

Chapter 13

Bioactive Scaffolds with Multifunctional Properties for Hard Tissue Regenerations

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Abstract The impact of bone/dental diseases and trauma in the whole world has increased significantly in the past decades. It is of great importance to develop bioactive scaffolds with multifunctional properties, such as osteogenesis, angiogenesis, cementogenesis, drug delivery and antibacterial property for hard tissue regeneration. Conventional bioactive scaffolds cannot efficiently combine these functions. A new class of bioactive glass, referred to as mesoporous bioactive glass (MBG), was developed several years ago, which possesses highly ordered mesoporous channel structure and high-specific surface area. Due to their special nanostructure, MBG scaffolds show multifunctional potential for hard tissue regeneration application. In this chapter, we review the recent research advances of multifunctional MBG scaffolds, including the preparation of different forms of MBG scaffolds, osteogenesis, angiogenesis, cementogenesis, drug delivery and antibacterial property. The future perspective of MBG scaffolds was further discussed for hard tissue regeneration application by harnessing their special multifunction.

Keywords Bioactive scaffolds • Mesoporous bioactive glass • Multifunction • Osteogenesis • Angiogenesis

13.1 Introduction

The treatment of hard tissue defects, especially large bone and periodontal defects resulting from trauma, infection, tumour or genetic malformation, represents a major challenge for clinicians [1]. To solve these problems, bioactive porous

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scaffolds have been widely studied to regenerate the lost/damaged hard tissues [2]. For better regeneration of large hard tissue defects, bioactive scaffolds should possess not only osteoconductivity (for guidance of new bone growth) but also the ability to stimulate both osteogenesis (for promoting new bone formation) and angiogenesis (for inducing vascularisation) [1, 3–5]. In addition, in bone reconstruction surgery, osteomyelitis caused by bacterial infection is an ever-present and serious complication. Conventional treatments include systemic antibiotic administration, surgical debridement, wound drainage and implant removal [6]. These approaches, however, are rather inefficient and may result in additional surgical interventions for the patients.

A new approach for solving this problem is to introduce local drug release system into the implant site. The advantages of this treatment include high delivery efficiency, continuous action, reduced toxicity and convenience to the patients [6, 7]. For this reason, bioactive scaffolds with an in-built drug delivery and antibacterial property would be very useful for bone and periodontal tissue regeneration and could solve the risk of osteomyelitis incidences caused by infection of the bone. Therefore, ideal bioactive porous scaffolds for the treatment of large bone and periodontal defects should possess multiple functions by combining angiogenesis, osteostimulation and drug delivery with antibacterial properties. However, to our best knowledge, few bioactive porous scaffolds possess such ‘real’ multifunctional properties [8].

Conventional bioactive calcium phosphate (Ca-P)-based bioceramic scaffolds, such as hydroxyapatite (HAp) and β -tricalcium phosphate (β -TCP), possess osteoconductivity, but lack osteostimulation and drug delivery function due to few nanopores in the sintered scaffolds treated by high temperature. Recent study has shown that the sintered Ca-P-based bioceramics, especially HAp, lack full biodegradability after implantation [9, 10]. Although β -TCP ceramics have been regarded as biodegradable materials, their degradation kinetics tends to be slow [11].

Bioactive glasses have played an increasingly important role in bone tissue regeneration applications by virtue of their generally excellent osteoconductivity, osteostimulation and degradation rate [1, 12–16]. Typically, the melt bioactive glass, called 45S5 Bioglass[®], was pioneered by Hench [17, 18] and was first developed using traditional melt method at high temperature (1300–1500 °C). The 45S5 Bioglass[®] has been regarded as bioactive bone regeneration materials which are able to bond closely with the host bone tissue [17]. Further studies have also showed that the Ca and Si containing ionic products released from the 45S5 contribute to its bioactivity, as both Ca and Si are found to stimulate osteoblast proliferation and differentiation [19–24]. Xynos et al. further found that 45S5 Bioglass[®] is able to enhance the expression of a potent osteoblast mitogenic growth factor, insulin-like growth factor-2 (IGF-2) [23, 25]. However, the 45S5 Bioglass[®] has a number of limitations [25]. One of them is the fact that it needs to be melted at a very high temperature (>1300 °C), and the other is its lack of microporous structure inside the materials with low specific surface area; therefore, the bioactivity of melt bioactive glasses will mainly depend on the contents of SiO₂

[25]. Generally, the bioactivity of melt bioactive glasses will decrease with the increase in the SiO_2 contents [1, 18, 26]. In the early 1990s, in an effort to overcome the limitation of melt bioactive glasses, Li et al. prepared sol-gel bioactive glasses [27]. Although sol-gel bioactive glasses have better compositional range and bioactivity than melt bioactive glasses, the micropore distribution is not uniform and inadequate for efficient drug loading and release [25, 28, 29].

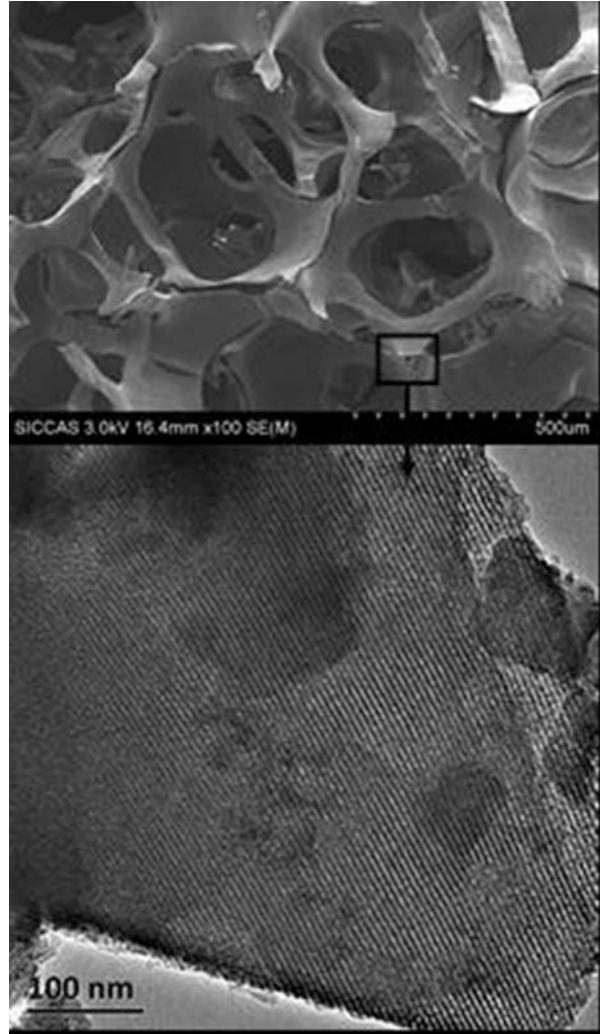
To overcome the limitations of conventional bioactive glasses (without well-ordered mesopore structures for drug delivery), it is of great importance to design and develop a new class of biomaterials which combine multifunctional properties, such as osteogenesis, angiogenesis, drug delivery and antibacterial characteristics. Yu et al., for the first time, prepared the mesoporous bioactive glass (MBG) particles in 2004 by the combination of sol-gel method and supramolecular chemistry of surfactants [30, 31]. Their study has paved a new avenue for applying nanotechnology to regenerative medicine by coupling drug delivery with bioactive materials. Their materials are based on a $\text{CaO-SiO}_2\text{-P}_2\text{O}_5$ composition and have a highly ordered mesopore channel structure with a nanoscale pore size ranging from 5 to 20 nm. Compared to conventional non-mesopore bioactive glasses (NBG), the MBG possesses a more optimal surface area, pore volume, ability to induce in vitro apatite mineralisation in SBF and excellent cytocompatibility [31–35]. However, for bone and periodontal tissue regeneration, MBG particles are not always ideal. It is of great interest and importance to develop MBG scaffolds with multifunctional properties. Therefore, in this chapter, we review the recent advances of MBG scaffolds, as multifunctional materials for hard tissue regeneration.

13.2 Preparation and Characterisation of Multifunctional MBG Scaffolds

MBG scaffolds could be prepared by three approaches [35]. The first is the porogen method, in which Yun et al. applied methyl cellulose as the porogen to prepare porous MBG scaffolds with large pore size of 100 μm and mesopore size of around 5 nm; however, the prepared large pores are not fairly uniform and interconnective though the mesoporous channels are well ordered [36].

The second is the polymer template method, which has been widely used. We, for the first time, prepared the MBG scaffolds with a large pore size of 400 μm by using polyurethane sponge as a porous template [37]. At the same time, Li et al. also prepared the MBG scaffolds using the same technique [38]. After that, we have developed a series of MBG scaffolds with varying compositions for drug delivery and bone tissue engineering applications as shown in Fig. 13.1 [7, 39–41]. The advantages of the MBG scaffolds prepared by the polyurethane sponge template method are their highly interconnective pore structure and controllable pore size (porosity), while the disadvantage is the low mechanical strength of the material

Fig. 13.1 SEM and TEM analysis for the MBG scaffolds prepared by spongy template method. SEM (*upper image*) showed highly interconnective porous scaffolds with large pore size of 300–500 μm ; TEM (*lower image*) showed that the large pore walls of scaffolds had well-ordered mesoporous channel structure (around 5 nm)



[42]. The compressive strength of the MBG scaffolds prepared by the polymer template method is lower than 200 kPa.

To better control the pore morphology, pore size, porosity and mechanical strength, 3D plotting technique (also called additive fabrication, direct writing or printing) has been developed to prepare porous MBG scaffolds. 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 [43–45]. Yun and Garcia, et al. prepared the hierarchical 3D porous MBG scaffolds using a combination of double polymer template and rapid prototyping techniques [46, 47]. In their study, they mixed the MBG gel with methylcellulose and then printed, sintered

at 500–700 °C to remove polymer templates for obtaining the MBG scaffolds. The main limitation of their method for preparing MBG scaffolds is the need of methylcellulose and the additional sintering procedure. Although the obtained MBG scaffolds have uniform pore structure, their mechanical strength is compromised because of the incorporation of methylcellulose which results in some micropores. Recently, we reported a new facile method to prepare hierarchical and multifunctional MBG scaffolds with controllable pore architecture, excellent mechanical strength and mineralisation ability for bone regeneration application by a modified 3D printing technique using polyvinyl alcohol (PVA) as a binder as shown in Fig. 13.2. The 3D-printed MBG scaffolds obtained possess a high mechanical strength which is about 200 times of that of the MBG scaffolds prepared using traditional polyurethane foam as templates. The compressive strength could reach 16 MPa. Such scaffolds have highly controllable pore

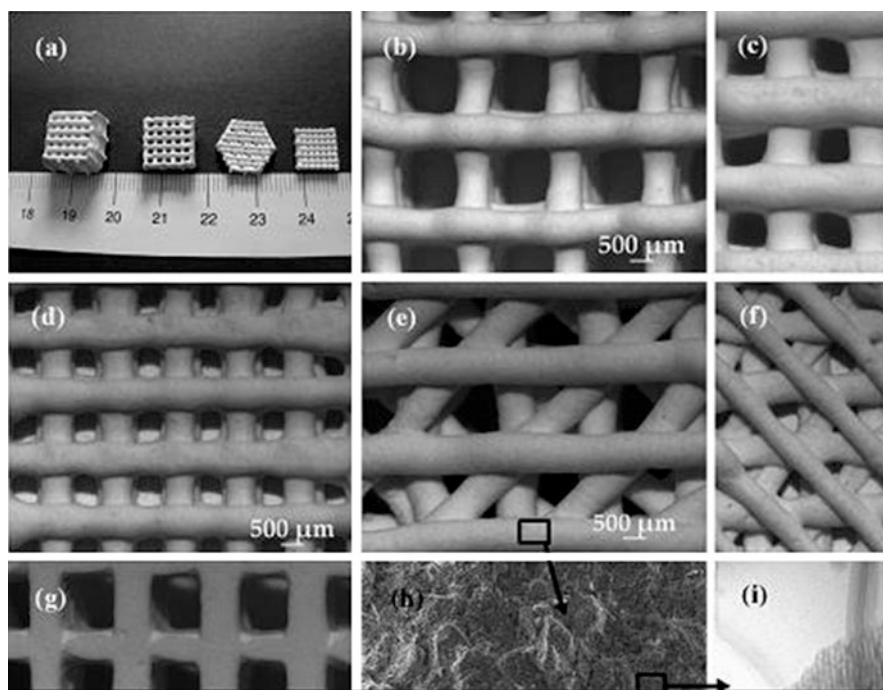


Fig. 13.2 MBG scaffolds, pore morphology and microstructure. (a) MBG scaffolds, obtained by the 3D printing method with different sizes, shapes and morphologies. (b, c and d) MBG scaffolds with different pore sizes (varying from $1307 \pm 40 \mu\text{m}$ (b) and $1001 \pm 48 \mu\text{m}$ (c) to $624 \pm 40 \mu\text{m}$ (d)). (d, e and f) MBG scaffolds with different pore morphologies. (g) Pore morphology of the bottom side for MBG scaffolds. The pores on the bottom side keep 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 scaffolds; the size of mesopore channel is about 5 nm (Reprinted with permission from Ref. [48] copyright 2011 by Elsevier)

Table 13.1 The mesoporous structure characteristics of MBG scaffolds

| Scaffold compositions | Surface area (m ² /g) | Pore volume (cm ³ /g) | Pore size (nm) | References |
|---|----------------------------------|----------------------------------|----------------|------------|
| CaO-P ₂ O ₅ -SiO ₂ | 350 | 0.33 | 4.8 | [37] |
| CuO-CaO-P ₂ O ₅ -SiO ₂ | 310–330 | 0.36–0.44 | 3.8–4.7 | [8] |
| CoO-CaO-P ₂ O ₅ -SiO ₂ | 127–180 | 0.15–0.19 | 4.1–4.5 | [49] |
| SrO-CaO-P ₂ O ₅ -SiO ₂ | 88–200 | 0.11–0.27 | 5.0 | [50] |
| MgO-CaO-P ₂ O ₅ -SiO ₂ | 269 | 0.34 | 3.6 | [51] |
| B ₂ O ₃ -CaO-P ₂ O ₅ -SiO ₂ | 194–234 | 0.21–0.24 | 5.1–5.3 | [41] |
| Fe ₂ O ₃ -CaO-P ₂ O ₅ -SiO ₂ | 268 | – | 4.5 | [40] |
| ZrO ₂ -CaO-P ₂ O ₅ -SiO ₂ | 227–287 | 0.27–0.32 | 3.4–4.1 | [7] |

architecture, excellent apatite mineralisation ability and sustained drug delivery property [35, 48].

Currently, the prepared MBG scaffolds are mainly based on the compositions of CaO-P₂O₅-SiO₂-MxOy (M: Cu, Co, Zr, Zn, Sr and B) system. The incorporation of functional elements of Cu, Co, Zr, Zn, Sr or B decreased the surface area and mesopore volume of MBG scaffolds. However, they still maintain high mesoporous level, and their surface area and mesopore volume are generally in the range of 200–400 m²/g and 0.2–0.4 cm³/g, respectively. The well-ordered mesopore size of the prepared MBG scaffolds is in the range of 4–5.5 nm. The typical structural characteristics of the prepared MBG scaffolds with varied compositions are shown in Table 13.1.

13.3 The In Vitro and In Vivo Osteogenesis, Angiogenesis and Cementogenesis of Multifunctional MBG Scaffolds

In the past 5 years, we have systematically investigated the in vitro and in vivo osteogenesis, angiogenesis and cementogenesis of the MBG scaffolds incorporated with varied functional elements. It was found that these functional elements in the MBG scaffolds play an important role in improving their multifunctional properties. The functional effects of different elements in MBG scaffolds are summarised in Table 13.2.

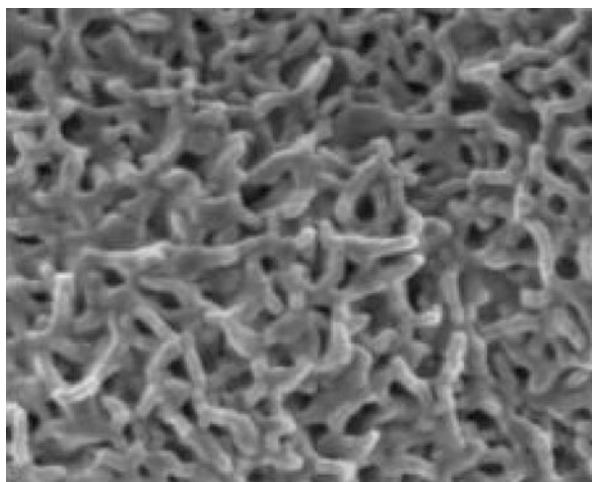
13.3.1 The Osteogenesis of MBG Scaffolds

It was found that the MBG scaffolds possess excellent apatite mineralisation ability as shown in Fig. 13.3, which was regarded one of the critical factors to contribute to

Table 13.2 The multifunctional properties the MBG scaffolds with varied compositions

| Scaffold compositions | Multifunctional properties |
|---|---|
| CaO-P ₂ O ₅ -SiO ₂ | Osteogenesis, drug delivery |
| MgO-CaO-P ₂ O ₅ -SiO ₂ | |
| B ₂ O ₃ -CaO-P ₂ O ₅ -SiO ₂ | |
| ZrO ₂ -CaO-P ₂ O ₅ -SiO ₂ | |
| CoO-CaO-P ₂ O ₅ -SiO ₂ | Osteogenesis, angiogenesis, drug delivery |
| CuO-CaO-P ₂ O ₅ -SiO ₂ | |
| Fe ₂ O ₃ -CaO-P ₂ O ₅ -SiO ₂ | Osteogenesis, hypothermia, drug delivery |
| SrO-CaO-P ₂ O ₅ -SiO ₂ | Osteogenesis, cementogenesis, drug delivery |
| Li ₂ O-CaO-P ₂ O ₅ -SiO ₂ | |

Fig. 13.3 The MBG scaffolds possess excellent apatite mineralisation ability in the simulated body fluids



the *in vivo* bioactivity of bone regeneration materials. Compared with non-mesoporous bioactive glass (NBG) scaffolds, the MBG scaffolds have significantly improved apatite mineralisation ability in simulated body fluids attributable to their high surface area and mesoporous volume [35]. In this regard, Garcia et al. studied the mechanism of apatite mineralisation of MBG by using nuclear magnetic resonance spectroscopy [52]. The significant difference of the apatite formation mechanism between MBG and conventional NBG is that MBG does not require the typical ‘first three stages’ [52], but conventional NBG does [26]. In the first three stages, conventional NBG releases M⁺ ions and form Si-OH groups, and then Si-OH groups form networks by repolymerisation. However, the surface of MBG is already inherently ‘prepared’ to accelerate the first three stages of the conventional NBG [52].

In addition to the excellent apatite mineralisation ability in the simulated body fluids, the MBG scaffolds possess excellent *in vitro* osteogenesis, which is evidenced by the attachment, proliferation and differentiation of bone-forming cells in the scaffolds as shown in Fig. 13.4. The incorporation of Mg, Sr, B, Fe

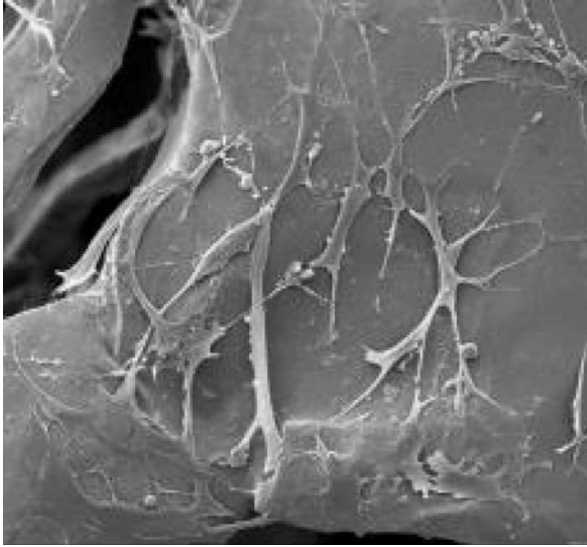


Fig. 13.4 BMSCs growing in MBG scaffolds

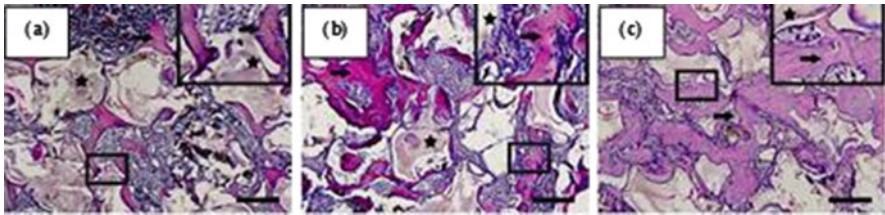


Fig. 13.5 The in vivo osteogenesis of the Sr-containing MBG scaffolds after implanted for 8 weeks. (a) MBG, (b) 2.5 % Sr-MBG and (c) 5 % Sr-MBG

and Zr significantly enhanced the cell proliferation and bone-related gene expression of osteoblasts or bone marrow stromal cells (BMSCs) [41, 42, 51].

Critical-sized femur defects in ovariectomised rats were created to simulate an osteoporotic phenotype. After implanted for 8 weeks, the results showed that the MBG scaffolds induced new bone formation in the osteoporotic bone defects, and the incorporation of Sr into the MBG scaffolds significantly stimulated new bone formation in the osteoporotic bone defects, indicating that the MBG scaffolds possess excellent in vivo osteogenesis (see Fig. 13.5).

13.3.2 The Angiogenesis of MBG Scaffolds

It is known that angiogenesis of porous scaffolds is of great importance to stimulate the tissue formation. Some studies have suggested that insufficient level of oxygen,

a condition known as hypoxia, plays a critical role in cell recruitment, cell differentiation and vessel formation, linking osteogenesis closely to angiogenesis [53–56]. Hypoxia-inducible factor-1 (HIF-1), a transcriptional factor consisting of α - and β -subunits (HIF-1 α and HIF-1 β), has already been identified as one of the critical proteins directly reacting to hypoxia [57]. Under a hypoxic condition, HIF-1 α binds to HIF-1 β and initiates the transcription of hypoxia-sensitive genes that include vascular endothelial growth factor (VEGF) among others [58, 59]. Hypoxia can be artificially mimicked by stabilising HIF-1 α expression and has been suggested as a potential strategy to promote neovascularisation [60–62].

To prepare the hypoxia-mimicking MBG scaffolds, we have incorporated Cu or Co into the MBG scaffolds to induce the pro-angiogenesis of BMSCs. The results showed that incorporation of chemical Cu (Fig. 13.6) and Co (Fig. 13.7) ions into MBG scaffolds is a viable way to inducing hypoxia effect on the BMSCs. Both Cu-MBG scaffolds and their ionic extracts could stimulate hypoxia-inducible factor (HIF)-1 α and vascular endothelial growth factor (VEGF) expression in human bone marrow stromal cells (hBMSCs). In addition, both Cu-MBG scaffolds and their ionic extracts significantly promoted the osteogenic differentiation of hBMSCs by improving their bone-related gene expression (alkaline phosphatase (ALP), osteopontin (OPN) and osteocalcin (OCN)) [8]. Similarly, low amounts of Co (<5%) incorporated into the MBG scaffolds had no significant cytotoxicity, and their incorporation significantly enhanced VEGF protein secretion, HIF-1 α expression and bone-related gene expression in BMSCs, and also the Co-MBG scaffolds support BMSC attachment and proliferation [49]. The study suggested that parts of Cu or Co in MBG scaffolds significantly improved both the angiogenesis and osteogenesis of BMSCs with a multifunctional effect for bone tissue engineering.

13.3.3 The Cementogenesis of MBG Scaffolds

In addition to the osteogenesis and angiogenesis of MBG scaffolds in vitro and in vivo, Sr- or Li-containing MBG scaffolds significantly stimulated the proliferation and bone-/cementum-related gene expression of periodontal ligament cells. Sr plays an important role in influencing the mesoporous structure of MBG scaffolds in which high contents of Sr decreased the well-ordered mesopores as well as their surface area/pore volume. Sr²⁺ ions could be released from Sr-MBG scaffolds in a controlled way. The incorporation of Sr into MBG scaffolds has significantly stimulated alkaline phosphatase (ALP) activity and osteogenesis-/cementogenesis-related gene expression (ALP, Runx2, Col I, OPN and CEMP1) of PDLCs. Furthermore, the Sr-MBG scaffolds in simulated body fluid environment still maintain their excellent apatite mineralisation ability. The study suggested that the incorporation of Sr into MBG scaffolds is a viable way to stimulate the biological response of PDLCs [50].

The Li-MBG scaffolds with hierarchically large pores (300–500 μ m) and well-ordered mesopores (5 nm) were also successfully prepared by incorporating Li⁺ ions into the scaffolds. We further investigated the cell proliferation and

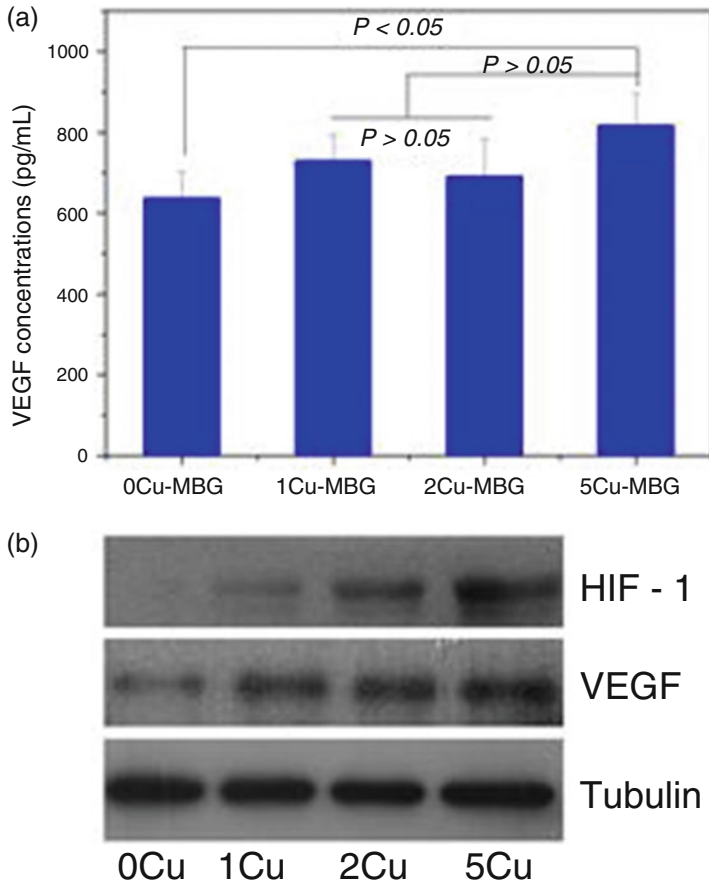


Fig. 13.6 VEGF secretion by ELISA (a) and HIF-1 α and VEGF expression by western blotting (b) for hBMSCs on Cu-MBG scaffolds (Reprinted with permission from Ref. [8] copyright 2013 by Elsevier)

cementogenic differentiation, including Wnt- and Shh-related gene expression of hPDLCs cultured with the Li-MBG scaffolds and Li⁺ ion-containing medium. It was found that the incorporation of 5% Li⁺ into MBG scaffolds significantly enhanced cell proliferation and cementogenic differentiation, as well as activation of Wnt and Shh signaling pathways in hPDLCs (see Fig. 13.8). Li⁺ by itself was sufficient to promote the cell proliferation, differentiation and cementogenic-related gene expression in hPDLCs. These results suggested that the Li⁺ ions released from such bioactive MBG scaffolds play an important role in enhancing cementogenesis of PDLCs on bioactive scaffolds and this biological reaction may be via activation of Wnt and Shh signaling pathways [63].

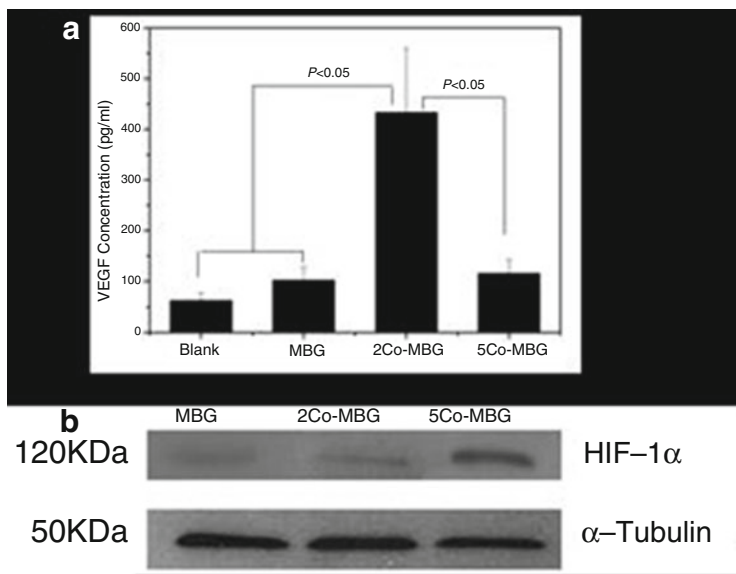


Fig. 13.7 VEGF secretion by ELISA (a) and HIF-1 α expression by western blotting (b) for hBMSCs on Co-MBG scaffolds (Reprinted with permission from Ref. [49] copyright 2012 by Elsevier)

13.4 The Drug Delivery, Antibacterial Property and Tissue Stimulation of Multifunctional MBG Scaffolds

One of the significant advantages for MBG scaffolds is that they possess higher specific surface area and pore volume than conventional bioactive glasses. The loading efficiency of drug and growth factors in MBG is significantly higher than that in conventional bioactive glasses [64, 65]. The drug release kinetics in MBG is lower than that in conventional bioactive glasses. These characteristics make MBG useful for drug delivery. In the past 5 years, the MBG scaffolds with varied compositions have been used for the study of drug delivery [25, 35]. Up to now, the MBG scaffolds have been used for delivery of gentamicin [7, 51, 65], ampicillin [49], dexamethasone [41], dimethylallyl glycine (DMOG) and vascular endothelial growth factor (VEGF) [66]. The studies have indicated that the controllable delivery of ampicillin in the MBG scaffolds significantly inhibited the viability of bacteria (see Fig. 13.9).

Dexamethasone was loaded into MBG scaffolds, and it was found that the MBG scaffolds could efficiently load dexamethasone and release it in a controllable way. The sustained release of dexamethasone from the MBG scaffolds significantly enhanced alkaline phosphatase activity and gene expressions (Col I, Runx2, ALP and BSP) of osteoblasts in the scaffolds (see Fig. 13.10), suggesting that dexamethasone-loaded MBG scaffolds show great potential as a release system to

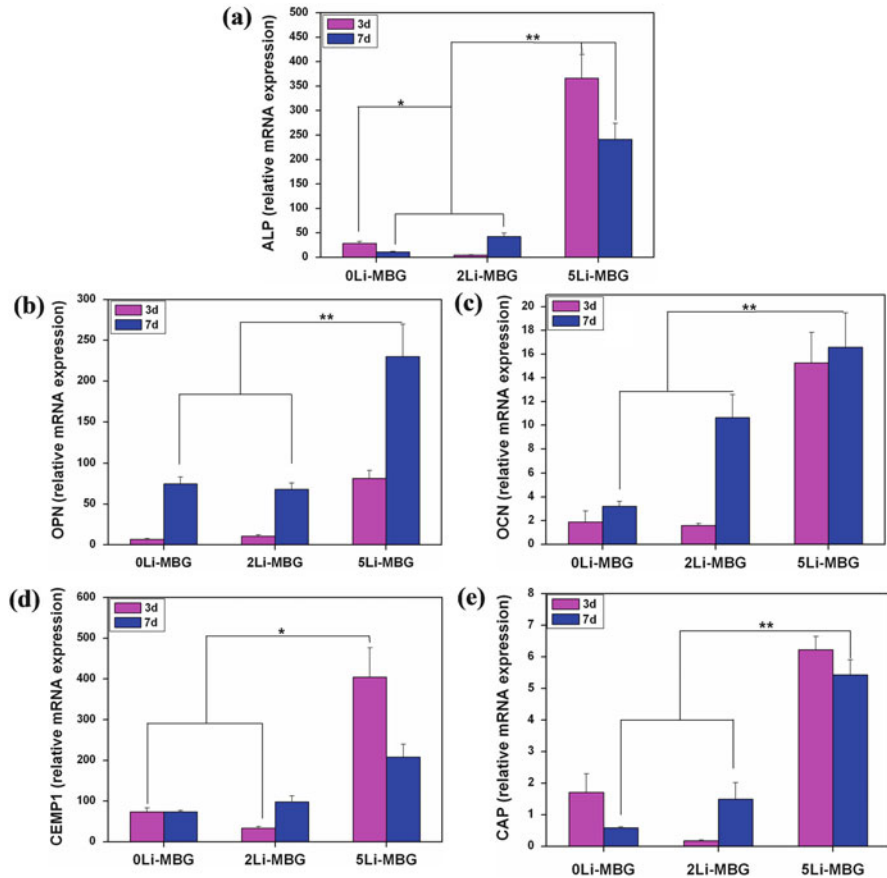


Fig. 13.8 The effect of Li contents in MBG scaffolds on bone-related gene expression of ALP (a), OPN (b), OCN (c) and cementum-specific markers of CEMP1 (d) and CAP (e) for hPDLs (Reprinted with permission from Ref. [63] copyright 2012 by Elsevier)

enhance osteogenesis and may be used for bone tissue engineering applications in the future [35, 41].

Recently, we have loaded DMOG in MBG scaffolds. The results showed that the loading and release of DMOG in the MBG scaffolds can be efficiently controlled by regulating their mesoporous properties via the addition of different contents of mesopore-template agent. DMOG delivery in the MBG scaffolds had no cytotoxic effect on the viability of hBMSCs. DMOG delivery significantly induced HIF-1 α stabilisation, VEGF secretion and bone-related gene expression of hBMSCs in the MBG scaffolds. Furthermore, it was found that the MBG scaffolds with slow DMOG release significantly enhanced the expression of bone-related genes than those with instant DMOG release. The results suggested that the controllable delivery of DMOG in the MBG scaffolds can mimic a hypoxic microenvironment,

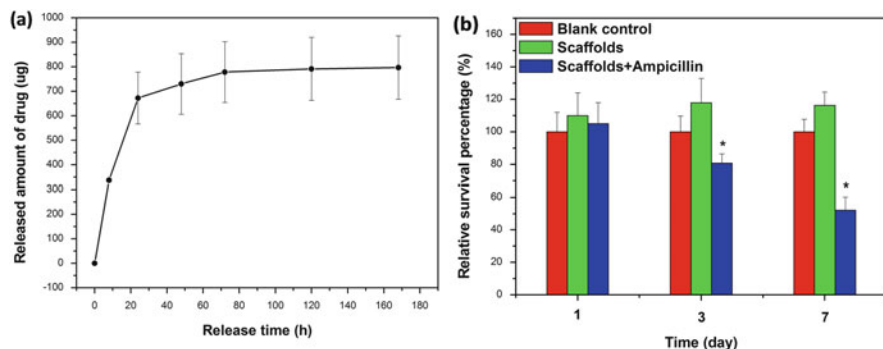


Fig. 13.9 Sustained release of ampicillin from the MBG scaffolds (a) and its antibacterial effect (b). *significant difference for the group of scaffolds loaded with ampicillin, compared to blank control and scaffolds groups not loaded with ampicillin ($P < 0.05$) (Reprinted with permission from Ref. [49] copyright 2012 by Elsevier)

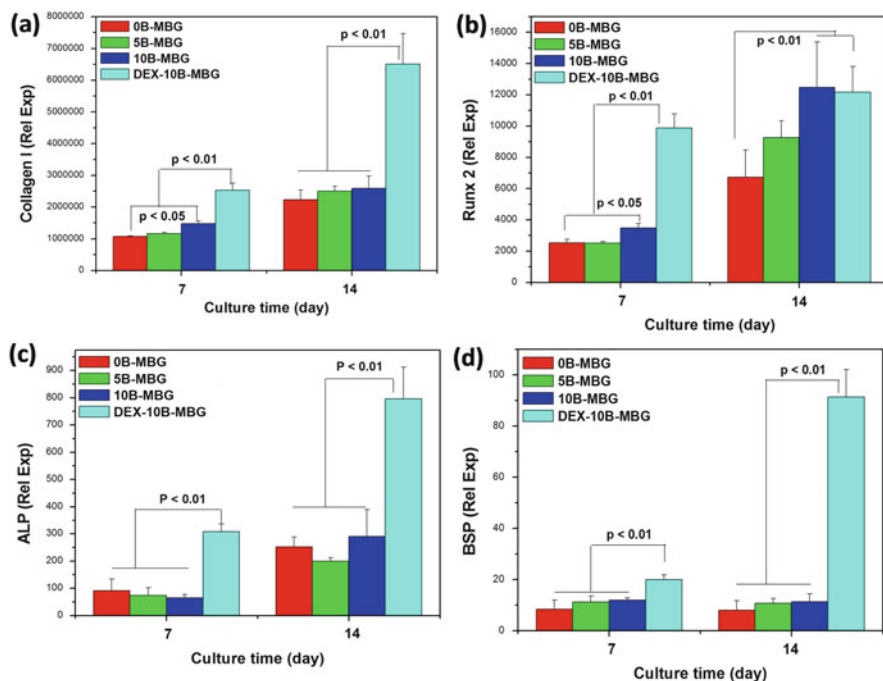


Fig. 13.10 The bone-related gene expression of Collagen I (a), Runx 2 (b), ALP (c) and BSP (d) for osteoblasts by RT-qPCR. The incorporation of 10% of B into MBG scaffolds enhanced the expression of Collagen I (at day 7) and Runx 2 (at day 7 and 14). DEX-loaded B-MBG scaffolds significantly enhanced the expression of Collagen I, Runx2, ALP and BSP of osteoblasts, compared to non-DEX-loaded B-MBG scaffolds (Reprinted with permission from Ref. [41] copyright 2011 by Elsevier)

which not only improves the angiogenic capacity of hBMSCs but also enhances their osteogenic differentiation.

In addition to the drug delivery, MBG scaffolds could be used for the delivery of vascular endothelial growth factor (VEGF). It was found that tMBG scaffolds have significantly higher loading efficiency and more sustained release of VEGF than non-mesoporous bioactive glass (NBG) scaffolds; and VEGF delivery from the MBG scaffolds improved the viability of endothelial cells. The study suggested that the mesopore structures in the MBG scaffolds play an important role in improving the loading efficiency, decrease the burst effect and maintain the bioactivity of VEGF, indicating that MBG scaffolds are an excellent carrier of VEGF for potential bone tissue engineering application [35, 66].

13.5 Conclusions and Perspective

In this chapter, we reviewed the recent research advances of multifunctional MBG scaffolds for hard tissue regeneration. Three preparation methods, characterisation, *in vitro/in vivo* osteogenesis, angiogenesis, cementogenesis, drug delivery and the corresponding functional effect on antibacterial, tissue stimulation have been reviewed. The MBG scaffolds allow combining the multifunctional properties as outlined above by varying their chemical compositions and delivering different drug and growth factors. The multifunctional properties were regarded to be of great importance to the improvement of the bone/periodontal regeneration and anti-infection ability. Therefore, multifunctional MBG scaffolds showed great potential for hard tissue regeneration. However, up to now, most of the studies have been focused on the *in vitro* experiments, and only few *in vivo* studies have been conducted to confirm their osteogenesis. Other functions of MBG scaffolds, such as angiogenesis, cementogenesis and antibacterial properties, should be further confirmed *in vivo* by using large animal models.

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