ESRF	***Experiment title:*** Study of newly formed bone induced by Bone Marrow Stromal Cells by means of microdiffraction	***Experiment number***: MD67
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Report:

The tissue engineering approach for the reconstruction of damaged large bony segments takes advantage of the patient's own cells, which are isolated, expanded in vitro, loaded onto a bioceramic scaffold and reimplanted into the lesion site. Bone marrow stromal cells (BMSC) are the most commonly used cell type.
A structural characterization of the engineered bone is largely desirable. An important point is to evaluate if the BMSC extracellular matrix deposition on a bioceramic scaffold recapitulates the ontogeny of the natural bone development. Moreover the investigation of the interaction between the newly deposited bone and the scaffold results particularly interesting. Indeed the chemistry and the geometry of the scaffold used to deliver BMSC in the lesion site determine spatial organization of the new bone and the bone-biomaterial integration.
In this experiment we investigated for the first time the local interaction between the newly formed mineral crystals in the engineered bone and the biomaterial by means of microdiffraction with sub-micron spatial resolution. We found out that the newly formed bone is well organized inside the scaffold pore, following the growth model of natural bone, and that there is a good adhesion with the scaffold. Combining Wide Angle (WAXS) and Small Angle (SAXS) X-ray Scattering with high spatial resolution, we were able to determine the orientation of the crystallographic c-axis inside the bone grains, and the orientation of the mineral crystals and collagen micro-fibrils with respect to the scaffold. From a quantitative analysis of both the SAXS and WAXS patterns the grain size appears to be compatible with the model for early stage mineralization.

Moreover with a special software we obtained microscopic images displaying the spatial variation of different structural features, thus allowing to map the mineralization intensity and bone orientation degree around the pore. We compared the results obtained for two different scaffolds with different composition and morphology for two different implantation times. In all the cases we found out similar results with respect to the organization of the mineral crystals and collagen micro-fibrils.

We evaluated in particular the performance of SkeliteTM (Millenium Biologix Corp., Kingston, Canada), a clinically available scaffold based on hydroxyapatite (HA) and Silicon-stabilized Tricalcium Phosphate (Si-TCP). Previous analysis by computed tomography (CT) revealed a progressive disappearance of this scaffold with the implantation time and its subsequent replacement with highly mineralized lamellar bone. However the resorption mechanism was not clearly understood in details. A preliminary analysis of the measured diffraction spectra in this experiment, suggest that the ratio TCP/HA could change in proximity of the interface scaffold/new bone and with the implantation time. These results could give important indication about the resorption mechanism, but they have to be validated by systematic analysis taken at different implantation time, and by comparison with samples not loaded with stromal cells, in order to understand their role in the structural change of scaffold.

References

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