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Long-term bone regeneration, mineralization and angiogenesis in rat calvarial defects implanted with strong porous bioactive glass (13–93) scaffolds☆

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ABSTRACT

There is growing interest in the use of bioactive glass scaffolds for repairing structural bone defects but data on the capacity of the scaffolds to regenerate bone in vivo, particularly over a long-term duration, are limited. In this study, bone regeneration in rat calvarial defects implanted with strong porous scaffolds of silicate 13–93 glass (porosity = 47 ± 1%) was investigated at 12 and 24 weeks post-implantation and compared with previous results from a similar study at 6 weeks. Three groups of implants, composed of as-fabricated scaffolds, scaffolds pretreated in a phosphate solution to convert a thin surface layer (5 μm) to hydroxyapatite (HA) and pretreated scaffolds loaded with bone morphogenetic protein-2 (BMP2) (1 μg/defect) were used. Bone regeneration, bioactive glass conversion to HA and blood vessel formation in the defects implanted with the three groups of scaffolds were evaluated using histology, histomorphometric analysis and scanning electron microscopy. When compared to the as-fabricated scaffolds, the pretreated scaffolds enhanced bone regeneration at 6 weeks but not at 12 or 24 weeks. In comparison, the BMP2-loaded scaffolds showed a significantly better capacity to regenerate bone at all three implantation times and they were almost completely infiltrated with lamellar bone within 12 weeks. The amount of glass conversion to HA at 24 weeks (30–33%) was not significantly different among the three groups of scaffolds. The area and number of blood vessels in the new bone that infiltrated the BMP2-loaded scaffolds at 6 and 12 weeks postimplantation were significantly greater than those for the as-fabricated and pretreated scaffolds. However, there was no significant difference in blood vessel area and number among the three groups of scaffolds at 24 weeks. The results indicate that these strong porous bioactive glass (13–93) scaffolds loaded with BMP2 are promising candidate implants for structural bone repair.

1. Introduction

There is growing interest in the development of bioactive glass scaffolds for healing large (critical size) bone defects, particularly defects in structural bone. The attractive properties of bioactive glasses as a scaffold material for bone repair, such as their ability to stimulate osteogenesis, convert to hydroxyapatite (HA) (the mineral constituent of bone) and bond strongly to bone, have been well documented for 30–40 years [1–4]. However, most previous studies have targeted bioactive glass in the form of particles, granules or low strength porous scaffolds that have inadequate mechanical properties for structural bone repair [5]. Furthermore, while several investigations have been performed to study the capacity of bioactive glass scaffolds to regenerate bone in osseous defects in vivo, the available data covering longer-term implantation times, such as times longer than 6–12 weeks, are limited.

Recent studies have shown the ability to create strong porous scaffolds of silicate 13–93 or 6P53B bioactive glass by robotic deposition techniques such as freeze extrusion fabrication [6] and robocasting [7,8]. Scaffolds with a grid-like microstructure (porosity ~50%; pore width ~300 μm) showed compressive strengths (~140 MPa) that were comparable to human cortical bone (100–150 MPa) [5]. Strong porous scaffolds of 13–93 glass created by robocasting also showed excellent fatigue resistance in vitro under compressive stresses that were higher than normal physiologic stresses on the femur of humans [9].

When implanted for 6 weeks in rat calvarial defects (4.6 mm in diameter), strong porous scaffolds of 13–93 bioactive glass were infiltrated with new bone and they integrated with host bone [10]. Pretreating the as-fabricated scaffolds in an aqueous phosphate solution to convert a thin surface layer (~5 μm) of the glass to HA prior to implantation

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significantly improved their capacity to regenerate bone. Loading the pretreated scaffolds with bone morphogenetic protein-2 (BMP2) (1 μg/defect) prior to implantation further enhanced their capacity to regenerate bone. Approximately 65% of the pore volume of the BMP2-loaded scaffolds was infiltrated with new bone at 6 weeks.

More recent studies have shown promising results for the use of strong porous 13–93 bioactive glass scaffolds in healing structural bone defects in small animals [11]. When implanted for 12 weeks in rat femoral segmental defects (6 mm long) using intramedullary pin fixation, the ends of the 13–93 scaffolds were infiltrated with new bone, resulting in integration. The percent new bone in the defects implanted with the 13–93 scaffolds (25%) was not significantly different from that in the defects implanted with autografts (38%) ($n = 6$; $p < 0.05$). In another study, cylindrical scaffolds of 13–93 glass (porosity = 55–67%; compressive strength = 40 MPa) were prepared by selective laser sintering and evaluated in rat femoral segmental defects (5 mm long) [12]. The scaffolds contained drill holes in the sides of the cylinder that were either filled with dicalcium phosphate dihydrate (DCPD) used as a carrier for BMP2 (10 μg/defect) or left unfilled (control group). X-ray radiography and micro-computed tomography (micro-CT) showed the formation of bridging calluses around both groups of implants but faster healing and better callus formation were found for the BMP2-loaded scaffolds.

In view of the capacity of strong porous bioactive glass (13–93) scaffolds to regenerate bone in rat calvarial defects at 6 weeks and to heal segmental bone defects in rodents at 12 weeks, this study was undertaken to evaluate longer term bone regeneration in osseous defects implanted with those strong porous 13–93 scaffolds. Implantation times longer than 6–12 weeks are necessary to better evaluate the capacity of the scaffolds to heal the defect, maintain healthy bone growth and convert to HA. Two groups of scaffolds, pretreated for three days in an aqueous phosphate solution or loaded with BMP2, were implanted in rat calvarial defects for 12 and 24 weeks. The as-fabricated scaffolds (no pretreatment or BMP2) were used as the control group. Bone regeneration, glass conversion to HA and blood vessel formation in the defects implanted with the scaffolds were evaluated using histology, histomorphometric analysis and scanning electron microscopy. The results were compared with those obtained in a previous study for similar scaffolds implanted for 6 weeks in the same animal model [10].

2. Materials and methods

2.1. Preparation of scaffolds

Scaffolds with a grid-like microstructure were prepared using a robotic deposition (robocasting) method, as described in detail elsewhere [9,10]. Briefly, the as-received 13–93 glass (Mo-Sci Corp., Rolla, MO) was ground to form particles (~1 μm), mixed with a 20 wt.% Pluronic-127 binder solution to form a paste (40 vol.% glass particles) and extruded using a robocasting machine (RoboCAD 3.0; 3-D Inks, Stillwater, OK). After drying at room temperature, the scaffolds were heated in O_2 at a rate of 0.5 °C/min to 600 °C to burn out the processing additives, and sintered for 1 h at 700 °C (heating rate = 5 °C/min) to densify the glass filaments. The as-fabricated scaffolds were sectioned and ground to form thin discs (4.6 mm in diameter × 1.5 mm), washed twice with deionized water and twice with anhydrous ethanol, dried in air and sterilized by heating for 12 h at 250 °C. For reference, images of the as-fabricated scaffolds, taken from Ref. [10], are shown in Fig. 1.

Twenty-eight of the as-fabricated scaffolds were reacted for 3 days in an aqueous phosphate solution (0.25 M K_2HPO_4 solution) at 60 °C and a starting pH of 12.0 to convert a thin surface layer of the glass to HA (or amorphous calcium phosphate, ACP), as described previously [10]. The mass of the glass scaffolds to the volume of the K_2HPO_4 solution was kept constant at 1 g per 200 ml and the solution was stirred gently each day. Fourteen of the pretreated scaffolds were loaded with BMP2 prior to implantation using a procedure described previously [10]. Briefly, a solution of BMP2 (Shenandoah Biotechnology Inc., PA, USA) in citric acid was prepared by dissolving 10 μg of BMP2 in 100 μl sterile citric acid (pH = 3.0). Then 10 μl of the BMP2 solution was pipetted on to each bioactive glass scaffold. The BMP2 solution was completely absorbed within the converted surface layer of the scaffolds and there was no visible evidence for any of the solution flowing out of the scaffolds. After loading with BMP2, the scaffolds were kept for ~24 h in a refrigerator at 4 °C to dry them prior to implantation. The release profile of the BMP2 from the scaffolds into a solution composed of equal volumes of fetal bovine serum (FBS) and phosphate-buffered saline (PBS) plus 1 vol.% penicillin was measured previously as a function of time in vitro [10].

2.2. Animals and surgical procedure

All animal experimental procedures were approved by the Animal Care and Use Committee, Missouri University of Science and Technology, in compliance with the NIH Guide for Care and Use of Laboratory Animals (1985). The three groups of scaffolds (described as as-fabricated, pretreated and BMP2-loaded) were implanted in rat calvarial defects for 12 and 24 weeks. Seven scaffolds from each group were implanted for each implantation time. This sample size ($n = 7$) was selected on the basis of the results of a previous study for an implantation time of 6 weeks [10] and a power analysis. The implants were assigned randomly to the defects, but scaffolds with and without BMP2 were not mixed in the same animal.

Twenty-two male Sprague–Dawley rats (3 months old; weight = 350–400 g, Harlan Laboratories Inc., USA) were maintained in the animal facility for 2 weeks to become acclimated to diet, water and housing. The rats were anesthetized with a combination of ketamine (72 mg/kg) and xylazine (6 mg/kg) and maintained under anesthesia with ether gas in oxygen. The surgical site was shaved, scrubbed with iodine and draped.

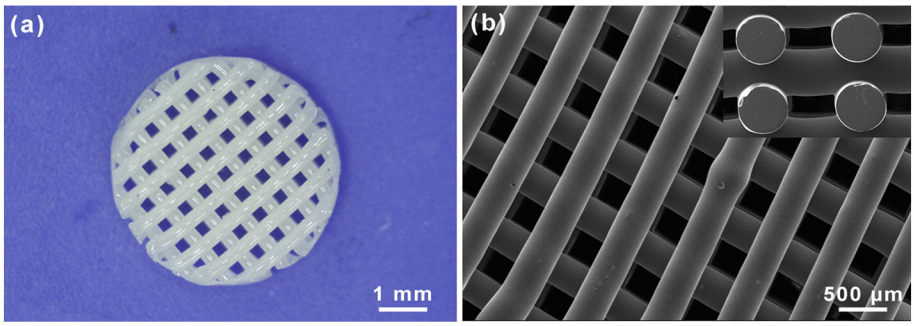

Fig. 1. (a) Optical image of 13–93 bioactive glass scaffold prepared by robocasting for implantation in rat calvarial defects. (b) Higher-magnification SEM image of the scaffold showing dense glass filaments and porous grid-like architecture in the plane of deposition (xy plane). Inset: SEM image in z direction. The scaffolds had a porosity of 47 ± 1%, a pore width of 300 ± 10 μm in the xy plane and 150 ± 10 μm in z direction (from Ref. [10]).

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