

Porous hydroxyapatite/gelatine scaffolds with ice-designed channel-like porosity for biomedical applications

Elena Landi *, Federica Valentini, Anna Tampieri

ISTEC-CNR, Institute of Science and Technology for Ceramics, National Research Council, via Granarolo 64, 48018 Faenza (RA), Italy

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Abstract

A cryogenic process, including freeze-casting and drying has been performed to obtain hydroxyapatite (HA) scaffolds (approx. diameter 10 mm, height 20 mm) with completely lamellar morphology due to preferentially aligned channel-like pores. Changing the process parameters that influence the cold transmission efficiency from the bottom to the top of the poured HA slurry, lamellar ice crystals with different thickness grew throughout the samples. After sintering, scaffolds with porosity features nearly resembling the ice ones were obtained. The interconnection of pores and the ability of the scaffolds to be rapidly penetrated by synthetic body fluid has been proven. Biohybrid HA/gel composites were prepared, infiltrating HA lamellar scaffolds (45–55 vol.% of porosity) with a 10wt.% solution of gelatine. Colouring genipine was used to cross-link gelatine and clearly show the distribution of the protein in the composite. The compressive mechanical properties of lamellar scaffolds improved with the addition of gelatine: the strength increased up to 5–6 times, while the elastic modulus and strain approximately doubled. The effectiveness of the cross-linkage has been preliminarily verified following scaffold degradation in synthetic body fluid.

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1. Introduction

Research concerning bone substitutes is focusing more and more on biohybrid composite materials that follow a biomimetic strategy. Hydroxyapatite (HA) is suitable for substituting or integrating diseased or damaged bone tissues since it resembles the mineralized phase of bone and during resorption supplies fundamental ions for the new forming bone.

Several techniques have been used to realize porous scaffolds [1]. They are based on replication, use of a sacrificial template, foaming, including gas bubbling, the addition of porogens and salt leaching, and have in common a homogeneous structure with isotropic globular and/or cellular porosity [2–4]. Considering the biomedical application of

the scaffold as a bone substitute, a porous cellular structure allows good cell ingrowth and proliferation if the pore dimension is adequate (at least 80–100 μm ; larger dimensions are desirable, but depend also on the bioactivity of the material constituting the scaffold), but can also be responsible for poor mechanical properties that limit the application to bone sites that do not have load-bearing functions. The use of anisotropic scaffolds could be a promising solution for sites in which the weight loading is mainly unidirectional. Freeze-drying [5,6] can be used to develop scaffolds with aligned channel-like meso- and macropores and good micro-interconnections. In addition, the porogen phase being ice, the production process is more simple and “clean” since it avoids the use of templates or porogens that need to be eliminated in a successive step by burning out or leaching, complicating the production and increasing the risk of failure. Further, the brittleness of porous ceramic materials and their tendency to form debris can be reduced by adding a polymer. Gela-

* Corresponding author. Tel.: +39 0546 699711; fax: +39 0546 46381.
E-mail addresses: elena@istec.cnr.it, elena.landi@istec.cnr.it (E. Landi).

tine is a natural polymer that has been shown to be useful as a reinforcing coat for porous cellular isotropic scaffolds [7,8] and also exhibits several bioattractive features [9,10]. It is a non-toxic, biodegradable, inexpensive and non-immunogenic material that has been widely applied in tissue engineering, thanks also to its readiness to chemically cross-link, thereby reducing its solubility in contact with body fluid at 37 °C. Good cell-interactive properties of gelatine have been reported [10] and have been shown to be independent of the pore size and geometry: gelatine scaffolds with channel-like pores, prepared by a cryogenic treatment, allow the adhesion, spreading and proliferation of many types of human cells, including osteoblasts, similar to gelatine cellular scaffolds [10].

The present work focuses on the preparation of lamellar anisotropic porous scaffolds having a cell-interactive gelatine [10] reinforcing coating that at the same time give channel-like guidance patterns for cells, modulate the resorption of apatite and control the release of a drug being loaded into the composite system. Local drug delivery, which can potentially be associated with the bone regeneration function, is a more effective and less costly approach to bone disease therapy or the control of inflammation in the prosthesis revision operation.

Such scaffolds represent an interesting objective for the development of bone synthetic substitutes since the substratum satisfies bone cell requirements in terms of morphology and composition, and the scaffold architecture can potentially be designed to achieve anisotropic load-bearing properties (thus improved load-bearing properties in preferential directions).

2. Materials and methods

Commercial HA powder (Riedel de Häen) was calcined at 1000 °C for 5 h and then sieved at 150 µm. Aqueous slurries of the powder, with HA loading in the range 15–40 vol.%, were prepared using 1 wt.% of ammonium polymethacrylate anionic dispersant (Darvan C, R.T. Vanderbilt Co., Norwalk, CT) and 1 wt.% of polyvinyl alcohol (organic binder) (Sigma-Aldrich, Steinheim, Germany). Slurries were ball-milled for 20 h with zirconia balls, deaired in a vacuum desiccator and then poured into cylindrical pre-cooled plastic moulds (plastic wells usually used for cell culture) and finally freeze-dried (Edwards Mod. MFD01, Crawley, UK).

The green bodies were sintered at 1300 °C for 2 h in an air furnace using a heating rate of 50 °C h⁻¹ up to 700 °C and 200 °C h⁻¹ thereafter.

X-ray diffraction analysis (Cu K_α radiation, Rigaku Miniflex, Tokyo Japan) was used to check the absence of secondary phases besides apatite.

The density of the porous bodies was measured by geometrical weight/volume evaluation (so-called apparent density) and the total porosity was consequently calculated. The open micro- and macroporosity distribution was evaluated by Hg-porosimetry (Carlo Erba Porosimeter

2000 and Macropores Unit 120) on 2 g specimens; the instrument automatically performs the sample outgassing at room temperature before the analysis.

The morphological and microstructural characterization of the porous sintered bodies was performed by scanning electron microscopy (SEM; Stereoscan 360, Leica, Cambridge, UK). The specimens were made electroconductive before the analysis using a sputter-coater with an Au target.

Some of the sintered porous HA samples were deaerated and infiltrated with 10% gelatine solution (Gelatine Type A Pig Skin, Italgelatine, Italy). After drying in an air-circulating oven at room temperature, gelatine was cross-linked with 10 ml of 0.67% genipine solution (Wako, Japan) for 24 h. Genipine was chosen among other cross-linking agents to show the presence of a proteic component up to the inner parts of the final HA/gel samples, thanks to the blue colouring obtained by spontaneous reaction of genipine with amino acids and proteins. Moreover, genipine-fixed tissue has a resistance against enzymatic degradation comparable to the glutaraldehyde-fixed tissue but genipine is less cytotoxic than glutaraldehyde [9,11].

The effectiveness of the genipine cross-linking was preliminarily tested by immersing the scaffolds in Hanks balanced solution (Sigma H6648) at 37 °C and following the degradation behaviour for up to 30 days in comparison with uncross-linked composites.

The compressive strength of the HA and HA/gel porous scaffolds was measured on cylindrical specimens of 10 mm × 10 mm (height × diameter) using a Zwick/Roell Z050 instrument (Ulm, Germany) with a crosshead speed of 1 mm min⁻¹. The specimen size was not normalized, as the diameter equals the height; however, the test can be used to compare the mechanical performance of the anisotropic lamellar scaffolds with that of previously developed isotropic cellular structures. On the other hand, mechanical data of bone tissues and of synthetic bone substitutes are frequently obtained by non-standardized procedures due to processing difficulties, and this is one of the main causes of the scattering of the data reported in literature. The compressive strength was calculated from the maximum load registered during the test divided by the original area; six specimens were tested and the average strength value together with the standard deviation value were calculated. The elastic modulus was calculated focusing on the stress–strain curve related to the 45–60% range of the ultimate strength, following the method reported in Ref. [12].

3. Results and discussion

The XRD analysis detected no secondary phases besides HA in the scaffolds sintered at 1300 °C (Fig. 1). The scaffold shrinkage due to sintering was in the range 20–30%, depending on the solid loading used for the slurry.

The microstructure, morphology and mechanical properties of the porous samples were strictly dependent on

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