3D-Printing of hierarchical and tough mesoporous bioactive glass scaffolds with controllable pore architecture, excellent mechanical strength and mineralization ability

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Abstract

New-generation biomaterials for bone regenerations should be highly bioactive, resorbable and mechanically strong. Mesoporous bioactive glass (MBG), as a novel bioactive material, has been used for the study of bone regeneration due to its excellent bioactivity, degradation and drug-delivery ability; however, how to construct a 3D MBG scaffold (including other bioactive inorganic scaffolds) for bone regeneration still maintains a significant challenge due to its/their inherit brittleness and low strength. In this brief communication, we reported a new facile method to prepare hierarchical and multifunctional MBG scaffolds with controllable pore architecture, excellent mechanical strength and mineralization ability for bone regeneration application by a modified 3D-printing technique using polyvinylalcohol (PVA), as a binder. The method provides a new way to solve the commonly existing issues for inorganic scaffold materials, for example, uncontrollable pore architecture, low strength, high brittleness and the requirement for the second sintering at high temperature. The obtained 3D-printing MBG scaffolds possess a high mechanical strength which is about 200 times for that of traditional polyurethane foam template-resulted MBG scaffolds. They have highly controllable pore architecture, excellent apatite-mineralization ability and sustained drug-delivery property. Our study indicates that the 3D-printed MBG scaffolds may be an excellent candidate for bone regeneration.

Keywords: 3D-printing scaffolds; Mesoporous bioglass; High strength; Bioactivity

1. Introduction

New-generation biomaterials for bone regeneration should be highly bioactive (enabling good tissue growth), resorbable and mechanically strong [1]. Bioactive inorganic materials, such as hydroxyapatite [2], β-tricalcium phosphate (β-TCP), and bioactive glasses have been designed as 3D porous scaffolds for bone regeneration due to their excellent osteoconductivity; however, their inherent brittleness and generally low mechanical strength (of porous specimens) are the main disadvantages for developing 3D scaffolds, limiting their further application in the clinic [3-6]. Traditionally, polyurethane foam templating, porogen-created pores and gas foaming are the main methods to prepare porous bioceramic and bioactive glass scaffolds. Although polyurethane foam templating and gas foaming methods are able to create highly interconnective pores, the mechanical strength of the prepared porous scaffolds is low [7-9]. Porogen-based methods can produce porous scaffolds with higher mechanical strength; however, the pores are not always interconnective [10]. In addition, with these traditional methods it is difficult to control the pore morphology, pore size and overall porosity of the scaffolds. Another issue is that the present bioactive ceramic and glass scaffolds are quite brittle and not easy to handle. Ceramic particles can be released in the process of handling and implantation, which may be detrimental to cells and tissues [11,12]. To better control the pore morphology, pore size and porosity, 3D plotting technique (also called direct writing or printing) was developed to prepare porous scaffolds in the past several years [13-15]. The significant advantage of this technique is that the architectures of the scaffolds can be concisely controlled by layer-by-layer plotting under mild conditions. Recently, HAp and β-TCP ceramic scaffolds with controllable pore structure and improved

mechanical strength have been prepared by this method; however, they need a second sintering process at high temperature after the plotting and the obtained ceramic scaffolds are still brittle [16-18].

Mesoporous bioactive glass (MBG) has a highly ordered mesopore channel structure with a pore size ranging from 5–20 nm [19]. Compared to non-mesopore bioactive glass, MBG possesses a more optimal surface area and pore volume, evident by greatly enhanced drug-delivery capability, *in vitro* apatite mineralization and suitable degradation behavior [19-22]. For this reason, MBG has received much attention for the applications of bone tissue engineering [7,22-25]. We have recently shown that MBG scaffolds prepared by polyurethane foam template method can support cell adhesion; however, the MBG scaffolds prepared by this method are quite brittle and the mechanical strength is low [7]. Yun and Garcia, et al. prepared hierarchical 3D porous MBG scaffolds using a combination of sol-gel, double polymer template and rapid prototyping techniques [26,27]. In their study, they mixed MBG gel with methylcellulose and then printed, sintered at 500-700ºC to remove polymer templates and obtain MBG scaffolds. This method for preparing MBG scaffolds is inconvenient, because of the need of methylcellulose and the additional sintering procedure. Although the obtained MBG scaffolds have uniform pore structure, they are still brittle and not easy to handle. In addition, the mechanical strength of the obtained MBG scaffolds is unknown, but it is speculated that the strength is low as the scaffolds were sintered only at 500-700ºC, and therefore at a low temperature. Furthermore, the incorporation of methylcellulose created some micropores with diameters of several micrometers, which will further decrease the mechanical strength of those MBG scaffolds.

Herein, we report a new facile method to prepare hierarchical and multifunctional MBG scaffolds with controllable pore architecture, excellent mechanical strength and calcium phosphate mineralization ability for bone regeneration by a modified 3D printing method using polyvinyl alcohol (PVA) as a binder. The method provides a new way to solve the commonly existing issues for inorganic scaffold materials, for example, uncontrollable pore architecture, low strength, high brittleness and the requirement for a second sintering at high temperature.

2. Materials and methods

2.1 Synthesis of MBG powders

Mesoporous bioactive glass (MBG) powder (molar ratio: Si/Ca/P = 80/15/5) was synthesized according to previous publications [19,22]. In a typical synthesis, 4.0 g of P123 (Mw=5800, Sigma-Aldrich, Germany), 6.7 g of tetraethyl orthosilicate (TEOS, 98%, Sigma-Aldrich), 1.4 g of $Ca(NO_3)_2 \cdot 4\ H_2O$ (Sigma-Aldrich), 0.73 g of triethyl phosphate (TEP, 99.8%, Sigma-Aldrich) and 1.0 g of 0.5 M HCl were dissolved in 60 g of ethanol and stirred at room temperature for 24 h. The resulting sol was introduced into a petri dish for an evaporation-induced self-assembly process for 24 h, and then the dry gel was calcined at 700ºC for 5 h to obtain MBG powders. The obtained MBG powders were ground and sieved through 300-meshes (300 micropores for each square inch), resulting in a particle size lower than 45 μm.

2.2. Preparation of MBG scaffolds by 3D printing

The injectable MBG paste was prepared by mixing 3 g of MBG powder with 3.3 g of an aqueous polyvinyl alcohol solution (15 wt.%, PVA, Sigmar-Aldrich). The printing device (3D scaffold printer) was developed by the Fraunhofer Institute for Materials Research and Beam Technology (Dresden, Germany), based on a precision three-axis positioning system (Nano-Plotter NP 2.1, GeSiM, Grosserkmannsdorf, Germany). The dosing pressure to the syringe pump was 520-590 kPa and the moving speed of the dispensing unit was 3 mm/s. To control the scaffold morphology, pore structure, pore size and porosity, different plotting parameters and nozzle sizes were selected. The obtained MBG scaffolds were dried at 40ºC for overnight and heated at 150ºC for 30 min for heat-crosslinking of PVA. The final dry weight of MBG in the obtained scaffolds is 86%, and PVA is 14%. As a control, MBG scaffolds were prepared by polyurethane foam template method according to our previous publication [7] to compare their mechanical behavior.

2.3. Characterization and mechanical testing

The scaffold morphology, pore structure and pore size were observed by optical microscopy (Stemi 2000-C, Zeiss, Germany). The microstructure of pore walls was investigated by scanning electron microscopy (SEM, DSM982-Gemini, Zeiss) and transmission electron microscopy (TEM, FEI, Eindhoven, NL). The compressive strength and modulus of the obtained scaffolds (10×10×10 mm) were tested using a computer-controlled universal testing machine (Instron 5566, Instron Wolpert, Darmstadt, Germany) at a crosshead speed of 0.5 mm/min. Four samples were used for the repeats of this experiment.

2.4. In vitro mineralization and ion release

SBF containing ion concentrations similar to those in human blood plasma was prepared according to the method described by Kokubo [28]. MBG scaffolds were soaked in SBF at 37°C for 1 and 3 d, and the ratio of the solution volume to the scaffold mass was 200 mL/g. 0.2g of scaffolds were soaked in 40mL SBF and three samples were used for repeated experiment. Apatite mineralization of scaffolds was determined by SEM, energy dispersive spectrometry (EDS) (Jeol JSM6510, Tokyo, Japan) and Fourier transforms infrared spectroscopy (FTIR) (Spectrum 2000, Perkin Elmer, USA).

To investigate the ion release and weight loss of MBG scaffolds, 0.2g of scaffolds were soaked in PBS for 1, 3 and 7 days. The concentration of Ca^{2+} and SiO_4^{4-} ions were tested by by atomic emission spectrometry (Perkin-Elmer Optima 7000DV). As MBG contains 80% molar of SiO_2, therefore, the weight loss of MBG scaffolds were calculated by the release SiO_4^{4-} ions.

2.5. Drug loading and release from MBG scaffolds

Dexamethasone (DEX) as model drug was dissolved in ethanol with a concentration of 0.5 mg/mL. 2 g of MBG powder were added to 24 mL of DEX/ethanol solution under stirring and the ethanol was evaporated. MBG powders loaded with DEX were obtained after drying at 50°C for 5 h. Then, DEX-loaded MBG powders were used for preparing MBG scaffolds by 3D printing. The obtained MBG scaffolds loaded with DEX were heated again at 50ºC for 24 h.

DEX release was evaluated by placing the DEX-loaded MBG scaffolds into 4 mL of PBS (pH 7.4) at 37 ºC for 3, 6, 9, 24, 48, 96, 168 and 240 h. DEX release was determined by UV analysis (UV min-1240, Shimadzu, Japan) and the accumulative release rate of DEX (%) was

calculated with the following equation: DEX (%) = (total amount of DEX released / total loading amount of DEX in scaffolds) × 100%. Three samples were used for the repeated experiment and UV analysis for drug release was tested by three times for each sample.

2.6. Proliferation and alkaline phosphatase (ALP) activity of BMSCs in scaffolds

The culture of human bone marrow stromal cells (hBMSCs) was carried out according to our previous publication [29]. 3D-printed MBG scaffolds (6×6 mm) were used for cell culture. 2×10^5 cells were added to each scaffolds and then cultured for 1, 3 and 7 days. The proliferation of MBSCs in scaffolds were tested by measuring the DNA content and the ALP activity was also tested according to our previous publication [29]. Cell culture plates were used for the control.

3. Results and discussion

3.1. Preparation and characterization of 3D-printed MBG scaffolds

In this communication, PVA was selected as a binder because it is generally biocompatible, degradable and water-dissolvable. No toxic solvents have to be used in the process of preparation. In addition, PVA can be crosslinked to improve its crystallinity and to control its dissolution by a simple heat treatment at low temperature (50-180ºC) [30]. Thus, the formed MBG scaffolds bound by PVA after heat crosslinking will maintain their structure and will not collapse in the biological environment. Our study shows that it is very efficient to mix MBG powders with an aqueous PVA solution to form an injectable MBG paste (Fig. 1). The final dry weight of MBG in the obtained scaffolds is 86%, and PVA is 14%. This small amount of PVA, added to the MBG scaffolds, will not decrease the bioactivity of MBG.

By 3D printing of MBG scaffolds with PVA as binder the size (from millimeters to centimeters) as well as the morphology (from cube to hexahedral) can be controlled in a wide range (Fig. 2a). In our study, the pore size of MBG scaffolds was varied from 1307±40 μm (Fig. 2b), 1001±48 μm (Fig. 2c), to 624±40 μm (Fig. 2d) and even smaller (200 μm) (Fig. 2f). The pore structure is quite uniform and pore morphology was chosen as square or parallelogram as the morphology is much easier to control and prepare by a simple program. The pores on the bottom side are still open, even in the case of bigger samples in which the weight might deform the structure (Fig. 2g). SEM image shows that MBG particles were bound together by PVA and formed a dense pore-wall surface (Fig. 2h). TEM image shows that the pore walls contain well-ordered mesopore channel structure with a size of about 5 nm (Fig. 2i). The obtained MBG scaffolds therefore possess a hierarchical pore structure: large pores (several hundred micrometers to 1.3 millimeters) as well as well-ordered mesopores (5 nm). The easily controllable large pore structure will benefit cell and tissue ingrowth [31], and the well-ordered mesopore structure makes the MBG scaffolds a potential drug carrier. Compared with the previous method to prepare MBG scaffolds, described by Yun and Garcia et al.[26,27], our method is much easier to control.

3.2. Mechanical properties of 3D-printed MBG scaffolds

Most importantly, MBG scaffolds obtained by our method possess excellent mechanical strength, a significant advance in comparison to the material developed by Yun et al., which seems to be mechanically too weak [26]. The compressive strength and modulus of the novel MBG scaffolds with square pore morphology and pore size of 1001 μm are 16.10±1.53 and 155.13±14.89 MPa, respectively. The corresponding porosity is 60.4% (calculated according

to the pore and pore wall size). The mechanical profiles of 3D-printed MBG scaffolds and those prepared by polyurethane templating are shown in Figure 3. The compressive strength of 3D-printed MBG scaffolds increases almost linearly with the deformation (Fig. 3a). However, the compressive strength of polyurethane templated MBG scaffolds increases with a waving curve until the maximum value of only 0.08 MPa (Fig. 3e). The compressive strength of 3D-printed MBG scaffolds is about 200 times that of polyurethane templated ones. After compressive testing, 3D-printed MBG scaffolds still maintain their bulk-scaffold morphology (Fig. 3c); however, those fabricated by polyurethane templating are crashed and became powders (Fig. 3g). Our results indicate that novel 3D-printed MBG scaffolds have significantly improved mechanical strength and toughness, compared to polyurethane templated ones. There are two possible reasons to explain the significantly improved mechanical strength. One is that PVA, as a binder, reinforces the MBG scaffolds by binding the particles together and decreases the brittleness of MBG, which can be seen from Fig. 3d. After the compressive testing, lots of PVA fibers can be seen inside of 3D-printed MBG scaffolds which bind the MBG particles together (Fig. 3d, see white arrows); the other is that 3D-printing method produces a more uniform and continuous pore structure. Generally, a uniform and continuous pore structure benefits the improvement of the mechanical strength [32]. In this study, the high brittleness of MBG scaffolds prepared by conventional methods resulted in more noncontinuous pores (or pore defects), which is detrimental to their mechanical strength; however, MBG scaffolds prepared by 3D-printing method had more uniform and continuous pore structures, which made them possess significantly improved mechanical strength. The average compressive strength of human trabecular bone is in the

range of 2-12 MPa; the compressive strength of 3D-printed MBG scaffolds is higher than that of trabecular bone, which makes them easy to handle and utilize. The obtained 3D-printed MBG scaffolds possessed significantly higher mechanical strength than other inorganic scaffolds prepared by traditional methods, for example, HAp (lower than 0.29MPa)[3], 45S5 Bioglass® (lower than 0.4MPa)[5] and $CaSiO_3$ (lower than 0.4MPa)[32] scaffolds. In this study, since the porosity of the 3D-printed MBG scaffolds is controllable, it is believed that their mechanical strength could be further improved by tailoring their porosity and pore structure. In addition, the method described here does not require a second sintering at high temperature, which can be used also for preparing other bioceramic scaffolds with improved mechanical strength.

3.3. In vitro mineralization, weight loss, drug delivery and cell response of 3D-printed MBG scaffolds

Previous studies have shown that apatite mineralization on the surface of biomaterials for bone replacement applications in simulated body fluid (SBF) plays an important role to improve osteoblast growth and differentiation, which further influences their *in vivo* bone-forming ability [4,10,28,33]. Our study shows that the 3D-printed MBG scaffolds possess excellent apatite mineralization ability in SBF (Fig. 4). After 1 and 3 days of soaking, platelet-like apatite crystals with 50 nm in diameter and 200 nm in length formed on the surface of MBG scaffolds (Fig. 4 a, b and c). EDS and FTIR analysis further confirmed the newly formed apatite on the surface of MBG scaffolds. Our result indicate that 3D-printed MBG scaffolds are highly bioactive and the incorporation of small amount of PVA as binder into MBG scaffolds does not decrease their bioactivity. Our study has further shown that

MBG scaffolds has a very quick release of Si and the weight loss reached 10% after soaking in biological solution for 7 days (Fig. 4e). It is known that the quick ion release (dissolution) is one of important factor to contribute to the degradation of materials. Therefore, it is speculated that 3D-printed MBG scaffolds still maintain quick degradation.

Another important characteristic of the obtained 3D-printed MBG scaffolds is that they possess a well-ordered mesopore channel structure with a size of 5 nm within their pore walls (see Fig. 2i), which suggest that they could be used for drug delivery purposes. In this study, dexamethasone (DEX) was selected as model drug as it is commonly used for stimulating cell differentiation and treating rheumatoid arthritis by virtue of its anti-inflammatory function [34]. We could demonstrate that in the first two days a burst release of DEX (about 75%) from MBG scaffolds occurs (Fig. 5a). After two days, DEX is released with a slow kinetic until ten days (Fig. 5a and b). The result indicates that the 3D-printed MBG scaffolds can carry some anti-inflammatory drugs with a sustained release for treating the inflammatory reaction after implantation.

Our study further showed that BMSC proliferation on MBG scaffolds is lower than controls; however, the ALP activity of BMSCs on MBG scaffolds is significantly higher than that for controls (Fig. 6). Further study will be carried out to evaluate the gene-expression of human bone marrow mesenchymal stem cells on our scaffolds as well as their *in vivo* osteogenesis.

4. Conclusions

In conclusion, novel multifunctional MBG scaffolds with hierarchical pore architecture and well-ordered mesopores were successfully prepared using the method of 3D-printing

combined with utilization of PVA as binder. The obtained scaffolds possess a high compressive strength which is about 200 times higher than that of scaffolds prepared by polyurethane foam templating. The use of PVA as a binder in the MBG scaffolds decreases their brittleness and significantly improves their toughness. The method described in this study provides a new way to solve the commonly existing issues (uncontrollable pore architecture, low strength, high brittleness and the requirement for second sintering) for inorganic biodegradable scaffold materials. 3D-printed MBG scaffolds possess excellent apatite-mineralization ability and sustained drug-delivery property. These significant advantages concerning architecture, mechanical strength, bioactivity and the capability to act as drug carriers suggest that the 3D-printed MBG scaffolds may be an excellent candidate for bone regeneration.

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