

Fabrication of bioactive glass–ceramic foams mimicking human bone portions for regenerative medicine

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Abstract

A technique for the preparation of bioglass foams for bone tissue engineering is presented. The process is based on the in situ foaming of a bioglass-loaded polyurethane foam as the intermediate step for obtaining a bioglass porous monolith, starting from sol–gel synthesized bioglass powders. The obtained foams were characterized using X-ray diffraction analysis, Fourier transform infrared spectroscopy, and field emission scanning electron microscopy observations. The material was assessed by soaking samples in simulated body fluid and observing apatite layer formation. Diagnostic imaging taken from human patients was used to reconstruct a human bone portion, which was used to mould a tailored scaffold fabricated using the in situ foaming technique. The results confirmed that the obtained bioactive materials prepared with three-dimensional processing are promising for applications in reconstructive surgery tailored to each single patient.

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1. Introduction

Bone reconstruction is mandatory in several clinical issues involving orthopaedics and dentistry. Since the use of autogenous graft is often impossible due to the scarcity of bone tissue or difficulties in modelling it according to a specific geometry, a possible solution to these problems is to provide biomaterials able to enhance the body's own reparative capability [1–4].

Bioglasses are silica-based materials that show bioactivity. Among the materials used for bone tissue engineering, the ability of bioactive glasses and glass–ceramics to bond with bones is well documented [5–7]. Bioglasses should be both osteoconductive and osteoproduative, enhancing both the proliferation and the differentiation of progenitor cells.

It has also been demonstrated that degradation products from bioactive glasses exert a genetic control on the osteoblast cell cycle, promoting bone tissue growth [8]. A common characteristic of the bioactive glasses is the formation of a layer of biologically active hydroxylapatite (HA) and carbonated hydroxylapatite (HCA) on the surface in contact with body fluids, which provides the bonding interface with surrounding tissues [1,9]. Such bonding ability allows the clinical use of bioactive glasses in the treatment of periodontal defects or other clinical applications [10,11]. New applications in bones bearing larger loads might exist for stronger bioactive glasses, providing that materials could easily be fashioned into clinically relevant shapes.

For bioglass synthesis, sol–gel technique represents an improvement in the conventional melting technique, allowing the preparation of high-purity materials at low temperatures and in various shapes, such as powders and thin films [11–13]. Moreover, sol–gel glasses exhibit a higher

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bioactivity in a wider compositional range with respect to conventional glasses [14].

For the production of an appropriate scaffold, the material's microstructure must be tailored to allow attachment and growth of bone cells and provide a proper interface with the extracellular matrix. For this reason, it is fundamental to produce a scaffold with a large and suitable porosity. Even though the porosity ranges suggested in the literature are very variable [15–18], it is widely accepted that an interconnected network of pores of about 150 μm in diameter [18] should be adequate not only to allow cell penetration, but also to promote neo-angiogenesis, which is one of the critical issues in bone healing [19–21]. Several techniques have been investigated for the preparation of bioglass, glass–ceramic and composite scaffolds, such as phase separation, the use of pore formers, replication from polymeric foams and direct foaming [22–28]. In particular, direct foaming of polyurethane systems has been widely investigated for the preparation of ceramic foams in several engineering applications [28–30], including bioceramics [31,32].

Another paramount aspect of scaffold fabrication is the possibility of shaping the material accordingly to the patient's anatomical features. Segmentation from diagnostic imaging data can be applied to the reconstruction of the bones of each patient [33]. As an example, we focused our attention on mandibular bone. The loss of mandibular functionality as a consequence of the resection of a malignancy or traumatic injury can result in heavy impairment while negatively affecting the patient's appearance, leading to a very poor quality of life. Thus, proper mandible reconstruction is mandatory to obtain the complete rehabilitation of the patient [34,35]. For this purpose, *in situ* foaming of a bioglass-loaded polyurethane system was coupled to reconstruction from computer tomography (CT) data. Diagnostic data were used to obtain a three-dimensional (3D) model of a mandibular bone portion, which was used as a mould for the foaming reaction, in order to prepare a porous monolith mimicking a specific bone portion.

The obtained material was submitted to morphologic and spectroscopic characterization and evaluated in terms of bioactivity *in vitro* according to the Kokubo protocol [9].

2. Materials and methods

For the preparation of the bioglass, a sol–gel technique was used, according to Vallet-Regí et al. [13]. Tetraethyl-orthosilicate (TEOS, Aldrich, 99%), triethylphosphate (TEP, Aldrich, 99%) and calcium nitrate hexahydrate (Aldrich, 99%) were mixed in a Teflon beaker, in the stoichiometric ratio $\text{SiO}_2:\text{CaO}:\text{P}_2\text{O}_5 = 70:26:4$. For a batch containing 25 ml of TEOS, 19 ml of 1 M nitric acid was added as a catalyst. Gelation was performed at 70 °C for 3 days. After desiccation, performed at 150 °C for 24 h, the product obtained was crushed to obtain a powder.

Foams were prepared by dispersing 1 g of the bioglass powder in 0.85 ml of poly(methylenephényldiisocyanate) (PMDI, Voranate M220, Dow Chemicals, industrial

grade). Then 0.4 ml of poly(ethylene glycol) (PEG200, average MW = 200, Aldrich, 99%), 0.1 ml of polyoxyethylene sorbitan monooleate (Tween 80, Fluka, 97%), 1 mg of diazabicyclooctane (DABCO, Aldrich, 99%) and 10 μl of distilled water were added, keeping the dispersion under mild stirring. Complete polymerization occurred in 12 h into a Teflon mould. A 10 mm cubic sample was cut from the bioglass-loaded polyurethane foam. The foam was sintered at 1000 °C for 10 h, with an intermediate step at 600 °C for 30 min to perform the burnout of the polymer. The size of sintered samples was measured to determine the shrinkage. Porosity was measured by Hg porosimetry (Carlo Erba Porosimeter 2000). The foam was characterized by field emission scanning electron microscopy (FE-SEM; Leo Supra 1535) and X-ray diffraction analysis (XRD; Philips X'pert 1900).

The bioactivity of the material was assessed following the Kokubo protocol in a simulated body fluid (SBF) solution [9]. This test is usually performed on flat surfaces for thin film investigation. Therefore, pellet samples were prepared by pressing the bioglass powder uniaxially at 300 MPa (Specac, Atlas T25). Pellets were then sintered according to the foam's sintering cycle, described above (sample S0). Sintered pellets were soaked into SBF solution for 1, 3 and seven days at 36.5 °C, obtaining S1, S3 and S7 samples, respectively. Samples were rinsed in distilled water followed by drying at room temperature in order to be characterized by thin-film XRD, FE-SEM observation and attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR; Nicolet 8700, Thermo Electron, equipped with GoldenGate ATR cell, Specac). Once bioactivity was assessed, the foaming process was used to produce a scaffold mimicking a bone portion.

CT data of a mandibular bone were obtained from a public library (Casimage database, Geneva University Hospital). A 3D model of the neck of the condyloid process was obtained by scripting in Matlab (The Math Works). A 3D print of the model (Z printer 310 plus, Zcorp) was used as a template to obtain a silicone rubber mould. A liquid silicone rubber system (RTV 246/75, Prochima, industrial grade) was cast in a beaker containing the template. Curing of the rubber occurred in 12 h. The foaming reaction then took place in the silicone rubber mould, followed by sintering according to the above-described thermal cycle, to produce a foam scaffold mimicking the reconstructed bone portion.

The foam scaffold was also tested according to the Kokubo protocol [9]. Foam was soaked in SBF for seven days, producing sample F7, which was characterized by FE-SEM observations.

3. Results and discussion

3.1. Foam preparation

Ceramic-loaded polyurethane foams were obtained by dispersing the ceramic powder in the precursors of a

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