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Report:

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X-Ray Microtomography and micromechanical testing of Composite Scaffolds

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Introduction

A thorough understanding of the three-dimensional (3D) microstructure of porous scaffolds is crucial in order to know the biomechanical environment cells will sense after seeding. Phase contrast X-Ray Microtomography allows the visualization of the scaffolds' morphology in 3D. The equipment available at beamline ID22 at the European Synchrotron Radiation Facility (ESRF), executes microtomographies and also offers the possibility of performing micromechanical tests, thus obtaining 3D images of the strained microstructure at high resolutions (0.7μm). These

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images, coupled with the mechanical data from the tests, may also be converted into finite element (FE) models which could be used to predict the scaffolds' mechanical behaviour.

Materials and Methods

The scaffolds used in this study are made of polylactic acid (PLA) and a bio-absorbable titania-stabilised calcium phosphate glass. They are produced by a solvent and casting method, using chloroform and NaCl as the solvent and porogen agent respectively. The size of the glass particles was less than 40 μm, and the size of the NaCl particles ranged between 80 and 210 μm.

Phase contrast X-ray microtomographies were obtained of three compositions of scaffolds, containing 0%, 20% and 50% weight percent of sieved glass particles. Each composition was strained at levels varying between 0 and 50% strain using the microtomographic press available at beamline ID22 at the ESRF. The sample shape was cylindrical, with a diameter (ϕ) of 1mm and a height (h) of 2mm according to the dimensional requirements of the facility.

Tomographic projections of the scaffolds were reconstructed into 3D using High Speed Tomography. These images were treated using the image processing programs ImageJ and Mimics 8.11. The images were meshed superficially using Magics, and solid 3D meshes were obtained with MSC Marc-Mentat2003 software.

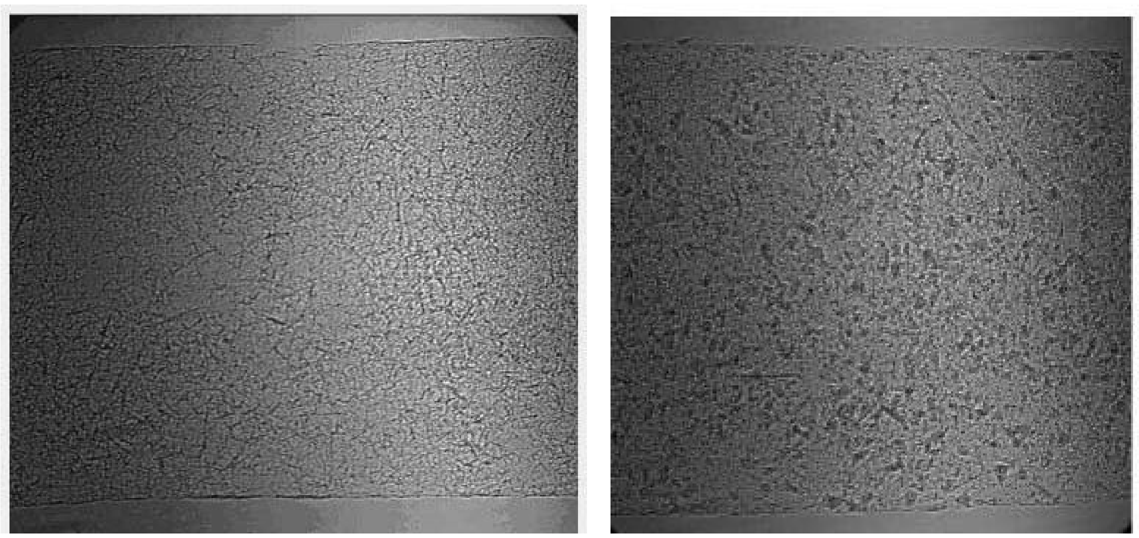

Figure 1: Microtomographic projections of scaffolds with a) 0 wt% glass and b) 50wt% of glass

In order to compare the microtomographic results with conventional tests, larger samples, with ϕ = 12mm and h = 10mm, of the same compositions were also produced. These samples were characterised using Scanning Electron Microscopy, pycnometry, and compression tests were performed on an Adamel Lhomargy tensile testing machine.

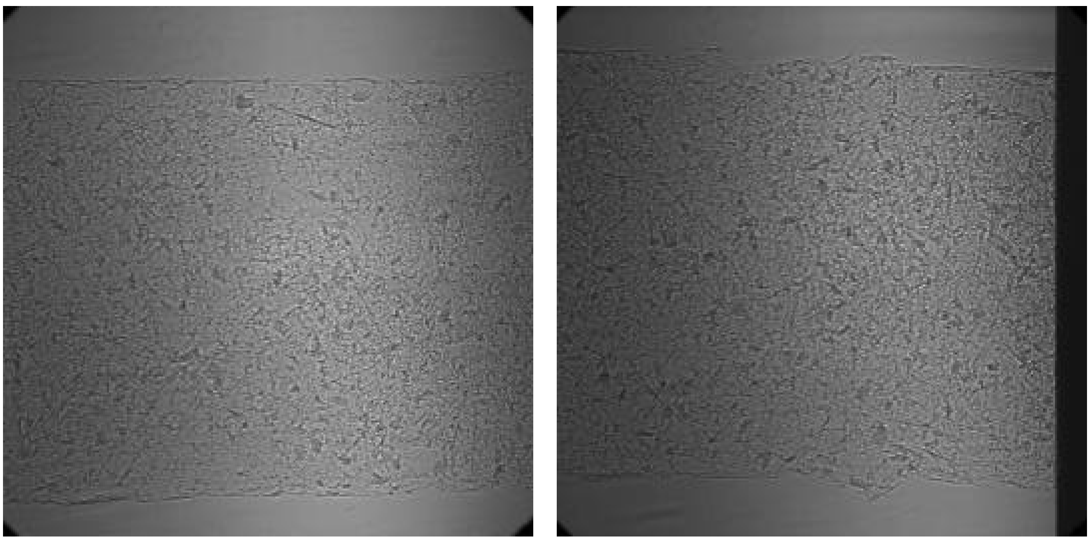

Figure 2: Microtomographic projections of a scaffold with 20 wt% of glass at a) 0% strain and b) 20% strain.

Results and Discussion

Phase-contrast microtomography was used instead of absorption-contrast due to the practically null X-ray absorption of the composite material. Projections of the microtomographic images show a very homogeneous distribution of glass particles within the polymer matrix (Figure 1). The collapse of the pore walls is evident in the projections taken under strain (Figure 2).

The images must be treated in order to enhance the contrast between the background and the material. The treatment involved converting the grey-scale image into binary mode and removing small artefacts in order to reduce the noise of the images. The 3D image obtained using the Magics software, allows the quantification of the porosity and the interconnectivity. A porosity of approximately 94% was found for the reconstructed microtomographies. Pycnometry measurements performed on the conventional samples gave values of 95% porosity.

Finally, sections of the 3D images were converted into finite element meshes (Figure 3). Cylinders measuring approximately ϕ = 1mm, and h = 0.1 mm were cut out of the 3D images to reduce the size of the FE files. The meshes contained some 500,000 elements, and will be used to perform simulations which can be validated with the in-situ mechanical tests. The memory of our hardware limits the size of the meshed sample, but we believe it is representative.

The information obtained from the technique will allow the characterisation of the scaffolds' microstructure, this knowledge will help interpret the phenomena occurring during static and dynamic cell culture assays.

Acknowledgements

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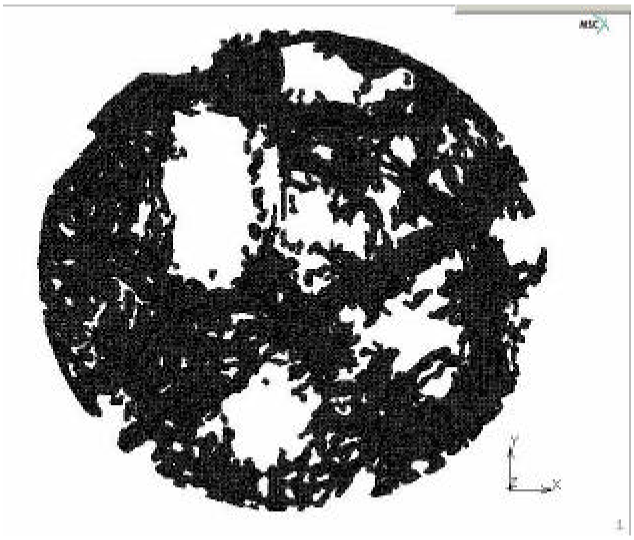

Figure 3: Finite element mesh of a scaffold with 0 wt% of glass