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Biodegradable borosilicate bioactive glass scaffolds with a trabecular microstructure for bone repair

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ABSTRACT

Three-dimensional porous scaffolds of a borosilicate bioactive glass (designated 13-93B1), with the composition $6Na_2O–8K_2O–8MgO–22CaO–18B_2O_3–36SiO_2–2P_2O_5$ (mol%), were prepared using a foam replication technique and evaluated in vitro and in vivo. Immersion of the scaffolds for 30 days in a simulated body fluid in vitro resulted in partial conversion of the glass to a porous hydroxyapatite composed of fine needle-like particles. The capacity of the scaffolds to support bone formation in vivo was evaluated in non-critical sized defects created in the femoral head of rabbits. Eight weeks post-implantation, the scaffolds were partially converted to hydroxyapatite, and they were well integrated with newly-formed bone. When loaded with platelet-rich plasma (PRP), the scaffolds supported bone regeneration in segmental defects in the diaphysis of rabbit radii. The results indicate that these 13-93B1 scaffolds, loaded with PRP or without PRP, are beneficial for bone repair due to their biocompatibility, conversion to hydroxyapatite, and in vivo bone regenerative properties.

1. Introduction

Large bone defects resulting from trauma, resection for tumors, and congenital diseases are a common clinical problem. Whereas microdefects in bone can heal over time, large defects are difficult to repair without external intervention such as bone grafting. At present, bone autograft, bone allograft, and prosthetic implants are widely used to repair large bone defects, but they suffer from limitations. Autologous bone grafts suffer from problems such as limited availability and donor site morbidity, whereas bone allografts and prosthetic implants show uncertain healing to bone, unpredictable long-term durability, and also suffer from high costs. Porous synthetic scaffolds that mimic bone would be ideal bone substitutes, but such porous scaffolds should have the capacity to support tissue ingrowth and integration with host bone and surrounding soft tissues, and they should degrade at a rate compatible with new bone formation.

Bioactive glasses have several attractive properties as a scaffold material in bone repair. Apart from being biocompatible, bioactive glasses degrade and convert to hydroxyapatite (HA) in vivo, which promotes osseous healing [1]. Calcium ions and soluble silicon released during the conversion of silicate bioactive glass such as 45S5 further promote osteogenesis [2,3]. Bioactive glasses are also amenable to fabrication into porous three-dimensional (3D) architectures that are capable of supporting tissue ingrowth and integration.

Our previous work has shown that by replacing varying amounts of SiO_2 in silicate 45S5 or 13-93 glass ($6Na_2O–8K_2O–8MgO–22CaO–54SiO_2–2P_2O_5$; mol%) with B_2O_3, borosilicate and borate bioactive glasses with a controllable degradation rate can be produced [4,5]. In particular, the degradation rate of the glass and its conversion to HA increase with the replacement of higher amounts of SiO_2 in silicate bioactive glass with B_2O_3. A borate glass (designated 13-93B3) with the composition $6Na_2O–8K_2O–8MgO–22CaO–54B_2O_3–2P_2O_5$ (mol%), obtained by replacing all the SiO_2 in 13-93 glass with B_2O_3, was shown to convert completely to HA when immersed in an aqueous phosphate solution, at a rate that was ~3–4 times faster than 13-93 bioactive glass [4,5]. However, the rapid degradation in strength which results from the conversion process limits the application of borate 13-93B3 scaffolds to the repair of non-loaded bone defects. Borate 13-93B3 glass has been successfully used as a drug delivery system for vancomycin [6,7] and teicoplanin [8,9] in the treatment of osteomyelitis in a rabbit tibial model. In addition, bone regeneration was observed in the sites implanted with the borate glass carrier but not with a carrier composed of commercial calcium sulfate beads [10].

A borosilicate glass (designated 13-93B1) with the composition $6Na_2O–8K_2O–8MgO–22CaO–18B_2O_3–36SiO_2–2P_2O_5$ (mol%), obtained by replacing one-third of the molar concentration of SiO_2 in 13-93 glass with B_2O_3, was observed to degrade and convert faster to HA

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than silicate 13-93 glass but slower than borate 13-93B3 [4,5]. Consequently, scaffolds of 13-93B1 glass could retain a greater fraction of their strength over a longer period of time in an aqueous phosphate solution when compared to borate 13-93B3 scaffolds. Borosilicate 13-93B1 glass also showed the capacity to support the proliferation and function of osteogenic MLO-A5 cells in vitro [11]. When implanted subcutaneously in the dorsum of rats for 6 weeks, porous 13-93B1 scaffolds showed the capacity to support tissue infiltration into the interior pores [11].

Based on the promising in vitro and in vivo results described above, this study was undertaken to evaluate the capacity of 13-93B1 glass scaffolds to repair bone defects in vivo. Scaffolds with a "trabecular" microstructure, similar to that of dry human trabecular bone, were prepared using a foam replication technique. The conversion of the glass to HA was studied in simulated body fluid (SBF) in vitro. The capacity of the scaffolds to support bone regeneration in vivo was evaluated using a rabbit femoral defect model. Since growth factors are often required to stimulate bone formation [12], the capacity of the scaffolds to serve as a carrier for platelet-rich plasma (PRP) was also evaluated in segmental defects created in the diaphysis of rabbit radii.

2. Materials and methods

2.1. Preparation of borosilicate (13-93B1) bioactive glass scaffolds

Scaffolds of borosilicate (13-93B1) bioactive glass scaffolds were prepared using a polymer foam replication technique, as described previously [13]. This technique was used because of its ability to produce scaffolds with a microstructure similar to dry human trabecular bone. Briefly, glass frits with the composition $6Na_2O$–$8K_2O$–$8MgO$–$22CaO$–$18B_2O_3$–$36SiO_2$–$2P_2O_5$ (mol%) were prepared by melting the required quantities of Na_2CO_3, K_2CO_3, $MgCO_3$, $CaCO_3$, H_3BO_3, SiO_2, and $NaH_2PO_4 \cdot 2H_2O$ (analytical grade; Sinopharm Chemical Reagent Co., Ltd., China) in a platinum crucible for 30 min at 1150 °C in air, and quenching the melt in cold water. Glass particles of size <50 μm were obtained by crushing the glass frits with a hardened steel mortar and pestle and sieving through a stainless steel sieve.

A slurry for the polymer foam infiltration step was prepared by dispersing the 13-93B1 particles in ethanol using ethyl-cellulose (EC; analytical grade, Sinopharm Chemical Reagent Co., Ltd., China) as a dispersant and binder. Cylindrical samples (5 mm in diameter × 15 mm) of a polyurethane foam (Shanghai No. 6 Plastic Co., Ltd., China), with open porosity of ~50 pores per inch, were immersed in the slurry to coat them with a layer of glass particles. After drying overnight at room temperature, the coated foams were heated in air for 1 h at 500 °C (heating rate = 1 °C/min) to burn off the foam and polymeric additives in the glass coating, and then for 2 h at 560 °C (heating rate = 5 °C/min) to sinter the glass particles into a dense network.

2.2. Characterization of 13-93B1 scaffolds

The as-fabricated scaffolds were coated with Au/Pd and their microstructure was examined in a field-emission scanning electron microscope, SEM (Quanta 200 FEG; FEI Co., The Netherlands) at an accelerating voltage of 10 kV and a working distance of 7.5 mm. The porosity in the scaffold was measured using the Archimedes method, while the size distribution of the open pores was measured using a liquid extrusion porosimeter (LEP-1100 AX, Porous Materials Inc., NY) with water as the wetting liquid. According to the manufacturer's instruction, the distribution of pore size is defined as [−(dV / d log D)], where V is the pore volume in 1 g porous scaffold and D is the pore diameter. The compressive strength of cylindrical samples (6 mm in diameter × 12 mm) was measured using an Instron testing machine (Model 4881; Instron Co., Norwood, MA) at a crosshead speed of 0.5 mm/min. Five samples were tested, and the strength was expressed as a mean ± standard deviation.

2.3. Evaluation of the bioactivity of 13-93B1 glass in vitro

The bioactivity of the 13-93B1 glass was assessed in vitro from the conversion of as-fabricated scaffolds and disks (10 mm in diameter × 2 mm) in simulated body fluid (SBF) at 37 °C. One gram of glass was immersed in 100 ml SBF, as described in previous studies [5,14,15], and immersion times of up to 30 days were used. At selected times, the scaffolds and disks were removed from the SBF, washed twice with deionized water and then twice with ethanol, and dried at 90 °C. The weight loss of the scaffolds and disks was determined as $\Delta W = (W_o - W_t) / W_o$, where W_o is the initial mass of the sample, and W_t is the mass after immersion for time t in the SBF. Four replicates were used for each time point, and the weight loss was determined as a mean ± standard deviation (SD). After the samples were removed at each time point, the SBF was cooled to room temperature and its pH was measured.

The crystalline phase formed on the surface of the glass disks by conversion in SBF was analyzed using thin-film XRD (X'Pert Pro; PANalytical, Almelo, The Netherlands) using Ca Kα1 radiation ($\lambda = 0.15405$ nm; incident radiation = 45 kV) at a scanning rate of 0.03°/min in the 2θ range 10–80°C. The surface morphology and microstructure of the scaffolds after immersion in SBF were examined in a field-emission SEM (Quanta 200 FEG) using the conditions described earlier.

2.4. Evaluation of scaffolds in rabbit femoral head defect model

Three male New Zealand white (NZW) rabbits weighing 2.5–3.0 kg were used in the experiments. The animals were obtained from the Shanghai Laboratory Animal Center (Shanghai, China, Certificate number SCXK: 2002-0010). Animal care and surgical procedures were in accordance with guidelines issued by the Department of Science and Technology of China in 2006 (Guidance Suggestions from the Act for Care and Use of Laboratory Animals).

Under intramuscular anesthesia (3% pentobarbital sodium; 30 mg per kg mass of rabbit), lateral approaches were performed in both shaved front knees to expose the distal femoral diaphysis. One defect (5 mm diameter × 5 mm deep) was created in each of those femoral heads using a medium speed burr under constant irrigation with sterile saline. The defects were implanted with the scaffolds or left unfilled (control), and the wounds were sutured. At 4 and 8 weeks post-implantation, the femoral heads with the implants or unfilled defects were harvested and fixed in 10% neutral formalin–saline solution. The samples were sectioned in the center to expose the cross section of the implants, and decalcified in 10% formic acid. After the samples were dehydrated in a graded series of alcohol, rinsed in xylene, and embedded in paraffin, 5 μm thick sections were cut and stained with hematoxylin and eosin (H&E). The stained sections were examined in an optical microscope (AX80T, Olympus, Japan).

2.5. Evaluation of scaffolds in critical-sized segmental defects in rabbit radii

Two groups of 13-93B1 scaffolds were evaluated in critical-sized segmental defects created in the diaphysis of rabbit radii: (1) as-fabricated 13-93B1 scaffolds and (2) as-fabricated 13-93B1 scaffolds loaded with platelet-rich plasma (PRP). The unfilled defects served as the negative control group.

Loading the scaffolds with PRP was performed as follows. Rabbits to be implanted with the PRP-loaded scaffolds were anesthetized by intravenously injecting 25 g/l pentobarbital sodium at a dose of 1 ml per kg body weight. Then 5 ml of blood was aspirated from the central arteries of the rabbit ears. The platelets in the blood were enriched by a two-step centrifugation process. First, the erythrocytes were removed at 8.40 N (centrifugal force at 2500 rev/min; 10 min) in a centrifuge (Heraeus Labofuge 300, Kendro Lab Products, Langenselbold, Germany) at 20 °C. Second, the leukocytes were sedimented at 13.10 N (centrifugal