Brief communication

Three-dimensional printing of hierarchical and tough mesoporous bioactive glass scaffolds with a controllable pore architecture, excellent mechanical strength and mineralization ability

Chengtie Wu a,b,*, Yongxiang Luo a, Gianaurelio Cuniberti a,c, Yin Xiao b, Michael Gelinsky a,b

* Institute for Materials Science and Max Bergmann Center of Biomaterials, Dresden University of Technology, Budapester Strasse 27, D-01069 Dresden, Germany
b Institute for Health and Biomedical Innovation, Queensland University of Technology, Brisbane, Queensland 4059, Australia

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A B S T R A C T

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

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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 (HAp) [2], β-tricalcium phosphate (β-TCP), and bioactive glasses, have been designed as three-dimensional (3-D) porous scaffolds for bone regeneration due to their excellent osteoconductivity, however, their inherent brittleness and generally low mechanical strength (of porous specimens) are major disadvantages in developing 3-D scaffolds, limiting their clinical application [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 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 interconnected [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 a 3-D plotting technique (also called direct writing or printing) has been developed to prepare porous scaffolds in recent 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 structures and improved mechanical strengths have been prepared by this method, however, they require a second sintering process at high temperature after the plotting and the ceramic scaffolds obtained are still brittle [16–18].
Mesoporous bioactive glass (MBG) has a highly ordered mesopore channel structure with pore sizes ranging from 5 to 20 nm [19]. Compared with non-mesopore bioactive glass, MBG possesses a more optimal surface area and pore volume, evident as a greatly enhanced drug delivery capability, in vitro apatite mineralization and suitable degradation behavior [19–22]. For this reason MBG has received much attention for applications in bone tissue engineering [7,22–25]. We have recently shown that MBG scaffolds prepared by the 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 et al. [26] and García et al. [27] prepared hierarchical 3-D porous MBG scaffolds using a combination of sol–gel, double polymer template and rapid prototyping techniques. In their study they mixed a MBG gel with methylcellulose, followed by printing then sintering at 500–700 °C to remove the polymer templates and obtain the MBG scaffolds. This method for preparing MBG scaffolds is inconvenient, because of the need for methylcellulose and the additional sintering procedure. Although the MBG scaffolds obtained had uniform pore structures, they were still brittle and not easy to handle. In addition, the mechanical strength of the MBG scaffolds obtained, although unknown, is speculated to be low as the scaffolds were sintered at low temperature at only 500–700 °C. Furthermore, the incorporation of methylcellulose created some micropores with diameters of several micrometers, which will further decrease the mechanical strength of those MBG scaffolds.

Fig. 1. Photograph showing the injectable MBG paste composed of MBG powder and aqueous PVA solution (15 wt.%). The weight ratio of MBG powder and PVA solution is 1:1. The final dry weight of MBG in the obtained scaffolds is 86%, and PVA 14%. PVA is used to bind the MBG powder to form stable 3-D scaffolds.

Fig. 2. MBG scaffolds, pore morphology and microstructure. (a) MBG scaffolds produced by 3-D printing of different sizes, shapes and morphologies. (b–d) MBG scaffolds with different pore sizes varying from (b) 1307 ± 40, to (c) 1001 ± 48, to (d) 624 ± 40 μm. (e–f) MBG scaffolds with different pore morphologies. (g) Pore morphology of the bottom side of the MBG scaffolds. The pores on the bottom side remain open. (h) SEM image of the microstructure of pore walls. (i) TEM micrographs demonstrating the well-ordered mesopore channel structure of the pore walls in the scaffolds. The size of the mesopore channel is about 5 nm. The 3-D printed scaffolds obtained have controllable large pores (from several hundred micrometers to more than a millimeter) for cell seeding and tissue in-growth and nanopores (5 nm) suitable for drug loading and delivery.
In a typical synthesis 4.0 g of P123 (according to techniques detailed in previous publications[19,22].

2.1. Synthesis of MBG powders

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

MBG powder (Si/Cal/P molar ratio 80/15/5) was synthesized according to techniques detailed in 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% purity, Sigma–Aldrich), 1.4 g of Ca(NO3)2·4H2O (Sigma–Aldrich), 0.73 g of triethyl phosphate (TEP) (99.8% purity, 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 evaporation-induced self-assembly for 24 h, and then the dry gel was calcined at 700 °C for 5 h to obtain MBG powders. The MBG powders obtained were ground and passed through 300 mesh (300 micropores per square inch) sieves, resulting in a particle size of less than 45 μm.

2.2. Preparation of MBG scaffolds by 3-D printing

The injectable MBG paste was prepared by mixing 3 g of MBG powder with 3.3 g of an aqueous PVA solution (15 wt.%, Sigma–Aldrich). The printing device (3-D 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 speed of the dispensing unit was 3 mm s⁻¹. To control the scaffold morphology, pore structure, pore size and porosity different plotting parameters and nozzle sizes were selected. The MBG scaffolds obtained were dried at 40 °C overnight, then heated at 150 °C for 30 min to induce heat-activated cross-linking of PVA. The final dry weight of MBG in the scaffolds obtained is 86%, and PVA 14%. As a control, MBG scaffolds were prepared by a polyurethane foam template method detailed in a 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 the pore walls was investigated by scanning electron microscopy (SEM) (DSM982-Gemini, Zeiss) and transmission electron microscopy (TEM) (FEI, Eindhoven, The Netherlands). 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 cross-head speed of 0.5 mm min⁻¹. Four samples were used for replicates of this experiment.

2.4. In vitro mineralization and ion release

Simulated body fluid (SBF) with 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 days, at 200 ml g⁻¹ solution volume to scaffold mass. 0.2 g of scaffold were soaked in 40 ml of SBF. Three samples were used for replicate experiments. Apatite mineralization of scaffolds was determined by SEM, energy-dispersive spectrometry (EDS) (Jeol JSM6510, Tokyo, Japan) and Fourier transform infrared spectroscopy (FTIR) (Spectrum 2000, Perkin Elmer, USA).
To investigate ion release by and weight loss of MBG scaffolds 0.2 g of scaffold were soaked in PBS for 1, 3 and 7 days. The concentration of Ca\(^{2+}\) and SiO\(_4\)^{4–} ions were tested by atomic emission spectrometry (Perkin-Elmer Optima 7000DV). As MBG contains 80 mol.% SiO\(_2\) the weight loss of the MBG scaffolds was calculated from the release of SiO\(_4\)^{4–} ions.

2.5. Drug loading and release from MBG scaffolds

Dexamethasone (DEX), as a model drug, was dissolved in ethanol at a concentration of 0.5 mg ml\(^{-1}\). 2 g of MBG powder were added to 24 ml of DEX/ethanol solution under stirring and the ethanol evaporated. MBG powders loaded with DEX were obtained after drying at 50 °C for 5 h. Then the DEX-loaded MBG powders were used to prepare MBG scaffolds by 3-D printing. The MBG scaffolds obtained loaded with DEX were heated again at 50 °C for 24 h.

DEX release was evaluated by placing the DEX-loaded MBG scaffolds into 4 ml of phosphate-buffered saline (PBS) (pH 7.4) at 37 °C 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 cumulative release of DEX (%) was calculated with the equation: DEX (%) = (total DEX released/total loading of DEX in scaffolds) \times 100%. Three samples were used for replicate experiments.

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**Fig. 4.** SEM images of 3-D printed MBG scaffolds soaked in simulated body fluid for (a) 1 and (b, c) 3 days. (b, insert) EDS analysis and (d) FTIR spectrum of MBG soaked in simulated body fluid for 3 days. (c) Platelet-like apatite 50 nm in diameter and 200 nm in length was formed on the surface of the MBG scaffolds. (b) EDS analysis shows a ratio of Ca/P of 1.70, similar to that of hydroxyapatite (Ca/P = 1.67). (d) An additional P–O peak assigned to bending vibration in the PO\(_4\) tetrahedron appears at around 598 cm\(^{-1}\) in the FTIR pattern after soaking the MBG scaffolds in SBF. (e) Ca and Si ion release from and weight loss of 3-D printed MBG scaffolds in PBS.
and UV analysis for drug release was carried out 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 a previous publication [29]. 3-D 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, also according to our previous publication [29]. Cell culture plates were used as the control.

3. Results and discussion

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

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

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

3.2. Mechanical properties of 3-D 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 a 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 sizes). The mechanical profiles of 3-D printed MBG scaffolds and those prepared by polyurethane templating are shown in Fig. 3. The compressive strength of 3-D printed MBG scaffolds increases almost linearly with deformation (Fig. 3a). However, the compressive strength of polyurethane templated MBG scaffolds increases irregularly up to maximum value of only 0.08 MPa (Fig. 3e). The compressive strength of 3-D printed MBG scaffolds is about 200 times that of polyurethane templated ones. After compressive testing the 3-D printed MBG scaffolds still maintain their bulk scaffold morphology (Fig. 3c), however, those fabricated by polyurethane templating were crushed to powders (Fig. 3g). Our results indicate that novel 3-D printed MBG scaffolds have significantly improved mechanical strength and toughness compared with 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 in Fig. 3d. After compressive testing numerous PVA fibers can be seen inside the 3-D printed MBG scaffolds which bind the MBG particles together (Fig. 3d, see white arrows). Another is that the 3-D printing method produces a more uniform and continuous pore structure. Generally, a uniform and continuous pore structure improves the mechanical strength [32]. In this study the high brittleness of MBG scaffolds prepared by conventional methods resulted in more non-continuous pores (or pore defects), which is detrimental to the mechanical strength, however, MBG scaffolds prepared by the 3-D printing method had more uniform and continuous pore structures, which imbued them with significantly improved mechanical strength. The average compressive strength of human trabecular bone is in the range 2–12 MPa; the compressive strength of 3-D printed MBG scaffolds is higher than that of trabecular bone, which makes them easy to handle and utilize. The 3-D printed MBG scaffolds obtained possessed significantly higher mechanical strength than other inorganic scaffolds prepared by traditional methods, for example HAp (lower than 0.29 MPa) [3], 4555 Bioglass™ (lower than 0.4 MPa) [5] and CaSiO₃ (lower than 0.4 MPa) [32] scaffolds. In this study, since the porosity of the 3-D 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, and can also be used to prepare other bioceramic scaffolds with improved mechanical strength.

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

Previous studies have shown that apatite mineralization on the surface of biomaterials for bone replacement applications in SBF plays an important role in improving osteoblast growth and differentiation, which further influences their in vivo bone-forming ability [4,10,28,33]. Our study shows that the 3-D printed MBG scaffolds possess excellent apatite mineralization ability in SBF (Fig. 4). After 1 and 3 days soaking, platelet-like apatite crystals 50 nm in diameter and 200 nm in length formed on the surface of MBG scaffolds (Fig. 4a–c). EDS and FTIR analyses further confirmed the newly formed apatite on the surface of MBG scaffolds. Our results indicate that 3-D printed MBG scaffolds are highly bioactive and the incorporation of small amounts of PVA into MBG scaffolds as binder does not decrease their bioactivity. Our study has further shown that MBG scaffolds display rapid release of Si, with the weight loss reaching 10% after soaking in biological solution for 7 days (Fig. 4e). It is known that rapid ion release (dissolution) is one important factor contributing to the degradation of materials. Therefore, it is speculated that 3-D printed MBG scaffolds will be rapidly degraded.

Another important characteristic of the 3-D 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 suggests that they could be used for drug delivery purposes. In this study DEX was selected as the model drug as it is commonly used to stimulate cell differentiation and treat rheumatoid arthritis by virtue of its anti-inflammatory action [34]. We have demonstrated that in the first 2 days burst release of DEX (about 75%) from MBG scaffolds occurs (Fig. 5a). After 2 days DEX is released with slow kinetics up to 10 days (Fig. 5a and b). The results indicate that the 3-D printed MBG scaffolds can carry anti-inflammatory drugs with sustained release to treat the inflammatory reaction after implantation.

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

4. Conclusions

In conclusion, novel multifunctional MBG scaffolds with a hierarchical pore architecture and well-ordered mesopores were successfully prepared using the method of 3-D printing combined with utilization of PVA as a binder. The scaffolds obtained possess a high compressive strength, 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 a second sintering) for inorganic biodegradable scaffold materials. 3-D printed MBG scaffolds possess excellent apatite mineralization ability and sustained drug delivery properties. These significant advantages concerning architecture, mechanical strength, bioactivity and the capability to act as drug carriers suggest that 3-D printed MBG scaffolds may be an excellent candidate for bone regeneration.

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Appendix A. Figures with essential colour discrimination

Certain figures in this article, particularly Figures 1, 2, and 4, are difficult to interpret in black and white. The full colour images can be found in the on-line version, at doi:10.1016/j.actbio.2011.03.009.

References


