Magnetic mesoporous bioactive glass scaffolds with hierarchical pore structure and multifunction

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Abstract: Hyperthermia and local drug delivery have been proposed the potential therapeutic approaches for bone defects resulting from malignant bone tumors. Development of bioactive materials with magnetic and drug-delivery properties may potentially meet this target. The aim of this study is to develop a multifunctional mesoporous bioactive glass (MBG) scaffold system for both hyperthermia and local-drug delivery application potentially. For this aim, Iron (Fe) containing MBG (Fe-MBG) scaffolds with hierarchically large pores (300-500 μm) and fingerprint-like mesopores (4.5 nm) have been successfully prepared. The effect of Fe on the mesopore structure, physiochemical, magnetism, drug delivery and biological properties of MBG scaffolds has been systematically investigated. The results showed that the morphology of the mesopore varied from straight channels to curved fingerprint-like channels after incorporated parts of Fe into MBG scaffolds. The magnetism magnitude of MBG scaffolds can be tailored by controlling Fe contents. Furthermore, the incorporating of Fe into mesoporous MBG glass scaffolds enhanced the mitochondrial activity and bone-relative gene (ALP and OCN) expression of human bone marrow mesenchymal stem cells (BMSCs) on the scaffolds. The obtained Fe-MBG scaffolds also possessed high specific surface areas and sustained drug delivery. Therefore, Fe-MBG scaffolds are magnetic,

degradable and bioactive. The multifunction of Fe-MBG scaffolds indicates that there is a great potential for Fe-MBG scaffolds to be used for the therapy and regeneration of large-bone defects caused by malignant bone tumors through the combination of hyperthermia, local drug delivery and their osteoconductivity.

Key words: Magnetic scaffolds; Mesoporous bioactive glasses; Drug delivery; Hyperthermia

1. Introduction

Malignant bone tumors are some of the main non-trauma related factors resulting in critical sized bone loss/defects. The treatment of such bone diseases typically proceed in two stages: the diseased bone tissue is first surgically removed, resulting in the bone defect, which is then remedied with a bone graft material [1]. Although such implantations in most cases result in the successful repair of the defect, the treatment of malignant bone disease and regeneration of the large bone defects still represents a significant clinical challenge. Hyperthermia therapy by means of biocompatible magnetic materials has emerged as a potential treatment option against malignant tumors and a number of recent studies have reported the development of such materials, for example, magnetic Ca-P ceramics[2,3], bioactive glass-ceramics[4,5] and composites [6,7]. These biomaterials were designed to be magnetic which, when exposed to a magnetic field, produce heat within the diseased tissue region; cancer cells are destroyed at temperatures higher than 43^{o}C, whereas the normal cells can survive these temperatures [1,5,8]. Since blood vessels and the nervous system are poorly developed in the diseased tissues they tend to be heated more than the surrounding normal tissues. Hyperthermia treatment is therefore considered an effective treatment option for malignant bone disease without adverse side effects [9]. Another method that has come under consideration in treating malignant bone disease is the targeted delivery of drugs through biomaterials, in which the drug will be efficiently released at the sites of bone disease from loaded biomaterials to either eradicate the disease or stimulate bone healing [10-12]. Methods such as these are, to some extent,

efficient for the therapy of malignant bone disease but a “one-size-fits-all” method of hyperthermia or drug-delivery therapy is far from ideal, since a combination of adjunctive treatments modalities, for example, radio and chemo therapies are often required [13]; such therapies, however, are nearly always accompanied by side effects. Hyperthermia treatment is used for killing the local cancer cells around bone defects; however, the limitation of this method is that if some cancer cells have migrated to a far location from local bone defects, hyperthermia treatment to local bone defects may not kill those cancer cells migrated from bone defects. Drug-delivery treatment is able to kill cancer cells in the whole body system; however, the limitation of drug-delivery treatment is that the side effects will be accompanied, in which normal tissue cells will be killed if the too much drug dose is used. Furthermore, how to maintain a sustained drug-delivery from biomaterials is another challenging issue. We therefore propose a new concept for the treatment of bone disease and bone regeneration defects: a bioactive material that combines hyperthermia and local drug delivery. This new concept has the potential to overcome the shortcomings of the simple therapy method; it will combine the physical (hyperthermia) and chemical (drug delivery) modalities via a multifunctional scaffold. To satisfy these requirements, the scaffolds have to be biodegradable and bioactive (for regenerative bone defects); magnetic (for hyperthermia); and possess a special nanostructure with sustained drug-delivery capacity (for drug therapy). As far as we know there are no traditional scaffolds suitable for bone tissue regeneration that meet these requirements.

Mesoporous bioglass (MBG), as a new bioactive material, has attracted significant attention in recent years [14-18]. A significant feature of MBG, compared with non-mesopore bioglass (NBG), is that it possesses a significantly improved surface area and nanopore volume which is evident by greatly enhanced bioactivity and degradation. We have recently demonstrated that the MBG has improved *in vitro* and *in vivo* bioactivity, degradation and drug delivery properties, compared to NBG [19,20]. MBG is a very promising bioactive material with respect to bone regeneration and drug delivery; however, MBG is not magnetic. Iron (Fe) plays a vital role in the functioning of the body and the total Fe pool in humans is found in the red blood cell mass, to a lesser extant in the

tissues and a small amount circulating in the plasma [21]. We hypothesized that incorporating ferrous ions into MBG would make it magnetic and, at the same time, influence its mesopore structure, physiochemical and biological properties. To our best knowledge, there are no reports detailing the development of magnetic MBG scaffolds for bone tissue repair, nor are there any systematic studies of how Fe influences cellular response to MBG scaffolds. Therefore, the aims of this study were to develop a multifunctional scaffold system which could potentially be used for hyperthermia and drug-delivery therapy of malignant bone diseases. For this purpose, Fe-containing MBG scaffolds were prepared and the effect of Fe on the mesopore structure, physiochemical and biological properties of Fe-MBG scaffolds was systematically investigated.

2. Materials and Methods

2.1 Preparation of porous Fe-MBG scaffolds

Porous Fe-containing mesopore-bioglass (Fe-MBG) scaffolds were prepared by incorporating parts of Fe (5 and 10%) into MBG using co-templates of nonionic block polymer P123 (EO20-PO70-EO20) (Sigma Aldrich, Germany) and polyurethane sponges. P123 is used to produce mesoporous structures (mesopore size: several nanometers) and polyurethane sponges are used to create large pores (large pore size: several hundred micrometers) as described in our previous publications [20]. To prepare MBG scaffolds containing of 10% Fe, typically, 6.67 g of P123 (Mw=5800, Sigma Aldrich, Germany), 11.17 g of tetraethyl orthosilicate (TEOS, 98%, Sigma Aldrich, Germany), 0.78 g of $Ca(NO_3)_2 \cdot 4H_2O$ (Sigma Aldrich, Germany), 1.12 g of $FeCl_3$ (Sigma Aldrich, Germany), 1.22 g of triethyl phosphate (TEP, 99.8%, Sigma Aldrich, Germany) and 1.67 g of 0.5 M HCl were dissolved in 100 g of ethanol (Fe/Ca/P/Si/ = 10/5/5/80, molar ratio, named 10Fe-MBG) and stirred at room temperature for 1 day. The polyurethane sponges (25ppi) were cleaned and completely immersed into this solution for 10 min, then transferred to a Petri dish; excess solution was removed and the remainder allowed evaporating at room temperature for 24 h. This procedure was repeated for 5 times. Once the samples were completely dry, they were calcined at 700^{o}C for 5 h yielding the

10Fe-MBG scaffolds. MBG scaffolds without Fe (named: 0Fe-MBG) and with 5% Fe (named: 5Fe-MBG) were prepared by the the same method except for their Fe content. The chemical composition and the amounts of reagents for 0Fe-MBG, 5Fe-MBG and 10Fe-MBG scaffolds are listed in Table 1.

2.2 Characterization of Fe-MBG scaffolds

The phase composition, surface morphology, and inner microstructure of the calcined Fe-MBG scaffolds were analyzed by wide-angle X-ray diffraction (XRD), small-angle XRD, transmission electron microscopy (TEM) and scanning electron microscopy (SEM) and energy dispersive spectrometer (EDS). TEM of the samples was performed using a FEI Tecnai 10 electron microscope (FEI Company, Eindhoven, NL) with a LaB_6-source at 100 kV acceleration voltage. Images were recorded with a Tietz slow scan CCD F224HD TVIPS camera (2k x 2k pixels, pixel size 24 μm, digitization 16 bit) with an active area of 49 mm × 49 mm. (Tietz Video and Image Processing Systems GmbH, Gauting, Germany). Brunauer-Emmett-Teller and Barret-Joyner-Halenda analyses were used to determine the specific surface area, the nano pore size distribution and the pore volume by N_2 adsorption-desorption isotherms.

The porosity of the prepared scaffolds was measured using Archimedes' principle: scaffolds with a size of 6×6×6 mm were used for the measurement and water was used as liquid medium. The porosity (P) was calculated according to the following formulation $P = (W_2-W_1)/(W_2-W_3) \times 100\%$, where W_1 is the dry weight of the scaffolds, W_2 is the weight of scaffolds saturated with water, and W_3 is the weight of scaffolds suspended in water.

2.3 The mechanical strength and magnetic property of Fe-MBG scaffolds

The compressive strength of 10×10×10 mm sized scaffolds were tested using an Instron 5566 computer-controlled universal testing machine (Instron Wolpert, Darmstadt, Germany) at a crosshead speed of 0.5 mm/min.

To investigate the effect of Fe on the magnetic property, the obtained scaffolds were crushed, ground and sieved through 300 meshes to obtain powder samples. Magnetic measurements were carried out with a vibrating-sample magnetometer (LakeShore VSM-7401) at room temperature.

2.4 Ion release and mineralization of Fe-MBG scaffolds

To investigate the ion release and mineralization of Fe-MBG scaffolds, simulated body fluids (SBF) were prepared according to Kokubo [22]. Fe-MBG scaffolds were soaked in SBF at 37°C for 1, 2 and 3 d, and the ratio of the solution volume to the scaffold mass was 200 mL/g. The concentrations of Fe^{2+} (or Fe^{3+}), SiO_4^{4-}, Ca^{2+} and PO_4^{3-} ions in the SBF were determined by atomic emission spectrometry (Perkin-Elmer Optima 7000DV). After soaking, the surface morphology and composition of Fe-MBG scaffolds were determined by SEM and EDS.

2.5 Drug loading and release from Fe-MBG scaffolds

Dexamethasone (DEX, Sigma Aldrich, Germany) served as a model drug and was dissolved in ethanol to a concentration of 0.5 mg/mL. 20 mg of Fe-MBG scaffolds (4 × 4 × 4 mm) were added to 5 mL of DEX/ethanol solution and soaked for 24 h at room temperature. Fe-MBG scaffolds loaded with DEX were obtained after drying at 40°C for 24h.

DEX release was evaluated by placing one DEX-loaded Fe-MBG scaffolds into 4 mL of PBS (pH 7.4) at 37 ºC for 3, 9, 24, 48, 96, 168 and 240 h. DEX release was determined by UV analysis (UV min-1240, Shimadzu, Japan). Then, Fe-MBG scaffolds were crushed and transferred into 5 mL of PBS. The DEX was solubilized by ultrasonic shaking for 30 min and then the solution was centrifuged and assayed by UV spectrometry at 240 nm. This was repeated 3 times to ensure the complete dissolution of the DEX into the PBS. The total amount of DEX loaded in the scaffolds was the sum of the total release at 240 h and the final release by three readings after ultrasonic shaking. 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%.

2.6 Morphology and mitochondrial activity of BMSCs on Fe-MBG scaffolds

Isolation and culture of BMSCs was conducted following previously published protocols [23]. Bone marrow aspirates were obtained from patients (mean age, 65 years) undergoing elective knee and hip replacement surgery. Informed consent was given by all patients involved and the research protocol had been approved by the Human Ethics Committees of Queensland University of Technology and The Prince Charles Hospital.

BMSCs were cultured on 4×4×4 mm scaffolds placed in 96-well culture plates, at an initial density of 1×10^5 cells/scaffold. The cells were cultured for 1, 3 and 7 days in DMEM culture medium (GIBCO) supplemented with 10% FCS, after which the scaffolds were removed from the culture wells, rinsed in PBS, and then fixed with 1.25% glutaraldehyde, 4% paraformaldehyde, and 4% sucrose in PBS for 1 hr. The fixative was removed by washing with buffer containing 4% (w/v) sucrose in PBS and post fixed in 1% osmium tetroxide in PBS followed by CO_2 critical-point drying. The specimens were coated with gold and the morphological characteristics of the attached cells determined using SEM.

To assess cell mitochondrial activity, an MTT assay was performed [24] by adding 0.5 mg/mL of MTT solution (Sigma-Aldrich, Australia) to each scaffold and incubated 37ºC to form formazan crystals. After 4 h, the media was removed and the formazan solubilized with dimethyl sulfoxide (DMSO). The absorbance of the formazan-DMSO solution was read at 495 nm on a plate reader. Results were expressed as the absorbance reading from each well minus the optical density value of blank wells.

2.7 Reserve transcription and real-time quantitative RT-PCR analysis

The osteogenic differentiation of BMSCs on Fe-MBG scaffolds was further assessed by real-time quantitative RT-PCR (RT-qPCR) to measure the mRNA expression of ALP and osteocalcin (OCN). Scaffolds were transferred into 24-well plastic culture plates and a total of 10^6 BMSCs were placed onto each scaffold. The cells were incubated at 37ºC in 5% CO_2 for 7 days and the medium changed every 3 days. On day 7, the samples were removed and total RNA isolated using Trizol Reagent® (Invitrogen) according to the manufacturer's instructions. Complementary DNA was synthesized

from 1 μg of total RNA using SuperScript III reverse transcriptase (Invitrogen) following the manufacturer's instructions. RT-qPCR was performed in 25 μL reaction volume containing 12.5 μL 2X SYBR Green Master Mix (Roche, Castle Hill, NSW, Australia), 2.5 μL each of 10 μM forward and reverse primers, 2.5 μL of cDNA template diluted 1:10, and 5 μL of RNase free water. All samples were performed in triplicates and the house keeping gene, 18s rRNA, was used as a control. The reaction was carried out using ABI Prism 7000 Sequence Detection System (Applied Biosystems). Melting curve analysis was performed to validate specific amplicon amplification without genomic DNA contamination. Relative expression levels for each gene were normalized against the Ct value of the house keeping gene and determined by using the delta Ct method.

2.8 Statistical analysis

All data were expressed as means ± standard deviation (SD) and were analyzed using One-Way ANOVA with a Post Hoc test. A p-value < 0.05 was considered statistically significant.

3. Results

3.1. Characterization, mechanical strength and magnetic property of Fe-MBG scaffolds

Small-angle and Wide-angle XRD patterns for the MBG scaffolds with different Fe contents are shown in Figure 1. There is an obvious diffraction peak around 2θ 1.25 degree for 0Fe-MBG, 5Fe-MBG and 10Fe-MBG scaffolds (Fig. 1a). Wide-angle XRD analysis showed that none of the three Fe-MBG species had sharp diffraction peaks (Fig. 1b). Their diffraction patterns had a wide SiO_2 peak with low intensity.

All three Fe-MBG scaffold species had a highly porous structure with a similar large pore size ranging from 300 to 500 μm (Fig. 2a, c and e). EDS analysis showed the main composition of three species. The ratio of Fe/Ca/Si was 0/16/79, 4.7/8.4/79, and 9.4/5.8/79 for the 0Fe-MBG, 5Fe-MBG and 10Fe-MBG scaffolds, respectively (Fig. 2b, d and f). The porosity of open pores is 81.6±2.6; 83.3±4.6; and 82.9±2.9 for 0Fe-MBG, 5Fe-MBG and 10Fe-MBG, respectively.

TEM analysis revealed 0Fe-MBG scaffolds to have a well-ordered mesoporous structures with straight channel (pore size: about 5nm) (Fig. 2a). When 5 and 10% Fe was incorporated, curved channels with fingerprint-like mesopores could be seen (Fig. 3b and c). The 5Fe-MBG scaffolds maintained a well-ordered hexagonal structure with a mesopore size of 5 nm (Fig. 3b). Although the 10Fe-MBG scaffolds also had a mesoporeous channel structure, the order of the mesopores seems appeared more random (Fig. 3c). The results of N_2 adsorption–desorption analysis of 10Fe-MBG scaffolds showed a type of IV isotherm pattern and pore distribution at the 4.5 nm, a typical characteristic of a mesoporous structure (Fig. 3d). BET analysis showed that the specific surface area of 10Fe-MBG scaffolds was 268 m^2/g.

Incorporating Fe into the MBG scaffolds did not influence their mechanical strength. The compressive strength is 50±4.7; 48±2.5 and 46±5.4kPa for the 0Fe-MBG, 5Fe-MBG and 10Fe-MBG scaffolds, respectively.

The magnetization curves of the MBG scaffolds are shown in Figure 4. With the increase of Fe content, the magnetic magnitude of scaffolds increased. 10Fe-MBG scaffolds had a more obvious hysterisis loop than the other two scaffolds.

3.2. Ion release and mineralization of Fe-MBG scaffolds

Generally, with the increase of soaking time, the released SiO_4^{4-} and Ca^{2+} ions increased; however, the concentrations of PO_4^{3-} ions in SBF decreased (Fig. 5). The incorporation of Fe did not change the release profile of SiO_4^{4-} ions (Fig. 5a). However, due to Ca being replaced of by Fe, the release of Ca^{2+} ions decreased inversely with the increase of Fe contents in MBG scaffolds (Fig. 5b). Interestingly, as the amount of Fe content increased, the concentrations of PO_4^{3-} ions in the SBF decrease (Fig. 5c), the pH of SBF on the other hand fell (Fig. 5d). The detection limit of ICP-AES is 0.143 ppm; however, the Fe ions were not detectible, which indicates that the released Fe from 5Fe-MBG and 10Fe-MBG scaffolds were below the detection limit.

SEM analysis showed that all three scaffold species had a smooth surface before being soaked in SBF (Fig. 6a, c and e); however, after soaking, some microparticles were deposited on the surface

of the scaffolds due to the mineralization in SBF (Fig. 6b, d and f). The morphologies of these microparticles differed significantly between the three scaffold species. Lath-like crystals, ranging in size from 2–5 μm were formed on 0Fe-MBG scaffolds (Fig. 6b). On the 5Fe-MBG scaffolds there was a deposition of flake-like particles (Fig. 6d) whereas on the 10Fe-MBG scaffolds very fine nanosized microparticles were deposited (Fig. 6f). EDS analysis (the inset in Fig. 6b, d and f) shows that the ratio of Ca/P for 0Fe-MBG, 5Fe-MBG and 10Fe-MBG is 1.70, 1.77 and 1.92, respectively.

3.3. Drug release from Fe-MBG scaffolds

All three MBG scaffolds species had a sustained release of DEX (Fig. 7). Incorporation of Fe into MBG scaffolds did not change the release kinetics of DEX; however, the Fe-containing MBG scaffolds still maintained a sustained release even after 240 h (The total release is around 80%).

3.4. Attachment, mitochondrial activity and bone-relative gene expression of BMSCs on Fe-MBG scaffolds

BMSC attachment and morphology on the three scaffolds species were examined by SEM (Fig. 8). After 7 days in culture, BMSCs spread on the pore walls of the scaffolds. Cells had a close contact with the substrates by numerous filopodia. The cells grew well on all three scaffolds species (Fig. 9). There was no obvious difference in the mitochondrial activity between days 1 and 3; however, by day 7, there was greater cell mitochondrial activity on the 5Fe-MBG and 10Fe-MBG scaffolds compared to the 0Fe-MBG scaffolds ($p<0.05$) (Fig. 9). The incorporation of 5% of Fe into MBG significantly enhanced the bone-related gene expression of OCN and ALP ($p<0.05$) (Fig. 10).

4. Discussion

We have successfully prepared magnetic MBG scaffolds with hierarchically large pores and fingerprint-like mesopores, and investigated what effects Fe have on mesopore structure, physiochemistry, magnetism, drug delivery and biological properties. Incorporating Fe into

mesoporous MBG glass scaffolds made them magnetic, as was expected and also significantly improved cell mitochondrial activity and bone-related gene expression of BMSCs on the scaffolds. The Fe-MBG the scaffolds were biodegradable, bioactive, magnetic, and at the same time capable of sustained drug delivery. The multifunction of Fe-MBG scaffolds suggests that they can be used for the therapy of malignant bone disease by a combination of hyperthermia and local drug delivery, and for the regeneration of bone defects by virtue of the material's excellent osteoconductivity (See Figure 11. the potential application of Fe-MBG scaffolds), in which the magnetic property of bioactive MBG scaffolds when exposed to a magnetic field**,** will contribute to the production of the required heat and temperature to kill cancer cells in bone defects.

We have been able for the first time to prepare magnetic MBG scaffolds using a polymer sponge method. This is a conventional approach used to prepare porous ceramic scaffolds, and is particularly useful since it allows for a simple way with which to prepare highly interconnective pore structure in the scaffolds [25,26]. Our data shows that Fe-MBG scaffolds produced by this method had high porosity of 83% with large pore sizes ranging from 300 to 500 μm. The porous properties of these scaffolds greatly benefit tissue ingrowths and nutrient transportation [27]. In addition, the incorporation of Fe into MBG scaffolds did not influence their overall mechanical strength, although the mechanical strength of Fe-MBG scaffolds could be described as frail. The scaffolds were, however, able to maintain their structural integrety when handled for the in vitro cell biological experiments. In terms of bone tissue engineering applications, the main role of scaffolds is to provide initial mechanical support for cells, meaning that they must be strong enough to be handled for this work. In this respect the Fe-MBG scaffolds were able to meet the requirements as a cell carrier for bone tissue engineering. Our previous studies have shown that the mechanical strength of mesoporous bioglass scaffolds could be significantly improved by the modification of silk fibrin [23] or by 3D-printing preparation method [28]. Therefore, it is believed that the mechanical strength of Fe-MBG scaffolds could be further improved by using two methods described above.

Mesoporous structure of biomaterials is important for the loading and delivery of drugs [18,28]. Our previous study has shown that the surface area of MBG without Fe is around 350 m^2/g [20]. It is generally thought that a surface area of 200 to 350 m^2/g with a mesopore size of 3 to 5 nm would provide the right environment to adsorb a range of biomolecules, such as drugs, antibiotics, and growth factors [29]. 10Fe-MBG scaffolds have high surface area for 268 m^2/g, which still falls well within the optimum surface area range mentioned above, which still falls well within the optimum surface area range mentioned above. The mesopore size was not affected be the incorporation of Fe, being approximately 4.5 nm, although it was interesting to note that the incorporation of Fe into the MBG scaffolds seemed to affect the shape of the mesopores from straight channels to twirled fingerprint-like channels. These results suggest that the replacement of Ca^{2+} with Fe^{3+} may disrupt the ordered orientation of SiO_4^{4-} during the self-assembly reaction. The likely reason for this may be that valence difference between Ca^{2+} by Fe^{3+} cause defects of atomic array which manifests itself by altering the shape of the mesopore channels and surface area. Although the incorporation of 10% of Fe into MBG scaffolds partially destroyed the ordered mesoporous structure; however, they still have a high specific surface area, indicating they are capable for adsorbing drugs and subsequently maintain a sustained release. Earlier studies have incorporated Fe into traditional glass-ceramic and Ca-P ceramic bulks (not scaffolds) to make them magnetic, but these materials are unsuitable for drug delivery due to the absence of nanopore structure [2,4,6]. Our results show that the Fe-MBG scaffolds are well suited for topical drug delivery to bone defect areas and potentially also the therapy of malignant bone disease.

The magnetic biomaterials are of great value for hyperthermia treatment. In this study, it was possible to produce magnetism for MBG scaffolds with ferromagnetic properties by replacing Ca^{2+} with Fe^{3+}; in addition to this, the magnetic magnitude of MBG scaffolds can be tailored by altering Fe contents, and this is very useful means to control and produce temperature needed to destroy diseased tissue cells by the process of hyperthermia. The hyperthermia temperature mainly depends on the magnetic magnitude of materials and external magnetic field. The temperature to kill bone

cancer cells is around 43 ^{o}C. It is believed that we can adjust the Fe contents (for different magnetic magnitude) of MBG scaffolds and external magnetic field to reach this temperature. Therefore, Fe-MBG scaffolds can combine the hyperthermia therapy and local-drug delivery to treat bone disease, which may be a more efficient way to improve healing ability compared to a single-therapy method. The rate of degradation and bioactivity of Fe-MBG scaffolds is an important aspect of their role in regeneration of bone defects. Fe-MBG scaffolds contain 80% of SiO_2 and we found that the incorporation of Fe into MBG did not influence the release of SiO_4^{4-} from scaffolds in SBF (Fig. 5). It is recognized that rates of dissolution play an important role in the degradation of biomaterials[30]; the Fe-MBG scaffolds we therefore predict will have good degradation properties. Osteoblast growth and differentiation is influenced by Ca-P mineralization on the surface of biomaterials which affect their *in vivo* bone-forming ability [22,30-32]. Although the incorporation of Fe into MBG modified the morphology of the mineralized Ca-P particles on the surface of scaffolds in SBF, it did not suppress the Ca-P mineralization, which may indicate that they are bioactive according to Kokubo's views [22]. The ionic environment, including ionic concentration and pH value of SBF, plays an important role to influence the morphology of apatite on bioactive materials. In this study, the incorporation of Fe into MBG by replacing parts of Ca results in the decrease of Ca^{2+} release and pH value in SBF (see Figure 5b and d), which may influence the morphology of the formed apatite on three scaffolds [33,34]. The ratio of Ca/P for 10Fe-MBG scaffolds after soaking in SBF is 1.92, which is much higher, compared to that of hydroxyapatite (1.67). The reason is that the formed Ca-P layer on 10Fe-MBG scaffolds is quite thin (see Figure 6f). When EDS testing was carried out, some Ca and Si signals were also detected from the substrate of 10Fe-MBG scaffolds themselves, which led to a higher ratio of Ca/P for the formed Ca-P microparticles. Similar phenomenon was observed for 5Fe-MBG scaffolds (Fig. 6d). However, for 0Fe-MBG scaffolds, there is a thick Ca-P layer. The ratio of Ca/P for the apatite on 0Fe-MBG scaffolds is about 1.70, which is much close that of hydroxyapatite (1.67).

To further investigate the *in vitro* bioactivity of Fe-MBG scaffolds, the attachment, mitochondrial activity and differentiation of BMSCs on the scaffolds were investigated. Our study has found that no cytotoxicity was observed in the Fe-MBG scaffolds as cell mitochondrial activity was observed from day 1 to day 7 with significantly increased cell number in the higher Fe scaffold (5Fe-MBG and 10Fe-MBG) compared with 0Fe-MBG scaffold. Furthermore, 5% of Fe incorporated into MBG significantly improved both mitochondrial activity and bone-related gene expression (ALP and OCN), which indicates that a proper amount of Fe will not bring potential cytotoxicity, on the contrary, it will improve the osteoconductivity of MBG scaffolds. In this study, the incorporation of Fe into MBG did not influence the large-pore structure of scaffolds, but modified the chemical composition and further modified the ionic environment of cell culture medium, including the ionic composition and pH value. Therefore, it is speculated that the ionic composition and pH value are the direct factors to improve cell viability and differentiation. Previously, Meng et al. found that the Fe_2O_3-added poly-lactide/HAp composite film significantly enhanced the proliferation, differentiation and extracellular matrix secretion of the osteoblast cells [35]. Parsons also found that the Fe contents play an important role to influence the response of osteoblasts [36]. In our study, it is speculated that trace amount of Fe^{3+} released from scaffolds may contribute to the effect of improved cell response. In addition, pH plays an important role for the cell response [37,38]. In this study, Fe was incorporated into MBG scaffolds by replacing parts of Ca. The replacing of Ca^{2+} by Fe^{3+} led to a decrease of Ca^{2+} release, which therefore decreased the pH value of biological solution (See Figure 5b and d). It can be seen that MBG scaffolds without Fe induced a high pH value (8.0 after 3 days) in biological solution, which is higher than the normal physiological level (pH: 7.4). However, after incorporating of parts of Fe into MBG scaffolds, Fe-MBG scaffolds induced a closer pH value to 7.4. Therefore, the beneficial pH environment for Fe-MBG scaffolds may be the other important factor to benefit the mitochondrial activity and gene expression of BMSCs. Although the incorporation of Fe into MBG scaffolds decreased the order mesopore structure, they still have a high surface area compared to traditional non-mesoporous bioglass. From our current results of

magnetic magnitude and cell culture, it is speculated that the optimal Fe contents in Fe-MBG scaffolds should be between 5 and 10%. Further study will be carried out by evaluating the effect of Fe contents in MBG scaffolds on hyperthermia function.

5. Conclusions

Magnetic MBG scaffolds with high porosity, hierarchically large pores, fingerprint-like mesopores and multifunction have been successfully prepared. The incorporation of parts of Fe into MBG scaffolds plays an important role to influence the mesopore structure, and enhance the magnetic property and the mitochondrial activity and gene expression of BMSCs. The prepared Fe-MBG scaffolds are magnetic, degradable, bioactive, and at the same time possess a sustained drug delivery, which indicates that they have potential for the regeneration of bone defects from malignant bone disease by the combination of hyperthermia and local-drug delivery therapy. Further study will be conducted to investigate how the magnetism-resulted heat and drug delivery of Fe-MBG scaffolds influences the behavior of cancer cells, and the *in vivo* osteogenesis.

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