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Unique microstructural design of ceramic scaffolds for bone regeneration under load

S.I. Roohani-Esfahani [a], C.R. Dunstan [a], J.J. Li [a], Zufu Lu [a], B. Davies [a], S. Pearce [b], J. Field [c], R. Williams [d], H. Zreiqat [a,*]

[a] Biomaterials and Tissue Engineering Research Unit, School of AMME, The University of Sydney, Sydney 2006, Australia
[b] University of Ballarat B, Victoria 3350, Australia
[c] Flinders University, Adelaide 5001, Australia
[d] Adelaide Microscopy, The University of Adelaide, South Australia 5005, Australia

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ABSTRACT

During the past two decades, research on ceramic scaffolds for bone regeneration has progressed rapidly; however, currently available porous scaffolds remain unsuitable for load-bearing applications. The key to success is to apply microstructural design strategies to develop ceramic scaffolds with mechanical properties approaching those of bone. Here we report on the development of a unique microstructurally designed ceramic scaffold, strontium–hardystonite–gahnite (Sr–HT–gahnite), with 85% porosity, 500 μm pore size, a competitive compressive strength of 4.1 ± 0.3 MPa and a compressive modulus of 170 ± 20 MPa. The in vitro biocompatibility of the scaffolds was studied using primary human bone-derived cells. The ability of Sr–HT–gahnite scaffolds to repair critical-sized bone defects was also investigated in a rabbit radius under normal load, with β-tricalcium phosphate/hydroxyapatite scaffolds used in the control group. Studies with primary human osteoblast cultures confirmed the bioactivity of these scaffolds, and regeneration of rabbit radial critical defects demonstrated that this material induces new bone defect bridging, with clear evidence of regeneration of original radial architecture and bone marrow environment.

1. Introduction

The gold standard for bone defect repair – bone grafting with autologous bone – has significant drawbacks, such as limited availability, second site surgery and donor site morbidity, leading to prolonged hospitalization [1]. Allografting also has several disadvantages which limit its use, including reduced bioactivity and increased risk of disease transmission. Consequently, the search for an alternative bone graft substitute that reproduces bone's structural properties combined with the necessary porosity, interconnectivity, bioactivity and mechanical strength is one of the key challenges facing scientists in the field [2,3]. A critical limitation in almost any biomaterial approach to the repair and regeneration of large bone defects in load-bearing applications is the balance between material properties, implant architecture and bioactivity to satisfy requirements for strength and toughness, as well as osteoconductivity and osteoinductivity. During the past 30 years, a variety of synthetic bone graft substitutes based on ceramics and glasses have become available, composed of materials such as bioactive glasses (modified and unmodified, beta-tricalcium phosphate (β-TCP), hydroxyapatite (HA) and TCP/HA). While they have excellent properties for bone regeneration and bioactivity, their mechanical properties are inadequate for load-bearing applications in the highly porous form (porosity >80%, pore size >300 μm and 100% interconnectivity between the pores) necessary for vascularization and bone ingrowth [4–8]. The composition and degradation of bioactive glasses are easily controlled, making them attractive scaffolds for use in bone regeneration. However, their lack of microstructure and long-range order contributes to their very low resistance to crack propagation and an extreme sensitivity to flaws, leading to catastrophic failure of the scaffolds under load [5,9–12]. Ceramics, on the other hand, exhibit ordered structures with micromorphological features (i.e. grains) that promote increased toughness compared to glasses. The fracture toughness (K_{1C}) for glass materials is inherently low (K_{1C} = 0.5–1) compared to crystalline ceramic materials, with typical K_{1C} values ranging from 0.5 to 5 (and from 6 to 15 for stabilized zirconia) [5,11,12]. However, ceramic scaffolds are inherently brittle, and are fabricated by sintering low-efficiency-packed powders, which is a contributing factor to their low strength [13–15]. This leads to the formation of a poorly sintered and weak scaffold. During the

* Corresponding author. Tel.: +61 293512392; fax: +61 2 9351 7060.
E-mail address: hala.zreiqat@sydney.edu.au (H. Zreiqat).

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past two decades, much effort has been directed towards improving the toughness and strength of dense ceramics, in order to prevent crack growth and formation of flaws [5,10,16–18]. The unsolved problem is that toughness is usually inversely proportional to strength, such that the design of a strong and tough ceramic material is inevitably compromised [10]. We believe that the key for producing a ceramic scaffold with optimal strength and toughness lies in the application of microstructural design strategies which can promote crack-tip shielding mechanisms, such as crack deflection and, most importantly, crack bridging [19]. The other main issue with current materials is their limited innate bioactivity relative to autologous bone grafts. Attempts to address this have included the addition of biologics such as bone morphogenetic protein or mesenchymal stem cells to enhance their bioactivity, both strategies substantially increasing the cost and complexity of their clinical development and use [20–22].

In the present study, we introduce a new ceramic with a devised microstructural design produced with the aim of developing a mechanically strong and tough material for use in highly porous scaffolds. We incorporated Ca, Sr, Zn and Si ions in this material with the added aim of enhancing its bioactivity [23,24]. In this study we assessed the mechanical properties of this scaffold and evaluated its in vitro and in vivo bioactivity.

2. Materials and method

2.1. Preparation of solid and porous ceramics

Sr–$Ca_2ZnSi_2O_7$ powders were prepared by the sol–gel process using tetraethyl orthosilicate ($(C_2H_5O)_4Si$, TEOS), zinc nitrate hexahydrate ($Zn(NO_3)_2{\cdot}6H_2O$), calcium nitrate tetrahydrate ($Ca(NO_3)_2{\cdot}4H_2O$) and strontium nitrate ($Sr(NO_3)_2$) as raw materials (all from Sigma–Aldrich, USA). The TEOS was mixed with water and 2 M HNO_3 (mol ratio: $TEOS/H_2O/HNO_3$ = 1:8:0.16) and hydrolyzed for 30 min under stirring. Then, the $Zn(NO_3)_2{\cdot}6H_2O$, $Ca(NO_3)_2{\cdot}4H_2O$ and $Sr(NO_3)_2$ (5 wt.%) solutions were added into the mixture (mol ratio: $TEOS/Zn(NO_3)_2{\cdot}6H_2O/Ca(NO_3)_2{\cdot}4H_2O$ = 2:1:2), and reactants were stirred for 5 h at room temperature. After the reaction, the solution was maintained at 60 °C for 1 day and dried at 120 °C for 2 days to obtain the dry gel. The dry gel was calcined at 1200 °C for 3 h. The resulting powders consisted mainly of Ca (25.55 wt.%), Si (17.90 wt.%) and O (35.69 wt.%). The ternary phase diagram of the Al_2O_3–CaO–SiO_2 system displays a number of invariant compositions with low melting points. The lowest is 1170 °C for the eutectic composition of 62.0% SiO_2, 23.3% CaO and 14.7% Al_2O_3. In order to provide a glass phase at the grain boundaries by forming a liquid phase during sintering, an optimum amount of aluminum oxide powder (15 wt.%) was added to the Sr–$Ca_2ZnSi_2O_7$ powder, and the powders were mixed and ground by a ball mill machine before preparation the scaffolds and disks for 2 h at 150 rpm. TCP/HA powder and scaffolds were prepared based on previous published work [25]. A polymer sponge replication technique was used for fabrication of Sr–HT–gahnite scaffolds according to a previous report [25]. For preparing the Sr–HT–gahnite disk samples, the powders were pressed by a steel die and sintered at 1250 °C for 3 h.

2.2. Physical and chemical properties of the scaffolds

The microstructure and fracture surface of the scaffolds and disks were evaluated by field emission scanning electron microscopy (FE-SEM; Carl Zeiss, Germany). Three-dimensional architecture of porous scaffolds was assessed by micro-computed tomography (μCT; SkyScan 1072, Belgium) (reconstructed images not shown). Chemical composition of the prepared scaffolds was analyzed by elemental analysis and mapping (EDS) and X-ray diffraction (XRD).

2.3. Degradation of scaffolds in simulated body fluid

In vitro biodegradation of the scaffolds was investigated by soaking the scaffolds in simulated body fluid (SBF). The SBF solution was prepared according to the procedure described by Kokubo and Takadama [26]. Cubic scaffolds (8 × 8 × 8 mm) were immersed in SBF solution at 37 °C for 1, 7, 14, 21 and 28 days at a solid/liquid ratio of 150 mg l^{-1}. All scaffolds were held in plastic flasks and sealed. At each time point the scaffolds were removed, rinsed with Milli-Q water and dried at 100 °C for 2 days, after which the final weight of each scaffold was measured. The concentration of ions in the SBF after soaking the scaffolds was tested using inductive coupled plasma atomic emission spectroscopy (ICP-AES; Perkin Elmer, Optima 3000DV, USA). The weight loss was calculated as a percentage of the initial scaffold weight. Three scaffolds from each sample group were used to measure the weight loss and pH changes and the results are expressed as means ± SD.

2.4. Mechanical properties of the scaffolds

Mechanical properties of the scaffolds were determined in both dry and wet conditions on five identical specimens from each sample group. For wet conditions, the scaffolds were first soaked in SBF for different time periods and excess liquid was carefully removed with filter paper prior to testing. Compressive strength was determined by crushing cubic scaffolds (7 mm × 7 mm × 7 mm) between two flat plates using a computer-controlled universal testing machine (Instron 8874, UK) with a ramp rate of 0.5 mm min^{-1}. Compressive strength and modulus of solid samples were determined according to ASTM C1424. Toughness value (amount of the energy per volume that a material can absorb before fracture (in unit of J m^{-3})) was determined by integrating the area under the stress–strain curve from zero to the point of maximum stress [27]. Vickers hardness values were calculated by use of the ASTM C1327. Fracture toughness was measured using two methods; the Anstis [28] and the single-edge notched beam (SENB) methods [29–31]. For the Anstis method, a radial crack from the corners of indentation was induced on the polished surface of the ceramics (n = 10). The crack lengths were measured in order to calculate the toughness value according to the following equation:

$$K_c = 0.16\left(\frac{E}{H}\right)^{1/2}\left(\frac{\rho}{C_0^{3/2}}\right) \quad (1)$$

H is the measured hardness using an applied load (ρ) of 9.8 N. Measurement of crack length (C_0) was achieved by creating reproducible radial cracks by applying a load of 98 N. E is the compressive modulus which was derived from the linear region of the stress–strain curve of solid samples. Fracture toughness was evaluated by the SENB method with a 31 mm span and cross-head speed of 0.05 mm min^{-1} using 3 mm × 4 mm × 40 mm test bars on a jig used for three-point bending tests. Each specimen was ground and polished down to 1 μm finish and its sharp edges were chamfered. The notches of the specimen were cut with a 0.2 mm diamond. The saw depth was nearly half of the specimen's height.

2.5. In vitro evaluation by cell culture

2.5.1. Scaffold sterilization

Cubic scaffolds with dimensions of 5 mm × 5 mm × 5 mm were sterilized by soaking twice in 70% ethanol for 30 min each time, followed by rinsing three times with phosphate-buffered saline