Study of the Biomineralization process using a multi-scale approach

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Tissue Engineering (TE) combines aspects of medicine, biology, and engineering to generate, repair, or replace human tissues. In particular, the TE approach may be used to regenerate bone by implanting a porous ceramic scaffold combined with bone marrow stromal cells (BMSC) *in vivo* [1]. A deeper comprehension of the biomineralization (BM) process is at the basis of TE and will be instrumental to the further development of regenerative medicine. However, the achievement of a complete understanding of a process as complex as BM will require the synergy of different advanced experimental techniques. We present a multi-scale approach based on the coupling of complementary X-ray experimental techniques (X-ray phase contrast Tomography, XPCT, microdiffraction, XuD, and microfluorescence, XuF) with new analytical tools, which has allowed us to obtain structural and morphological information on the engineered tissues, from the atomic to the micrometric scale. In particular, we studied the mechanism of mineralized matrix deposition in a TE approach, wherein bone tissue is formed when porous ceramic constructs are loaded with BMSC implanted in vivo. High resolution XPCT provided the 3D spatial distribution of the different tissues participating to the BM process (Bone, B, Scaffold, SC, Soft Tissue, ST; see Fig. 1a). High resolution scanning XuD, on the other hand, is a powerful tool to distinguish and monitor the evolution of the different 'players' of the regeneration process (Collagen, Organic Matrix, Hidroxy Apatite, HA, and Amorphous Calcium Phosphate, ACP) in the regenerated bone at the organic–mineral interface within a porous scaffold (Fig. 1b) [2]. Finally, XuF allows for verifying the chemical evolution of the different growing phases, and to study the distribution of Ca in the regenerated bone (Fig. 1c). This approach provides information on the first steps of BM. In addition, we imaged the 3D vascularization network [3] inside the scaffold (Fig. 1d). The control of the angiogenesis of the micro-vascular network is a key step to obtain tissue regeneration and repair, while a full understanding of the morphology and functionality of the BM process will be of key importance for developing new drugs for preventing and healing bone diseases and for the development of bio-inspired materials.

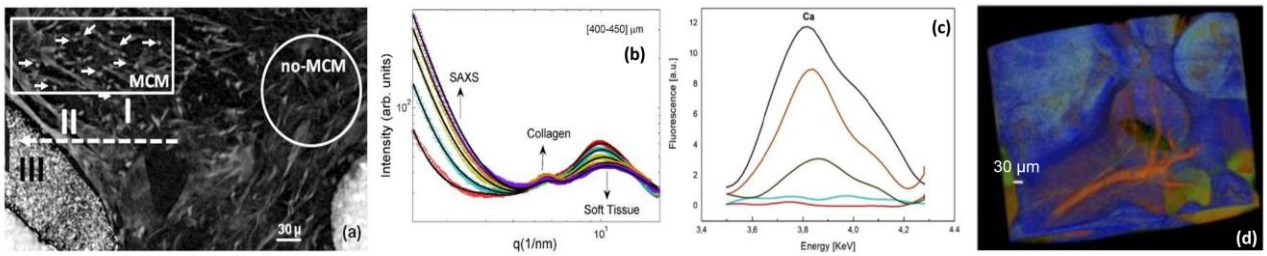

Figure 1: (a) 3d phase recostruction of a skelite scaffold implanted for 8 weeks in the animal, obtained by XPCT. We can distinguish the collagenous soft tissue, ST (I), the bone, B (II), and the scaffold, SC (III). (b) XuD profiles acquired at varying distance from the SC (the distance increases on going from the red to the purple curve). (c) Ca XuF spectra measured at the same positions at which the XuD profiles reported in (b) were acquired. (d) 3D volume of the sample.

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