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Stimulation of osteogenesis and angiogenesis of hBMSCs by delivering Si ions and functional drug from mesoporous silica nanospheres

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ABSTRACT

Multifunctional bioactive materials with the ability to stimulate osteogenesis and angiogenesis of stem cells play an important role in the regeneration of bone defects. However, how to develop such biomaterials remains a significant challenge. In this study, we prepared mesoporous silica nanospheres (MSNs) with uniform sphere size (~90 nm) and mesopores (~2.7 nm), which could release silicon ions (Si) to stimulate the osteogenic differentiation of human bone marrow stromal cells (hBMSCs) via activating their ALP activity, bone-related gene and protein (OCN, RUNX2 and OPN) expression. Hypoxia-inducing therapeutic drug, dimethyloxaloylglycine (DMOG), was effectively loaded in the mesopores of MSNs (D-MSNs). The sustained release of DMOG from D-MSNs could stabilize HIF-1α and further stimulated the angiogenic differentiation of hBMSCs as indicated by the enhanced VEGF secretion and protein expression. Our study revealed that D-MSNs could combine the stimulatory effect on both osteogenic and angiogenic activity of hBMSCs. The potential mechanism of D-MSN-stimulated osteogenesis and angiogenesis was further elucidated by the supplementation of cell culture medium with pure Si ions and DMOG. Considering the easy handling characteristics of nanospheres, the prepared D-MSNs may be applied in the forms of injectable spheres for minimally invasive surgery, or MSNs/polymer composite scaffolds for bone defect repair. The concept of delivering both stimulatory ions and functional drugs may offer a new strategy to construct a multifunctional biomaterial system for bone tissue regeneration.

1. Introduction

Multifunctional bioactive materials for the management of bone defects caused by trauma, tumors, infections or genetic malformations, have attracted much attention in the past several years [1–4]. In order to repair large-bone defects, this kind of biomaterials should combine both osteostimulation (for promoting new bone formation) and angiostimulation capacity (for inducing vascularization) [5–7]. Most of the recent studies focus on the optimization of chemical compositions of biomaterials to enhance cell response to the materials [8] and demonstrate that the therapeutic ions, such as Sr, Mg, Zn and Cu, have important effects on stimulating the osteogenic and angiogenic differentiation of stem cells [4]. Some studies have incorporated growth factors such as bone morphogenetic proteins (BMPs), transforming growth factor (TGF) and vascular endothelial growth factor (VEGF) into the biomaterials to improve the osteogenic and angiogenic differentiation of stem cells [9–11]. However, most of these approaches only harness either the ions or functional drugs to induce the differentiation of stem cells toward a single lineage. To achieve the multifunctional properties of biomaterials for bone repair, we proposed that the combination of released Si ions and functional drugs in a single biomaterial system could induce the multidirectional differentiation of bone marrow stromal cells (BMSCs).

In 1970, Carlisle suggested that Si element might play an important role in the initiation of preosseous tissue mineralization [12]. Since then, many studies on silicate bioactive materials, including silicon-substituted calcium phosphates [13], silicate-based bioceramics [14] and bioactive glasses [1], have been studied for bone regeneration. Inspired by the silicate-based bioactive materials, it is speculated that Si ions may be used as modulatory ions with osteostimulation property. However, due to the relatively complex degradation products of these Si-containing bioactive materials,

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which always contain other ions, such as Na, Ca and P, it is difficult to elucidate whether pure Si ions possess the ability to stimulate stem cells toward an osteogenic lineage. Meanwhile, mesoporous silica has been widely studied as a promising drug/growth factor carrier in biomedicine and diagnosis [15–18]. Attention has been paid to studies on mesoporous silica used for bone regeneration in recent years. However, most studies focused on the improved mechanical properties of polymer/mesoporous silica composite biomaterials [19–21]. These studies suggested the good cytocompatibility of mesoporous silica, but none of them specifically addressed the osteogenic effect induced by mesoporous silica themselves [22]. Therefore, it is of great interest to study the interaction between mesoporous silica nanospheres (MSNs) and hBMSCs to elucidate the possible mechanism of Si ions in bone regeneration.

MSNs have been used to deliver drugs or growth factors in a controllable manner due to their high pore volume [23]. The loading and release of vascularization-inducing drugs by MSNs may be a promising strategy to stimulate the angiogenesis of stem cells in the process of bone regeneration. Dimethyloxaloylglycine(DMOG), a relatively small molecular drug, is regarded as an ideal candidate [24,25]. It is a cell permeable (molecular weight: 175.1) competitive inhibitor of hypoxia inducible factor-prolyl hydroxylase (HIF-PH) and can stabilize the HIF-1α expression under normoxic conditions [26]. DMOG is therefore expected to induce a hypoxic microenvironment and act as a pro-angiogenic compound. For these reasons, we speculate that it is feasible to design degradable MSNs with mesoporous channel structure and high specific surface area to release Si ions for osteogenic stimulation and at the same time to deliver DMOG that may further enhance the angiogenesis of hBMSCs. Therefore, the aim of this study is to prepare degradable MSNs and to explore the effect of both the Si ions and functional drug DMOG released from MSNs on the osteogenic and angiogenic differentiation of hBMSCs. It is expected that the study will deliver a new concept by combining both Si ions and functional drugs to construct multifunctional biomaterial system for bone tissue regeneration.

2. Materials and methods

2.1. Synthesis of mesoporous silica nanospheres (MSNs)

In a typical synthesis procedure, 1.82 g of cetyltrimethylammonium bromide (CTAB) and 3.0 g of ammonium fluoride (NH_4F) were dissolved in 500 mL of distilled water at 80 °C under vigorous stirring, and then 9 mL of tetraethoxysilane (TEOS) was added drop by drop slowly (CTAB, NH_4F, TEOS, Sinopharm Chemical Reagent Co. Ltd. Shanghai). After stirring for 2 h, the solution was cooled at room temperature overnight. The as-synthesized materials were centrifuged, washed with distilled water and ethanol, and then dried under vacuum. To remove the surfactant CTAB, the solid products were calcined at 600 °C in air for 6 h at a heating rate of 1 °C min^{-1}.

The morphology and microstructure characteristics of MSNs were tested by scanning electron microscopy (Magellan400, FEI, USA), transmission electron microscopy (JEM-2100F, JEOL, Japan) and small-angel X-ray diffraction using CuKα radiation (40 kV and 40 mA) at a scanning rate of 0.4° min^{-1} over the range of 0.5–6° with a step width of 0.02° (Rigaku D/Max-2550V, Geigerflex, Japan). N_2 adsorption–desorption experiments were carried out at 77 K to obtain the isotherm and mesopore size distribution of MSNs and DMOG-loaded MSNs (D-MSNs) (Micromeritics ASAP 2010 analyzer, Micromeritics, USA). Zeta-potential test was carried out to determine the surface charges of MSNs and D-MSNs. Both the nanoparticles were washed twice gently by PBS solution before the test. (Zeta sizer Nanoseries. Nano ZS90, UK).

2.2. Drug loading and release of DMOG in MSNs

To load drug molecules into the pores of the particles, 0.4 g of MSNs was soaked in 2 mL of phosphate buffered solution (PBS, pH = 7.4) containing 6 mg/4 mg/2 mg of dimethyloxaloylglycine (DMOG, Sigma–Aldrich Co. USA). After 24 h, the mixture was centrifuged and the supernatant was removed. The drug-loaded MSNs were washed twice with PBS solution to remove DMOG that was adsorbed on the surface and then dried under 40 °C. Drug-loaded MSNs in different concentrations of DMOG-PBS solutions (3 mg mL^{-1}, 2 mg mL^{-1} and 1 mg mL^{-1}) were referred to as 3D-MSNs, 2D-MSNs and 1D-MSNs, respectively.

The MSNs loaded with the drug DMOG (hereafter referred to as D-MSNs) were immersed into PBS solution at 37 °C on a shaker with the shaking frequency of 120 r/min. The release medium was collected at defined time intervals and replaced with fresh PBS solution. The released DMOG from MSNs was monitored by UV–Vis analysis at 230 nm (Epoch, BioTek Instruments, Gene Co. Ltd., USA).

2.3. Isolation and culture of human bone marrow stromal cells (hBMSCs)

Human bone marrow was sourced from patients undergoing knee replacement surgery at the Prince Charles Hospital in Queensland, Australia. Informed consent was given by all participants, and the research protocol was approved by the Ethics Committee of the Prince Charles Hospital and the Queensland University of Technology. The hBMSCs used in the present study [27] were isolated from the bone marrow by density gradient centrifugation using Lymphoprep™ (Axis-Shield PoC AS, Oslo, Norway) according to the manufacturer's instructions. The cells were then seeded into the culture flasks containing low glucose Dulbecco's Modified Eagle's Medium (DMEM; Life Technologies Pty Ltd., Australia) supplemented with 10% fetal bovine serum (FBS; In Vitro Technologies, Australia) and 1% penicillin/streptomycin (complete medium) at 37 °C, 5% CO_2. The medium was changed twice weekly to wash out all non-adherent cells. Upon reaching 70–80% confluence, the attached hBMSCs were further expanded. Only early-passage cells (2–3 passage) were used in this study.

2.4. The effect of MSNs and D-MSNs on the metabolic activity and alkaline phosphatase (ALP) activity of hBMSCs

The in vitro metabolic activity was assessed using 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay (Sigma–Aldrich, USA). Cell culture flasks and multiple well plates were purchased from Nunc, Thermo Scientific, USA. 3×10^3 hBMSCs per well were seeded in 96-well plates (2800 cells cm^{-2}) for 24 h to allow the cells to attach, and then exposed to complete medium supplemented with serial concentrations of MSNs and D-MSNs (31.25, 125, 500 μg mL^{-1}). The medium was changed every two days during cell culture. After the cells were incubated for1, 3 and 7 days, 20 μL of MTT solution in a final concentration of 0.5 mg ml^{-1} was added to each well and incubated at 37 °C to form formazan. After 4 h, the mixture was removed and the formazan product was dissolved with 100 μL of dimethyl sulfoxide (DMSO). The absorbance value was measured at λ = 495 nm on a microplate reader (SpectraMax, Plus 384, Molecular Devices, Inc., USA). All the results were demonstrated as the optical density values minus the absorbance value of blank wells.

ID	Title	Pages
302	Stimulation of osteogenesis and angiogenesis of hBMSCs by delivering Si ions and functional drug from mesoporous silica nanospheres	12