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Healing of critical-size segmental defects in rat femora using strong porous bioactive glass scaffolds

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ABSTRACT

The repair of structural bone defects such as segmental defects in the long bones of the limbs is a challenging clinical problem. In this study, the capacity of silicate (13-93) and borate (13-93B3) bioactive glass scaffolds (porosity = 47–50%) to heal critical-size segmental defects in rat femurs was evaluated and compared with autografts. Defects were implanted with 13-93 and 13-93B3 scaffolds with a grid-like microstructure (compressive strength = 86 MPa and 40 MPa, respectively), 13-93B3 scaffolds with an oriented microstructure (compressive strength = 32 MPa) and autografts using intramedullary fixation. Twelve weeks post-implantation, the defects were harvested and evaluated using histomorphometric analysis. The percentage of new bone in the defects implanted with the three groups of glass scaffolds (25–28%) and the total von Kossa-positive area (32–38%) were not significantly different from the autografts (new bone = 38%; von Kossa-positive area = 40%) ($p > 0.05$). New blood vessel area in the defects implanted with the glass scaffolds (4–8%) and the autografts (5%) showed no significant difference among the four groups. New cartilage formed in the 13-93 grid-like scaffolds (18%) was significantly higher than in 13-93B3 grid-like scaffolds (8%) and in the autografts (8%) ($p = 0.02$). The results indicate that these strong porous bioactive glass scaffolds are promising synthetic implants for structural bone repair.

1. Introduction

Bone defects are a common occurrence in orthopedic practice, resulting from trauma, malignancy, infection and congenital disease. Clinically, these defects can be reconstructed through the use of various bone grafts. While contained bone defects can be repaired using autografts, allografts and biocompatible synthetic materials [1,2], the reconstitution of structural bone loss, such as segmental defects in the long bones of the limbs, is challenging. Current treatments for repairing structural bone loss suffer from limitations. Autografts are the gold standard for treatment while allografts are the most widely used bone graft but they suffer from limited tissue availability, increased surgery time, donor site morbidity (autografts), high cost, uncertain healing to bone, increased risk of disease transference and undesirable host immune reactions (allografts) [3,4]. Porous metals cannot be readily shaped to fit the anatomy of the patient, are bioinert, and can serve as a surface for long-term bacterial infection. Because of these disadvantages, the need for synthetic bone grafts continues to increase.

Synthetic bone grafts could become the ideal bone graft provided that they can replicate the structure and function of bone and have the requisite mechanical properties for reliable long-term load bearing. Reconstructive bone grafts should be biocompatible, osteoconductive and osteoinductive, and they should have a three-dimensional (3D) microstructure capable of supporting new bone ingrowth and angiogenesis to sustain and augment new bone growth [5,6]. As fabricated, synthetic grafts should have the ability to support the physiologic stresses of the bone to be replaced [7]. They should also have the ability to resorb or to convert to a hydroxy-carbonate-apatite (the mineral constituent of bone), referred to simply as hydroxyapatite (HA), at a rate that is comparable with the rate of new bone growth [8]. As the scaffold resorbs or converts to HA, the reduction in its strength should be compensated by an increase in strength due to new bone formation within the defect.

Bioactive glass has attractive properties as a scaffold material for bone repair, such as its ability to react with the body fluid and convert to HA which promotes osseous healing [9–11]. Calcium ions and soluble silicon released during the conversion of silicate 45S5 glass have been shown to promote osteogenesis and activate gene expression [9]. Bioactive glass can be doped with trace amounts of elements such as Sr, Zn and Cu that are known to stimulate osteogenesis and angiogenesis [12,13]. As the glass degrades and converts to HA in vivo, those elements are released at therapeutically acceptable rates.

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Most previous studies have targeted silicate bioactive glass compositions (such as 45S5 and 13-93) in the form of particulate fillers and implants for repairing small contained defects [11]. Recent years have seen the development of new bioactive glasses such as borate compositions [10,14]. A borate glass designated 13-93B3, obtained by replacing all the SiO_2 in 13-93 glass with B_2O_3, converts faster to HA than silicate 13-93 glass [10]. Scaffolds of 13-93B3 glass with a fibrous microstructure formed by thermally bonding short glass fibers, have also shown a greater capacity to regenerate bone in rat calvarial defects when compared to 13-93 scaffolds with a similar microstructure [15]. There has also been a heightened interest in advancing the use of bioactive glasses to the challenging case of healing structural bone loss [16]. Scaffolds of 13-93 and 13-93B3 glass, created using techniques such as unidirectional freezing of suspensions and solid freeform fabrication have shown compressive strengths that are much higher than scaffolds created by more conventional techniques. Scaffolds of 13-93 glass with a grid-like microstructure (porosity ~50%) created by robocasting showed compressive strengths comparable to cortical bone [17,18] while 13-93B3 scaffolds with a similar microstructure showed strengths that were several times the highest strength reported for trabecular bone [19].

In view of the ability to create strong porous bioactive glass scaffolds and the capacity of those scaffolds to heal non-loaded osseous defects [20,21], this study was undertaken to assess the capacity of 13-93 and 13-93B3 scaffolds to reconstitute loaded bone defects in a small animal model. Scaffolds of 13-93 and 13-93B3 glass with a grid-like microstructure created by robotic deposition and scaffolds of 13-93B3 glass with an oriented microstructure created by unidirectional freezing of suspensions were implanted in segmental defects in rat femurs. Autologous bone was used as the positive control. Twelve weeks post-implantation, new bone, mineralization, cartilage formation and blood vessel area in the defects were evaluated using histology and histomorphometric analysis.

2. Materials and methods

2.1. Fabrication of bioactive glass scaffolds

The silicate 13-93 and borate 13-93B3 bioactive glasses used in this study were provided by Mo-Sci Corp., Rolla, MO. The composition of the 13-93B3 glass (Table 1) is obtained by replacing all the SiO_2 in 13-93 with B_2O_3. Three groups of scaffolds were used in this study. Scaffolds of 13-93 and 13-93B3 glass with a grid-like microstructure were prepared using a robocasting technique while scaffolds of 13-93B3 glass with an oriented microstructure of columnar pores were prepared by freezing a suspension of glass particles unidirectionally.

The preparation of the 13-93 and 13-93B3 grid-like scaffolds followed the methods described elsewhere [18,19]. Briefly, a mixture of bioactive glass particles and processing additives with the consistency of a paste was loaded into a robocasting machine (3D Inks, Stillwater, OK) and extruded through a nozzle (inner diameter = 330 μm) to form a pre-designed grid-like pattern (center-to-center spacing between filaments = 830 μm). An aqueous-based paste (40 vol.% glass particles of size ~1 μm and 20 wt.% aqueous Pluronic® F-127 solution) was used to form the 13-93 scaffolds. Because of the higher reactivity of the borate glass, an organic-based paste composed of 40 vol.% glass particles (~1 μm), 12.8 vol.% ethyl cellulose, 4.3 vol.% poly(ethylene glycol) (PEG) and 42.9 vol.% ethanol was used to prepare the 13-93B3 scaffolds. After drying, the constructs were heated at a rate of 0.1–1 °C/min up to 500 °C to burn out the processing additives and sintered to convert the glass phase into dense filaments. Sintering was performed for 1 h at 700 °C for the silicate 13-93 scaffolds and for 1 h at 560 °C for the borate 13-93B3 scaffolds (heating rate = 5 °C/min).

Table 1
Composition (in wt.%) of silicate 13-93 and borate 13-93B3 glasses used in this study.

Glass	Na_2O	K_2O	MgO	CaO	SiO_2	B_2O_3	P_2O_5
13-93	6.0	12.0	5.0	20.0	53.0		4.0
13-93B3	6.0	12.0	5.0	20.0		53.0	4.0

The method used to create the 13-93B3 oriented scaffolds was similar to that described previously for 13-93 [22]. A mixture composed of 10 vol.% glass particles (~1 μm), 2 wt.% isostearic acid ($C_{18}H_{36}O_2$; MP Biomedicals LLC; Solon, OH) and camphene ($C_{10}H_{16}$; CAS 5794-04-7; Alfa Aesar, Ward Hill, MA) was ball-milled for 24 h at 55 °C in a closed polypropylene bottle. Then the slurry was poured into a mold placed on a cold stage and frozen unidirectionally at a rate of 7 °C/min. The frozen samples were annealed for 24 h at 34 °C in an incubator and placed in a fume hood at room temperature to sublime the camphene. The porous constructs were heated in flowing O_2 at a rate of 0.1–1 °C/min to 500 °C to burn out the processing additives and sintered in air for 1 h at 565 °C (heating rate = 5 °C/min) to densify the glass phase.

Scaffolds for implantation in rat femoral segmental defects were prepared with a central hole for intramedullary fixation. The hole was drilled in the direction of deposition of the grid-like scaffolds (z direction) and in the direction of freezing (pore orientation direction) for the oriented scaffolds. After thermal treatment to burn out the processing additives (as described above), the constructs were infiltrated with camphene and a hole was drilled in the axial direction using a drill bit of diameter = 5/64 inch. Then the camphene was removed by sublimation at room temperature and the constructs were thermally treated as described above to densify the glass phase. The sintered constructs were sectioned and ground to produce implants with the geometry and dimensions shown in Fig. 1.

2.2. Characterization of scaffolds

Characterization of the scaffolds was performed using the as-fabricated scaffolds without the central hole. The porosity was measured using the Archimedes method according to ASTM C830. Scanning electron microscopy, SEM (S-4700; Hitachi; Tokyo, Japan) was used to examine the microstructure of the scaffolds. The width or diameter of the pores was determined from SEM images of the cross sections using ImageJ software (National Institutes of Health, USA). The scaffolds were ground into a powder (particle size <45 μm) and analyzed using X-ray diffraction (XRD) (X'Pert Pro; PANalytical, Almelo, the Netherlands) and Fourier transform infrared (FTIR) spectroscopy (NEXUS 670 FTIR; Thermo Nicolet, Madison, WI) to check for any crystalline phases and compositional changes due to the fabrication process. XRD was performed using Cu K_α radiation ($\lambda = 0.15406$ nm) at a scan rate of 1.8°/min in the 2θ range 10–80°. In the FTIR analysis, 2 mg of the glass powder was mixed with 198 mg KBr, pressed to form pellets and analyzed in the wavenumber range 400–2000 cm^{-1} at a resolution of 4 cm^{-1}.

2.3. Mechanical testing

The compressive strength and elastic modulus of as-fabricated 13-93 and 13-93B3 scaffolds (6 mm × 6 mm × 6 mm) were measured using an Instron testing machine (model 5881, Norwood, MA). Testing was performed at a cross-head speed of 0.5 mm/min using a 10 kN load cell. The load was applied in the z direction of the grid-like scaffolds, perpendicular to the plane of deposition (xy plane), and in the pore orientation direction of the oriented scaffolds. This loading direction was used because it matched the compressive loading direction of the scaffolds in the bone defects. The elastic modulus was determined from the linear region of the stress vs. strain curve. Prior to testing, the contact surfaces of the scaffolds were ground using a surface grinder (FSG-618; Chevalier Machinery Inc., Santa Fe, NM) to produce parallel surfaces.

The flexural strength of the 13-93 grid-like scaffolds (3 mm × 5 mm × 25 mm) was measured in four-point bending on a fully articulated fixture (outer span = 20 mm; inner span = 10 mm) according to ASTM C1674-11. Testing was performed at a crosshead speed of 0.2 mm/min