Acta Biomaterialia 10 (2014) 2269–2281

Contents lists available at ScienceDirect

Acta Biomaterialia

journal homepage: www.elsevier.com/locate/actabiomat

Three-dimensional printing of strontium-containing mesoporous bioactive glass scaffolds for bone regeneration

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ARTICLE INFO

Article history:
Received 25 September 2013
Received in revised form 26 December 2013
Accepted 2 January 2014
Available online 10 January 2014

Keywords:
3-D printing
Mesoporous bioactive glass
Scaffolds
Strontium
Bone regeneration

ABSTRACT

In this study, we fabricated strontium-containing mesoporous bioactive glass (Sr-MBG) scaffolds with controlled architecture and enhanced mechanical strength using a three-dimensional (3-D) printing technique. The study showed that Sr-MBG scaffolds had uniform interconnected macropores and high porosity, and their compressive strength was ~170 times that of polyurethane foam templated MBG scaffolds. The physicochemical and biological properties of Sr-MBG scaffolds were evaluated by ion dissolution, apatite-forming ability and proliferation, alkaline phosphatase activity, osteogenic expression and extracellular matrix mineralization of osteoblast-like cells MC3T3-E1. The results showed that Sr-MBG scaffolds exhibited a slower ion dissolution rate and more significant potential to stabilize the pH environment with increasing Sr substitution. Importantly, Sr-MBG scaffolds possessed good apatite-forming ability, and stimulated osteoblast cells' proliferation and differentiation. Using dexamethasone as a model drug, Sr-MBG scaffolds also showed a sustained drug delivery property for use in local drug delivery therapy, due to their mesoporous structure. Therefore, the 3-D printed Sr-MBG scaffolds combined the advantages of Sr-MBG such as good bone-forming bioactivity, controlled ion release and drug delivery and enhanced mechanical strength, and had potential application in bone regeneration.

1. Introduction

Bioactive glass (BG) is a well-known biomaterial that has been used as bone tissue regeneration since the discovery of 45S Bioglass by Hench and Wilson [1]. Studies demonstrated that the incorporation of mesoporous silica in BG scaffolds could endow the scaffolds with both bioactivity and local drug delivery capability due to the bioactivity of BG and drug release ability of mesoporous silica, which is promising for bone regeneration [2–4]. Recently, mesoporous bioactive glass (MBG) has been synthesized, and was found to exhibit enhanced bone-forming bioactivity, degradation and drug delivery properties compared to conventional BG [5–11], because MBG has high specific surface area, large pore volume and mesoporous structure. Therefore, there has been a growing interest in MBG as a bioactive material in bone regeneration [12,13]. To date, many efforts have been made to fabricate three-dimensional (3-D) interconnected porous MBG scaffolds and MBG/polymer composite scaffolds [14–37], which are beneficial for cell migration, nutrient delivery, bone ingrowth and eventually vascularization.

Using traditional methods of fabricating 3-D porous scaffolds, such as polyurethane foam templating, porogen templating, solvent casting and freeze drying, it is difficult to control the pore interconnection, pore size and overall porosity of the scaffolds [38,39]. For example, the polyurethane foam templating method can create highly interconnected macroporous MBG scaffolds with a porosity of 90%, but the mechanical strength is only ~50 kPa [16–23]. Porogen templating methods are able to fabricate porous MBG scaffolds with higher mechanical strength, but it is difficult to control their mechanical stability and both the structure and interconnectivity of pores [28].

Recently, a 3-D printing technique has been developed to fabricate more ideal porous scaffolds with better control of pore morphology, pore size and porosity, with materials such as bioactive glass, hydroxyapatite, tricalcium phosphate and polymer scaffolds [33–37,40–47]. The significant advantage of this new technique for scaffold fabrication is that scaffolds with precisely controlled architectures can be printed from computer-assisted

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http://dx.doi.org/10.1016/j.actbio.2014.01.001

design (CAD)/computer-aided manufacturing (CAM) under mild conditions. For example, Yun et al. fabricated the 3-D porous MBG scaffolds with tailored hierarchical meso-macroporosity and pore geometry using a 3-D printing technique [33,34]. García et al. developed 3-D scaffolds in the SiO_2–P_2O_5 system with tailored hierarchical meso-macroporosity by the combination of methylcellulose as a porogen and a 3-D plotting method [40]. Fielding et al. evaluated the SiO_2/ZnO-doped tricalcium phosphate scaffolds made by 3-D printing, and found that a maximum compressive strength of 10.21 ± 0.33 MPa was achieved in doped scaffolds with 500 μm interconnected macropores [43]. Wu et al. prepared calcium silicate ($CaSiO_3$) scaffolds with controllable pore structure by 3-D printing, and found that these scaffolds had enhanced mechanical strength and showed excellent in vivo osteogenesis [46].

Studies demonstrated that the physicochemical and biological properties of the MBG scaffolds could be improved by the incorporation of additional inorganic components with specific biological activity, such as strontium (Sr), zinc (Zn), magnesium (Mg) or copper (Cu) [21–24]. Among them, Sr is an important trace element in human bone and could be used for the treatment of osteoporosis, because Sr can promote bone formation and osteoblast replication while inhibiting bone resorption by osteoclasts [48]. On the other hand, Sr can potentially be substituted for Ca in hydroxyapatite, tricalcium phosphate, calcium silicate and bioactive glasses to further improve their biological performance due to the similarities in their charge and ionic radius [49–52]. Gentleman et al. reported the effects of Sr-substituted BG on osteoblasts and osteoclasts in vitro, and the results suggested that Sr-substituted BG may promote an anabolic effect on osteoblasts and an anti-catabolic effect on osteoclasts, which is similar to the osteoporosis drug strontium ranelate [49]. Zhang et al. modified hydroxyapatite (HA), calcium silicate ($CaSiO_3$) and borosilicate (BS) with Sr, and found that the Sr-containing HA, $CaSiO_3$ or BS stimulated osteoblast proliferation and alkaline phosphatase (ALP) activity with an optimum Sr dose [50]. Recently, Wu et al. reported Sr-containing MBG scaffolds and mesoporous SrO–SiO_2 scaffolds by using the polyurethane foam templating method [21,53]. The results indicated that the incorporation of Sr in both scaffolds enhanced the biological properties and showed potential application in tissue engineering, but the mechanical strength was much lower due to the polyurethane foam templating method.

Therefore, Sr-containing MBG (Sr-MBG) scaffolds could deliver a controlled dose of Sr ion into the biological medium, thereby enhancing bone cell activity. Also, the high surface area, large pore volume and mesoporous structure of Sr-MBG scaffolds could be beneficial for the enhancement of bone-forming bioactivity and local drug delivery. At the same time, the 3-D printing technique could better control the macroporous structure and mechanical property of Sr-MBG scaffolds. It is reasonable to expect that 3-D printing of Sr-MBG scaffolds for bone regeneration would be promising. In this study, the aims were to fabricate Sr-MBG scaffolds with enhanced mechanical strength using 3-D printing and to investigate their potential in bone regeneration. The structures, physicochemical and biological properties of Sr-MBG scaffolds were systematically investigated. Furthermore, dexamethasone (DEX) was used as a model drug to evaluate the drug delivery property of Sr-MBG scaffolds.

2. Materials and methods

2.1. Materials

Nonionic block copolymer $EO_{20}PO_{70}EO_{20}$ (P123, Mw = 5800) was purchased from BASF. Nitric acid (HNO_3, ⩾65%), tetraethyl orthosilicate (TEOS, 98%), triethyl phosphate (TEP, 99.8%), calcium nitrate ($Ca(NO_3)_2{\cdot}4H_2O$, 99%), and polyvinyl alcohol (PVA, degree of polymerization: 1700 ± 50, 99%) were purchased from Sinopharm Chemical Reagent Co. Ltd. Strontium chloride ($SrCl_2{\cdot}H_2O$, 99.5%) was purchased from Aibi Chemistry Preparation Co. Ltd. Dexamethasone (DEX, 98%) was purchased from Sigma–Aldrich. All chemicals were used without further purification.

2.2. Preparation of Sr-MBG powders

Sr-MBG powders were prepared by using nonionic block copolymer $EO_{20}PO_{70}EO_{20}$ (P123) as a structure-directing agent, according to the previously reported method, with some modification [9]. Sr-MBG powders containing $57.2SiO_2$:$7.5P_2O_5$:35.3 (SrO+CaO) (mol.%), where either no Ca or 5% or 10% or 20% of Ca was substituted with Sr, were named as MBG, 5Sr-MBG, 10Sr-MBG and 20Sr-MBG, and the amounts of the reactants for the synthesis of Sr-MBG powders are listed in Table 1. As an example, in a typical synthesis for 10Sr-MBG, 3.0 g of P123 was dissolved in 120 ml of 2 M HNO_3 and 30 ml of distilled water while stirring at 37 °C in a water bath until the solution became clear. 8.5 g of TEOS, 0.98 g of TEP, 5.35 g of $Ca(NO_3)_2{\cdot}4H_2O$ and 0.67 g of $SrCl_2{\cdot}6H_2O$ were then added into the solution. The mixture was stirred at 37 °C for 12 h, and then was transferred into the autoclaves for hydrothermal treatment at 100 °C for 48 h. Without any filtering and washing, the resulting precipitate was directly dried at 100 °C for 10 h in air. The as-synthesized powders were calcined from room temperature to 650 °C with a heating rate of 1 °C min^{-1} in air, and maintained at 650 °C for 6 h to remove the organic structure-directing agents completely.

2.3. Fabrication of Sr-MBG scaffolds by 3-D printing

In this study, the 4th 3-D Bioplotter™ (EnvisionTEC GmbH, Germany) was used to print 3-D Sr-MBG scaffolds. Before printing the scaffolds, the injectable Sr-MBG paste was prepared as follows. Typically, Sr-MBG powders were ground and passed through 300 mesh sieves, resulting in a particle size of less than 45 μm. Subsequently, 10% of PVA solution was prepared by dissolving PVA in distilled water at 95 °C. Finally, Sr-MBG powders were added to aqueous PVA solution ($W_{Sr\text{-}MBG}$:W_{PVA} = 50:50), and the mixture was stirred at room temperature until it had formed a paste with the right consistency for injection.

Sr-MBG scaffolds were fabricated by introducing the prepared paste into a polyethylene injection cartridge that was fixed onto the 3-D Bioplotter™ printing device. Square block models were loaded on the 3-D Bioplotter software, and the scaffold was printed layer-by-layer through the extrusion of the paste as a fiber. Typically, the dosing pressure to the syringe pump was 1.5–3.8 bar and the speed of the dispensing unit was 9–12 mm s^{-1}. The nozzle size was 0.4 mm. The projected area of each Sr-MBG scaffold was

Table 1
The amounts of the reactants for the synthesis of MBG and Sr-MBG powders.

Samples	P123 (g)	TEOS (g)	TEP (g)	$Ca(NO_3)_2{\cdot}4H_2O$ (g)	$SrCl_2{\cdot}6H_2O$ (g)	HNO_3 (2 M, ml)	H_2O (ml)
MBG	3	8.5	0.98	5.94	0	120	30
5Sr-MBG	3	8.5	0.98	5.64	0.34	120	30
10Sr-MBG	3	8.5	0.98	5.35	0.67	120	30
20Sr-MBG	3	8.5	0.98	4.75	1.34	120	30