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Mechanical and structural characterisation of completely degradable polylactic acid/calcium phosphate glass scaffolds

Montse Charles-Harris[a,b], Sergio del Valle[b], Emilie Hentges[b], Pierre Bleuet[c], Damien Lacroix[a,b], Josep A. Planell[a,b,*]

[a]*Institute of Bioengineering of Catalonia, C/ Josep Samitier 1-5, 08028 Barcelona, Spain*
[b]*Department of Material Sciences, Universitat Politècnica de Catalunya, Avda. Diagonal 647, 08028 Barcelona, Spain*
[c]*European Synchrotron Research Facility (ESRF), BP 220, 38043 Grenoble Cedex, France*

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Abstract

This study involves the mechanical and structural characterisation of completely degradable scaffolds for tissue engineering applications. The scaffolds are a composite of polylactic acid (PLA) and a soluble calcium phosphate glass, and are thus completely degradable. A factorial experimental design was applied to optimise scaffold composition prior to simultaneous microtomography and micromechanical testing. Synchrotron X-ray microtomography combined with in situ micromechanical testing was performed to obtain three-dimensional (3D) images of the scaffolds under compression. The 3D reconstruction was converted into a finite element mesh which was validated by simulating a compression test and comparing it with experimental results. The experimental design reveals that larger glass particle and pore sizes reduce the stiffness of the scaffolds, and that the porosity is largely unaffected by changes in pore sizes or glass weight content. The porosity ranges between 93% and 96.5%, and the stiffness ranges between 50 and 200 kPa. X-ray projections show a homogeneous distribution of the glass particles within the PLA matrix, and illustrate pore-wall breakage under strain. The 3D reconstructions are used qualitatively to visualise the distribution of the phases of the composite material, and to follow pore deformation under compression. Quantitatively, scaffold porosity, pore interconnectivity and surface/volume ratios have been calculated. Finite element analysis revealed the stress and strain distribution in the scaffold under compression, and could be used in the future to characterise the mechanical properties of the scaffolds.

Keywords: Synchrotron X-ray microtomography; Mechanical test; Biodegradable glass; Scaffold; Finite element analysis

1. Introduction

Although porous degradable scaffolds are used extensively in tissue engineering to regenerate musculoskeletal tissue [1–3], some aspects of their characterisation remain a challenge. Scaffolds are designed to be colonised by cells whose viability will depend on the porosity and the mechanical and chemical stimuli they receive within [4–6]. An ideal tissue engineering scaffold should offer appropriate mechanical, structural, chemical, and surface properties. Thus, a thorough characterisation of the scaffolds is crucial in order to assess their suitability and to understand the biomechanical environment the cells will sense upon seeding. This study focuses on the mechanical and structural aspects of the scaffolds.

The mechanical properties of scaffolds are generally measured by way of their compressive properties. Compression is thought to be one of the prevailing loading modes they will experience in vivo [7]. Many authors have measured and discussed these properties [8–10]. However, testing procedures described in the literature are generally incompletely defined and have been adapted from testing standards that are not always applicable [11–13]. This is due to the fact that sample dimensions are limited and that the interpretation of the compression curves of these foamed materials is complex. A rigorous compression

*Corresponding author. Institute of Bioengineering of Catalonia, C/ Josep Samitier 1-5, 08028 Barcelona, Spain.
E-mail address: josep.a.planell@upc.edu (J.A. Planell).

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testing protocol has been developed in this study, in order to evaluate the stiffness of the scaffolds. A novel approach combining microtomography with simultaneous compression has also been tested as a complement to conventional mechanical assays.

The microstructure of the scaffolds can be imaged using two-dimensional (2D) or three-dimensional (3D) microscopic techniques. 2D imaging is insufficient to assess some 3D characteristics such as porosity, pore interconnectivity or phase distributions within composite materials. Furthermore, some 2D techniques require transparent or translucent materials. Microtomography is a 3D non-destructive technique which provides isotropic data on material morphology. Synchrotron X-ray microtomography offers high-resolution 3D images thanks to the monochromatic beam and the high-photon flux (approximately 10^{12} photons/s).

Microtomography has been used previously to assess cell-scaffold morphology after in vivo and in vitro implantation. Gauthier et al. [14], Weiss et al. [15], and Mastrogiacomo et al. [16] apply Synchrotron X-ray microtomography to study bone ingrowth in calcium phosphate cements at various in vivo implantation times. Thurner et al. [17] perform an elegant comparison between fibroblast- and osteoblast-like cell growth on poly-ethyleneterephthalate yarns by using Synchrotron X-ray imaging, addressing, in addition, the crucial issue of taking cell biology into the "third dimension". Tuan and Hutmacher [18] use microcomputed tomography to quantify porosity, interconnection and osteochondral repair in polycaprolactone meshes.

Synchrotron X-ray microtomography has also been used to perform thorough characterisations of biomaterial morphologies. Rustichelli et al. [19] apply this technology to evaluate the properties of hydroxyapatite coatings deposited on titanium surfaces. Maspero et al. [20] and Müller et al. [21] study the microstructure of porous poly(D,L-lactic-co-glycolic) acid scaffolds. The authors compare the accuracy of their 2D and 3D data in characterising the porosity and develop a methodology to quantify a mean pore diameter value.

This study combines Synchrotron X-ray microtomography with simultaneous in-situ mechanical tests to analyse the microstructure and the deformation of completely degradable composite polylactic acid/calcium phosphate glass scaffolds. First the influence of the composition on the porosity and mechanical properties of the scaffolds is assessed by means of a factorial experimental design. Then, scaffolds with optimised composition are imaged at different strains, in order to study the scaffolds' porosity, pore deformation under strain and the distribution of the glass within the polymeric matrix. Finally, the tomographical images are converted into a finite element mesh which will be used to evaluate the mechanical properties of the scaffolds numerically.

2. Materials and methods

2.1. Scaffold fabrication

The scaffolds are a composite of Poly-95%LL,5%DL-lactic acid (PLA) and a titania-stabilised, completely degradable, calcium phosphate glass [22,23]. They are manufactured by means of a solvent-casting salt-leaching method. Briefly, PLA pellets are dissolved in chloroform. Sieved sodium chloride (NaCl) and glass particles are then added to the mixture, forming a thick paste. The paste is spread on a Teflon sheet until complete chloroform evaporation. Cylindrical samples are punched out of the sheets and placed in distilled water for 48 h. By this time the NaCl particles would have leached out of the cylinders and leave behind a porous network.

2.2. Characterisation

Prior to the microtomography experiment, a 2^3 factorial experimental design was used in order to optimise the scaffolds' composition. This methodology allows the analysis of the effect of the scaffolds' composition on their properties, in this case morphology, porosity and mechanical properties, by varying three factors between two levels. The three factors were: (a) size of the NaCl particles, (b) weight percent (wt%) of glass, and (c) size of the glass particles. Each factor was tested at two levels: a maximum (+) and a minimum (−) (Table 1), testing a total of 8 different compositions.

2.2.1. Morphology

A qualitative study of the scaffold morphology was performed by scanning electron microscopy (SEM).

2.2.2. Porosity

The porosity of the scaffolds was measured by mercury pycnometry. The apparent density of the scaffold (ρ_{scaffold}) was measured by means of the volume of mercury it displaced when submerged. The porosity of the scaffold is calculated by dividing its apparent density by the density of the solid composite (ρ_{solid}):

$$\%\text{Porosity} = 100\left(1 - \frac{\rho_{\text{scaffold}}}{\rho_{\text{solid}}}\right). \quad (1)$$

Measurements are presented as an average of five samples.

2.2.3. Compression tests

Compression tests were performed on an Adamel Lhomargy tensile testing machine, with a 100 N load cell at a constant cross-head speed of 2 mm/min until 50% strain of the sample or a maximum of 70 N compressive stress. All compression samples had a diameter of 12 mm. The height of the samples was approximately 10 mm. The sample dimensions

Table 1
The three factors and two levels used in the experimental design

	NaCl particle size (μm)	Glass (wt%)	Glass particle size (μm)
Low level (−)	80–210	20	<40
High level (+)	297–590	50	40–80