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# Three-dimensional printed strontium-containing mesoporous bioactive glass scaffolds for repairing rat critical-sized calvarial defects

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ABSTRACT

The development of a new generation of biomaterials with high osteogenic ability for fast osseointegration with host bone is being intensively investigated. In this study, we have fabricated three-dimensional (3-D) strontium-containing mesoporous bioactive glass (Sr-MBG) scaffolds by a 3-D printing technique. Sr-MBG scaffolds showed uniform interconnected macropores (~400 μm), high porosity (~70%) and enhanced compressive strength (8.67 ± 1.74 MPa). Using MBG scaffolds as a control, the biological properties of Sr-MBG scaffolds were evaluated by apatite-forming ability, adhesion, proliferation, alkaline phosphatase activity and osteogenic gene expression of osteoblast-like cells MC3T3-E1. Furthermore, Sr-MBG scaffolds were used to repair critical-sized rat calvarial defects. The results showed that Sr-MBG scaffolds possessed good apatite-forming ability and stimulated MC3T3-E1 cell proliferation and differentiation. Importantly, the in vivo results revealed that Sr-MBG scaffolds had good osteogenic capability and stimulated new blood vessel formation in critical-sized rat calvarial defects within 8 weeks. Therefore, 3-D printed Sr-MBG scaffolds with favorable pore structure and high osteogenic ability have more potential applications in bone regeneration.



## 1. Introduction

The reconstruction of bone defects caused by trauma, severe infection, tumor resection and congenital skeletal abnormalities is a major treatment challenge in orthopedic surgery. Autologous and allogeneic bone grafts are widely used for repairing osseous defects. However, problems associated with their use include tissue availability, disease transmission, donor morbidity and high cost [1]. To overcome these drawbacks, bone tissue engineering has emerged as a promising technique. Within this technique, synthetic three-dimensional (3-D) porous biomaterial scaffolds are of great importance, because 3-D porous scaffolds act as a temporary framework for providing a suitable environment for cell adhesion, growth and phenotype maintenance, and thereby help tissue regeneration [2]. Generally, the relevant properties of ideal scaffolds and the requirements for their successful application in bone tissue engineering include excellent osteoconductivity, controlled biodegradability, a highly interconnected porous structure, the ability to deliver cells and therapeutic agents, appropriate mechanical properties and the ability to fabricate irregular shapes [3,4]. With traditional methods for fabricating 3-D porous scaffolds, such as gas foaming, solvent casting, melt molding and freeze drying, it is difficult to control the pore interconnection, pore size and overall porosity of the scaffolds. Recently, a 3-D printing technique was developed to overcome these problems for fabricating ideal scaffolds [5–7]. The significant advantage of this new method for bone-graft fabrication is that customized implants with precisely controlled architectures could be printed from computer-assisted design (CAD) based on computerized tomography (CT) or magnetic resonance imaging (MRI) 3-D data files of patients.

As a trace element in the human body, Sr plays a dual role in bone metabolism by stimulating bone formation and inhibiting bone resorption [8–11]. The most recognized underlying mechanism is that Sr ions have the ability not only to increase cell response of mesenchymal stem cells (MSCs), but also to inhibit the differentiation of osteoclasts by inhibiting the expression of receptor activator of nuclear factor kappa-B ligand (RANKL) in MSCs [12,13]. Additionally, Sr ions can stimulate the expression of osteoprotegerin (OPG),

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which blocks the interaction of RANK with its ligand, RANKL, and inhibits the activity of osteoclasts [14,15]. On the other hand, Sr ions might also stimulate the expression of the angiogenic factors, such as *vascular endothelial growth factor* (VEGF) [16]. Clinically, strontium ranelate (SrR) has already been used as a commercial anti-osteoporotic oral drug to reduce the incidence of hip and vertebral fractures in osteoporotic patients [8,17]. However, the use of SrR often induces the relatively low bioavailability of the drug (only <20% by oral administration) and the production of systemic drug complications, such as chronic renal failure and osteomalacia [18,19]. Therefore, a controlled, local release of Sr ions in the bone defect site might be preferential to systemic administration. Thus doping Sr into synthetic bone graft substitutes for treatment of bone defects, particularly critical-sized bone defects, is of great interest.

Recently, mesoporous bioactive glass (MBG) has been synthesized, and exhibited enhanced bone-forming bioactivity, degradation and drug delivery properties compared to conventional BG, because MBG has high specific surface area, large pore volume and mesoporous structure [20–22]. Therefore, MBG as a bioactive material has stimulated growing for use in bone regeneration. Furthermore, previous studies demonstrated that the physicochemical and biological properties of the MBG scaffolds could be further altered or improved by the incorporation of additional trace inorganic components with specific biological activity, such as strontium (Sr), lithium (Li), copper (Cu) or zirconium (Zr) [23–26]. For example, the incorporation of Sr or Li into MBG scaffolds offered a favorable composition, microstructure and mesopore properties for attachment, proliferation and cementogenic differentiation of human periodontal ligament-derived cells (hPDLCs) [23,24]. We found that Zr-MBG scaffolds could significantly enhance bone marrow stromal cell attachment and proliferation [25]. Wu et al. fabricated Cu-containing MBG scaffolds and found that Cu-MBG scaffolds exhibited enhanced angiogenesis capacity, osteostimulation and antibacterial activity due to the addition of Cu into MBG [26].

In our previous study, MBG scaffolds with different Sr content (0, 5, 10 or 20 mol.% of Sr substituted for Ca) were fabricated by a 3-D printing technique [27]. Our results demonstrated that the prepared Sr-MBG (10 mol.% Sr) scaffolds possessed controlled architectures, exhibited good apatite-forming bioactivity and sustained drug delivery properties, and stimulated osteoblast cell proliferation and differentiation, which indicated the potential bone-generation ability for the Sr-MBG scaffolds. Recently, Wei et al. incorporated Sr into MBG scaffolds and investigated their bone formation capacity in the femurs of rats induced by ovariectomy [28]. They found that the local release of Sr from bone scaffolds improved fracture repair. Furthermore, Zhang et al. showed that Sr-MBG scaffolds promoted alveolar bone defect regeneration in an osteoporotic animal model carried out by bilateral ovariectomy [29]. Although these polyurethane foam templated Sr-MBG scaffolds showed their potential in bone tissue engineering applications, the mechanical strength of the scaffolds is low and the pore structure of the scaffolds is difficult to control. Therefore, the aim of this study was to fabricate porous Sr-MBG scaffolds by 3-D printing, which had excellent mechanical strength and controlled pore structure and perfectly filled in a critical-sized rat calvarial defect model. Furthermore, the in vivo bone regeneration and blood vessel formation ability was examined by using micro-CT imaging, sequential fluorescent labeling and histological analysis.

## 2. Materials and methods

### 2.1. Preparation of Sr-MBG powders and fabrication of Sr-MBG scaffolds by 3-D printing

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 [27]. Sr-MBG powders containing $57.2SiO_2$:$7.5P_2O_5$:35.3(SrO + CaO) (wt.%), where either no Ca or 10 mol.% of Ca was substituted with Sr, were named as MBG and Sr-MBG. In a typical synthesis of Sr-MBG powder, 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 tetraethyl orthosilicate (TEOS), 0.98 g of triethyl phosphate (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 structure-directing agents, nitrates, chlorides and organic materials form of Si and P sources completely.

Sr-MBG scaffolds were fabricated using a 3-D BioplotterTM printing device (EnvisionTEC GmbH, Germany) according to the previously reported method [27]. Cylinder models were loaded on the 3-D Bioplotter software, and the scaffolds were printed layer-by-layer through the extrusion of the paste as a fiber.

### 2.2. Characterization of Sr-MBG powders and scaffolds

Transmission electron microscopy (TEM) was performed using a JEM-2010 electron microscope operated at an acceleration voltage of 200 kV. $N_2$ adsorption–desorption isotherms were obtained on a Micromeritics Tristar 3020 at −196 °C under continuous adsorption conditions. Brunauer–Emmett–Teller (BET) and Barrett–Joyner–Halenda (BJH) methods were used to determine the surface area, the pore size distribution and the pore volume. Energy-dispersive X-ray spectroscopy (EDS) was also carried out to verify the composition of as-synthesized powders.

Thermogravimetric (TG) analysis was carried out on a Perkin–Elmer Diamond thermobalance between 50 °C and 800 °C under a 20 ml $min^{-1}$ $N_2$ flow and at a heating rate of 10 °C $min^{-1}$. Scanning electron microscopy (SEM) was carried out with a FEI Quanta 450 field emission scanning electron microscope. The porosity of Sr-MBG scaffolds (5 mm × 5 mm, diameter × height) was measured using Archimedes' principle according to our previous study [27]. To demonstrate the pore structure of Sr-MBG scaffolds, micro-CT (Skyscan 1176, Kontich, Belgium) was further used with a resolution of 18 μm. The compressive strength of Sr-MBG scaffolds with a size of 10 mm × 5 mm (diameter × height) was tested using a Zwick static materials testing machine (5 kN) at a cross-head speed of 0.5 mm $min^{-1}$.

### 2.3. In vitro bioactivity of Sr-MBG scaffolds in simulated body fluid (SBF)

In vitro bioactivity of the as-fabricated scaffolds was carried out in SBF according to our previously reported method [27]. In a typical procedure, Sr-MBG scaffolds were soaked in SBF with a $V_{SBF}/M_{Sr\text{-}MBG}$ of 200 ml $g^{-1}$ in a polyethylene bottle at 37 °C for 3 days. After soaking, Sr-MBG scaffolds were collected from SBF solution, rinsed with ethanol and distilled water and then dried at 37 °C. The formation of apatite on the surface of the scaffolds was determined using SEM (FEI Quanta 450) and EDS.

### 2.4. In vitro cellular responses of MC3T3-E1 cells to Sr-MBG scaffolds

#### 2.4.1. Cell attachment and proliferation

In order to assess cell attachment on MBG and Sr-MBG scaffolds, $1 \times 10^5$ MC3T3-E1 cells were seeded on each scaffold in a 24-well plate and allowed to adhere to the scaffold for 3 h. Subsequently, the cells were incubated in alpha minimum essential medium