Mode of failure of a biomaterial composite scaffold for bone tissue engineering using synchrotron micro-tomography and finite element analysis

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Abstract

Computer reconstructions of tissue engineered scaffolds from micro-tomography images allow to study the architecture of the materials and to make biomechanical analyses using the finite element method. Scaffolds of PLA and glass composite material were used in this study. Mechanical compressive loads were applied on the scaffolds up to a total deformation of 50 %, and *in situ* synchrotron transversal images were obtained during compression. A FE model was made to simulate the compression test at 5 % of deformation. The highest local stress appears on thin pore walls leading to maximal strain of 20 %. The *in situ* compression of the scaffold indicates that it fails by buckling as typical thin-walled porous materials. These results have important implications in the deformations felt by the cells when attached in the material in bone tissue engineering experiments.

1. Introduction

Bone tissue engineering is currently investigated as an alternative to regenerative and reparative medicine. In that field the design of biodegradable scaffolds opens new perspectives. For instance the so-called PLA-Glass scaffold developed by Navarro et al. (2006) constitutes an original design made of a bi-material composite of Poly Lactic Acid (PLA) which forms a polymeric framework in which calcium phosphate glass particles are embedded [1]. Criteria of scaffold for bone tissue engineering are biocompatibility of the material, surface properties which promote cells adhesion, level of porosity and pore interconnectivity which allows colonisation by cells and transport of nutriments and waste products through the material. Moreover, the mechanical properties of the scaffold are crucial since the synthesis of an appropriate bone tissue depends on the mechanical environment at the scale of a cell [2,3]. In that way cells may be stimulated mechanically via the scaffold. Thus, optimizing the scaffold microstructure as well as the stimulation path could be needed to control bone formation into the scaffold. So to determine the cell-scale mechanical environment generated by external loading, we propose here a study that investigates by experimental micromechanical testing and finite-element numerical approach the mechanical response of the scaffold, especially the mode of deformation of the PLA-glass scaffold when it is submitted to global compression.

2. Methods

A biodegradable porous scaffold made of a composite material of Polylactic acid (PLA95L/5DL) + glass (Titanium-stabilised Calcium Phosphate glass) was prepared [4]. The material contains equal quantities of PLA and glass in mass. The material was made by means of the solvent-casting salt-leaching method. Briefly PLA and 40µm-diameter glass particles are mixed in chloroform with 80 - 210 µm-diameter salt particles as porogen and form a paste. The paste is spread to a final thickness of 7.2 mm and left until complete chloroform evaporation. Cylindrical samples whose diameter is 6 mm are punched out from the paste and placed in distilled water for 48 h. By this time the salt particles leach out of the cylinders and leave behind pores in the material. This method gives a highly porous material with a controlled pore-size and total pore interconnectivity.

The cylindrical samples of PLA-Glass material were submitted to compression tests treated by micro computed tomography (micro-CT). These experiments were carried out at the European Synchrotron Radiation Facility (ESRF, Grenoble, France) using the micro-tomographical press available at beamline ID22 with a resolution of 0.7 µm (Figure 1). Seven compression levels ranging from 1.6 % to 50 % were imposed on each sample and the internal structure of the PLA-Glass were reconstructed at deformed states using filtered-back projection.

A finite element model was developed to reproduce the complete scaffold cylinder sample whose diameter and height are respectively 6 mm and 7.2 mm (Figure 2). The volume of the sample was rendered by micro computed tomography then reconstructed in 3D using Mimics software and meshed using MSC Patran software. The model of the scaffold is composed of 1,800,000 tetrahedral, linear and isotropic elements. The PLA phase and glass particles were identified in micro-CT by scaling the gray values. By relating micro-CT and finite element model, PLA and glass parts were created in the model and relative material properties were assigned to elements. The mechanical properties of PLA and Glass are respectively, 3.6 GPa and 71.1 GPa for the Young's moduli and 0.167 and 2.9 for the Poisson's ratio [1]. Nonetheless the complete model was constituted by solid elements which are not able to take into account the microporosity of the real PLA-Glass material. The material properties were adapted in the model: E_{Glass} = 10.2 GPa, E_{PLA} = 517 MPa so that the global stiffness was equal to experimental measurement, i.e. E_{global} = 130 kPa. A static compression was performed on the complete finite element model until 5 % of deformation. Fixed boundary conditions were applied on the nodes of the bottom plane circular side while an axial displacement of 0.36 mm was applied on the top nodes. The structure remained unconfined at lateral side.

Distribution of local strain and stress are expected as results in terms of principal stress and strain, as well as shear stress and strain. The computation was linear elastic with large deformation and performed using DS Simulia Abaqus.

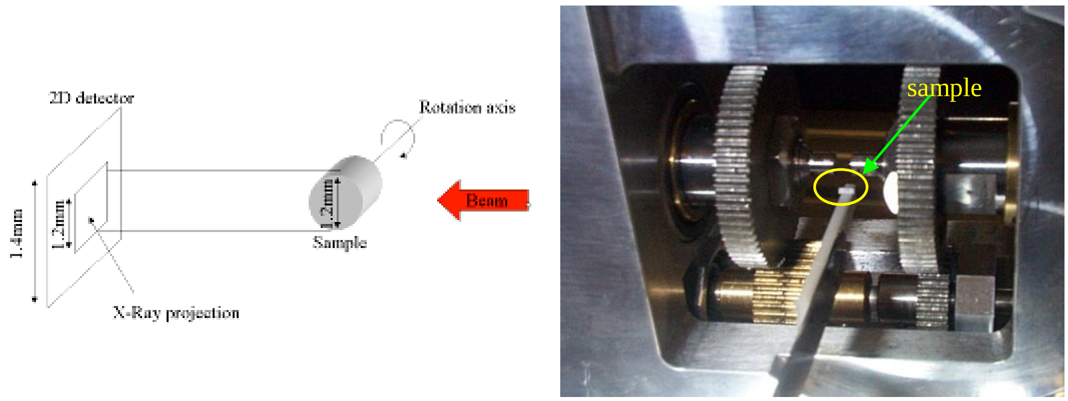


Figure 1: Conventional Tomographies. In-situ mechanical tests with strain controlled.

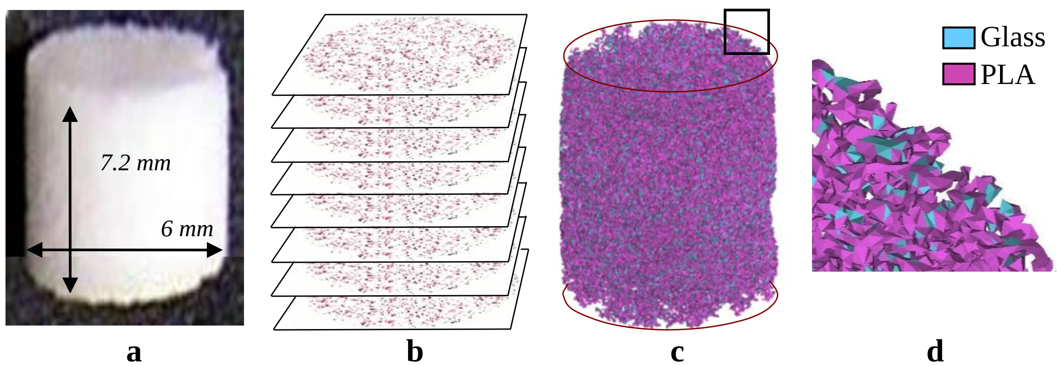


Figure 2: Reconstruction of a complete scaffold by micro-CT segmentation. a) PLA-glass scaffold. b) Micro-CT collection. c) Finite element model. d) Material property assignment.

3. Results

The X-ray radiographs of the samples at different compositions revealed the pore and glass particle distribution. Thin PLA pore walls and randomly distributed glass particles were observed in the scaffold material by computational imaging (Figure 3a). Porosity of PLA-glass material was measured by image treatment to 94 % which is consistent with porosity of 95 % measured by mercury porosimeter. The volume proportions within PLA-Glass material are 76.2 % of PLA and 23.8 % of Glass. The ratio between surface and volume is about 1000 mm2/mm3. Structure deformation was observed at different levels of compression by superposition of transversal sections (Figures 3a and b). Higher compression levels were performed using the micro-tomographical press to determine the mechanical response of the PLA-Glass material at large deformations. Figure 3c shows a pore at 0 % and at 20 % of compression. At 20 % of compression the pore walls deform largely by evident buckling mode. When 50 % of global compression was applied on the material, pore-wall breakage appeared.

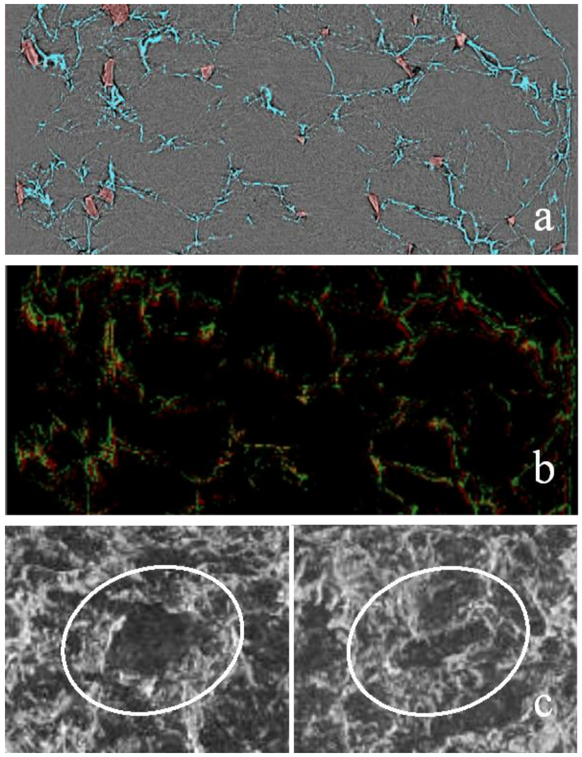
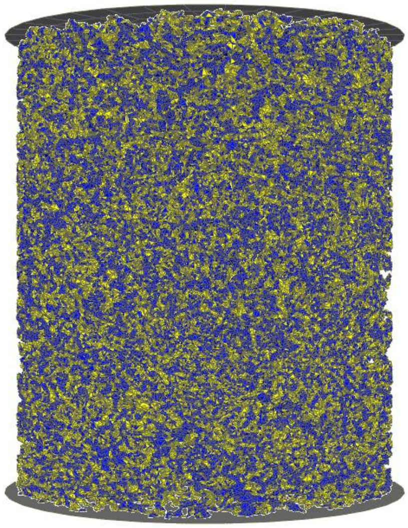


Figure 3: Microstructure under compression; coronal view. a) undeformed; pore walls in cyan, glass particles in red. b) undeformed (green) and 1.6 %-deformed (red) state. c) pore geometry at 0 % (left) and at 20 % of compression (right).

The finite element model of the scaffold has a porosity of 90 % and all the pores are interconnected. The volume of the material is about 25 mm^3 and the total wall pore surface is 1790 mm^2 giving a ratio between surface and volume about 73 mm^2/mm^3. The porosity of the material is equal 90% and all the pores are interconnected. At 5 % of compression the reaction force of the complete model is 180 mN giving a global stiffness of 130 kPa.

Figure 4a shows the complete scaffold whose elements are marked in blue or yellow depending if they undergo respectively compressive or tensile stress. In fact there are in the model almost as much compressed elements (50.7 %) as stretched ones (49.3 %). This suggests that the mechanical response of the PLA-Glass scaffold under compression involves equilibrium between internal tension and compression.

Figure 4b indicates that an axial compression of 5 % involves low lateral deformation or widening of the model structure. Figures 4c shows that under global compression some parts of the model undergo high strain (5 - 20 %). It is also found that some parts undergo high strain and low displacement while neighbouring parts move more being low strained (0.1 %). These results suggest that when the scaffold is compressed, most of internal parts are only displaced without being significantly stressed or deformed while the thinnest pore wall undergo high stress and strain. Moreover, the distribution of strain and stress is very heterogeneous within the structure. Indeed if most part of the scaffold undergoes average major principal stress equal to 1.5 MPa with a large standard deviation of 3.4 MPa and average major principal strain equal to 660 µstrain with large standard deviation of 2600 µstrain, some extreme values of stress and strain can be found in thinnest pore walls and range until maxima of 130 Mpa and 200,000 µstrain respectively (Table 1).



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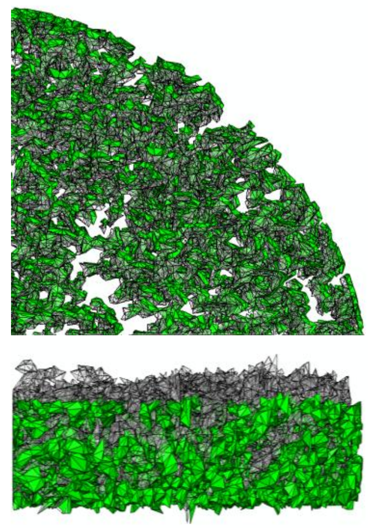

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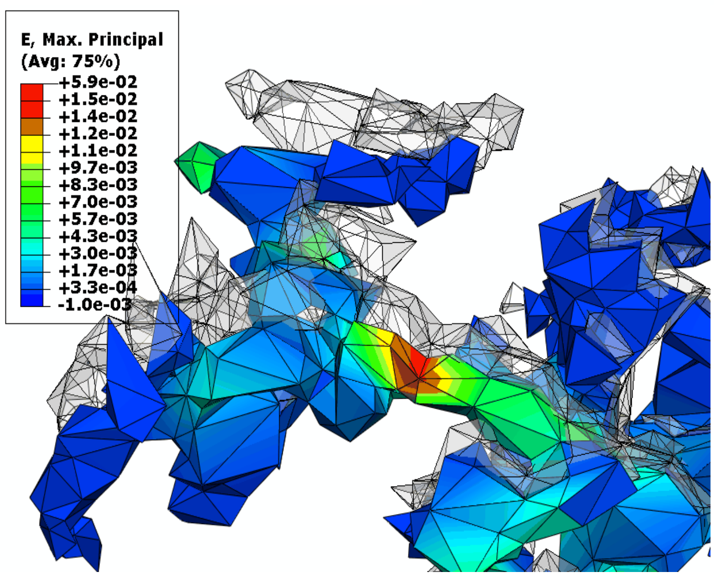

c

Figure 4: The complete model under compression. a) The yellow elements are under tensile stress and the blue ones are under compressive stress. b) Section of the model took in the middle represented at deformed (green) and undeformed (gray) states. c) Local strut displacement with strain concentration in thinnest part of a wall pores.

Means ± sd	**σ Glass (MPa)**	**Σ PLA (MPa)**	**ε Glass (µstrain)**	**ε PLA (µstrain)**
Minimum principal	-1.51 ± 3.42	-0.36 ± 1.46	-151 ± 340	-664 ± 2600
Maximum principal	1.20 ± 2.71	0.31 ± 1.40	126 ± 276	537 ± 2072
Octahedral shear	0.74 ± 1.65	0.30 ± 0.83	97 ± 215	790 ± 2137

Table 1: Means of principal stresses (σ) and strains (ε) and octahedral shear stress and strain in PLA and Glass phases at 5 % of compression of complete model.

Figure 5 shows two modes of deformation within the scaffold submitted to global compression. The strut of the model which is depicted in Figure 5a is oriented in compression direction and deforms following a buckling mode. Both extremities of the struts which are very bent while the center remains undeformed, seem to sustain the whole deformation. Figure 5b shows that in a highly reticulated part of the scaffold, the deformation is taken by few struts oriented radially which deform following a bending mode.

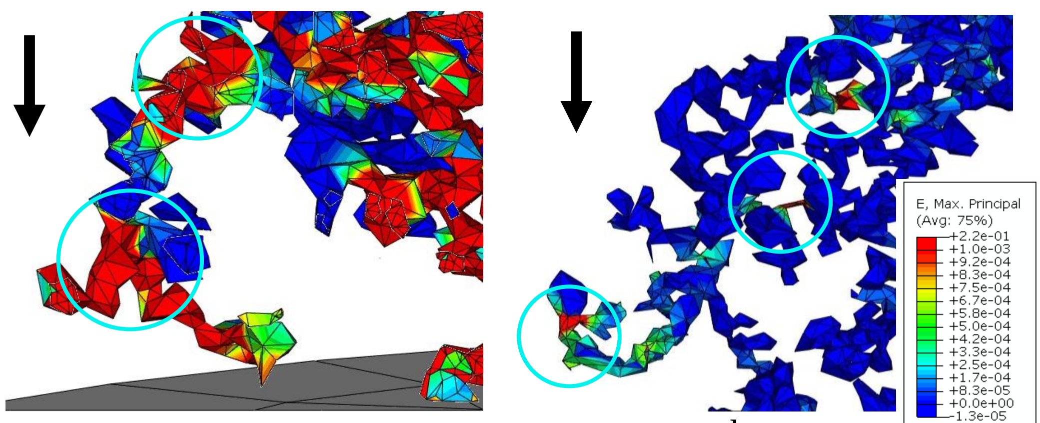

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Figure 5: a) Buckling and b) bending deformation modes circled in cyan within the finite element model in response to external compression in arrow direction.

4. Discussion

This study is an innovative approach to the mechanical and structural characterization of PLA-Glass scaffold for bone tissue engineering applications. Synchrotron X-Ray Micro-tomography and in-situ micromechanical testing have been applied to obtain 3D images of polymer/glass composite material. These experiments allow for a thorough qualitative and quantitative analysis of the microstructure of the scaffolds as well as offering valuable insight into their mechanical behaviour. The 3D images display the distribution of the different phases of the composite materials and the pore deformation under strain. The quantification of the porosity and pore interconnectivity by means of image analysis overcomes a challenging hurdle in tissue engineering biomaterial science. In situ micro-tomography combined to finite element analysis has permitted to determine the mode of deformation of the PLA-Glass material. Under compressive loading, PLA-Glass material deformation involves a spatial reorganization by displacement of the internal structure which tends to relax strain energy. Most of internal parts are mainly displaced being low stressed or strained while the stress and strain concentrate on thinnest pore walls. The present study indicates that the PLA-Glass material fails by buckling and bending in some parts of the structure which is typical from thin-walled porous materials.

Few parts of the structure undergo extreme mechanical environment with a maximal stress of around 130 MPa in both PLA and Glass phases and maximal strains of around 20 % in PLA and 1.5 % in Glass.

Unless the PLA part in the studied PLA-Glass material have different mechanical properties than those of pure PLA material (tensile and compressive strength of 50 – 80 MPa, ultimate elongation of 2.4 – 10 % [5,6]), this maximal stress and strain values would lead to material breakage in the few concerned points of the structure. Mesh refinement and introduction of more realistic plastic laws in PLA material properties could help in managing damage processes possibly occurring at rare locations.

It is worth to note that loading a PLA-Glass scaffold in such a simple way involves a large range of local stress and strain magnitudes which may correspond in adherent cells to different thresholds of stimulation and may lead to the formation of a heterogeneous bone tissue whose phenotype depends on the location in the scaffold. For instance, through the whole scaffold model submitted to 5 % of compression, 850,000 elements (45 % of total elements) present a resultant octahedral shear strain which ranges in [100;10,000]µstrain, values known stimulating bone formation [2,3]. The model suggests that compressing the PLA-Glass scaffold by 5% involves throughout the structure very numerous sites which may promote bone formation.

These results may have important implications in the deformations felt by the cells which adhere on the pore walls of the PLA-Glass material in bone tissue engineering experiments.

5. Acknowledgment

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