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Enhanced bone regeneration in rat calvarial defects implanted with surface-modified and BMP-loaded bioactive glass (13-93) scaffolds

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ABSTRACT

The repair of large bone defects, such as segmental defects in the long bones of the limbs, is a challenging clinical problem. Our recent work has shown the ability to create porous scaffolds of silicate 13-93 bioactive glass by robocasting which have compressive strengths comparable to human cortical bone. The objective of this study was to evaluate the capacity of those strong porous scaffolds with a grid-like microstructure (porosity = 50%; filament width = 330 μm; pore width = 300 μm) to regenerate bone in a rat calvarial defect model. Six weeks post-implantation, the amount of new bone formed within the implants was evaluated using histomorphometric analysis. The amount of new bone formed in implants composed of the as-fabricated scaffolds was 32% of the available pore space (area). Pretreating the as-fabricated scaffolds in an aqueous phosphate solution for 1, 3 and 6 days to convert a surface layer to hydroxyapatite prior to implantation enhanced new bone formation to 46%, 57% and 45%, respectively. New bone formation in scaffolds pretreated for 1, 3 and 6 days and loaded with bone morphogenetic protein-2 (BMP-2) (1 μg per defect) was 65%, 61% and 64%, respectively. The results show that converting a surface layer of the glass to hydroxyapatite or loading the surface-treated scaffolds with BMP-2 can significantly improve the capacity of 13-93 bioactive glass scaffolds to regenerate bone in an osseous defect. Based on their mechanical properties evaluated previously and their capacity to regenerate bone found in this study, these 13-93 bioactive glass scaffolds, pretreated or loaded with BMP-2, are promising in structural bone repair.

1. Introduction

The repair of large bone defects is a challenging clinical problem [1]. While contained bone defects are repairable with commercially available, osteoconductive and osteoinductive filler materials [2,3], there is no ideal biological solution to reconstitute structural bone loss, such as segmental defects in the long bones of the limbs. Available treatments, such as bone allografts, autografts, porous metals and bone cement, have limitations related to costs, availability, longevity, donor site morbidity and uncertain healing to host bone. Consequently, there is a great need for porous biocompatible implants that can replicate the structure and function of bone and have the requisite mechanical properties for reliable long-term cyclical loading during weight bearing.

As described previously [4–6], bioactive glasses have several attractive properties as a scaffold material for bone repair, such as their biocompatibility, their ability to convert in vivo to hydroxyapatite (HA; the mineral constituent of bone) and their ability to bond strongly to hard tissue. Some bioactive glasses, such as the silicate glass designated 45S5, also have the ability to bond to soft tissue [5,6]. Most previous studies have targeted bioactive glass scaffolds with relatively low-strength three-dimensional (3-D) architectures, such as strengths in the range of human trabecular bone (2–12 MPa) [7]. Recent studies have shown that silicate bioactive glass scaffolds (13-93 and 6P53B) created by solid freeform fabrication techniques such as freeze extrusion fabrication [8] and robocasting [9,10] have compressive strengths (~140 MPa) comparable to human cortical bone (100–150 MPa) [7].

Our recent work showed that strong porous bioactive glass (13-93) scaffolds created using robocasting had excellent mechanical reliability (Weibull modulus = 12) and promising fatigue resistance under cyclic stresses far greater than normal physiological stresses [11], but the capacity of those strong porous bioactive glass (13-93) scaffolds to regenerate bone has not yet been studied. Our recent studies also showed that the elastic (brittle) mechanical response of the 13-93 bioactive glass scaffolds in vitro changed to an "elasto-plastic" response after implantation for longer than 2–4 weeks in vivo as a result of soft and hard tissue growth into the pores of the scaffolds [11,12]. However, concerns still remain about the

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low fracture toughness, flexural strength and torsional strength of the as-fabricated bioactive glass scaffolds.

In addition to material composition and microstructure [13], scaffold healing to bone in vivo can be markedly affected by other variables, such as surface composition and structure, the release of osteoinductive growth factors and the presence (or absence) of living cells. Interconnected pores of size ~100 μm are recognized as the minimum requirement for supporting tissue ingrowth [14], but pores of size ~300 μm or larger may be required for enhanced bone ingrowth and capillary formation [15]. Surface modification of macroporous bioactive glass scaffolds has targeted the creation of fine pores (nanometers to a few microns in size) to modify the surface roughness and increase the surface area of the scaffolds [16–18]. Conversion of a surface layer to HA, by reaction in an aqueous phosphate solution, has been shown to improve the capacity of borate and silicate bioactive glass to support cell proliferation and differentiation in vitro [19]. Treatment of B_2O_3-doped silicate bioactive glass scaffolds with a fibrous microstructure in simulated body fluid (SBF) to create a rough surface layer of carbonated HA was shown to improve the capacity of the scaffolds to support cell proliferation in vitro and to enhance bone formation in vivo [20].

Osteoinductive growth factors such as bone morphogenetic protein-2 (BMP-2) and BMP-7 are well known to stimulate bone formation [21,22]. However, the use of porous 3-D bioactive glass scaffolds as delivery devices for growth factors has so far received little attention. In a recent study [23], the surfaces of three silicate bioactive glasses were functionalized by a silanization technique using 3-amino-propyl-triethoxysilane; then BMP-2 was immobilized on the glass surfaces. However, the release of the BMP-2 and the effect of the BMP-2 on bone regeneration in vivo were not studied.

Particles of 13-93 glass have a lower tendency to crystallize prior to appreciable sintering when compared to 45S5 glass [4]. Consequently, 13-93 glass particles can be more readily sintered into a dense and strong network. As described earlier, our recent work showed that strong porous 3-D scaffolds of 13-93 glass, prepared with a grid-like microstructure using robocasting, had promising mechanical properties for loaded bone repair. The bioactivity of 13-93 glass and the capacity of 13-93 glass scaffolds with a "trabecular" and an "oriented" microstructure to support bone ingrowth in vivo were shown in our previous studies [12,24].

The objective of the present study was to evaluate the capacity of those strong porous 13-93 bioactive glass scaffolds fabricated by robocasting to regenerate bone in an osseous defect model. The effects on bone regeneration of pretreating the scaffolds for various times in an aqueous phosphate solution, to convert the glass surface to HA prior to implantation, and loading the pretreated scaffolds with BMP-2 were studied. After implantation for 6 weeks in rat calvarial defects, new bone formation in the implants was evaluated using histomorphometric techniques and scanning electron microscopy.

2. Experiments

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

Scaffolds of 13-93 bioactive glass (composition $6Na_2O$, $12K_2O$, 5MgO, 20CaO, $53SiO_2$, $4P_2O_5$, wt.%) with a grid-like microstructure were prepared using a robotic deposition (robocasting) method, as described in our previous work [11]. Briefly, a slurry was prepared by mixing 40 vol.% glass particles (~1 μm) with a 20 wt.% aqueous Pluronic® F-127 solution in a planetary centrifugal mixer (ARE-310, THINKY U.S.A. Inc, Laguna Hills, CA, USA). The slurry was then loaded into a robotic deposition device (RoboCAD 3.0, 3-D Inks, Stillwater, OK) and extruded through a syringe (tip diameter = 410 μm) onto an Al_2O_3 substrate to form a 3-D scaffold. The extruded filaments were deposited at right angles to the filaments in the adjacent layer, with a center-to-center spacing between the filaments of 910 μm in the plane of deposition. After forming, the scaffolds were dried for 24 h at room temperature and heated for 2 h at 100 °C to remove any residual water. The scaffolds were then heated slowly in flowing oxygen to 600 °C (heating rate = 0.5 °C min^{-1}, with isothermal holds for 2 h each at 150, 200, 250 and 300 °C) to burn out the polymer processing aids, and sintered in air for 1 h at 700 °C (heating rate = 5 °C min^{-1}) to densify the glass filaments. The as-fabricated constructs were sectioned and ground into thin discs (4.6 mm in diameter × 1.5 mm), washed twice with deionized water and twice with ethanol, dried in air and then sterilized by heating for 12 h at 250 °C.

2.2. Surface modification of scaffolds

Some of the as-fabricated scaffolds were modified prior to implantation by reacting them in an aqueous phosphate solution to convert a surface layer of the glass to an amorphous calcium phosphate (ACP) or HA material. In the surface modification process, the scaffolds were immersed for 1, 3 and 6 days in 0.25 M K_2HPO_4 solution at 60 °C and a starting pH of 12.0 (obtained by adding the requisite amount of 2 M NaOH solution). 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 system was stirred gently each day. These reaction conditions were based on our previous studies on the conversion of bioactive glasses to HA [25,26] and the ability to enhance the dissolution rate of the silicate glass network at higher pH [27]. In general, the reaction conditions were selected to accelerate the conversion of 13-93 glass to HA because of the slow conversion of the glass in SBF at body temperature (~37 °C) [4,24]. After each reaction time, the scaffolds were removed from the solution and washed twice with deionized water and twice with anhydrous ethanol to displace residual water from the scaffolds. The scaffolds were removed from the ethanol, dried for at least 24 h at room temperature and stored in a desiccator.

2.3. Characterization of converted surface layer

The surface-treated scaffolds were sputter-coated with Au/Pd and examined in a scanning electron microscope (SEM; S-4700, Hitachi, Tokyo, Japan), using an accelerating voltage of 15 kV and a working distance of 8 mm. Some surface-treated scaffolds were also mounted in epoxy resin, sectioned, polished to expose the cross-sections of the glass filaments and examined in the SEM. The thickness of the converted surface layer was determined from more than 15 measurements in the SEM images using the ImageJ software (National Institutes of Health, USA) and expressed as a mean value ± standard deviation (SD).

The converted surface layer was removed by vigorously shaking the scaffolds and used in determining its surface area and phase composition. Surface area measurements were made using nitrogen gas adsorption (Nova 2000e; Quantachrome, Boynton Beach, FL, USA). The volume of nitrogen adsorbed and desorbed at different gas pressures was measured and used to construct adsorption–desorption isotherms. Eleven points of the adsorption isotherm, which initially followed a linear trend implying monolayer formation of the adsorbate, were fitted by the Brunauer–Emmett–Teller equation to determine the surface area.

The presence of crystalline phases in the converted surface layer was determined using X-ray diffraction (XRD; D/mas 2550 v, Rigaku, The Woodlands, TX). The material was ground into a powder and analyzed using Cu K_α radiation (λ = 0.15406 nm) at a scanning rate of 1.8°/min^{-1} in the 2θ range 10–80°.

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