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Short communication

Gelatin-bioactive glass composites scaffolds with controlled macroporosity

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HIGHLIGHTS

- Highly bioactive glass particles have been dispersed in a gelatin matrix.
- Composite scaffolds with controlled and >90% opened porosity are obtained at RTP.
- They are as bioactive as pure bioactive glass, but own higher compressive strengths.

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ABSTRACT

Scaffolding materials are often needed for bone regeneration: their role is to act as temporary templates for the reconstruction of bone tissues either *in situ* or in the laboratory. Suitable properties for such scaffolds are bioactivity and mechanical toughness, which can both be combined in composite matrices based on bioceramics and biopolymers. Among possible combinations, bioactive glasses possess unique bone-bonding ability compared to other bioceramics, while gelatin is a biocompatible polymer naturally derived from collagen. Here we report the synthesis of bioactive glass–gelatin composite scaffolds with well-controlled porosity, which is a major concern as it can deeply influence osteogenesis. Compared to pure bioactive glass scaffolds, enhanced mechanical properties were observed, while the composite scaffolds still own promising *in vitro* bone-like apatite forming ability.

1. Introduction

Numerous approaches in the field of bone regeneration are directed by the concept of biomimetic systems with materials close to the natural phase of bone, which is apatite, and more generally with bioceramics [1,2]. Among them, bioactive glasses are of high interest because of their high bioactivity, especially sol–gel derived ones. Nevertheless, bioactive glasses exhibit poor mechanical properties, a weakness that removes them from the list of candidates for bone regeneration in load-bearing applications. An interesting idea to solve this problem is to go further in the imitation of nature. Indeed, mechanical properties of bone come from its composite structure which consists in apatite crystals and organic fibers of collagen. As a consequence, there are lots of studies dedicated to composites for bone regeneration [3,4] and among them gelatin, which is a derivative from collagen, naturally finds its place as an organic part suitable for such materials.

Nevertheless, to improve its efficiency, an ideal implant should possess a porous structure to allow cell invasion and vascularization which would facilitate its integration with surrounding bone tissue. A method that allows synthesis of 3D macroporous implants with pores of few hundreds of micrometers and interconnections has to be used [5]. Moreover, the high variety of possible applications for such materials, used in different sites of the body, requires a process that allows tailoring porosity and shape of the implant. There are already examples of associations of gelatin and bioceramics, based e.g. on hydroxyapatite [6], β-tricalcium phosphate [7] and bioactive glasses [8] into promising 3D macroporous composite implants for bone regeneration. However, the synthesis of gelatin – bioactive glass composites is generally made by freeze-drying processes [9,10] and leads to limited and uncontrolled pore sizes with irregular pore shapes.

To avoid such shortcomings, we have used the microsphere leaching technique [11]. The technique allows a fine and easy control of the porosity and relies on the leaching of polymer spheres used as porogen agents. Hence porosity is largely determined by the particle size of the sacrificial polymer spheres. However, when processing composites based on bioactive glasses, careful attention

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has to be paid to preserve the glassy matrix, which is highly reactive and soluble in aqueous media. If not, leaching of alkali and alkaline-earth elements from the glass can occur simultaneously to leaching of the porogen.

Here to generate porosity inside bioactive glass–gelatin composites, we used PMMA (PolyMethylMethAcrylate) micropheres as a porogen agent [12]. PMMA has the advantage of being readily dissolved in acetone, a common and non cytotoxic solvent, at ambient temperature. Importantly, acetone is a non-solvent of both gelatin and bioactive glass particles, thereby preserving the composite matrix. Finally, PMMA is a biocompatible polymer implying no risk of toxic effects in case of incomplete elimination with acetone.

2. Materials and methods

2.1. Fabrication of the composite scaffolds

Sol–gel derived bioactive glass powders with a composition of 75% SiO_2–25% CaO (weight percent) was synthesized. Briefly 13.48 mL of water and 13.48 mL of ethanol were mixed with 2.25 mL of hydrochloric acid (HCl at 2 N in water before hydrolysis). After 5 min of stirring, 13.94 mL of TEOS (TetraEthyl OrthoSilicate: $Si(OCH_2CH_3)_4$) were added and left for hydrolysis for 30 min. Then 5.2637 g of calcium nitrate ($Ca(NO_3)_2{\cdot}4H_2O$) are introduced and the mixture is left under stirring for 1 h. The obtained sol is poured into PTFE containers for ageing during 24 h at 60 °C and is then dried at 125 °C for 24 h. Calcination at 700 °C during 24 h allowed complete elimination of the nitrates and incorporation of calcium into the glass network.

The glass powder was grinded and sieved so that only particles smaller than 50 μm were selected. 0.025 g of the obtained powder fraction was then mixed with 0.2 g of PMMA microspheres of 200–400 μm diameter. A gelatin solution was obtained by dissolution of type A gelatin (porcine) in distilled water at 35 °C, the concentration of gelatin in water being 0.1 g/mL. 0.15 mL of the gelatin solution were added to the bioactive glass/PMMA microspheres blend, and the mixture was compacted into an open mold to allow drying of gelatin. The compacting stage was necessary to improve the interconnectivity of the resulting structure. The reason why the gelatin/bioactive glass blend was not directly infiltrated into a stack a polymer beads is the high viscosity of the solution when gelatin is mixed with the bioactive glass particles; a highly concentrated gelatin solution is however required to avoid bioactive glass dissolution. After 24 h in ambient air, the gelatin–PMMA–glass composites are immersed in acetone baths for 6 h under continuous stirring, and then for 24 h in renewed acetone to allow proper dissolution of the PMMA microspheres. Finally, the glass/gelatin composite scaffolds were immersed in a 1% glutaraldehyde solution for 24 h to allow crosslinking of gelatin.

2.2. Characterization of the composite scaffolds

The morphology of the composite was characterized by Scanning Electron Microscopy (SEM) at an acceleration voltage of 5 kV. The pore and interconnection sizes are estimated by measurements on the SEM pictures with a minimal number of 50 measurements for each parameter. Pore diameters and interconnections were extracted from SEM pictures thanks to the Image J software. This method of measurement is here preferable to traditional mercury intrusion porosimetry which is limited to the characterization of pores under 250 μm [13]. Total porosity of the scaffolds was deduced from apparent density of weighed cylindrical scaffolds and helium pycnometry measurement (1.5 g/cm^3 scaffold skeletal density).

The mechanical behavior of the composite scaffolds was tested in compression using a TA Instruments 2980 Dynamic Mechanical Analyser. Scaffolds of 5.8 mm diameter and 5 mm height were compressed at a fixed rate of 1 N/min until 18 N. Scaffolds were tested both in the dry and in the wet state. Here scaffolds in the wet state correspond to scaffolds that were immersed during 1 h in Simulated Body Fluids (SBF) at 37 °C prior to testing, then removed from the fluids and immediately tested in compression.

2.3. In vitro apatite forming ability in SBF

The composite scaffolds were immersed in SBF, a fluid that mimics the ionic composition of blood plasma, for periods between 1 h and 5 days. Immersion in SBF is the most widely used test for evaluating the *in vitro* bioactivity (apatite-forming ability) of a material [14]. The SBF was prepared in two separate solutions (one of them contained all salts with the exception of $CaCl_2$ which was into the second solution) [15]. The pH was buffered with Tris(hydroxymethyl)amino-methane. The solutions were filtered and mixed just before the beginning of the test. Silicon, calcium and phosphorus concentrations in the SBF were measured by Inductively Coupled Plasma–Atomic Emission Spectroscopy (ICP–AES). On the other hand, after reaction with SBF the composites were removed from the fluids, washed with ethanol and embedded into resin (AGAR, Essex). Cross-sections of 20 μm thickness were cut with a microtome. Elemental microanalysis of the sections was carried out using the micro-PIXE (Particle Induced X-ray Emission) technique, which is very similar to SEM-EDS in its principle, but has the advantage of being much more sensitive due to the use of ions rather than electrons as incident particles. Micro-PIXE was performed using a 3 MeV proton beam of 1 μm diameter at the AIFIRA nanobeam line, CENBG, France. Key features of the PIXE technique for the study of biomaterials and in particular scaffolds for bone tissue engineering have already been described [16].

3. Results and discussion

The obtained macroporous scaffolds which skeleton consists of bioactive glass powder dispersed into gelatin are presented in Fig. 1. Different shapes can easily be obtained using appropriate molds. SEM pictures of the scaffold (Fig. 2) show that highly interconnected macroporous structures are obtained. The total porosity is calculated as 91 ± 1%. The average pore size is of 187 ± 6 μm, with pores ranging from 105 to 295 μm, and the average interconnection diameter is of 74 ± 4 μm, with diameters ranging from 25 to 115 μm. Fig. 3a summarizes the distributions of pore sizes and interconnections inside composite scaffolds. Fig. 3b demonstrates

Fig. 1. Optical picture of gelatin-bioactive glass composite scaffolds with different shapes.