EUROPEAN **S**YNCHROTRON **R**ADIATION **F**ACILITY

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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 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:** **Synchrotron X-ray micro-tomography characterization of bone ingrowth after implantation of injectable bone substitutes**	**Experiment number**: LS-1841
Beamline: 22	**Date of experiment**: from: 23-mai-2001 to: 29-mai-01	**Date of report**: 20-08-2002 *Received at ESRF:*
Shifts: 18	**Local contact(s)**: RAU Christoph	

Names and affiliations of applicants (* indicates experimentalists):

WEISS Pierre EMI INSERM 99-03
OBADIA Laetitia
BOURGES Xavier

Report:

The aim of this work was to study and compare with a micrometer resolution, using Synchrotron X-ray micro-tomography, the 3-dimensional bone osteoconduction after implantation of two injectable bone substitutes in our rabbit model. The use of phase-contrast imaging and tomography will provide detailed information of bone ingrowth-biomaterial interfaces and important 3-D information. Micro-diffraction could be combined and will bring additional information on the orientation and crystalline state of micro-crystalline phases at interface.

A first article using several eperimental methods is in press : O. Gauthier, I. Khairoun, J. Bosco , L. Obadia, X .Bourges, C. Rau, D. Magne, J.M. Bouler , E. Aguado ,G. Daculsi and P. Weiss “Non-invasive bone replacement using a new injectable calcium phosphate biomaterial” *J Biomed Mater Res*.

Abstract

The use of injectable calcium phosphate (CaP) biomaterials in non-invasive surgery should provide efficient bone colonization and implantation. Two different kinds of injectable biomaterials are presently under development: ionic hydraulic bone cements that harden in vivo after injection, and an association of biphasic calcium phosphate (BCP) ceramic granules and a water-soluble polymer vehicle (a technique particularly investigated by our group), providing an injectable CaP bone substitute (IBS). This study compared these two

approaches, using physicochemical characterizations and in vivo evaluations in light microscopy, scanning electron microscopy and three-dimensional microtomography with synchrotron technology.
Three weeks after implantation in rabbit bone, both biomaterials showed perfect biocompatibility and bioactivity, but new bone formation and degradation of the biomaterial were significantly greater for BCP granules than ionic cement. Newly-formed bone developed, binding the BCP granules together, whereas new bone grew only on the surface of the cement, which remained dense with no obvious degradation three weeks after implantation.
This study confirms that BCP granules carried by a cellulosic polymer conserve bioactivity and are conducive to earlier and more extensive bone substitution than a carbonated-HA bone cement. The presence of intergranular spaces in the BCP preparation, as shown on microtomography imaging, seems particularly favorable, allowing body fluids to reach each BCP granule immediately after implantation. Thus, the IBS functions as a completely interconnected ceramic with total open macroporosity. This new bone replacement approach should facilitate micro-invasive bone surgery and local delivery of bone therapy agents.

A second article more specific on the 3D Synchotron method will be send to Bone and an oral communication will be presented at : ESB 2002 – Annual Meeting of the European Society for Biomaterials – 11th-14th September 2002 – Barcelona, Spain

Synchrotron hard X-Ray Micro Tomography 3D representation of bone in growth in Calcium Phosphate Biomaterial at the micron scale.

Weiss Pierre[1], Laetitia Obadia[1], Bourges Xavier[1], Rau Christophe[3],Khairoun Ibrahim[1], Gauthier Olivier [1-2] and Daculsi Guy[1].
[1]Laboratoire de Recherche sur les Matériaux d'Intérêt Biologique, Inserm 99-03, Faculté de Chirurgie Dentaire, 1 place Alexis Ricordeau, BP 84215, 44042 Nantes cedex 01, France.
[2]Laboratoire de Chirurgie, Ecole Nationale Vétérinaire de Nantes, BP 40706, 44307 Nantes cedex 03, France
[3]ESRF, 6 rue Jules Horowitz, BP 220, F-38043 Grenoble CEDEX, France

Introduction

Two bioactive injectable material families are currently in development in laboratories. The first ones are (Ionic) hydraulic cements which harden in vivo after injection. They require an extemporaneous preparation just before the injection. The second is calcium phosphate ceramic in suspension in a vector phase who can be proposed ready to use but without initial hardening.
The aim of this work is to study and compare with a micrometer resolution, using Synchrotron X-ray micro-tomography (ESRF), the 3-dimensional bone bone ingrowth after implantation of calcium phosphate bone substitutes in a rabbit model. The use of phase-contrast imaging and tomography will provide detailed information of bone ingrowth-biomaterial interfaces and important 3-D information. An in vivo study investigated bone substitution of two different calcium-phosphate injectable biomaterials, a cement material

(CPC : calcium phosphate cement) and injectable bone substitute (IBS), and blocks of micro macro porous biphasic calcium phosphate (MBCP™ Biomatlante France) will be used as control.

Materiel and Method

We selected, from previous studies, various samples issued of bilateral femoral implantation performed on mature female New Zealand White rabbits was used. After 2,3,8 weeks of implantation, the femoral ends have been dissected, fixed in glutaraldehyde, dehydrated, then embedded in glycolmethylmethacrylate.. Absorption or phase-contrast micro-tomography can be achieved using the dedicated set-up at beamline ID22 using high resolution FreLon camera. The sample was mounted at a distance of 65 meters from the undulator source. The rotation axis of the sample was horizontal. The distance between sample stage and detector can be freely chosen in order to increase the phase contrast using the highly coherent light from the third generation synchrotron radiation source as it represents the ESRF. The distance between sample and detector was between 2-10 mm, where at larger distances features having only a weak absorption contrast are revealed. A microscope optic (magnification: x 10) projected the scintillation-light image onto a 2,048-by-2,048-pixel CCD camera with a 14-bit real dynamic range. For this configuration, the effective pixel size is 1.4 µm which corresponds to the resolution of the chosen scintillation screen.

Results and discussion

All the calcium phosphate tested showed a total surface osteoconduction with different degree of penetration inside the CaP implants. The Calcium phosphate cement was totally in contact with host bone, but bone penetration was superficial and occured by the surface fissures without any colonization of the implant body. The MBCP blocks have shown better bone colonization in the superficial macro porous but in the early steep of the substitution (3-8 weeks) the center was no colonized because of the weak degree of interconnection of these BCP blocks. The IBS material before implantation can be observed after 3D reconstruction as an interconnected bioactive biomaterial with total open macroporosity. After implantation since 3 weeks all the implant was totally colonized by a new bone with little free mineral space. After 8 weeks the space trabecular bones were again visible. This method is very precise and allows imaging of 3-D reconstruction of osseous in growth with osteocytes lacunae.

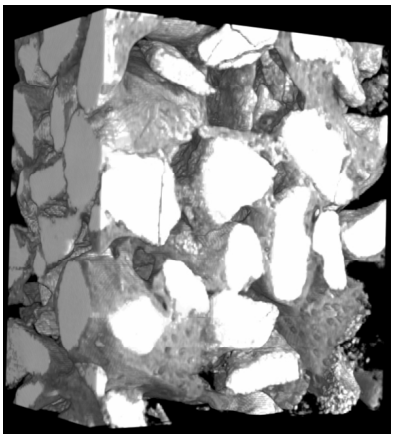

Figure 1 : 3 D microtomogra-hy reconstruction of IBS (BCP ceramics between 80-200 µm) after 8 weeks of implantation

In conclusion, the 3 D microtomography reconstruction was able to evaluate the bone architecture with a resolution of a couple of microns and it was demonstrate using various type of bone substitute the quality of bone ingrowth into and at the expense of the implants.