Hypoxia-mimicking mesoporous bioactive glass scaffolds with controllable cobalt ion release for bone tissue engineering

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Abstract

Low oxygen pressure (hypoxia) plays an important role in stimulating angiogenesis; there are, however, few studies to prepare hypoxia-mimicking tissue engineering scaffolds. Mesoporous bioactive glass (MBG) has been developed as scaffolds with excellent osteogenic properties for bone regeneration. Ionic cobalt (Co) is established as a chemical inducer of hypoxia-inducible factor (HIF)-1α, which induces hypoxia-like response. The aim of this study was to develop hypoxia-mimicking MBG scaffolds by incorporating ionic Co^{2+} into MBG scaffolds and investigate if the addition of Co^{2+} ions would induce a cellular hypoxic response in such a tissue engineering scaffold system. The composition, microstructure and mesopore properties (specific surface area, nano-pore volume and nano-pore distribution) of Co-containing MBG (Co-MBG) scaffolds were characterized and the cellular effects of Co on the proliferation, differentiation, vascular endothelial growth factor (VEGF) secretion, HIF-1α expression and bone-related gene expression of human bone marrow stromal cells (BMSCs) in MBG scaffolds were systematically investigated. The results showed that low amounts of Co (< 5%) incorporated into MBG scaffolds had no significant cytotoxicity and that their incorporation significantly enhanced VEGF protein secretion, HIF-1α expression, and bone-related gene expression in BMSCs, and also that the Co-MBG scaffolds support BMSC attachment and proliferation. The scaffolds maintain a well-ordered mesopore channel structure and high specific surface area and have the capacity to efficiently deliver antibiotics drugs; in fact, the sustained released of ampicillin by Co-MBG scaffolds gives them excellent anti-bacterial properties. Our results indicate that incorporating cobalt ions into MBG scaffolds is a viable option for preparing hypoxia-mimicking tissue engineering scaffolds and significantly enhanced hypoxia function. The hypoxia-mimicking MBG scaffolds have great

potential for bone tissue engineering applications by combining enhanced angiogenesis with already existing osteogenic properties.

Key words: hypoxia; mesoporous bioactive glass; bone tissue engineering; VEGF secretion, HIF-1α expression

1. Introduction

The treatment of many bone defects, especially large bone defects due to trauma, infections, tumors or genetic malformations, represents a major challenge for clinicians [1,2]. Autologous bone grafting is considered the most effective treatment; in practice, however, this approach is limited by insufficient amount of donor tissue, coupled with donor site morbidity. Allogeneic or xenogeneic bone grafts, on the other hand, have obvious clinical limitations due to immunological reactions in the host recipient. Bone tissue engineering approaches has come into focus as an alternative source for bone regeneration [3-5] as a substitute for bone grafts in order to repair defects and restore normal function [6]. This approach consists of applying a supportive matrix (a scaffold) to support osteogenic cells and bioactive molecules for bone reconstruction. A critical problem, implicit in using this approach, is that the nutrient supply and cell viability, at the centre of the scaffold, is severely hampered since the diffusion distance of nutrients and oxygen for cell survival is limited to 150–200μm [7]. Indeed, studies have shown that tissue engineered products formed tissue layers no thicker than 5 mm on the scaffold surface, and that in the centre of the scaffold, cell density tends to be low and necrosis may occur [7].

Low oxygen pressure (hypoxia) *in vivo* plays a pivotal role in coupling angiogenesis with

osteogenesis via progenitor cell recruitment, differentiation and angiogenesis [8-11]. Hypoxia activates a series of angiogneic processes mediated by the hypoxia inducing factor-1a (HIF-1α) transcription factor. HIF-1α initiates the expression of a number of genes associated with tissue regeneration and skeletal tissue development and has been shown to enhanced fracture repair. Hypoxia can the mimicked artificially by stabilizing HIF-1α expression , such as by the application of Co^{2+} ions, and has been suggested as a potential strategy to promote neovascularization [8,12,13]. Co is an essential element in human physiology and an integral part of B12, a vitamine the human body is unable to manufacture. Ionic Co^{2+} is known to chemically induce HIF-1α to promote a hypoxia-like response. Cells adapt to hypoxia by expressing a number of genes that are related to angiogenesis, mobility, and glucose metabolism, all via the HIF-1α pathway [14].

Mesoporous bioactive glass (MBG) has attracted significant attention for bone tissue engineering in the past several years [15-19]. Compared with non-mesopore bioactive glass (NBG) MBG has significantly improved specific surface area and nanopore volume, which is evidenced by greatly enhanced *in vitro* bioactivity and degradation [15,20-22] As a bioactive material, MBG has great potential for bone tissue engineering and drug delivery applications [23-25]. Cobalt ions are a well-established chemical inducer of HIF-1α which elicits a hypoxia-like response. It is expected that Co ions released from scaffolds could inactivate HIF-specific prolyl hydroxylase and consequently stabilize HIF-1α in a normoxic environment [26]. We hypothesized that MBG scaffolds with controllable Co ion release could mimic hypoxic condition and induce the coupling of osteogenesis and angiogenesis, which would be of great interest for applications in bone tissue engineering. Previously, although Azevedo, *et al*. prepared Co-containing bioactive glasses particles by high temperature melt method, however, they did not investigate whether Co ion release from

biomaterials could induce a hypoxia function [27]. To our best knowledge, no previous studies have prepared hypoxia-mimicking tissue engineering scaffolds. Therefore, the aims of this study were to prepare Co-containing MBG scaffolds and investigate whether the addition of Co could induce a hypoxia function in tissue engineering scaffold system. For this aim, the effect of Co on the physicochemical property of MBG scaffolds, and the proliferation, differentiation, VEGF secretion, HIF-1α expression, and bone-related gene expression of BMSCs in the scaffolds were systematically studied. The drug-delivery properties and anti-bacterial functions of the manufactured Co-MBG scaffolds are also investigated with respect to bone tissue engineering applications.

2. Materials and Methods

2.1. Preparation and characterization of porous Co-MBG scaffolds

Porous cobalt-containing mesopore-bioglass (Co-MBG) scaffolds were prepared by incorporating Co (molar: 2 and 5%) into MBG to replace parts of calcium (Ca) using co-templates of nonionic block polymer P123 (EO20-PO70-EO20) 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 [28,29]. To prepare MBG scaffolds containing of 2% cobalt, typically, 12 g of P123 (Mw=5800, Aldrich), 20.1 g of tetraethyl orthosilicate (TEOS, 98%), 3.64 g of $Ca(NO_3)_2 \cdot 4H_2O$, 0.31 g of $CoCl_2$ (Aldrich), 2.19 g of triethyl phosphate (TEP, 99.8%) and 3 g of 0.5 M HCl were dissolved in 180 g of ethanol (Co/Ca/P/Si/ = 2/13/5/80, molar ratio, named 2Co-MBG) and stirred at room temperature for 1 day. The polyurethane sponges (20ppi) were cleaned and completely

immersed into this solution for 10 min, then transferred to a Petri dish to allow evaporating at room temperature for 12 h. This procedure was repeated for 3 times. Once the samples were completely dry, they were calcined at 700^{o}C for 5 h yielding the 2Co-MBG scaffolds. MBG scaffolds without cobalt (Co/Ca/P/Si/ = 0/15/5/80, molar ratio, named: MBG) and with 5% cobalt (Co/Ca/P/Si/ = 5/10/5/80, molar ratio named: 5Co-MBG) were prepared by the same method except for their Co and Ca contents.

The large-pore structure, surface morphology, and inner microstructure of the calcined Co-MBG scaffolds were characterized by scanning electron microscopy (SEM), energy dispersive spectrometer (EDS), small-angle X-ray diffraction (XRD) and transmission electron microscopy (TEM). Brunauer-Emmett-Teller (BET) and Barret-Joyner-Halenda (BJH) analyses were used to determine the specific surface area, the nano-pore size distribution and the nano-pore volume by N_2 adsorption-desorption isotherms.

2.2. Ion release of Co-MBG scaffolds in DMEM

To investigate the ion release and mineralization of Co-MBG scaffolds, Co-MBG scaffolds (5×5×5 mm) were soaked in Dulbecco's Modified Eagle's Medium (DMEM) at 37°C for 1, 3 and 7d, and the ratio of the solution volume to the scaffold mass was 200 mL/g. The concentrations of Co^{2+}, SiO_4^{4-}, Ca^{2+} and PO_4^{3-} ions in the DMEM were determined by inductive coupled plasma atomic emission spectrometry (ICP-AES).

2.3. Morphology and proliferation of BMSCs on Co-MBG scaffolds

Isolation and culture of BMSCs were conducted following previously published protocols [30,31].

Bone marrow aspirates were obtained from patients (mean age, 60 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 5×5×5 mm scaffolds placed in 96-well culture plates, at an initial density of 1×10^5 cells/scaffold. The cells were cultured for 1 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 2.5% glutaraldehyde 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. Then the cells were dehydrated in a graded ethanol series (50, 70, 90, 95 and 100%) and hexamethyldisilizane (HMDS). The specimens were coated with gold and the morphological characteristics of the attached cells determined using SEM.

To assess cell proliferation, an MTT assay was performed by adding 0.5 mg/mL of MTT solution (Sigma-Aldrich) 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.

2.4. Alkaline phosphatase (ALP) activity of BMSCs on Co-MBG scaffolds

Osteogenic differentiation was assessed by measuring a time course of alkaline phosphatase (ALP) activity of BMSCs grown on the various scaffold types. Scaffolds were placed into 24-well plastic culture plates and seeded with $1x10^5$ BMSCs per 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 ALP activity was measured. The scaffolds were irrigated with PBS three times to remove as much residual serum as possible and then 0.5 mL of 0.02% Triton® X-100 were placed on the scaffold sample to dissolve the cells. The solution was transferred into a 1.5 mL tube, and sonicated after which the samples were centrifuged at 14,000 rpm for 15 min at 4°C. The supernatant transferred to fresh 1.5 mL tubes to which 100 µL 1 mol/L Tris-HCl, 20 µL 5 mmol/L $MgCl_2$, and 20 µL 5 mmol/L p-nitrophenyl phosphate was added. After 30 min incubation at 37°C the reaction was stopped by the addition of 50 µL of 1N NaOH. Using p-nitrophenol as a standard, the optical density was measured at 410 nm with a spectrophotometer. The ALP activity was expressed as the changed optical density (OD) value divided by the reaction time and the total protein quantity as measured by the BCA Protein assay kit (Thermo Scientific, Melbourne, Australia).

2.5. VEGF secretion and HIF-1α expression

To measure the VEGF secretion and HIF-1α expression of BMSCs on Co-MBG scaffolds, the scaffolds were transferred into 24-well plastic culture plates and a total of $1x10^6$ 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. The supernatant was collected and the release of VEGF expression was quantified using ELISA assay kits (R&D Systems Inc., Bio-Scientific Pty. Ltd., NSW, Australia) according to the manufacturer's instruction. The test was performed in triplicates and results were expressed as the amount (pg) of VEGF in per µl supernatant.

For direct detection of the HIF-1α protein, western blot analysis was performed. Briefly, after seeded onto scaffold for one week, whole cell lysates were obtained. 10µg protein from each

sample was separated on SDS-PAGE gels. The protein was then transferred onto a nitrocellulose membrane (Pall Corporation, East Hills, NY, USA) and blocked in 5% non-fat milk. The membranes were incubated with primary antibodies against HIF-1α (1:1000, mouse anti-human, Novus Biologicals, Sapphire Bioscience Pty. Ltd., NSW, Australia) and α-tubulin (1:5000, rabbit anti-human, Abcam, Sapphire Bioscience Pty. Ltd., NSW, Australia) overnight at 4 ^{o}C. The membranes were washed three times in TBS-Tween buffer, and then incubated with anti-mouse/rabbit HRP conjugated secondary antibodies at 1:2000 dilutions for 1hr. The protein bands were visualized using the SuperSignal West Femto Maximum Sensitivity Substrate (Thermo Fisher Scientific, VIC, Australia) and exposed on X-ray film (Fujifilm, Stafford, QLD, Australia).

2.6. Reserve transcription and real-time quantitative RT-PCR analysis

The osteogenic differentiation of BMSCs on Co-MBG scaffolds was further assessed by real-time quantitative RT-PCR (RT-qPCR) to measure the mRNA expression of VEGF and osteocalcin (OCN). Scaffolds were transferred into 24-well plastic culture plates and a total of 1x10^{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.

2.7. Antibiotics loading and release

As we have found that 2Co-MBG scaffolds have optional viability and VEGF secretion for BMSCs, 2Co-MBG scaffolds were selected for further drug delivery and *in vitro* anti-bacteria test. 2Co-MBG scaffolds (5×5×5 mm) were soaked in 2mL of 5mg/mL of ampicillin-phosphate buffer saline (PBS) solution at 4°C overnight. Then the scaffolds were taken out. The ampicillin-PBS solution was centrifuged at 10,000 rpm for 10 min and the supernatant were completely removed. The loading amount of ampicillin was determined by UV analysis (at wavelength 230nm) through calculating the difference of ampicillin-PBS concentration before and after loading. For ampicillin releasing test, the collected ampicillin-loaded Co-MBG scaffolds were soaked into 4mL fresh PBS at 37°C for different period of time. At each time point, 2mL of PBS solution was taken out to test the released ampicillin and 2mL fresh PBS was added back. The accumulative release of ampicillin from scaffolds was calculated.

2.8. In vitro anti-bacteria test

To test the ampicillin release from 2Co-MBG scaffolds on the anti-bacteria effect, one ampicillin-loaded scaffold was mixed with 5mL *E.coli* (DH5α)-LB culture media ($3.5\text{-}4.0\times10^4$

bacteria/mL) and maintained at 4°C for 1, 3 and 7 days. Then 10μL of mixture was plated into a 10mm culture dish and incubated at 37°C for 12 h. The bacteria without scaffolds (blank) or mixed with 2Co-MBG scaffolds (without loading with ampicillin) were used as controls. The test was carried out in triplicate for each group and the *E.coli* colonies on each dish were counted for group comparisons.

2.9. 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 of porous Co-MBG scaffolds

SEM analysis showed that the three Co-MBG scaffold types had a highly porous structure with a similarly large-pore size ranging roughly from 300 to 500μm (Fig. 1a, c and e). EDS analysis showed that Co had been incorporated into the scaffolds. There was no characteristic peak of Co in pure the MBG scaffolds (Fig. 1b). The ratio of Co/Ca in the 2Co-MBG and 5Co-MBG scaffolds was 0.14 and 0.49, respectively (Fig. 1d and f). Small-angle XRD patterns for the three Co-MBG scaffolds types are shown in Figure 2. There are obvious diffraction peaks around 2θ 1.25-1.30 degree for the MBG, 2Co-MBG and 5Co-MBG scaffolds.

TEM analysis reveals that both the 2Co-MBG and 5Co-MBG scaffolds have a well-ordered mesoporous channel structure (pore size: around 4.5-5nm) (Fig. 3). The results of N_2 adsorption–desorption analysis of the three Co-MBG scaffold types show a type IV isotherm pattern

(Fig. 4a) and pore distribution in the range of 4-5 nm (Fig. 4b), a characteristic typical of a mesoporous structure. After incorporating 2 and 5% of Co into MBG scaffolds, the specific surface area of MBG scaffolds decreased from 290 to 180 and 127 m^2/g, respectively, and the pore volume decreases from 0.30 to 0.19 and 0.15 cm^3/g, respectively (Table 1). The average mesopore size of 5Co-MBG scaffolds (4.1nm) was observably smaller than that of pure MBG scaffolds (4.97nm).

3.2. Ion release of Co-MBG scaffolds in DMEM

Generally, the release of Co^{2+}, SiO_4^{4-} and Ca^{2+} ions increased commensurate with increased soaking time and with a decrease in the concentration of PO_4^{3-} ions in DMEM (Fig. 5). There is a controlled release profile of Co^{2+} ions, in which high Co-containing MBG scaffolds have a quick release of Co^{2+} ions (Fig. 5a). The incorporation of Co into MBG scaffolds did not significantly change the release profile of SiO_4^{4-} and Ca^{2+} ions (Fig. 5b and c).

3.3. Attachment, morphology, proliferation and ALP activity of BMSCs on Co-MBG scaffolds

BMSCs attachment and morphology on the three Co-MBG scaffold types was examined by SEM (Fig. 6). After 1 and 7 days of culture, BMSCs were attached to the surface of the pore walls in all three scaffolds types (see arrows). Cells have close contact with the scaffolds by numerous filopodia.

MTT analysis shows that cell number increased on the Co-MBG scaffolds with increased culture time (Fig. 7a). The proliferation of BMSCs on 2Co-MBG scaffolds showed no difference with that of pure MBG scaffolds, whereas cell proliferation on 5Co-MBG scaffolds was slightly less than that on 2Co-MBG and pure MBG scaffolds. Overall the cell proliferation on the three scaffold types was significantly lower than that on blank control (cell culture plate) (Fig. 7a), which may be attributed

to the difference of 3D (scaffolds) and 2D (blank control) culture environment. The ALP activity of BMSCs on the three Co-MBG scaffold types showed no obvious differences ($p>0.05$) and was comparable with that of the blank control (Fig. 7b).

3.4. VEGF secretion, bone-relative gene and HIF-1α expression of BMSCs on Co-MBG scaffolds

Incorporating 2% of Co into MBG scaffolds significantly enhances the VEGF secretion of BMSCs at day 7 (Fig. 8). HIF-1α expression of BMSCs in Co-MBG scaffolds also increased with increased Co contents (Fig. 9). Interestingly, both 2Co-MBG and 5Co-MBG scaffolds have significantly enhanced bone-related gene expression of VEGF at day 7, compared to pure MBG scaffolds and the blank control (Fig. 10a). OCN expression of BMSCs on 5Co-MBG scaffolds was upregulated compared to pure MBG scaffolds and the blank controls (Fig. 10b).

3.5. A*ntibiotics release and in vitro anti-bacteria test*

2Co-MBG scaffolds showed a sustained release of ampicillin over the initial 72 h and the release then plateaued (Fig. 11a). Bacterial survival rate fell significantly in the ampicillin loaded scaffolds compared to blank controls and non-ampicillin loaded scaffolds (Fig. 11b).

4. Discussion

In this study, we have successfully prepared hypoxia-mimicking MBG scaffolds with hierarchically large pores (300-500μm) and well-ordered mesopores (5nm) by incorporating Co^{2+} ions into the scaffolds. We further investigated the effects of Co on the proliferation, differentiation, VEGF secretion, HIF-1α expression, and bone-related gene expression of BMSCs in MBG scaffolds. Our

results show that the incorporation of Co^{2+} ions into MBG scaffolds is a viable way to enhance VEGF secretion, HIF-1α expression, and bone-related gene expression of BMSCs; the prepared Co-MBG scaffolds had an obvious hypoxia inducing function. In addition to these properties, the Co-MBG scaffolds had well-ordered mesopore channel structures, making them well-suited to efficiently load and release antibiotics drugs; the sustained release of antibiotics from the scaffolds adds an anti-bacterial feature. These results suggest that these hypoxia-mimicking MBG scaffolds combine angiogenesis with osteogenesis, as well as an anti-bacterial function for bone tissue engineering applications.

Co-MBG scaffolds were prepared by a typical polymer sponge method, in which some fraction of the Ca in the scaffolds was substituted by Co. This resulted in the advantage of the Co-MBG scaffolds having a sustained release of Co^{2+} ions into the liquid medium; the amount released depending on the amount of Co incorporated into the scaffolds in the first place. Previous studies have indicated that cobalt at high concentrations of may cause cell toxicity [32-34]. A controlled ion release system is therefore vital for tissue engineering applications for vascularized bone regeneration. With this in mind, in the current study the Co-MBG scaffold system had to satisfy the requirement that the Co^{2+} ions had to have a controlled release by only loading limited amounts of Co into the scaffolds. Our results have shown that the concentration of the released Co^{2+} ions from 2Co-MBG and 5Co-MBG scaffolds did not exceed 20 ppm, a relatively low concentration. For this reason, we found that the manufactured Co-MBG scaffolds supported the attachment and growth of BMSCs, with no obvious cytotoxicity. It did appear, however, that 5% Co did have the effect of reducing BMSC viability. Incorporating ionic Co into the MBG scaffolds at 2 and 5% did not affect ALP activity of BMSCs; ALP is an early cell differentiation marker. Our results, therefore, suggests

that Co-MBG scaffolds with Co contents less than 5% can support the normal differentiation of BMSCs with no obvious cytotoxic effects.

It was most encouraging that incorporating Co into MBG scaffolds did, in fact, induce a significant hypoxic cascade, including increased VEGF protein secretion, HIF-1α and VEGF gene expression in BMSCs. Anginogenesis is directed by a variety of growth factors in a complex multistep process, in which VEGF has been identified as a key regulator [35,36]. VEGF activates endothelial cells in the surrounding tissue by stimulating their liberation, migration, proliferation, and finally the formation of tubular structures [37]. HIF-1α can initiate the expression of a number of genes associated with tissue regeneration and skeletal tissue development which are activated in fracture repair [13]. The incorporation of ionic Co into MBG scaffolds has scientific merit and is a promising strategy with which to induce the hypoxic cascade to promote neovascularization of scaffolds for bone tissue regeneration. Previous studies have demonstrated that Co^{2+} ions are a chemical inducer of HIF-1α that triggers a hypoxia-like response by causing an oxygen deficient microenvironment [12]. Under conditions of normal oxygen tension (normoxia) HIF-1α is degraded by ubiquitination in the proteasome, although it is constitutively expressed. In hypoxic conditions, HIF-1α is stabilized due to the lack of oxygen, and binds to DNA at a specific recognition of target genes. Although oxygen is present in normoxic conditions, Co ions can inactivate HIF-specific prolyl hydroxylases leading to the stabilization of HIF-1α, therefore, mimicking hypoxia conditions [26]. There are few reports as to whether Co^{2+} ion incorporation into tissue engineering scaffolds is capable of inducing a hypoxic response. Our study does indicate the induction of hypoxic functions is possible by doping Co^{2+} ions into biomaterials. We are of the view that this is an important study as it paves the way to prepare functional biomaterials with improved anginogenesis and

osteogenesis capacity.

In bone reconstruction surgery, osteomyelitis caused by bacterial infection is an everpresent and serious complication. Conventional treatments include systemic antibiotic administration, surgical debridement, wound drainage and implant removal [38]. These approaches are, however, rather inefficient and results in additional surgical interventions for the patient. A new method to solve this problem is to introduce system of 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 [38,39]. A 3D scaffold with an in-built drug delivery property would be very useful for bone tissue regeneration and can solve the risk of osteomyelitis incidences caused by infection of the bone. The incorporation of Co into the MBG scaffolds decreased the specific surface area and nano-pore volume. The likely cause of this may be due to the fact that replacing Ca^{2+} with Co^{2+} may somehow disrupt the ordered orientation of mesopore channels during the self-assembly reaction. However, the manufactured Co-MBG scaffolds still maintained well-ordered mesoporous structures, as well as a high surface area and nanopore volume, all of which are important for the loading and delivery of drugs [31,40,41]. The results from this study suggest that Co-MBG scaffolds are capable of being efficiently loaded with an antibiotics drug and then subsequently deliver that drug topically at the site of implant. Co-MBG scaffolds, therefore, have potential as a local drug delivery system with functional anti-bacterial effect for bone tissue engineering.

5. Conclusions

Hypoxia-mimicking MBG scaffolds with hierarchically large pores (300-500μm) and well-ordered mesopores (5nm) were successfully prepared by substituting parts of the Ca^{2+} ions for Co^{2+} ions in

the scaffolds. The prepared Co-MBG scaffolds significantly enhance VEGF protein secretion, HIF-1α expression, and bone-related gene expression of BMSCs, compared to pure MBG scaffolds. The incorporation of Co^{2+} ions into MBG scaffolds is an efficient way to induce the hypoxic cascade. The mesopore structure of the Co-MBG scaffolds gives them capacity for sustained release of antibiotics drug and, therefore, had considerable anti-bacterial properties. The study confirms that the application of the hypoxia concept to the tissue engineering scaffolds is possible. The hypoxia-mimicking MBG scaffolds have potential uses in bone tissue engineering applications as a result of a combination of improved hypoxia function, excellent osteogenesis and anti-bacteria property.

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