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Magnetic nanoparticles modified-porous scaffolds for bone regeneration and photothermal therapy against tumors

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Abstract

For effectively treating tumor related-bone defects, design and fabrication of multifunctional biomaterials still remain a great challenge. Herein, we firstly fabricated magnetic $SrFe_{12}O_{19}$ nanoparticles modified-mesoporous bioglass (BG)/chitosan (CS) porous scaffold (MBCS) with excellent bone regeneration and antitumor function. The as-produced magnetic field from MBCS promoted the expression levels of osteogenic-related genes (OCN, COL1, Runx2 and ALP) and the new bone regeneration by activated BMP-2/Smad/Runx2 pathway. Moreover, the $SrFe_{12}O_{19}$ nanoparticles in MBCS improved the photothermal conversion property. Under the irradiation of near-infrared (NIR) laser, the elevated temperatures of tumors co-cultured with MBCS triggered tumor apoptosis and ablation. As compared with the pure scaffold group, MBCS/NIR group possessed the excellent antitumor efficacy against osteosarcoma via the hyperthermia ablation. Therefore, the multifunctional MBCS with excellent bone regeneration and photothermal therapy functions has a great application for treating the tumor-related bone defects.

Key words: Magnetic nanoparticle; Scaffold; Tumor; Bone regeneration; Photothermal therapy

Primary malignant bone tumors such as osteosarcoma often take place in children and adolescents with poor early diagnosis and low 5-year survival rate.[1,2] Bone tumors can be eliminated from patients by surgical resection, but the removal of tumor tissues as well as surrounding healthy tissues may result in bone defects.[3] Moreover, the residual tumor cells tend to trigger the tumor recurrence. Chemotherapy and radiotherapy are often carried out before or after surgical resection, but they may cause severe side-effects on patients.[4,5] Therefore, it is urgent to develop the novel multifunctional bioactive scaffolds that not only can heal effectively bone defects due to tumor removal, but also kill the residual tumor cells without systemic toxicity.

Various bone scaffolds such as bioglass (BG), hydroxyapatite (HA), poly(methyl methacrylate) (PMMA) and chitosan (CS) possess excellent biocompatibility and osteoconductivity, but do not have desired osteoinductivity.[6–8] Interestingly, magnetic field can stimulate stem cell proliferation and osteogenic differentiation, improve the expression levels of growth factors, and accelerate new bone formation in bone defect sites.[9,10] Recently, magnetic particles with biocompatible and magnetic properties have been incorporated in bone biomaterials.[11–13] Notably, the common magnetic iron oxide nanoparticles (Fe_3O_4) show superparamagnetism or weak magnetism so that remarkable osteogenesis effects should be supported by external

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magnetic fields.[9,14,15] As we know, M-type hexagonal ferrites (such as $SrFe_{12}O_{19}$) exhibit high coercivity due to the magnetocrystalline anisotropy along easy magnetization axis.[16] It is reasonable to infer that the good magnetic property of bone scaffolds can be obtained by incorporating M-type ferrites.

To treat tumor-related bone defects, desired bone scaffolds should possess the ability to avoid postoperative tumor recurrence, too. Local drug delivery systems facilitate the release of anticancer drugs at target tumor sites, but the drugs may cause chemo-resistance and severe toxic risk.[4,5]Recently, low side-effect photothermal therapy has been developed to improve tumor therapeutic efficacy using gold nanoparticles, MoS_2 nanosheets, carbon materials, antiferromagnetic pyrite nanocubes, Na_xGdWO_3 nanorods and magnetic nanoparticles as photothermal agents.[4,17–21] Notably, magnetic nanoparticles are considered as promising photothermal agents because of their non-toxicity and good photothermal conversion efficiency.[19] Under the irradiation of near-infrared light (NIR), the elevated local temperatures of 42~50°C promote tumor hyperthermia ablation. To our knowledge, M-type ferrite nanoparticles were rarely incorporated to bone scaffolds for simultaneously enhancing bone regeneration capacity and photothermal therapy against bone tumors.

Both BGs and CS have been widely explored for bone repair applications due to their good biocompatibility, excellent osteoconductivity and appropriate biodegradability.[22] In this work, we designed and fabricated a novel magnetic nanoparticles modified-mesoporous BG/CS scaffold (MBCS). Interestingly, we firstly found that the magnetic $SrFe_{12}O_{19}$ nanoparticles in MBCS not only remarkably promoted stem cell osteogenic differentiation and new bone regeneration by activated BMP-2/Smad/Runx2 pathway, but also served as ideal photothermal agents to effectively kill residual tumor cells by NIR photothermal therapy.

Methods

Preparation of $SrFe_{12}O_{19}$nanoparticles and mesoporous BG microspheres

The magnetic $SrFe_{12}O_{19}$ nanoparticles were prepared by molten-salt method. Briefly, 61.09 g $FeCl_3{\cdot}6H_2O$ and 4.19 g Sr $(NO_3)_2$ were dissolved in 200 ml deionized water. The above mixed solution was added dropwise into 250 ml Na_2CO_3 solution. After reaction for 30 min, the precipitations were washed with deionized water and dried at 60°C for 48 h. The same amounts of the precursors and potassium bromide particles were ground, followed by calcinations at 900°C for 1 h. Finally, the products ($SrFe_{12}O_{19}$ nanoparticles) were washed and dried at 60°C.

BG microspheres with the molar ratio of SiO_2:CaO:P_2O_5 of 84:12:4were fabricated by a sol-gel self-assembly method. 1.22 g cetyltrimethlammonium bromide and 9.00 ml ammonium hydroxide were dissolved in a mixed solution of deionized water and ethanol. 9.00 ml tetrahydrate orthosilicatel, 0.69 ml triethylphosphate and 1.92 g calcium nitrate tetrahydrate were successively added into the above solution. After stirring for 12 h, the BG precursors were washed with water, collected by filtration, and dried at 60°C. The BG microspheres were obtained after calcinations at 650°C for 3 h. Mesoporous BG microspheres were converted from BG microspheres by alkaline treatment. 1.00 g BG microspheres were added into the Na_2CO_3 solution (2.70 g/l) and then stirred vigorously for 1 h. Finally, the mesoporous BG microspheres were washed with deionized water, and dried at 60°C for 48 h.

Preparation of MBCS

1.0 g CS powders were dissolved in an acetic solution under mechanical agitation. The magnetic $SrFe_{12}O_{19}$ nanoparticles (0.125 g or 0.25g) and mesoporous BG microspheres (0.875 g or 0.75 g) were added into CS solutions. After stirring for 2 h, the mixtures were transferred into the moulds of 24-well or 96-well plates. The samples were frozen at -20°C for 12 h under external strong magnetic fields, and then were freeze-dried freeze-drying equipment at -60°C for 48 h. MBCS was soaked in sodium hydroxide (10.0 wt%), washed with deionized water up to pH= 7.0 and freeze-dried. MBCS was magnetized by a magnetization machine. The products with the $SrFe_{12}O_{19}$/BG mass ratios of 1:7 and 1:3 were abbreviated to MBCS1:7 and MBCS1:3, respectively. In addition, the mesoporous BG/CS porous scaffold (BCS) as control group was prepared under the same condition without adding magnetic powders.

In vitro cell behaviours on MBCS

Both cell and animal experiments were approved by the Ethical Committee of Shanghai Jiao Tong University Affiliated Sixth People's Hospital. The hBMSCs (1.0×10^4) were co-cultured with the scaffolds in 24-well culture plates, and incubated in α-MEM containing 10% FBS at 37°C in 5% CO_2 for different days. The attachment and morphology of hBMSCs on scaffolds were observed by SEM after they were successively removed from the medium, fixed with 2.5% glutaraldehyde, washed with PBS, dehydrated by ethanol/hexamethyldisilizane, and freeze-dried at -80 °C.

Cell proliferation assays were performed by cell counting kit-8 (CCK-8). After 1.0×10^4 hBMSCs were co-cultured with scaffolds for different days, 500 μl CCK-8 were added in the medium and incubated for another 2 h. The absorbance was detected at wavelength of 450 nm by a plate reader. The mRNA expression levels of COL1, OCN, Runx2, and ALP were measured by real-time PCR. The hBMSCs were co-cultured with scaffolds for 14 d. The reverse transcription procedure was carried out by Easyscript one-step gDNA Removal and cDNA Synthesis Supermix (TransGen Biotech). The cells were lysed, and the protein concentrations were determined by Bradford reagent (Bio-Rad, Hercules, CA). Equal amounts of lysates resolved on 10% sodium dodecyl-polyacrylamide gel electrophoresis (SDS–PAGE) were transferred to a polyvinylidene fluoride (PVDF) membrane (Millipore), and the membrane was blocked with 1x TBS containing 0.05% Tween 20 and 5% skim milk or 2% bovine serum albumin for 1 h. After blocking, the membranes were incubated overnight at 4°C with the respective primary antibodies. The membranes were washed twice and incubated with diluted horseradish peroxidase (HRP)-conjugated secondary antibodies (1:10,000) for 1 h. The membranes were detected using the enhanced chemiluminescence reagents