

Synchrotron and non synchrotron X-ray microtomography three-dimensional representation of bone ingrowth in calcium phosphate biomaterials.

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INTRODUCTION: Numerous types of bone and dental surgery require bone substitutes. In recent years, our group has developed a new type of Injectable Bone Substitute (IBS), consisting of a mixture of BCP ceramic granules and a cellulosic polymer derivative. The resulting composite provides a sterile, ready-to-use, injectable material. Injectable CaP biomaterials should associate efficient bone colonization and implantation with non-invasive surgical techniques.

Typically the spatial resolution of conventional medical CT-scanners is in the range of 1-2.5 mm, which corresponds to 1-10 cubic mm voxel (volume element) size. Computerized X-Ray Micro Tomography gives possibilities to improve the spatial resolution by mm to μm . The purpose of the present study was to compare the 3-dimensional (3D) representation of bone ingrowth after implantation of injectable bone substitute suspension implanted in a rabbit model, using Synchrotron X-Ray Micro Tomography (SuT) and Laboratory Desktop Micro Tomography (LD μ T). 2D imaging performed with Scanning Electron Microscopy (SEM) is used as the reference method.

METHODS: Injectable bone substitute (IBS): A CaP aqueous suspension was developed to obtain an injectable biomaterial associating a biphasic CaP ceramic mineral phase (MBCP®, Biomatlante, Vigneux, France) sieved in two different ranges (BCP granules 40 to 80, or 80 to 200 μm in diameter) with a 3% aqueous solution of a cellulosic polymer (hydroxypropyl-methylcellulose) in a 50/50 (w/w) ratio. The CaP fillers used were composed of BCP ceramic (60% HA and 40% β -tricalcium phosphate). The IBS was sterile, ready-to-use and injectable (CNRS Patent WO 95/21634; (MBCP Gel®)Biomatlante, Vigneux, France).

In vivo study: These materials were implanted for 3 weeks into critical-sized bone defects at the distal

end of rabbit femura. Implantations were performed on New Zealand white rabbits, as detailed in previous studies, in aseptic conditions and under general anesthesia. The samples were embedded in methylmethacrylate with glycol. For each sample, rods (0.6 mm x 0.6 mm x 10 mm) were cut perpendicularly to the drilling axis of the implantation site, using an Isomet™ diamond saw (Buehler LTD, Germany).

Synchrotron X-Ray Micro Tomography (SuT): The experiment was carried out at the European Synchrotron Radiation Facility (ESRF) [1] at the Micro-Fluorescence, Imaging and Diffraction beamline ID22. The sample was set at a distance of 65 m from the undulator source with a horizontal rotation axis. The distance between the sample and the detector was 8 mm. In the CCD-based high-resolution X-ray image detectors commonly used in microtomography, a visible-light image is generated by a fluorescent screen and then projected by a microscope optic onto a CCD camera. The shadow projections of the object were recorded with a high-resolution CCD-based camera system. The scintillator was a lutetium aluminum garnet (LAG) single crystal with a 12 μm -thick europium-doped luminescent layer. An optical microscope (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 was 1.4 μm which corresponds to the resolution of the chosen scintillation screen. After darkfield correction and flatfield normalization of the projections, the tomograms were reconstructed using the filtered backprojection technique. 3D histomorphometric analysis was performed in voxels with Imaris™ (Bitplane AG, Zurich, Switzerland) software.

Laboratory Desktop Micro Tomography (LD μ T): We use a compact desktop system (SkyScan-1072) with a laboratory X-Ray source. It consists of the

combination of an x-ray shadow microscopic system and a computer with tomographic reconstruction software.

The system "SkyScan 1072" allows reaching a spatial resolution of 5 μm corresponding to near 1x10⁷ cubic mm voxel size.

The X-ray shadow projections digitized as 1024x1024 pixels with 4096 brightness gradations (12 bit) for cooled camera or 256 gradations (8 bit) for analog camera. The reconstructed cross-sections have a 1024x1024 (or 2048x2048, 512x512, 256x256...) pixels (float point) format. Typical cycle of data collection for reconstruction contains of shadow image acquisitions from 200 to 400 views over 180 or 360 degrees of object rotation.

Table 1. Experimental characteristics for the both methods of this study

	(SμT)	(LDμT)
Source size	Beam 800X30μm	Cone from 5μm to 22 mm
Camera CCD	2048X2048	1024 X 1024
Pixel size	0.7	1.8 μm
Resolution	1.4μm	5μm
exposure time	0.5 s	6.5 s
angular range	180 °	360 °
Projections	625	400

RESULTS:

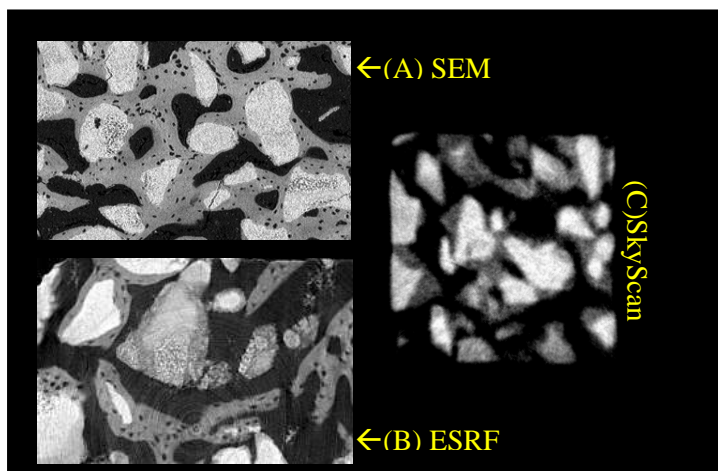


Fig. 1: Scanning electron microscopy (SEM, X 50) picture (A), synchrotron (B) and Skyscan (C) tomography reconstruction of bone ingrowth with IBS

The results show and can differentiate the mineral structures like bone and BCP ceramic with all the techniques used (Fig. 1). The SEM picture and the ESRF reconstructions are similar with the presence of osteocytic lacunae (osteoplasts). The desktop system (SkyScan-1072) don't show this small

structure and the limits of the mineral contours are less accurate (blurred).

The 3 Dimensional (3D) representations of both implanted biomaterials (Fig. 2) are similar with the same representation of the 3 D structures, BCP ceramics (white), Trabecular Bone (grey), and soft tissue (black) with the two μscan methods.

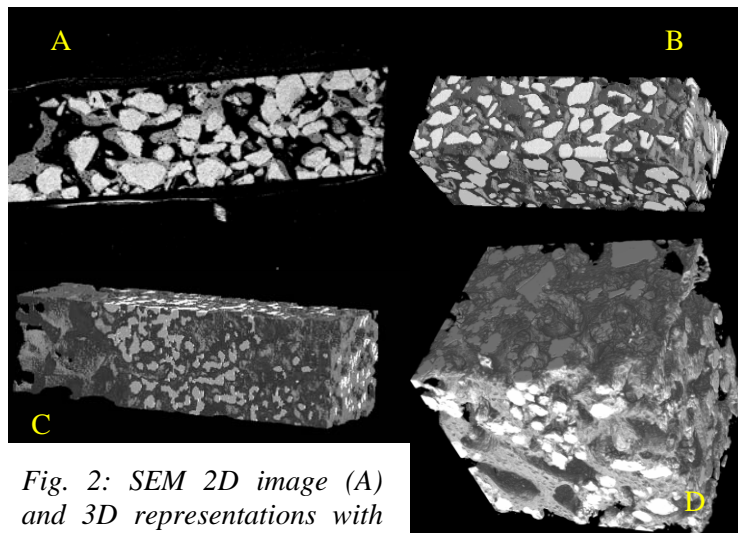


Fig. 2: SEM 2D image (A) and 3D representations with NSM (B,C) and SM (D) of bone ingrowth with IBS with BCP granules 80 to 200 μm (A,B) or 40 to 80 μm (CD) in diameter

DISCUSSION & CONCLUSIONS:

These systems allow making a non-destructive three-dimensional reconstruction of bone ingrowth within an injectable bone substitute with the same information on the structure. SM method allows visualisation of small structure getting near the μm, osteons, osteocytic lacunae but it's less easy to use than a compact desktop system. The NSM method shows good 3D results but the quantification of 2 mineral phases has to be improved because of the delimitation between these different grey levels (Fig. 1C)

This study shows the advantage and the limits of each technique for the qualitative representation and quantitative measurement of the different mineral phases into calcium phosphate implants.

REFERENCES: ¹ P. Weiss, L. Obadia, D. Magne et al. (2003) Synchrotron X-ray microtomography (on a micron scale) provides three-dimensional imaging representation of bone ingrowth in calcium phosphate biomaterials, *Biomaterials*, **24**: 4591-601.