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Short communication

Biocompatible β-$SrHPO_4$ clusters with dandelion-like structure as an alternative drug carrier

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ABSTRACT

Recent researches about calcium phosphate (CaP) biomaterials used as drug delivery systems are focusing on the better understanding of the microenvironment around the implant-host tissue interface, with the aim to provide a bone response in pathological ones. Towards the improvement of the osteogenic potential of CaP drug carriers, dandelion-like β-$SrHPO_4$ clusters (Φ10–20 μm) has been prepared by a homogeneous precipitation method under the hydrolysis of carbamide. Adhesion, spreading, proliferation, osteogenic differentiation and mRNA expression of bone mesenchymal stem cells (BMSCs) mediated by β-$SrHPO_4$ clusters were investigated. Highly osteoconductive and biodegradable octacalcium phosphate with similar structure was employed as the control. By contrast, β-$SrHPO_4$ clusters exhibited remarkably better affinity, enhanced proliferation and osteogenic differentiation of BMSCs, providing a promising alternative bioactive bone substitute and drug carrier for tissue repair. With the unique dandelion-like microstructure, we believe that our as-prepared material will open up new avenues for applicability of CaP drug delivery systems in the near future.

1. Introduction

Recently, hybrid materials and inorganic materials are widely recognized as upcoming materials for delivery, therapy, diagnosis, imaging, sensors, etc. in optical, electrical, biomedical, medical, clinical and other applications [1–12]. With the compositional similarities to bone minerals and excellent biocompatibility, calcium phosphates (CaPs) are widely used in bone regeneration. CaPs have become the research focus in simultaneous use as bone substitute and drug delivery vehicle [13]. As drug carriers, the biodegradation property usually prior to delivery ability of the drug. However, major concerns associated with biodegradable polymeric nanoparticles are the acidic degradation by-products that can alter the drug activity and adversely interact with tissue. Most of CaPs are relatively insoluble in physiological environments, and this nature occurrence is one of the primary advantages over other synthetic drug delivery systems [14]. Except for the drugs, CaPs have also proven to be effective for non-viral intracellular gene delivery or transfection [15–17]. The use of CaP particle systems for protein delivery has also been studied using model proteins (e.g. bovine serum albumin) [18,19].

Up to now, CaPs have been used successfully in various drug delivery applications in the form of nanoscale (particulate systems) to microscale (coatings) to macroscale (cements and scaffolds) for local delivery, and in some cases for targeted delivery [13]. The recent researches are focusing on the better understanding of the microenvironment around the implant-host tissue interface, aiming to improve the osteogenic potential of bone substitutes in healthy bone sites and to provide a bone response in pathological ones [20].

With the purpose of improving the osteogenic potential of CaPs delivery, ion substitution may be a more appropriate way, for example strontium (Sr). Sr, which is demonstrated as a trace element in the human body, regulates bone remodeling effectively by stimulating bone formation and inhibiting bone resorption [21–23]. Numerous Sr substitution strategies have been developed for the efficient controlled release of bioactive strontium ions (Sr^{2+}) [24–28]. For bone regeneration, a Sr carrier with appropriate biodegradability and biocompatibility can exactly suit the demands of dynamic uptake and release of bone mineral ions, since almost all of Sr exists in the mineral metabolism region of bone tissue [29]. Among these Sr carriers, strontium hydrogen phosphate ($SrHPO_4$, DSPA), as one of the main

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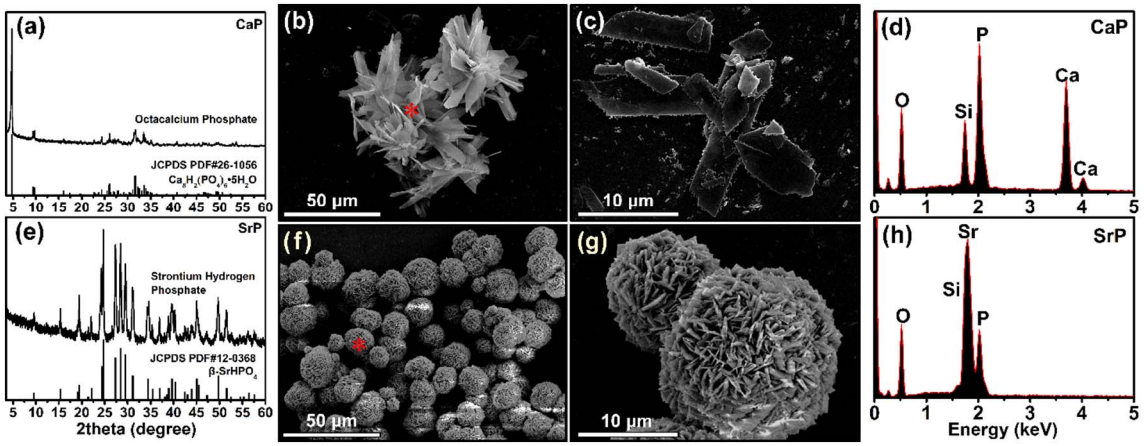

Fig. 1. XRD patterns, SEM images and EDS spectra of CaP (a–d) and SrP (e–h). EDS spectra were detected from the positions marked by red asterisks. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

compounds, has been recognized as an ion exchanger biomaterial for holding both HPO_4^{2-} and Sr^{2+} involved in the bone mineral metabolism [30].

$SrHPO_4$ has been mainly claimed as an important catalyst, proton conductor and surface conditioner as well as for application in batteries, fuel cells, flame proofing and thermal cathodes. $SrHPO_4$ is also usually used for the immobilization of heavy metal ions (e.g. Pb^{2+}) from the environmental sewage [31]. But in the biomedical fields, $SrHPO_4$ is merely reported as a kind of Sr^{2+} additives in the CaPs biomaterials. $SrHPO_4$ has been described with three types of modifications: α-$SrHPO_4$ contains two independent Sr sites with eightfold coordination to form a distorted quadratic antiprism; γ-$SrHPO_4$ includes two independent Sr sites coordinated by nine oxygen atoms; whereas little is known with concern to β-$SrHPO_4$.

In this work, β-$SrHPO_4$ clusters with dandelion-like structure and function would be prepared as a novel drug delivery system. Biocompatibility evaluation including cellular adhesion, spreading, proliferation, osteogenic differentiation and mRNA expression would be employed to characterize its superior biological response using a bioactive CaP as the control. We believe that β-$SrHPO_4$ clusters could be a suitable drug delivery system as bone substitute for the synergistic effects of bioactive Sr^{2+} release and unique dandelion-like structure.

2. Experimental procedure

Powder samples were prepared by a homogeneous precipitation method as described in our previous work [32]. Briefly, white precipitation was obtained by vigorously stirring a solution containing 20 mM $Ca(CH_3COO)_2$/$Sr(CH_3COO)_2$, 15 mM $NH_4H_2PO_4$ and 50 mM CO $(NH_2)_2$ at 90 °C for 2 h, followed by wash, centrifugation and air drying. Obtained samples were labelled as CaP and SrP, respectively. Phase component was determined by X-ray diffraction (XRD; X'Pert PRO, PANalytical, Netherlands). Morphology was observed by field emission scanning electron microscopy (FESEM; Nova NanoSEM 430, FEI, USA) with energy dispersive spectroscopy (EDS; Inca Energy, Oxford Instruments, UK).

The in vitro biocompatibility was assessed in terms of biological response of bone mesenchymal stem cells (BMSCs; CRL-12424, ATCC, USA). Cells were seeded onto the culture plates (37 °C, 5%CO_2), and powder samples were uniformly added after adhesion. Cellular morphology was observed by SEM after fixation and dehydration process. Cellular viability was observed with live/dead cell staining using an inverted fluorescence microscope (Eclipsc Ti-U, Nikon, Japan), and quantitatively assessed by CCK-8 assay (Dojindo, Japan) using a microplate reader (Varioskan Flash Multimode Reader, Thermo Scientific, USA). Osteogenic differentiation was assessed by the amount of alkaline phosphatase (ALP) expressed by BMSCs. Cells were harvested and lysed by Triton X-100 (1 vol%). The cell lysates were quantified with a protein assay kit (Pierce BCA Protein Assay Kit, Thermo Scientific, USA). ALP activity was determined by measuring the absorbance of the *para*-nitrophenol products using a *para*-nitrophenyl phosphate substrate (Sigma, USA). ALP staining was performed using BCIP/NBT substrate (SouthernBiotech, USA). Osteogenic differentiation mRNA expression of ALP, runt-related transcription factor 2 (Runx2), collagen 1 (Col1), osteocalcin (OCN) were detected by RT-PCR analysis using GAPDH as the housekeeping gene (Table S1). The total RNA in cells was isolated with HiPure Total RNA Micro Kit (Magen, China), subjected with iScript cDNA Synthesis Kit (Bio-Rad, USA), and then subjected to RT-PCR analysis conducted with SYBR green assay (iQ™ SYBR Green Supermix, Bio-Rad, USA). Cellular tests were repeated four replicate samples and quantitative data were compared by a one-way analysis of variance (ANOVA) analysis at a significance level of $P < 0.05$.

3. Results and discussion

OCP, which is proposed as an important bioactive precursor of biological apatite, has great potential to enhance new bone formation [33,34]. With the typical characteristics and structure of CaPs, its osteoconductive capacities were first demonstrated in granular form within the subperiosteal region of mouse calvaria, which demonstrated the rapid formation of new bone tissue more clearly than other CaPs (e.g. hydroxyapatite and Ca-deficient hydroxyapatite) [35]. OCP also has the potential to support the osteogenic differentiation of BMSCs [36,37]. In this work, bioactive OCP was employed as a control of CaPs for the biocompatibility evaluation of our $SrHPO_4$ material.

Phase component, morphology and structure of both powder samples are showed in Fig. 1. CaP and SrP were composed of exclusive OCP ($Ca_8H_2(PO_4)_6 \cdot 5H_2O$, PDF#26-1056) and β-$SrHPO_4$ (PDF#12-0368), respectively. OCP crystals displayed typical plate-like appearance, while dandelion-like β-$SrHPO_4$ cluster (Φ10–20 μm) consisted of numerous thin flaky crystals were observed. It was different from the general cube-shaped α-$SrHPO_4$ crystals (PDF#70-1215, Fig. S1) which were structurally like DCPD ($CaHPO_4 \cdot 2H_2O$) crystals. It was reported that the rapid cooling of the process temperature resulted in the formation of α-$SrHPO_4$, whereas a continuous slow-heating process led to the appearance of β-$SrHPO_4$ [38]. Here, carbamide could be another critical factor which could stabilize the newly formed β-$SrHPO_4$ crystals despite our rapid cooling process. OCP crystals formed via the in-situ structural transition of rapidly precipitated plate-like DCPD crystals under the continuous hydrolysis of carbamide as reported in our previous work [32]. Similarly, the uniform β-$SrHPO_4$ clusters could also epitaxially grow from the rapid formation of nuclei of strontium

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