ESRF	**Experiment title:** Investigation of new bone formation from bone marrow stem cells in porous hydroxyapatite and carbonate hydroxyapatite ceramics at a tridimensional level	**Experiment number**: **MD-22**
Beamline: ID19	**Date of experiment**: from: 28 June 2003 to: 30 June 2003 from: 16 November 2003 to: 18 November 2003	**Date of report**: 5 February 2004
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Report:

In the recent years innovative methods have been developed for the repair and regeneration of damaged and diseased tissues. Auto- or allografting are currently used therapeutic approaches.[1,2] Implantation of either inert or bioactive artificial substitutes have also attracted the interest of the scientific community.[3-5,10-11] Interestingly the highest expectancies, and possibly the best solutions for tissue-defect handling, rely upon a combination of both approaches, i.e. upon *in situ* tissue engineering.[3-6]

For instance, scaffolds in combination with Bone Marrow Stromal Cells (BMSC) have been used for the repair of bone lesions[6-9,12,20]. Ceramic scaffolds provide advantageous features as compared to other materials.[13]

Several studies[9,21-22] have shown that by using porous calcium phosphate ceramics loaded with BMSC, it is possible to generate new bone in a shorter time and with a higher efficiency with respect to cells or ceramics alone. In fact osteoprogenitor cells, properly expanded *in vitro* and associated with scaffolds, should lead to

the development of integrated bone substitutes, where a biologically controlled osteogenesis is combined with the intrinsic osteoconduction and osteointegration properties of the phosphate ceramics -based bioceramics.[15]

Qualitative analysis of the new bone formed in the implants can be performed relatively easily by conventional histology. On the contrary, 3D structure data and quantitative analysis are difficult to obtain. Recently Martin et al[23] described a computer-based method for the automated quantification of bone tissue in 2D histological sections of decalcified specimens. Although in principle a 3D structure of the new bone could be derived by the analysis of serial sections of the implant, this approach is not practical and due to the histological treatment that the sample undergo before the analysis (decalcification), the method doesn't allow to determine the volume of the remaining scaffold.

In the present experiments (MD22) the X-ray computed microtomography (Micro-CT) associated with X-ray synchrotron radiation[16-19,24] was used as possible experimental technique for investigating bone formation in porous scaffolds charged with BMSC. MicroCT experiments were performed at the ESRF on beamline ID19 with the following operating conditions: monochromatic beam with an energy of 28-40 KeV; detection system: Gadox scintillator associated to FReLoN CCDcamera. A typical scan includes 900 –1300 projections of the sample over 180 degrees. The field of view of images depends on the number and the size of pixels. In our experiment, images were recorded on a 2048 × 2048 CCD detector, with the pixel size set to 4.91 μm.

The research activities were splited in two parts. In particular while the first session (28 June 2003 / 30 June 2003) were investigated the different porous scaffolds such as hydroxyapatite, tricalcium-phosphate (TCP) and tricalcium-phosphate/hydroxyapatite composites of various composition, porosity and resorbibility rate before implantation. Then, they were loaded with *in vitro* expanded BMSC and were subcutaneously implanted in immunodeficient mice (CD-1 nu/nu, Charles River). After 8 weeks implants were harvested, fixed in paraphormaldehyde 4% in PBS for 2 h at 4°C and kept in Falcon tubes in PBS for the second session of MicroCT analysis (16 November 2003 / 18 November 2003).

At the present, it was shown that, by selecting a high X-ray energy, it is possible to obtain a 3D imaging of newly formed bone and of the scaffold, and a quantitative information. Details of 3D distribution of newly

formed bone into scaffold are shown in Fig. 1, the different phases were colored using 3D-display software in order to make them more easily recognizable.

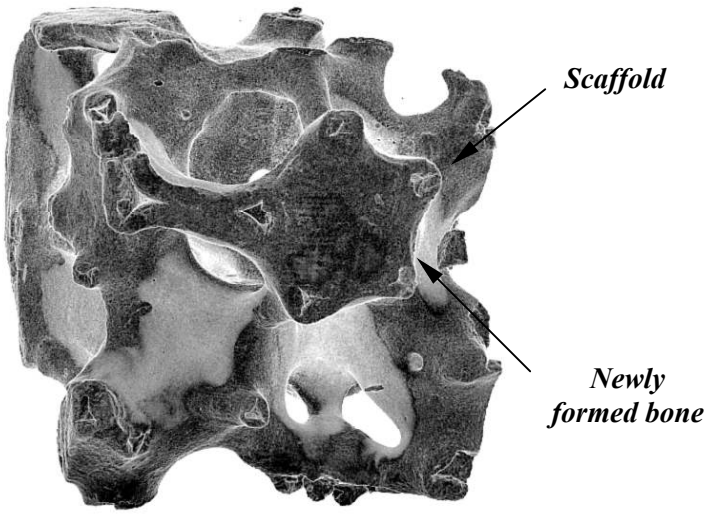

Fig. 1. *3D-section of bioceramics scaffold with Bone Marrow Stromal Cells after implantation 8 weeks in animal model.*

Furthermore, it is possible to use image processing to make one or more phases translucent or even "cancel" it in order to allow a more accurate observation of the spatial distribution of each phase. Fig.2 shows an example of this data treatment for TCP scaffold.

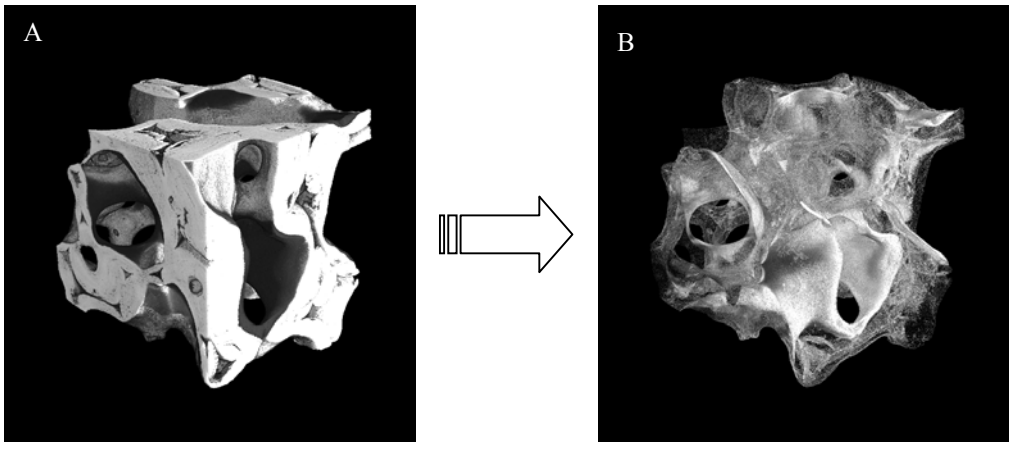

Fig. 2. *A. - 3D-detail image of TCP scaffold with BMSC at 8 weeks implantation; B. - 3D-section of newly formed bone.*

Moreover, it is possible to evaluate of the volume of the newly formed bone and of the remaining ceramics from the obtained data (Fig. 3).

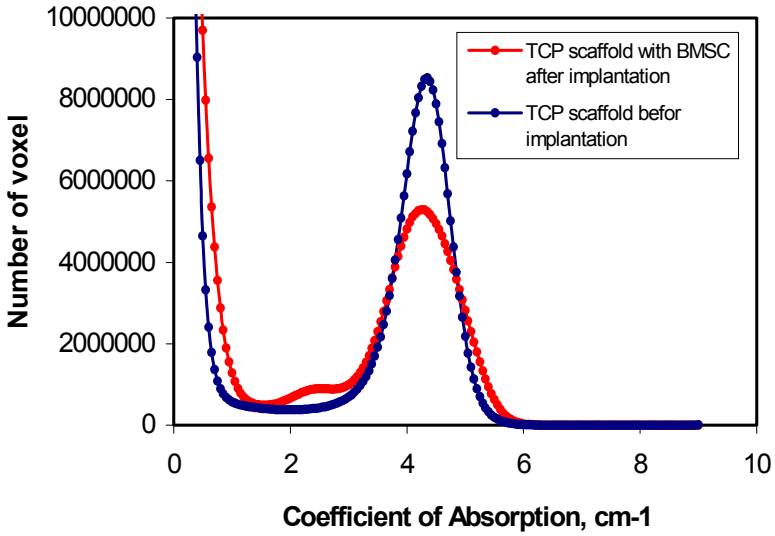

Fig. 3*. Gray level histogram of the whole reconstructed volume of TCP scaffold before and TCP scaffold with BMSC after implantation 8 weeks.*

In fact, the results of this study clearly indicate the possibility of further non-destructive, quantitative analysis of tissue engineering constructs to determine the volume distribution of newly formed bone into implant by using the MicroCT technique associated to X-ray synchrotron radiation. This approach offers the possibility of carrying out a complete experimental plan investigating, for instance, the influence of several parameters such as pore size and the spatial distribution on ingrowth tissues into implant. Eventually this should allow to determine which microstructural parameters of the utilized scaffolds are significant in the field of tissue engineering. Further analysis, for instance, the influence of several parameters such as pore size and the spatial distribution on ingrowth tissues into implant, is still in progress.

PUBLICATIONS

Please note below the references of all papers published during the past 18 months as a result of measurements which have done at the ESRF.

1. R.Cancedda, S. Casari, M.Hausard, V. Komlev, M.Mastrogiacomo, F. Peyrin, F. Rustichelli /Non-destructive three-dimensional evaluation of a bone formation in porous hydroxyapatite ceramics loaded

with bone marrow stromal cells by microtomography using synchrotron radiation/ *2nd Annual Meeting of the European Tissue Engineering Society*, Genova, Italy, 2003.

2. F. Rustichelli, R. Cancedda, S. Casari, M. Hausard, V. Komlev, M. Mastrogiacomo, F. Peyrin /Non – destructive three-bimensional evaluation of a bone formation in porous hydroxyapatite ceramics loaded with bone marrow stromal cells by microtomography using synchrotron radiation/ *The International Journal of Artificial Organs*, Vol. 26, № 9, 2003, p. 842.
3. Mastrogiacomo M, Komlev VS, Hausard M, Peyrin F, Turquier F, Casari S, Cedola A, Rustichelli F, Cancedda R, /New Bone Formation in Porous Hydroxyapatite Ceramics Loaded with Bone Marrow Stromal Cells: 3D Structure obtained by Microtomography using Synchrotron Radiation/ *Journal Tissue engineering*, (submitted).

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