Gelatin-bioactive glass composites scaffolds with controlled macroporosity for bone regeneration

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Abstract

This communication reports a process for the synthesis of bioactive glass – gelatin composites with controlled porosity. Very promising mechanical properties and in vitro bioactivity were observed, showing that this material is of high interest for the design of implants for bone regeneration.

Keywords

Scaffolds, bone regeneration, composites, bioactive glass, gelatin

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 dispersed in organic fibres 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 into surrounding bone tissue. A method that allows synthesis of 3D macroporous implants with pores of few hundreds of micrometers and interconnections5 has to be used. Moreover, the high variety of possible applications for such materials, used in different sites of the body, requires a process that allows the tailoring of porosity and shape of the implant. There are already examples of associations of gelatin and bioceramics in 3D macroporous composite implants (Hydroxyapatite [6], β-Tricalcium phosphate [7] and bioactive glasses [8]) into promising composites for bone regeneration. However, the synthesis of gelatin – bioactive glass composites is generally made by freeze-drying process [9, 10] and leads to limited and uncontrolled pore sizes with irregular pore shapes.

This communication reports a process based on a stack of polymeric beads as porogen agent [11], a technique that allows a better control of the porosity for synthesis of bioactive glass – gelatin porous composites. In this process, a judicious association of raw materials and solvents is proposed with PMMA (PolyMethylMethAcrylate) beads for the porogen and acetone for the solvent. Indeed,

acetone is a solvent of PMMA and a non-solvent of gelatin, it has the advantage of being an easy to use solvent and besides, it will not deteriorate the bioactive glass particles. Moreover, the choice of PMMA for the porogen is not innocent because it 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

In the first step of the process, bioactive glass powder with a composition of 75% SiO_2 – 25% CaO (in weight percent) was made with a commonly used sol-gel method. Briefly 13.48 mL of water and 13.48 mL of ethanol were mixed with 2.25 mL of hydrochloric acid (HCl at 2N in water before hydrolysis). After 5 minutes of stirring, 13.94 mL of TEOS (TetraEthyl OrthoSilicate: $Si(OCH_2CH_3)_4$) were added followed by 5.2637 g of calcium nitrate ($Ca(NO_3)_2.4H_2O$) after 30 minutes of stirring. The mixture was left under stirring for 1 hour and then the sol was poured into PTFE containers for drying during 24 hours at 60°C and for other 24 hours at 125°C. Calcination at 700°C (reached after 2 hours of heating from ambient temperature) allowed elimination of the nitrates and incorporation of calcium into the glass network.

In the second step, the glass powder was grinded and sieved with a 50 µm sieve. 0.025 g of the obtained powder was then mixed with 0.2 g of PMMA beads of 200-400 µm. A gelatin solution was made by dissolution of gelatin powder (porcine type A) 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 blend of glass and beads and the mixture was compacted into an open mold to allow drying of gelatin. The compacting stage was necessary for improving 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 viscosity of the solution, when gelatin is mixed with the bioactive glass particles. After 24 hours in ambient air, the gelatin-beads-powder composite was immersed into acetone for 6 hours under stirring, and then for 24 hours in renewed acetone to eliminate the PMMA beads.

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. The mechanical behavior of the composite scaffold was tested in compression (Dynamic Mechanical Analyser 2980, an increasing stress was applied at 1 N/min until 18 N)

3. Results

The obtained macroporous scaffolds with walls made of bioactive glass powder dispersed into gelatin are presented in Figure 1. Different shapes can be obtained with appropriate molds. SEM pictures of the scaffold (Figure 2) show a highly macroporous structure with interconnections. 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. The total porosity is of 91 ± 1%. The obtained porosity seem to be suitable for tissue engineering applications but if necessary, it would be really simple to tailor the porosity by tailoring the PMMA beads distribution. By using beads with increased diameters, larger pores and interconnections will be obtained.

The mechanical behavior was compared with a scaffold made through the sol-gel foaming process which synthesis has already been described [12, 13]. This bioactive glass foam has an average pore size of 185 ± 10 µm, with pores ranging from 74 until 475 µm. The average interconnection diameter is 63 ± 6 µm, with values ranging from 23 until 111 µm and the total porosity is of 88 ± 3%, that is to say similar to the one of the composite scaffold. If the pores and interconnections of the two scaffolds have same average diameters, the synthesis with the use of calibrated PMMA beads, compared with the sol-gel foaming process, allows a narrower distribution of pore diameters and avoids the biggest pores that could have a negative impact on the mechanical properties. The resulting curves of the mechanical testing are shown in Figure 3. A higher compressive strength is observed for the composite scaffold while the glass foam presents a step-by-step cracking. Moreover, the composite can be deformed without breaking; this property would be very useful for implantation of the composite which can be more easily manipulated than pure bioactive glass implants.

To assess its in vitro bioactivity, the composite implant was treated in a glutaraldehyde solution at 1% in water for 24 hours before being immersed for 5 hours and 5 days in Simulated Body Fluid (SBF), a fluid that mimics the ionic composition of blood plasma which is often used as a preliminary test for estimating the bioactivity of a material [14]. The SBF was prepared in two separated 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 into the SBF were measured by Inductively Coupled Plasma – Atomic Emission Spectroscopy (Table 1). SBF evolutions exhibit continuous increase in silicon concentration and an increase of the calcium concentration from 0 until 5 hours of interaction with the composite implant. Those results demonstrate that the glass dissolution process happens inside the composite walls and this is very important because silicon and calcium release is highly desirable as those elements have positive effects on bone regeneration [16]. Moreover, the decrease of calcium from 5 hours until 5 days and the continuous decrease of phosphorus suggest precipitation of calcium phosphates, which is the extension of the bioactive process [17].

In order to confirm that the calcium and phosphorus are really precipitating together at the surface of the material, an implant that was immersed 5 days into SBF was washed with ethanol and embedded into resin (AGAR, Essex). Sections of 20 µm thick were cut with a microtome and micro-PIXE (Particle Induced X-ray Emission spectroscopy) analysis was performed with a 3 MeV proton beam of 1 µm diameter at the CENBG on the nanobeam line of the AIFIRA platform. The procedure and the interest for the study of macroporous implants with the PIXE method has already been described [18]. For comparison, a piece of macroporous implant that was not immersed into SBF (0 day) was also prepared for PIXE analysis.

The elemental maps obtained for the un-reacted sample (Figure 4) show the particles of bioactive glass inside the walls of the macroporous composite scaffold. The organic part (gelatin) is not visible as it is composed of light elements that are on one hand difficult to detect because of few X-rays emissions (for S) and on the other hand not detectable due to the thickness of the Be window mounted on the X-rays detector (for O and C). After 5 days of immersion into SBF (Figure 5), elemental maps exhibit the presence of calcium phosphate precipitates deposited onto the composite implant. Concentration measurements were made on the CaP layer and gave a

composition of 37 ± 2% Ca – 14 ± 1% P – 6 ± 1% Si – 0.3 ± 0.03% Mg (in weight %). Those values are close to the values obtained by Lao et al. [19] on pure bioactive glass at the same time of immersion and suggest that the in vitro bioactivity was not deteriorated by the addition of gelatin.

4. Conclusion

This communication reports a novel synthesis of bioactive glass – gelatin scaffolds with controlled macroporous structure. Indeed, the pore size distribution can be defined by the proper choice of PMMA beads distribution. The association PMMA – gelatin- acetone allows the elimination of the template without risk of degradation for the biopolymer. The composite porous implant presents improved mechanical properties compared to a bioactive glass foam. The in vitro bioactivity test indicates that the glass dissolution occurs, releasing stimulatory ions while a precipitation of a calcium phosphate layer on the implant was evidenced by micro-PIXE. Moreover, the evolution of the calcium phosphate layer seems to be close to the one observed for bioactive glasses showing no decrease of the bioactivity.

Further investigations are in progress to better understand the reactivity of those materials that already seem to be very promising for bone healing applications.

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Table 1: Evolution of silicon, calcium and phosphorus concentrations into SBF during immersion of the composite porous implant for 0 day, 5 hours and 5 days.