Forsterite–hydroxyapatite composite scaffolds with photothermal antibacterial activity for bone repair

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Abstract: Bone engineering scaffolds with antibacterial activity satisfy the repair of bacterial infected bone defects, which is an expected issue in clinical. In this work, 3D-printed polymer-derived forsterite scaffolds were proposed to be deposited with hydroxyapatite (HA) coating via a hydrothermal treatment, achieving the functions of photothermal-induced antibacterial ability and bioactivity. The results showed that polymer-derived forsterite scaffolds possessed the photothermal antibacterial ability to inhibit *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*) *in vitro*, owing to the photothermal effect of free carbon embedded in the scaffolds. The morphology of HA coating on forsterite scaffolds could be controlled through changing the hydrothermal temperature and the pH value of the reaction solution during hydrothermal treatment. Furthermore, HA coating did not influence the mechanical strength and photothermal effect of the scaffolds, but facilitated the proliferation and osteogenic differentiation of rat bone mesenchymal stem cells (rBMSCs) on scaffolds. Hence, the HA-deposited forsterite scaffolds would be greatly promising for repairing bacterial infected bone defects.

Keywords: forsterite scaffolds; hydroxyapatite (HA) coating; bone repair; photothermal effect; antibacterial activity

1 Introduction

Bone defects caused by the postoperative infections,

injuries, osteoporosis, bone tumors, and other diseases are a serious problem in clinical [1]. Generally, the repair of critical-sized bone defects should be helped with bone repair materials [2]. Among them, silicate-based bioceramics, such as bioglass, calcium silicate, akermanite, and bredigite, have received increasing attention to treat bone defects due to their high bioactivity [3–6]. Forsterite (Mg_2SiO_4) as one type of silicate-based bioceramics also has great potential for load-bearing

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bone repair owing to their biocompatibility, excellent mechanical property, and slow biodegradation behavior [7]. Choudhary *et al.* [8] fabricated cylindrical forsterite scaffolds by molding forsterite powder and sintering, and the compressive strength and Young's modulus of forsterite scaffolds were 201 MPa and 4.8 GPa, respectively. Such mechanical property could match with the cortical bones. Hence, forsterite can be used as a potential material for bone repair in load-bearing areas [9,10].

Recently, the combination of organosilicon polymer-derived ceramics (PDCs) strategy with 3D printing has been demonstrated to fabricate silicate ceramic scaffolds [11]. We also developed to fabricate forsterite scaffolds by 3D printing of silicone resin/MgO precursor and sintering in an inert atmosphere [12]. Such forsterite scaffolds exhibited photothermal-induced antibacterial activity due to the embedded free carbon in the scaffolds [12,13]. However, the relatively poor bioactivity of forsterite would affect its osteogenesis ability [14]. To address this issue, an efficient strategy is developed to modify the surface of forsterite scaffolds with bioactive materials, which could stimulate cell responses of bone mesenchymal stem cells (BMSCs) including cell adhesion, proliferation, and osteogenic differentiation, thereby promoting bone repair [15,16].

HA, one main mineral composition of human bones, possesses the outstanding bioactivity and biocompatibility, which has become one of the most popular bioactive materials to modify the metal implants [17]. Nowadays, some coating techniques, such as electrophoretic deposition and plasma spraying, have been applied to produce uniform HA coatings on the implants and scaffolds [18,19]. Studies demonstrated that the coating morphology and bonding strength between the coating and matrix material are crucial for cell response and *in vivo* bone integration [20].

Recently, hydrothermal method was increasingly developed to fabricate HA coating on the scaffolds for improving the performance of scaffolds [21,22]. Studies demonstrated that hydrothermal method could controllably synthesize HA with different morphologies including whiskers, microspheres, nanorods, and nanowires [23,24]. Zhang *et al.* [25] reported the utilization of calcium nitrate and diammonium hydrogen phosphate as raw materials to synthesize HA by a hydrothermal method, and different morphological HA could be synthesized by controlling the pH values of the reaction solutions during hydrothermal treatments.

On the other hand, hydrothermal method can induce HA nucleation on the active sites of matrix materials, and further promote to grow and crystallize, which facilitates HA to bond on the surface of matrix materials [26].

Hence, hydrothermal method has been developed to deposit HA coating on the surface of scaffolds. Yang *et al.* [27], as an illustration, deposited an HA coating with a controllable nanostructure on the surface of a 3D-printed pure iron scaffold by a hydrothermal method. The results demonstrated that the mechanical performances of the composite scaffolds were comparable to natural bones. The HA coating was highly bonded to the substrate materials, and significantly promoted cell response of rBMSCs. Similarly, Hu *et al.* [28] used a hydrothermal method to deposit nano HA with rod-like morphology on porous biphasic calcium phosphate (BCP) scaffolds, and achieved better osteogenic differentiation of BMSCs compared with pure BCP scaffolds. It indicated that a hydrothermal method to deposit HA coating could be an efficient strategy to enhance the bioactivity of scaffolds for bone repair.

In this study, we proposed to deposit HA coating on the surface of 3D-printed polymer-derived forsterite scaffolds by a hydrothermal method to improve the bioactivity. On one hand, the morphology and size of HA coating could be regulated by changing the pH value and hydrothermal temperature. On the other hand, HA coating did not destroy the structure and influence the photothermal effect of scaffolds owing to the excellent stability of forsterite scaffolds. Hence, the HA-coated forsterite scaffolds would be promising for repair bone defects with bacterial infection.

2 Experimental

2.1 Materials

All raw materials were utilized without further purification including a commercial silicone resin (SilresMK, Wacker Chemie, Germany), isopropyl alcohol, magnesium oxide (MgO , $\geq 99.5\%$), ethylene diamine tetraacetic acid disodium salt (EDTA-2Na , $\geq 99.5\%$), diammonium phosphate ($(\text{NH}_4)_2\text{HPO}_4$, $\geq 99.0\%$), tetrahydrate calcium nitrate ($\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, 98.5%), urea ($\text{CO}(\text{NH}_2)_2$, $\geq 99.0\%$), and sodium hydroxide (NaOH , $\geq 96.0\%$), which were all purchased from Sinopharm Chemical Reagent Co., Ltd., China.

2.2 Fabrication of HA-coated forsterite (M_2S –HA) scaffolds

The 3D-printed silicone resin-derived forsterite (Mg_2SiO_4 , M_2S) scaffolds were fabricated after sintering at 1400 °C in a continuous argon atmosphere on the basis of our previous report [12]. HA deposition on forsterite scaffolds was successfully treated via a hydrothermal method. Typically, 11.8 g of $Ca(NO_3)_2 \cdot 4H_2O$ and 18.61 g of EDTA-2Na were completely dissolved in 500 mL deionized water, and 3.96 g of $(NH_4)_2HPO_4$ and 15.15 g of $CO(NH_2)_2$ were subsequently added. After stirring to dissolve completely, NaOH liquor (1 mol/L) was used to regulate the pH value of the mixed solution reaching 6. Next, the above mixed solution (45 mL) was transferred into a 70 mL Teflon autoclave, and five M_2S scaffolds were put in it. A hydrothermal treatment was conducted at 160 °C for 10 h before cooling to room temperature, and the HA-deposited M_2S (M_2S –HA) scaffolds were ultrasonically treated in deionized water bath for 30 s and rinsed with ethanol. The final scaffolds were then oven-dried at 100 °C. The pH values of the mixed solution (4, 6, 8, and 10) and hydrothermal temperatures (140, 160, 180, and 200 °C) were changed to investigate the phase, morphology, and structure of HA coating.

2.3 Characterization

The phases of scaffolds were characterized by wide-angle X-ray diffraction (XRD) measured with a Bruker D8 ADVANCE X-ray powder diffractometer (Bruker Corp, Billerica, MA, USA). The surface morphology of HA coating on forsterite scaffolds was observed and analyzed by a scanning electron microscope (SEM, FEI Quanta 450, USA). A static material testing machine (2.5 kN, Zwick Roell, Germany) was utilized to measure the compressive strength of scaffolds. The overall porosity of scaffolds was measured using traditional Archimedes principle according to Ref. [3].

2.4 Photothermal effect of scaffolds

The photothermal effect of scaffolds was detected with an infrared thermal imager (FLIRTM A325SC camera, USA) after an 808 nm laser irradiation. The real-time surface temperature changes of each M_2S or M_2S –HA scaffold were tested after laser irradiation at different power densities (0.5, 0.75, and 1.0 W/cm²) and in different environmental conditions (in air for dry condition and in phosphate buffer solution (PBS) for

wet condition), respectively. The photothermal stabilities of scaffolds were analyzed after three cycles with a laser “on and off” state at a power density of 1.0 W/cm².

2.5 Cell response of rBMSCs on M_2S –HA scaffolds

In this study, the third-generation rBMSCs were used to evaluate cell response on M_2S –HA scaffolds. Firstly, the M_2S and M_2S –HA scaffolds sterilized by dry heating at 200 °C for 2 h were put in 24-well plates, and 1.0×10^5 cells were seeded on each scaffold. Subsequently, the rBMSCs-seeded scaffolds were placed in an incubator (5% CO₂, 37 °C) for 2 h to allow the cell attachment on the scaffolds, and then added 1 mL culture medium in each well for continuing the culture.

The proliferation of rBMSCs on scaffolds was evaluated by a Cell Counting Kit-8 (CCK-8) assay, which was determined by measuring the absorbance at a wavelength of 450 nm with a microplate reader (Bio-Rad680, USA). Furthermore, the distribution of rBMSCs with fluorescence labeling was observed by a confocal laser scanning microscope (CLSM, Leica, Germany). Cell nuclei and cytoskeleton of rBMSCs were stained with 10 mg/mL 4',6-diamidino-2-phenylindole (DAPI, Yeasen, China) and 5 mg/mL rhodamine phalloidin (Yeasen, China) for 5 min, respectively.

The alkaline phosphatase (ALP) activity of rBMSCs on scaffolds was tested by an ALP assay kit. Firstly, 1.0×10^5 rBMSCs were seeded on each M_2S or M_2S –HA scaffold, and the culture medium was renewed every two days [29]. When cultured for 7 and 14 days, the culture medium was removed and the attached cells were gently rinsed with PBS for three times and with cold Tris buffer (50 mM) once. Next, the cells were lysed using a 0.2% Triton X-100 (200 µL), and the lysates were sonicated to disperse after being centrifuged for 15 min (14,000 rpm, 4 °C). Afterward, 50 µL of supernatant was mixed with 150 µL working solution on the basis of the supplier's protocol for determining. The conversion of p-nitrophenylphosphate into p-nitrophenol in the presence of ALP was determined by the absorbance at 405 nm measured with a microplate reader. The ALP activity was calculated from a standard curve after normalizing to the total protein content.

Real-time quantitative reverse transcription-polymerase chain reaction (qPCR) was utilized to detect the related osteogenic gene expression of rBMSCs on both types of scaffolds including genes collagen type I (COL-1), osteocalcin (OCN), runt-related transcription factor 2

(RUNX2), and ALP. The expression level of genes was normalized by the glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and the results were estimated with reference to $2^{-\Delta\Delta C_t}$ method according to Ref. [30].

2.6 *In vitro* photothermal antibacterial ability of scaffolds

Staphylococcus aureus (*S. aureus*) and *Escherichia coli* (*E. coli*), as the representative Gram-positive and Gram-negative bacteria, were used to investigate *in vitro* antibacterial ability of M₂S–HA scaffolds. M₂S scaffolds were used as a control. The antibacterial efficiency of M₂S or M₂S–HA scaffolds with or without an 808 nm laser irradiation was determined by a spread plate counting method [31], and the operation procedures were followed as Ref. [12].

2.7 Statistical analysis

The results were expressed as the mean±standard deviation (SD), which were based on the data from three rational parallel experiments. The one-way ANOVA and Student–Newman–Keuls post hoc tests were used to determine the level of significance and *P* values < 0.05 were commonly deemed to be significant.

3 Results and discussion

The structure, surface macro/micro morphology, and phase change can be directly reflected by simple physical and chemical characterization. Figure 1 shows the representative photographs and XRD patterns of M₂S and M₂S–HA scaffolds. It can be seen that M₂S scaffold became rougher and the scaffold color changed from black to gray after HA coating (Figs. 1(a) and 1(b)), suggesting the deposition of white HA on M₂S scaffolds was successfully achieved via a simple hydrothermal process. XRD technique was simultaneously utilized to further confirm HA coating on the surface of scaffolds by analyzing the phases of M₂S scaffolds before and after hydrothermal treatments. As shown in Fig. 1(c), only the diffraction peaks of forsterite phase (JCPDS Card 34-0189) were observed for M₂S scaffolds; however, the diffraction peaks of HA phase (JCPDS Card 09-0432) also appeared on M₂S–HA scaffolds except for the diffraction peaks of forsterite phase. It obviously revealed that HA could deposit on M₂S

Fig. 1 Optical photographs of (a) M₂S and (b) M₂S–HA scaffolds; (c) XRD patterns of M₂S and M₂S–HA scaffolds.

scaffolds well to form M₂S–HA scaffolds through a hydrothermal process.

To optimize the nucleation and growth state of HA coating on M₂S scaffolds, the effects of pH value of the reaction solution and hydrothermal temperature on HA coating were subsequently investigated in this study. Here, Ca(NO₃)₂·4H₂O and (NH₄)₂HPO₄ were utilized as the Ca and P sources for HA coating by a hydrothermal treatment, and the ratio of Ca to P was precisely adjusted to be 1.67 which is consistent with natural HA. Figure 2 shows the XRD patterns of the M₂S–HA scaffolds that were hydrothermally treated at 140–200 °C when pH values were from 4 to 10. The HA phase almost could not be observed for M₂S–HA scaffolds while the reaction condition was set to pH = 6 and hydrothermally treated at 140 °C, but M₂S–HA scaffolds that were hydrothermally treated at 160, 180, and 200 °C obviously exhibited the diffraction peaks of HA phase, indicating the HA deposition on M₂S scaffolds. On the other hand, the diffraction peak intensities of HA were similar for M₂S–HA scaffolds hydrothermally treated at 160, 180, and 200 °C, suggesting that the deposited HA with parallel crystallinity could be obtained at the hydrothermal temperatures of 160–200 °C. It can also be seen that HA could deposit on M₂S scaffolds at a hydrothermal temperature of 160 °C when pH value of the reaction solution was adjusted from 4 to 10, which was confirmed by the diffraction peaks of HA phase appeared in M₂S–HA scaffolds. However, the diffraction peak intensity increased with the increase of pH value of the reaction solution, suggesting that the pH environment could regulate the HA crystallinity during the hydrothermal treatments.

Figure 3 shows SEM images of the surfaces of the M₂S and M₂S–HA scaffolds treated at different hydrothermal temperatures. It can be observed that no

Fig. 2 XRD patterns of M_2S –HA scaffolds obtained under different hydrothermal conditions: (a) different hydrothermal temperatures at pH = 6 and (b) different pH values of the reaction solution at a hydrothermal temperature of 160 °C.

Fig. 3 SEM images of the surfaces of (a) M_2S scaffolds; and M_2S –HA scaffolds hydrothermally treated at different hydrothermal temperatures at (b) 140, (c) 160, (d) 180, and (e) 200 °C when the pH value of the reaction solution was set to 6.

HA was covered on the surface of the scaffolds hydrothermally treated at 140 °C, although the surface morphology suffered from a little change due to the dissolution occurrence by hydrothermal treatment. However, different morphological particles were obviously observed on the surfaces of the M_2S –HA scaffolds hydrothermally treated at 160, 180, and 200 °C, which could be identified to be HA particles based on the XRD analysis above. Interestingly, the regular changes of hydrothermal temperature induced different shapes and structures of HA coatings. It can be observed that the hydrothermal temperatures at 160, 180, and 200 °C induced the formation of the flower-

like, spherical, or rod-like HA particles on the scaffold surface, respectively. Here, during the hydrothermal process in this study, urea was utilized as an effectual precipitant to adjust the pH value of the hydrothermal reaction, which promoted the various HA formation. Low hydrothermal temperature (140 °C) was not able to decompose urea, and thereby did not form HA coating. When the hydrothermal temperature raised, the decomposition rate of urea could be accelerated. Thus, the pH value of the reaction solution slowly increased, which contributed to the formation of HA particles on the surfaces of scaffolds. The higher hydrothermal temperature induced rapid decomposition

of urea, resulting in the rapid increase of alkalinity in the solution, which accelerated the nucleus formation and growth of HA, and thereby formed diverse morphologies of HA particles on the surfaces of M₂S–HA scaffolds.

Figure 4 shows SEM images of the surfaces of M₂S–HA scaffolds hydrothermally treated at 160 °C with the pH values of the reaction solutions from 4 to 10. HA particles could be observed on the surfaces of M₂S–HA scaffolds treated with different pH values of the reaction solutions. However, the morphology of HA particles changed from spherical to flower-like and rod-like. Furthermore, irregular HA particles were deposited on the scaffolds when the pH value of the reaction solution was at 4 or 10, suggesting that the weakly acidic or alkaline environment of the original reaction solution facilitates to form regular HA particles, which was similar to other reported study [32]. On the other hand, the M₂S–HA scaffolds were ultrasonically treated in deionized water bath for 30 s and rinsed with ethanol, and SEM observation confirmed that the deposited HA particles have not been dropped off from the scaffolds, suggesting that HA particles could stably bond to the surface of the scaffolds.

To investigate the effects of HA coating on the physicochemical and biological performances of M₂S scaffolds, the M₂S–HA scaffolds hydrothermally treated with the reaction solution of pH = 6 and at hydrothermal temperature of 160 °C were used in this study. The porosity and compressive strength of the M₂S scaffolds before and after HA coating were summarized in Table 1. The compressive strength of M₂S–HA scaffolds was determined to be 32.4±4.6 MPa at a porosity of 51.5%±4.3%, and there are no significant decreases in the porosity and compressive strength of the M₂S scaffolds after HA coating, indicating that HA coating process via hydrothermal treatment did not destroy the structure of scaffolds.

Zhu *et al.* [12] demonstrated that polymer-derived forsterite scaffolds exhibited excellent photothermal performance due to the embedded free carbon. Here, whether the HA coating on the surface of the scaffold will affect the photothermal performance was further confirmed. Figure 5 shows the influence of HA coating on the photothermal effect of scaffolds. It can be seen that both M₂S and M₂S–HA scaffolds exhibited excellent photothermal effect under dry (in air) or wet (500 µL PBS) condition with an 808 nm laser irradiation, and

Fig. 4 SEM images of the surfaces of M₂S–HA scaffolds hydrothermally treated at different pH values of (a) 4, (b) 6, (c) 8, and (d) 10 when the hydrothermal temperature was set to 160 °C.

Table 1 Porosity and compressive strength of M₂S and M₂S–HA scaffolds

Scaffold	Porosity	Compressive strength (MPa)
M ₂ S	52.7%±3.8%	33.8±5.1
M ₂ S–HA	51.5%±4.3%	32.4±4.6

there are negligible changes after HA coating on M₂S scaffolds. After laser irradiation with an 808 nm laser at a power density of 0.75 W/cm² for 5 min, the surface temperatures of both M₂S and M₂S–HA scaffolds in dry condition increased from room temperature to above 100 °C, and the solution temperatures of both M₂S and M₂S–HA scaffolds in wet condition could still increase

to above 50 °C. Here, the photothermal effect of the M₂S and M₂S–HA scaffolds were determined by the free carbon content in the scaffolds. We have detected the carbon element of the cross section of the M₂S and M₂S–HA scaffolds by energy disperse spectroscopy (EDS) analysis, and the carbon contents were estimated to be 10.37% and 10.68%, respectively. It indicated that HA coating did not influence the photothermal effect of M₂S scaffolds. Hence, the M₂S–HA scaffolds had photothermal antibacterial ability. Furthermore, the temperature of M₂S–HA scaffolds caused by the photothermal effect increased with the increase of laser power density, suggesting that the photothermal

Fig. 5 Photothermal heating curves of both M₂S and M₂S–HA scaffolds in (a) dry and (b) wet conditions under an 808 nm laser irradiation with a power density of 0.75 W/cm². The photothermal heating curves of M₂S–HA scaffolds in (c) dry and (d) wet conditions under an 808 nm laser irradiation with different laser power densities (0.5, 0.75, and 1.0 W/cm²). The time–temperature curve of the laser “on–off” cycle (0.75 W/cm²) of M₂S–HA scaffolds in (e) dry and (f) wet conditions under an 808 nm laser irradiation.

temperature of M₂S–HA scaffolds could be controlled by adjusting the laser power density to meet the photothermal antibacterial temperature (Figs. 5(c) and 5(d)). On the other hand, an 808 nm laser with a power density of 0.75 W/cm² was utilized to irradiate the M₂S–HA scaffolds for 5 min (turn on), followed by a cooling process to room temperature (turn off), and three “on–off” cycles were performed. As shown in Figs. 5(e) and 5(f), the increased temperature of M₂S–HA scaffolds after each “on–off” cycle did not significantly decrease, whatever in dry or wet condition, which indicated that the M₂S–HA scaffolds had remarkable photothermal stability, and showed a great potential for long-term photothermal antibacterial function.

The cell adhesion and *in vitro* osteogenic activity of M₂S–HA scaffolds were investigated to confirm if HA coating could enhance the bioactivity of M₂S scaffolds. The adhesion of rBMSCs on both M₂S and M₂S–HA scaffolds were visualized by confocal laser scanning microscopy (CLSM). As shown in Fig. 6(a), the dense and uniform layers of rBMSCs with a typical cytoskeleton morphology were distributed on both

scaffolds after 3-day culture, which indicated that rBMSCs were well adhered on both scaffolds, and the interconnected porous structure facilitated cell migration and further proliferation.

Figure 6(b) shows the proliferation of rBMSCs on both M₂S and M₂S–HA scaffolds for 1, 3, and 7 days. With increasing culture time, the number of rBMSCs on both M₂S and M₂S–HA scaffolds increased significantly. However, both M₂S and M₂S–HA scaffolds showed similar level of cell proliferation at each culture time point, revealing the good biocompatibility for both M₂S and M₂S–HA scaffolds. Here, the OD value for the M₂S–HA scaffold group showed a little decrease compared to that for the M₂S scaffold group, but both groups had no significant difference, suggesting that HA coating did not influence the biocompatibility of the scaffolds. ALP activity of rBMSCs on both M₂S and M₂S–HA scaffolds was further evaluated to assess the early differentiation of osteoblasts. As shown in Fig. 6(c), the level of ALP activity increased with culture time, and both M₂S and M₂S–HA scaffolds showed higher level of ALP activity than the blank group.

Fig. 6 *In vitro* cellular response on the M₂S and M₂S–HA scaffolds. (a) CLSM images of rBMSCs seeded on the M₂S and M₂S–HA scaffolds for 3 days (scale bar: 50 μm); (b) proliferation of rBMSCs cultured on the M₂S and M₂S–HA scaffolds for 1, 3, and 7 days; (c) ALP activity of rBMSCs cultured on the M₂S and M₂S–HA scaffolds for 7 and 14 days (**P* < 0.05, ***P* < 0.01).

Furthermore, the ALP activity of rBMSCs on M₂S–HA scaffolds was much higher than that on M₂S scaffolds. It indicated that HA coating on M₂S scaffolds could improve the bioactivity of scaffolds and promote the early osteogenic differentiation of rBMSCs [33].

To further evaluate the osteogenic differentiation of rBMSCs on the M₂S and M₂S–HA scaffolds, the expression of osteogenic related genes of rBMSCs (COL-1, OCN, ALP, and RUNX2) on both M₂S and M₂S–HA scaffolds was tested in this study. GAPDH was used as the internal reference for standardization. Primers (BioTNT, Shanghai, China) used for the amplification reaction are listed in Table 2. Figure 7 shows the expression of osteogenic related genes of rBMSCs on the scaffolds after 7-day culture. The expression levels of COL-1, OCN, ALP, and RUNX2 genes on the M₂S and M₂S–HA scaffolds were much higher compared to blank group, suggesting that both scaffolds could promote the expression of osteogenic

related genes. More importantly, M₂S–HA scaffolds showed much higher expression level of osteogenic related genes than M₂S scaffolds, which indicated that HA coating on M₂S scaffolds could improve the osteogenic differentiation. Previous studies also demonstrated that HA coating as a bioactive surface could enhance cell attachment, proliferation, and osteogenic differentiation of stem cells *in vitro* [34].

It has demonstrated that M₂S scaffolds had excellent photothermal-induced antibacterial activity due to the embedded free carbon with photothermal effect [12]. Here, using *S. aureus* and *E. coli* as Gram-positive and Gram-negative bacteria, respectively, the photothermal-induced antibacterial activity of the M₂S–HA scaffolds was also evaluated by dilution plate counting method. As shown in Fig. 8, after 15 min of irradiation with an 808 nm laser irradiation, the number of viable bacterial colonies of the samples containing M₂S–HA scaffolds on the AGAR plates decreased significantly, and the

Table 2 Primer sequences used for qPCR

Gene	Forward primer sequence (5'-3')	Reverse primer sequence (5'-3')
ALP	CAAGGATGCTGGAAGTCCG	CTCTGGGCGCATCTCATTGT
RUNX2	CCGAGACCAACCGAGTCATTTA	AAGAGGCTGTTTGACGCCAT
OCN	TCAACAATGGACTTGGAGCCC	AGCTCGTCACAATTGGGGTT
COL-1	GGAGAGTACTGGATCGACCCTAAC	CTGACCTGTCTCCATGTTGCA
GAPDH	GAAGGTGAAGGTCGGAGTC	GAAGATGGTGATGGGATTTC

Fig. 7 Osteogenesis-related gene expression of (a) COL-1, (b) OCN, (c) ALP, and (d) RUNX2 for rBMSCs cultured on the M₂S and M₂S–HA scaffolds for 7 days by qPCR analysis (*P < 0.05, **P < 0.01).

Fig. 8 *In vitro* photothermal-induced antibacterial ability of the M₂S-HA scaffolds. (a) Optical images of *S. aureus* and *E. coli* colonies formed on AGAR plates after different treatments; bacterial colony viability of (b) *S. aureus* and (c) *E. coli* after different treatments.

antibacterial efficiency increased with increasing the laser power density. It can be observed that the antibacterial efficiencies of M₂S-HA scaffolds against *S. aureus* and *E. coli* were 57.7% and 51.9% after 0.5 W/cm² laser irradiation for 15 min, respectively. However, when the laser power density was increased to 1.0 W/cm², the antibacterial efficiencies of M₂S-HA scaffolds against *S. aureus* and *E. coli* were significantly enhanced, even up to 96.1% and 95.6%, respectively, which showed similar antibacterial efficiencies for the M₂S and M₂S-HA scaffolds at the same irradiation conditions [12]. Therefore, M₂S-HA scaffolds still had excellent photothermal antibacterial ability, and the antibacterial efficiency could be regulated by controlling the laser power density.

HA coating did not influence the photothermal effect of the M₂S scaffolds, and thereby endowed the M₂S-HA scaffolds with excellent photothermal-induced antibacterial activity. On the other hand, HA coating on the M₂S scaffolds promoted the osteogenic differentiation of

rBMSCs due to the enhanced bioactivity. Hence, M₂S-HA scaffolds show great potential for bone repair against bacterial infection.

4 Conclusions

In summary, forsterite-HA composite scaffolds with photothermal-induced antibacterial ability have been successfully fabricated through HA deposition on 3D-printed polymer-derived forsterite scaffolds via a hydrothermal method. The morphology of HA coating could be regulated by controlling the hydrothermal temperature and pH value of the reaction solution. The HA-coated forsterite scaffolds not only dramatically boosted the proliferation and promoted osteogenic differentiation of rBMSCs on the M₂S-HA, but also maintained excellent photothermal-induced antibacterial ability under an 808 nm laser irradiation. Therefore, such HA-coated forsterite scaffolds showed great

potential for bacterial infected bone repair.

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