mm² area of acquisition (a fourth of the area of interest, symmetrically representative of the entire circular defect).

3. Results

Figure 1 presents maps of both longitudinal deformations (i.e. along the loading direction) for the {00.2} plane family in mineral particles in and around the defect (Fig. 1.a) and the related mean orientation of HA particles platelets (Fig. 1.b), at 8 weeks of regeneration. The main result deduced from Fig 1.a stands in the reduction of heterogeneity of mechanical properties highlighted by a relatively uniform distribution of the internal strains between the natural (703 ± 90 µdef) and regenerated (630 ± 175 µdef) bone parts. This therefore reflects an elastic modulus E of the regenerated crystals close to that of the crystals initially present in the bone around the defect. However, these results should be qualified because of

the limited number of measuring points that can be used in the implanted region. Indeed, the hatched areas in figure 1 correspond to measurement points that are not usable (and therefore eliminated from the study). Indeed, the contribution of BCP to the diffraction signal in these areas is higher than that of HA crystals. As the H factor and crystalline strains are deduced from an analysis of the {002} diffraction peak in WAXS, their determination is made very delicate as soon as the intensity ratio observed between the BCP diffraction peaks and those of HA crystals becomes unfavorable.
Fig 1.b presents the map of the Hermans orientation factor at 8 weeks post implantation by BCP. The synthesized tissue (organic and mineral) in the new matrix is mostly well oriented as regard to the orientation met in the natural bone for this regeneration time. Perfect homogenization with the natural bone is observed from the second week of regeneration (results not presented here). This shows that the newly formed HA crystals on the edges of the defect and around the grains of BCP orient and organize themselves naturally along the major axis of the bone as soon as they are formed.

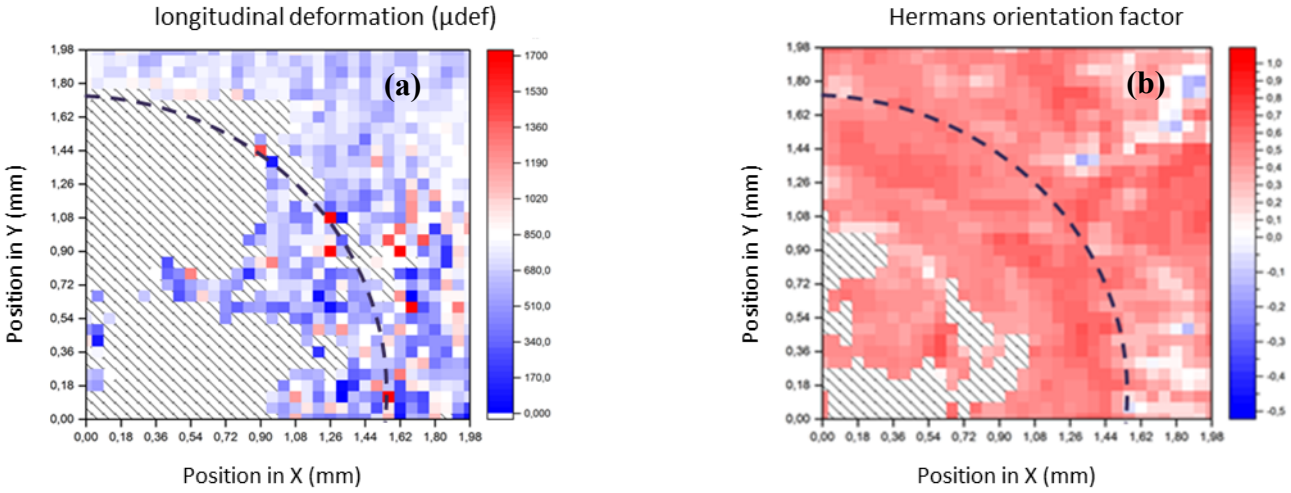

Figure 1: (a) Mapping of longitudinal deformations for the {00.2} plane family in HA particles in and around the defect implanted by BCP for a load of 30 N, (b) associated Hermans' orientation factor at 8 weeks post-implantation. (dotted black lines indicate the original defect position; lattice strains are given as micro-strain (με, units of 10^{-6}))

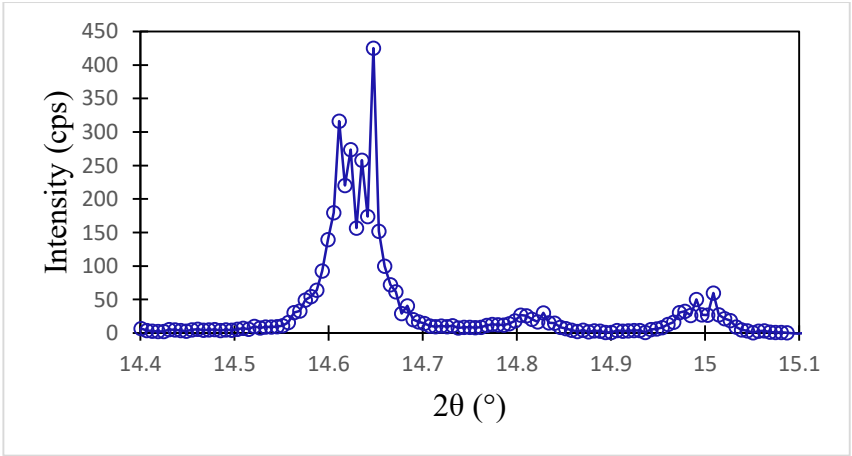

Figure 2: Integrated intensity vs 2θ in the defect implanted by BCP at 8 weeks post-implantation

As shown in Figure 2, the diffraction peak observed around 2θ ≈ 14.6° result in the convolution of different peaks: {00.2} reflection from the mineralized bone particles and diffraction peaks related to BCP phase. The analyses are therefore underway and a peak deconvolution procedure (feasible as regard to the strong peak shape difference, related to the diffraction domain size) is currently being applied to clearly separate reflection contributions.