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Micro-CT studies on 3-D bioactive glass–ceramic scaffolds for bone regeneration

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Abstract

The aim of this study was the preparation and characterization of bioactive glass–ceramic scaffolds for bone tissue engineering. For this purpose, a glass belonging to the system SiO_2–P_2O_5–CaO–MgO–Na_2O–K_2O (CEL2) was used. The sponge-replication method was adopted to prepare the scaffolds; specifically, a polymeric skeleton was impregnated with a slurry containing CEL2 powder, polyvinyl alcohol (PVA) as a binding agent and distilled water. The impregnated sponge was then thermally treated to remove the polymeric phase and to sinter the inorganic one. The obtained scaffolds possessed an open and interconnected porosity, analogous to cancellous bone texture, and with a mechanical strength above 2 MPa. Moreover, the scaffolds underwent partial bioresorption due to ion-leaching phenomena. This feature was investigated by X-ray computed microcomputed tomography (micro-CT). Micro-CT is a three-dimensional (3-D) radiographic imaging technique, able to achieve a spatial resolution close to 1 μm^3. The use of synchrotron radiation allows the selected photon energy to be tuned to optimize the contrast among the different phases in the investigated samples. The 3-D scaffolds were soaked in a simulated body fluid (SBF) to study the formation of hydroxyapatite microcrystals on the scaffold struts and on the internal pore walls. The 3-D scaffolds were also soaked in a buffer solution (Tris–HCl) for different times to assess the scaffold bioresorption according to the ISO standard. A gradual resorption of the pores walls was observed during the soakings both in SBF and in Tris–HCl.

Keywords: Glass–ceramic scaffolds; X-ray computed microtomography; Bioactivity; Bioresorption

1. Introduction

During the last decade progress in the chemical, physical, material and biological sciences has resulted in the possibility of bone tissue engineering, i.e. a biologically based method for the repair and regeneration of natural tissues [1–4]. A key component in tissue engineering for bone regeneration is the scaffold, which acts as a template for cell interactions and for the growth of bone extracellular matrix to provide structural support for the newly formed tissue [5].

Many researchers have tried to define which properties are required for the optimal synthetic scaffold, in particular for bone tissue replacement [6–14]. First of all, scaffolds need to be biocompatible. A three-dimensional (3-D) internal geometry, similar to bone morphology, and the retention of mechanical properties after implantation are required for scaffolds in order to maintain a tissue space of the prescribed size and shape for tissue formation. A

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porosity higher than 50–60 vol.% seems to be necessary. In the case of ceramic scaffolds, a macroporosity of 100–500 μm is needed to promote bone cell attachment, and a microporosity of less than 10 μm should favour ion and liquid diffusion [15].

Scaffold properties depend primarily on the nature of the biomaterial, on the fabrication process [16–19] and on the implant's 3-D microarchitecture. The nature of the biomaterial has been the subject of extensive studies concerning different materials, such as metals, ceramics, glasses, chemically synthesized polymers and natural polymers, and combinations of these materials to form composites. Moreover, several methods have been developed to create highly porous scaffolds, including fiber bonding [17], solvent casting/particulate leaching [20], gas foaming [19], phase separation [21] and the space holder technique [22,23]. Interstitial flow has been shown to have an important role in bone growth [24]. The scaffold's 3-D architecture determines the level of flow inducing shear stress on cells adhered to the scaffold walls both in vitro (for tissues cultured in bioreactors) and in vivo.

The replication process to produce 3-D scaffolds has interesting advantages. The 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 process parameters finally leads to a highly bioactive 3-D macroporous structure [25], characterized by an open and highly interconnected porosity, analogous to the one of the spongious bone [25–27]. These scaffolds can undergo a partial bioresorption due to the ion-leaching phenomenon.

The aim of this study was to characterize bioactive and bioresorbable glass–ceramic scaffolds. In particular, microarchitectural parameters were evaluated by X-ray microcomputed tomography (micro-CT). Micro-CT is known to be a unique technique for the non-invasive, non-destructive 3-D characterization of materials in medicine, material science and biology. It is a 3-D radiographic imaging technique, similar to the conventional computed tomography systems used in medical and industrial applications. Unlike such systems, which typically have a maximum spatial resolution of about 1 mm^3, micro-CT is capable of achieving a spatial resolution of the order of 1 μm^3. In particular, synchrotron radiation offers the possibility of selecting X-rays with a small energy bandwidth from the wide and continuous energy spectrum whilst, at the same time, guaranteeing a high enough photon flux for efficient imaging [28–30]. Moreover, the use of synchrotron radiation allows the selected photon energy to be tuned in order to optimize the contrast of the different phases in the investigated samples. This possibility sparks great interest for micro-CT since it allows high spatial resolution images to be generated from 10 to 1 μm, with a high signal-to-noise ratio [31–33]. The recent use of micro-CT in scaffold research has enabled accurate morphological studies to be carried out, yielding comprehensive data sets [34–42]. Very promising and advanced fields of investigations can be opened by micro-CT in tissue engineering [43], but in most studies its use has been limited to the visualization of the scaffold morphology and the determination of its porosity while the investigation of the newly formed phase is usually carried out only at the scaffold surface by scanning electron microscopy (SEM) and X-ray diffraction (XRD) [44,45].

In this work an accurate analysis of the scaffold's 3-D structure was performed in order to confirm and extend the promising results, from previous works, concerning the use of CEL2 glass–ceramic as effective biomaterial for scaffolding. In particular, micro-CT analysis was used to study the new phase 3-D distribution in the bulk material and its evolution as a function of the soaking time in a simulated body fluid (SBF) and Tris–HCl medium. These results are completed by SEM and XRD measurements.

2. Materials and methods

In this study, glass–ceramic macroporous scaffolds were prepared using a polyurethane (PU) sponge such as organic template and bioactive glass powders.

The chosen glass, belonging to the SiO_2–P_2O_5–CaO–MgO–Na_2O–K_2O system (CEL2), has been studied and characterized previously [23,25] due to its excellent biocompatibility and bioactivity [46]. It has the following molar composition: 45% SiO_2, 3% P_2O_5, 3%, 26% CaO, 7% MgO, 15% Na_2O, 4% K_2O.

Briefly, CEL2 was prepared by melting the raw products (SiO_2, $Ca_3(PO_4)_2$, $CaCO_3$, $4MgCO_3?Mg(OH)_2?5H_2O$, Na_2CO_3, K_2CO_3) in a platinum crucible at 1400 °C for 1 h in air and by quenching the melt in cold water to obtain a frit, which afterwards was ground by ball milling and sieved to a final grain size below 30 μm.

2.1. Scaffolds preparation

The organic template used in this work is an open-cell polyurethane sponge characterized by a highly interconnected macroporosity. The sponge was cut into 1.5 × 1.5 × 1.5 cm cubic blocks and then impregnated with a slurry containing CEL2 particles (weight composition: 25% CEL2, 6% PVA, 69% water). First PVA was hydrolyzed and stirred in distilled water at 60 °C for 1 h, before the glass powder was added to the solution. Then the polymeric template underwent the infiltration process: the sponge blocks were soaked in the glass slurry and taken back for several times, followed by cycles of compression to shrink the sponge in thickness along the three spatial directions, in order to remove the exceeding slurry. Afterwards the samples were thermally treated at 950 °C for 3 h (heating and cooling rate were 5 and 10 °C min^{-1} respectively) in order to remove the polymeric phase and to sinter the inorganic one, so that macroporous glass–ceramic scaffolds were produced.