ESRF	**Experiment title:** Investigation of Bone Formation in Porous Calcium Phosphate Base Ceramics Loaded with Bone Marrow Stromal Cells at Longer Time After the Implant	**Experiment number**: MD-95
Beamline: BM05 ID19	**Date of experiment**: from: 03 November 2004 to: 06 November 2004 from: 26 November 2004 to: 28 November 2004	**Date of report**: 20/02/2005
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Report:

During last decade the progress in chemical, physical, material and biological sciences resulted in the possibility of bone tissue engineering – a biologically based method for repair and regeneration of tissues. In a typical tissue engineering application, osteogenic cells would be harvested from the patient and seeded on a synthetic scaffold that acts as guide and stimulus for tissue growth in order to create a tissue engineering construct or a living biocomposite. The biocomposite would then be implanted back into the patient. Over time, the scaffold should resorb into the body as non-toxic degradated product at the same rate that the cells produce their own extracellular matrix [1-4]. It is conceivable that tissue engineering construct or living biocomposite are complex structures and in order to analyze them it is necessary to turn to the life sciences. In this way, X-ray computed microtomography (μCT) is known to be a unique technique for non-invasive,

nondestructive three-dimensional (3D) characterization of materials in medicine, material science, and biology [5-7].

Some previous applications of μCT for tissue engineering purposes have already produced fruitful results corresponding to the requirements of biological systems for engineering design: to investigate the cells feasibility within a 3D scaffold structure and to adapt rapid prototyping technology for the realization of a synthetic trabecular scaffold.

Experiments to study *in vivo* bone formation at long time of implant (64 weeks) for different porous ceramic scaffolds, loaded with bone marrow stromal cells (BMSC), have been performed by our group at BM05 and ID19 beamlines of the ESRF facilities. The research activities were divided on two parts. In particular while the first session at BM05, in order to optimize the contrast of the different phases of the investigated samples, we performed the experiment at different operating conditions such as beam energy and the distance between the sample and the detection system. For the second session at ID19, the following operating conditions were used: monochromatic beam with energy of 20 and 27 KeV; detection system: Gadox scintillator associated to FReLoN CCD camera. A typical scan includes 1000 –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, yielding a field of view of 10 mm. A 3D image of the sample was reconstructed from the series of obtained 2D projections using a 3D filtered back projection algorithm implemented at ESRF. A volume of interest of 4x4x4 mm^3, within each sample, was reconstructed.

The data obtained from these new experiments was compared with the previous one (MD22) that to evaluate bone formation within the scaffolds at long period. Here, we draw briefly the obtained results.

Details of 3D distribution of the newly formed bone into ceramic scaffolds at different time of the implant (8, 16 and 64 weeks) can be observed by 3D rendering. As shown in Fig.1 the subvolumes of the investigated samples, the different phases were colored using a 3D-display software in order to make them more easily recognizable.

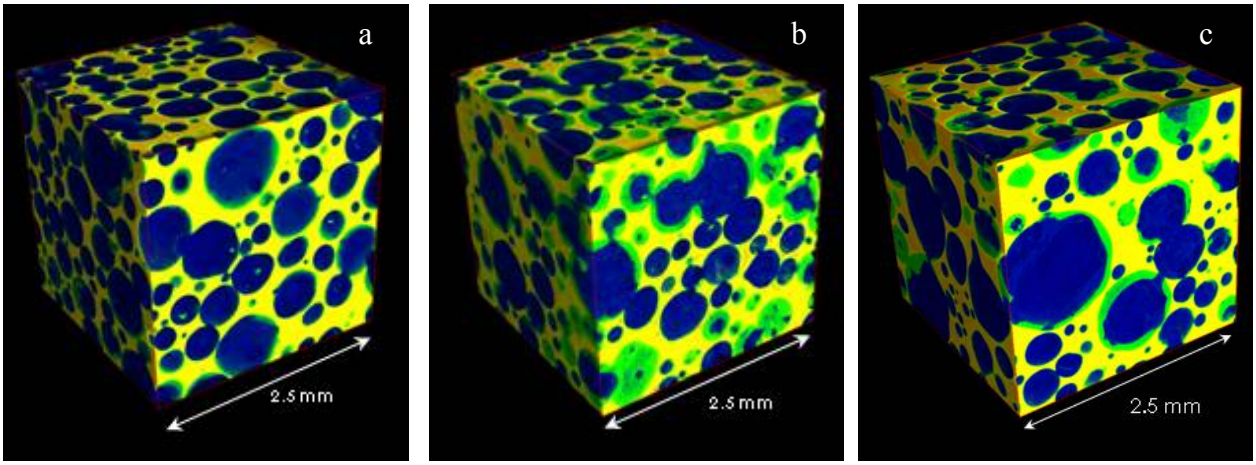

Fig. 1. 3D-images of ceramic scaffolds after loading *in vitro* expanded sheep BMSC and subcutaneously implantation in immunodeficient mice on 8 (a), 16 (b) and 64 (c) weeks. The images show newly formed bone (green) onto the inner surface of scaffold (yellow). The organic phase is blue.

To fully benefit of our 3D information, 3D quantitative parameters directly calculated from the 3D images using both a 3D Mean Intercept Length method, and a model independent techniques are presented in Table 1 and Fig. 2. The 3D MIL method was used to derive: scaffold surface/scaffold volume, mean pore thickness and mean wall thickness of scaffolds. The same model-independent techniques were applied to quantify the new bone region of interests. The percentage of new bone volume compared to total volume, the new bone thickness and distribution were also measured.

Porosity of scaffolds (%)	71%
Mean pore thickness of scaffolds (μm)	430
Mean wall thickness of scaffolds (μm)	107
Scaffold Surface/Scaffold Volume (mm-1)	32,0
New bone/Total Volume at 8 weeks *in vivo*	18,5%
New bone/Total Volume at 16 weeks *in vivo*	26,6%
New bone/Total Volume at 64 weeks *in vivo*	28,3%
Mean New bone thickness 8 weeks (μm) *in vivo*	34,59
Mean New bone thickness at 16 weeks (μm) *in vivo*	57,82
Mean New bone thickness at 64 weeks (μm) *in vivo*	57,29

Table 1 Measured histomorphometric parameters.

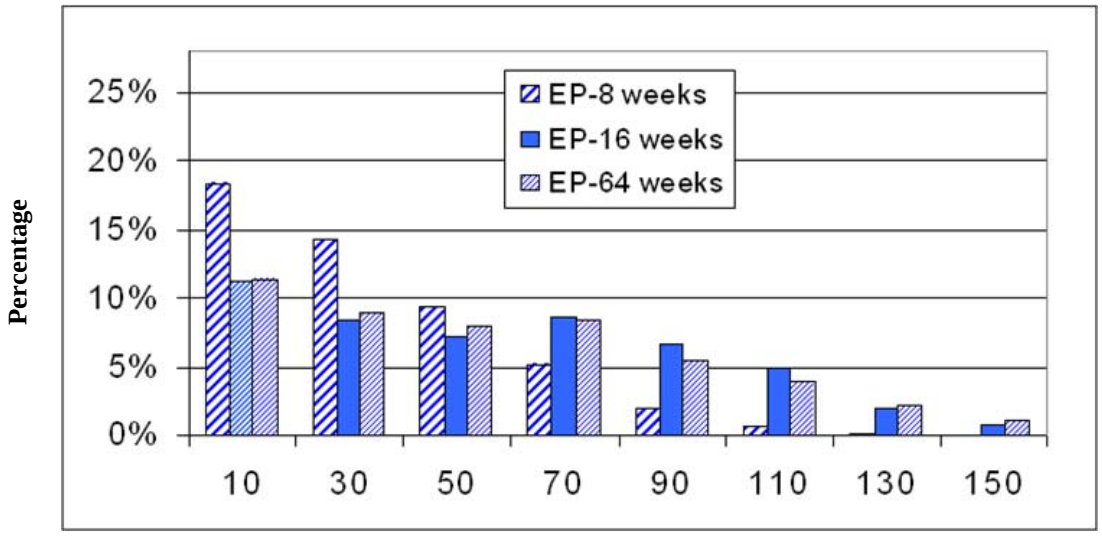

Fig. 2. The histogram of the distribution of the new bone within the scaffolds at 8, 16 and 64 weeks of implant.

Measured quantitative parameters of the scaffolds were in agreement with histological analysis which was performed complementary. When we analyzed the scaffolds at 8 and 16 weeks after the implant we observed a significant increase with the time of newly formed bone to compare for the scaffold at 64 weeks after the implant. However more bone was formed on the scaffold with higher porosity, higher bone surface to bone volume, higher isotropy, and thinner walls (data not shown). Interesting, in agreement with the fact that the scaffold chemistry was the same, the thickness of the newly formed bone did not vary in the two scaffolds (data not shown). Based on the above results, the scaffold characterized by an engineered structure with a higher total porosity, and a higher degree of interconnections among pores appeared to be a better material for bone reconstruction.

In conclusion, μCT offers major advantages to compare with other techniques including the possibility to investigate the influence of scaffold parameters such as pore size and spatial distribution with regard to growth of bone within the implant. At the present, μCT allows us to determine efficiency of bone formation in scaffolds with different microstructural parameters or under different circumstances.

Publications

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

Academic Journal Papers

1. A. Cedola, V. Stanic, M. Burghammer, S. Lagomarsino, F. Rustichelli, R. Giardino, N. Nicoli Aldini, M. Fini, **V. Komlev** and S. Di Fonzo /X-ray micro-diffraction analysis of reconstructed bone at Zr

prosthetic surface with sub-micrometre spatial resolution/ *Journal Physics in Medicine and Biology*, Vol. 48, 2003, p. 37-48.

2. A. Cedola, S. Lagomarsino, **V. Komlev**, F. Rustichelli, M. Mastrogiacomo, R. Cancedda, S. Milita, M. Burghammer /High spatial resolution X-ray microdiffraction applied to biomaterial studies and archeometry/ *Spectrochimica Acta Part B*, Vol. 59, 2004, p. 1557– 1564.
3. M. Mastrogiacomo, **V.S. Komlev**, M. Hausard, F. Peyrin, F. Turquier, S. Casari, A. Cedola, F. Rustichelli, R. Cancedda /Synchrotron Radiation Microtomography of Bone Engineered from Bone Marrow Stromal Cells / *Tissue engineering*, November, Vol. 10, No. 11-12, 2004, p. 1767-1774.
4. M. Mastrogiacomo, A. Muraglia, **V. Komlev**, F. Peyrin, F. Rustichelli, A. Crovace, and R. Cancedda /Tissue engineering of bone: search for a better scaffold/ *Journal Orthodontics and Craniofacial Research*, (in press).
5. A. Cedola, M. Mastrogiacomo, M. Burghammer, **V. Komlev**, P. Giannoni, A. Favia, R. Cancedda, F. Rustichelli and S. Lagomarsino /Engineered bone from bone marrow stromal cells: a structural study by advanced X-ray microdiffraction technique/ *Tissue Engineering*, 2004, (submitted).

Refereed Conference Proceedings

6. 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.
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8. M. Mastrogiacomo, A. Cedola, **V.S. Komlev**, F. Peyrin, M. Burghammer, P. Giannoni, R. Cancedda, F. Rustichelli, S. Lagomarsino /Advanced X-ray micro-analysis of bone regenerated by bone marrow stromal cells/ The proceedings of the 9thCCT on "Materials for Tissues Engineering - Chemistry and Microstructure: The Role for Ceramics", (in press).
9. **V.S. Komlev**, M. Mastrogiacomo, F. Peyrin, R. Cancedda, F. Rustichelli /X-ray computed microtomography for bone tissue engineering/ The proceedings of International School on Advanced Materials, Course V, Tissue Engineering, Edited by F. Rustichelli, Ancona, 2005, (in press).

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3. Jones, J.R. and Hench, L.L. Regeneration of trabecular bone using porous ceramics. Current Opinion in Solid State and Materials. *Science, 7* (2003), 301-307.
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