ESRF	**Experiment title:** Non-destructive studies of novel bone tissue-engineered scaffolds by X-ray microtomography	**Experiment number**: MD-255
Beamline: ID19	**Date of experiment**: from: 18-FEB-2007 at 8:00 to: 20-FEB-2007 at 8:00	**Date of report**: 10 April, 2007
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Report:

The aim was to investigate in detail the original 3D-porous structure a novel scaffolds in terms of pore size, struts thickness and degree of pore interconnection using the computed microtomography analysis.

Introduction

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 tissue [1-4]. A key component in tissue engineering for bone regeneration is the scaffold that serves as a template for cell interactions and the formation of bone-extracellular matrix to provide structural support to the newly formed tissue [5]. Scaffolds for bone regeneration should meet certain criteria to serve this function, including mechanical properties similar to those of bone repair site, biocompatibility and biodegradability at a rate commensurate with remodelling. Scaffolds serve primarily as osteoconductive moieties, since new bone is deposited by creeping substitution from adjacent living bone.

Scaffolds properties (porosity and pore size) depend primarily on the nature of the biomaterial and the fabrication process [6-9]. The nature of the biomaterial has been subject of extensive studies including different materials such as metals, ceramics, glass, chemically synthesized polymers, natural polymers and combinations of these materials to form composites. Moreover several methods have been developed to create highly porous scaffolds, including fiber bonding [7], solvent casting/particulate leaching [10], gas foaming [9], phase separation [11], space holder technique [12,13].

Among the different techniques that can be used to produce 3D-scaffolds, the replication process presents a few very interesting advantages. This technique involves the use of a macroporous polymeric skeleton that is impregnated with a slurry (suspension) containing the bioactive glass particles, the impregnated sponge is then thermally treated to remove the polymeric phase and to sinter the inorganic one. The optimization of the

processing parameters leads to a final 3D-macroporous structure with a highly open interconnected porosity analogous to the spongious bone one [14-16].

X-ray microCT image acquisition

MicroCT experiments were performed at the European Synchrotron Radiation Facility (ESRF, Grenoble, France) on Beamline ID19 (The experiment MD-255 on Beamline ID19 from 18-FEB-2007 at 8:00 to 20-FEB-2007 at 8:00). Subsequent to some initial acquisitions with different operating conditions and a monochromatic beam with energy between 15 to 35 KeV were performed. Sample-to-detector distance was 2 mm. The detection system was a Gadox scintillator associated to FReLoN CCD camera. The exposure time was 1 seconds per projection. Typical scans included 1500 projections of the sample over 180 degrees. Images were recorded on a 2048 × 2048 CCD detector, with the pixel size set to 0.7 from 5 µm, yielding a field of view about 1.5 and 6 mm, respectively.

Samples description

The samples produced at Imperial College London by the replication technique. Samples were based on polymer coated Bioglass® (W_SC_PDLLA_coated (porosity 85-90%) and B_SC_PDLLA_coated (porosity 75-80%). The samples were cubic scaffolds 5x5x5mm^3.

Results

Fig 1 illustrates the 3D images of B_SC_PDLLA_coated (up) and W_SC_PDLLA_coated (down) with pixel size 5 (A) and 0.7 microns (B).

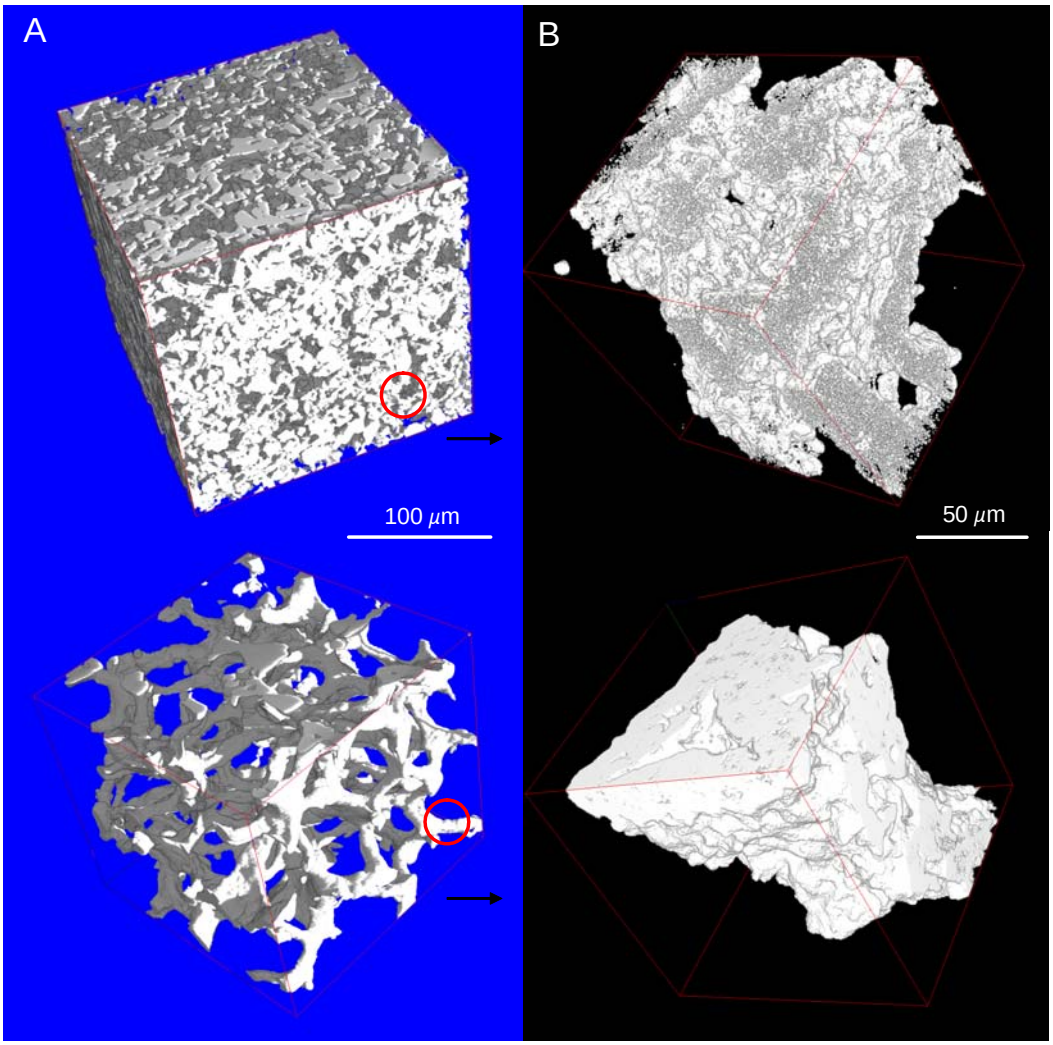

Fig 1 3D visualisation of a B_SC_PDLLA_coated (up) and W_SC_PDLLA_coated (down).

3D quantitative parameters were calculated directly from 3D images to characterize the scaffolds using 3D Mean Intercept Length method and of the 3D direct trabecular thickness method. The example of this analysis is reported in table 1.

Table 1

Scaffolds Analysis
==========
Mean: 78.1593
Standard Deviation: 50.9504
Total Volume: 2.8992 (mm^3)
Scaffold Volume: 0.3438 (mm^3)
Voxel: 2899232
BVF: 0.1186

Scaffolds Stereology
==========
Total # of points used: 2682030
Total volume of examined region (mm^3): 2.6820
The total number of voxels occupied by bone: 283851
The number of intersections (entering and leaving) for each direction:
20687 20762: 21894 23024: 24711 34018
The Euler # found using 27 surrounding voxels: -119.0
-Euler # / Volume analyzed ($1/mm^3$): 44.36938
Morphologic variables calculated from total planar intersection counts:

	SV/TV	SS/SV	Tb.Th	Tb.N	Tb.Sp
(x)	0.105834	29.204759	0.068482	1.545434	0.578585
(y)	0.105834	31.648999	0.063193	1.674776	0.533902
(z)	0.105834	41.380161	0.048332	2.189722	0.408347
(avg)	0.105834	34.077973	0.058689	1.803311	0.495847

Porosity was measured using IDL Virtual Machine (plug-in Blob3D). Blob3D is designed for efficient measurement of up to thousands of discrete features (e.g. clasts, mineral grains, porphyroblasts, voids) within a single sample. Blob3D is unique because it gives the program operator primary control over data interpretation and measurement, and all computations are carried out in 3D, rather than individually on a series of 2D slices. Example of typical processing operations in Separate module is shown on Fig 2.

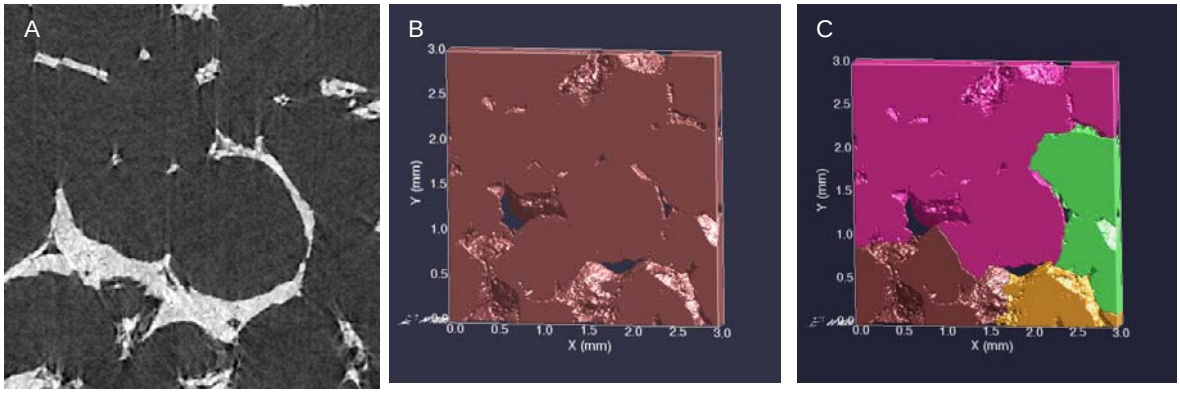

Fig 2 Part A shows a sample of computed tomography. A three-dimensional volume searching algorithm finds all voxels in contact, up to limits imposed by computer memory (B); faces truncated by the algorithm are marked in red. "Stair steps" on upper and lower portions of spheres are caused by 3:1 voxel aspect ratio (inter-slice vs. inter-pixel spacing). An erosion/dilation operator successfully separates most of spheres. Those contacting truncated faces have their processing postponed (C), allowing interior spheres to be processed

In addition, the following works are in progress. The microstructural effects of scaffold bioresorption are studied, simulated by *in vitro* soaking tests according to ISO standard. The gained experimental information will be completed by triaxial mechanical tests on the same samples, in order to reveal microstructure-mechanical property relationships, which are compared to those of natural bone, and which will be used in transport and promechanical computer simulations of the scaffolds [17,18]. Afterwards, different in vitro test will be performed in order to study the bioactivity and biodegradability of the developed scaffolds at various time frames. Specifically, the nucleation and growth of microstalline HAp on the pore walls will be studied to achieve a better understanding of the expected in vivo osteoproductive behaviour of the scaffolds. As far as the scaffolds bioresorption is concerned, in vitro soaking tests according to ISO standard will be carried out to study their effect on the scaffold microstructure and specifically on the pores size and morphology and of the scaffold struts thickness.

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