Chemical Engineering Journal 222 (2013) 49–59

Contents lists available at SciVerse ScienceDirect

Chemical Engineering Journal

journal homepage: www.elsevier.com/locate/cej

Strontium substituted hydroxyapatite porous microspheres: Surfactant-free hydrothermal synthesis, enhanced biological response and sustained drug release

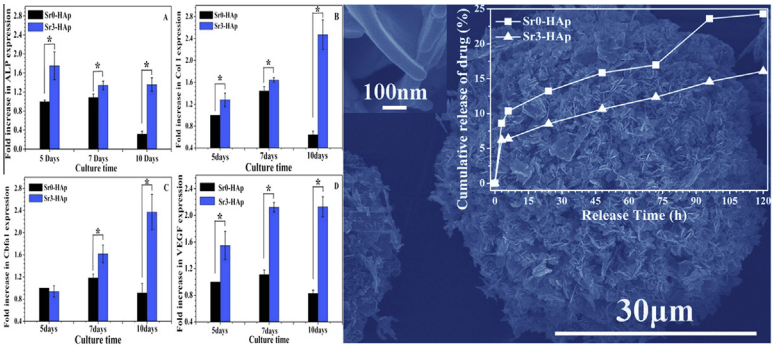
Kaili Lin [a,1], Peiyi Liu [b,1], Li Wei [b], Zhaoyong Zou [a], Weibin Zhang [b], Ying Qian [b], Yuhui Shen [b,*], Jiang Chang [a,*]

[a] State Key Laboratory of High Performance Ceramics and Superfine Microstructure, Shanghai Institute of Ceramics, Chinese Academy of Sciences, China
[b] Department of Orthopaedics, Shanghai Institute of Orthopaedics & Traumatology, Shanghai Ruijin Hospital, Shanghai Jiaotong University School of Medicine, China

HIGHLIGHTS

- The mesoporous strontium substituted hydroxyapatite (SrHAp) microspheres were fabricated.
- The ionic extracts of the microspheres promoted the biological responses.
- The architecture of SrHAp resulted in favorable drug-loading and sustained release properties.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 5 December 2012
Received in revised form 6 February 2013
Accepted 12 February 2013
Available online 21 February 2013

Keywords:
Hydroxyapatite
Strontium
Porous microspheres
Synthesis
Biological response
Drug delivery and release

ABSTRACT

In the absence of any surfactants, organic solvents or template-directing reagents, the strontium substituted hydroxyapatite (SrHAp) microspheres with hierarchically mesoporous structures and 1.17–5.60 mol.% of Sr substitution were successfully fabricated via hydrothermal method. The morphology observation showed that the fabricated SrHAp porous microspheres in diameters of 50–65 μm were assembled by two-dimensional single-crystal nano-sheets with 30–70 nm in thickness, and up to 3 μm in width and length. The ionic extracts of SrHAp porous microspheres promoted the proliferation, osteogenic differentiation and angiogenic factor expression of human osteoblast-like cells (MG-63), and the microspheres with 3.22 mol.% of Sr substitution showed the best potentially therapeutic applications in bone regeneration field. In addition, the novel 3D architectures of SrHAp resulted in favorable drug loading and sustained release properties. Our study indicated that the fabricated multifunctional SrHAp porous microspheres might be a potential candidate as bioactive bone-regeneration and drug-delivery material.

1. Introduction

The material with initiative stimulation capacity in tissue regeneration is the major character for next generation biomaterials [1]. Calcium phosphate (Ca–P) materials, such as hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$, HAp], β-tricalcium phosphate [β-$Ca_3(PO_4)_2$, β-TCP] and calcium phosphate cement (CPC), are widely used in biomedical fields due to their good biocompatibility, osteo-conductivity and similarity to the inorganic component of bone tissues [2]. However, the traditional Ca–P based materials, including the HAp, are lack of the ability to stimulate the formation of new bone, which hinder their clinical applications [3]. Several method are

* Corresponding authors. Tel.: +86 21 52412810; fax: +86 21 52413903.
E-mail addresses: yuhuiss@163.com (Y. Shen), jchang@mail.sic.ac.cn (J. Chang).
[1] These authors contributed equally to this article.

1385-8947/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.cej.2013.02.037

applied to solve this problem, such as loading bioactive growth factors [4], grain size and surface morphology/topography design [5], and the incorporation of the functional trace elements [6,7]. In which, the incorporation of the functional trace inorganic elements into the biomaterials to improve the biological responses has been aroused great interest due to its simplicity and low cost. Recently, strontium (Sr) ranelate, a newly developed drug treating osteoporosis, has been shown to have dual effects of stimulation osteoblast differentiation and inhibiting osteoclast activity and bone resorption, which could reduce the incidence of fractures in osteoporotic patients [8]. In addition, the partial substitution of Ca by Sr can apparently improve the biological properties of Ca–P materials [9].

Recently, the nano-structured HAp porous microspheres have attracted great interests due to their high specific surface area and novel three-dimensional (3D) hierarchical architectures, which make it possible to incorporate higher dosages of drugs into the materials and release them at a control rate [6,10]. Furthermore, the 3D architectured HAp microporous materials can be used as injectable bone regeneration biomaterials and cell/drug loaded implants, which is superior to particles [11,12]. Thus, comparing with traditional particles, the Sr-substituted HAp (SrHAp) porous microspheres might possess better biological properties for guiding bone tissue regeneration, and have great potential application for drug delivery. Up to now, the SrHAp porous microspheres with hierarchical nano-architectures are rarely reported.

Furthermore, the traditional strategy to synthesize the 3D architectured materials is focused on the organic solvent, surfactant and chelating ligand assistant assembled approach [13–20]. Ma and Zhu synthesized HAp hollow microspheres via solvothermal method using N,N-dimethylformamide (DMF) as the solvent [16]. Zhang et al. fabricated HAp microspheres and microflowers via hydrothermal method using hexadecyltrimethylammonium bromide (CTAB) as the surfactant [17]. He et al. reported that in the hexane–water–bis(2-ethylhexyl) sulfosuccinate (AOT) system hollow HAp microspheres were presented [18]. The HAp materials with flower-like morphology assembled from nanosheets consisting of nanorod building blocks was synthesized by Ma using potassium sodium tartrate as chelating ligand and template molecule [19]. Veljovic et al. prepared the HAp hollow microspheres assembled by rod-shaped nanoparticles using Na_2EDTA as chelating ligand and template molecule [20]. However, in most of these cases, large amounts of template and/or organic solvents were widely used, which might be hazardous to health and environment. Up to now, self-assembly of nano-units into the 3D architectures in the absence of any surfactants, template-supports or structure-directing reagents are still a major challenge [21]. Recently, Neira et al. developed a repeated stepwise hydrothermal method to synthesize micrometer-sized HAp particles with a sharp faceted hexagonal prism-like morphology [22].

Herein, the SrHAp porous microspheres with 1.17–5.60 mol.% of Sr substitution was synthesized via hydrothermal method. Then the effect of Sr substitution on osteoblast cell (MG-63) proliferation, osteogenic differentiation and angiogenic factor expression of the microspheres, and the effect of the 3D architectured nanostructures on drug loading and release capacities were further investigated.

2. Materials and methods

2.1. Synthesis and characterization of SrHAp porous microspheres

The SrHAp porous microspheres with designed $Sr^{2+}/(Ca^{2+} + Sr^{2+})$ molar ratios of 0.01, 0.03 and 0.05 were hydrothermally synthesized using urea ($(NH_2)_2CO$) as homogeneous precipitation reagent. The obtained products were labeled as Sr1-HAp, Sr3-HAp and Sr5-HAp, respectively. In the synthesis process, the aqueous solutions containing 0.05 mol ($Ca^{2+} + Sr^{2+}$), 0.03 mol HPO_4^{2-} and 0.15 mol urea were prepared by dissolving $Ca(NO_3)_2$, $Sr(NO_3)_2$, $NH_4H_2PO_4$ and urea in 100 mL distilled water, and 0.1 mol/L HNO_3 solution was used to adjust the pH to 2.41–2.45 to obtain clear solutions. Then 17 mL of the obtained solution were transferred into 20 mL Teflon autoclaves and heated at 120 °C for 1–72 h, followed by cooling to room temperature naturally. It is well known that, with the increase of the hydrothermal temperatures, the $(NH_2)_2CO$ continuously decomposes to form CO_2 and aqueous ammonia species based on the following equation:

$$(NH_2)_2CO + H_2O \rightarrow 2NH_3 + CO_2$$

In which, the released NH_3 easily dissolves in water and increases the pH value to alkaline condition in the reaction solution, wherein HAp becomes the more thermodynamically stable calcium orthophosphate compound, and is thus formed [22]. Therefore, the continuous and homogeneous decomposition of urea generates the driving force toward the nucleation and growth of HAp crystals under moderate supersaturation conditions [22]. In addition, the CO_2 dissolves in water to form HCO_3^- and CO_3^{2-}, and then incorporates as carbonate in the HAp lattice [23]. After hydrothermal reaction, the obtained suspension was filtrated and washed with distilled water and anhydrous ethanol for three times, respectively. The obtained powders were dried at 120 °C for 24 h.

To investigate the roles of the chemical composition (Sr-substitution) and porous morphologies on the biological performance and drug loading/release properties, respectively, the pure HAp nanoparticles labeled as Sr0-HAp were prepared as the control sample. The pure HAp nanoparticles can well play dual roles as chemical component control for biological response, and as morphology control for drug loading/release properties. First, 0.5 M $Ca(NO_3)_2$ solution and 0.3 M $(NH_4)_2HPO_4$ solution were obtained by dissolving $Ca(NO_3)_2$ and $(NH_4)_2HPO_4$ in distilled water, respectively, and the pH of both solutions was adjusted to 10.5 using ammonia solution. The reactant molar ratio of Ca/P was kept at 1.67. The solution of $(NH_4)_2HPO_4$ was added dropwise into the $Ca(NO_3)_2$ solution to obtain a suspension, and the pH of the suspension was maintained at 10.5 using ammonia solution. Then the suspension was transferred into autoclaves and heated at 120 °C for 72 h, followed by cooling to room temperature naturally. After the hydrothermal reaction, the obtained suspensions were filtrated and washed with distilled water and anhydrous ethanol for three times, respectively. The obtained powders were dried at 120 °C for 24 h.

The obtained powders were characterized by X-ray diffraction (XRD: D/max 2550 V, Rigaku, Japan) with mono-chromated Cu Kα radiation and Fourier transform infrared spectroscopy (FTIR: Nicolet Co., USA). The lattice constants were calculated from the well determined positions of the intense XRD diffractions that were processed by MDI Jade 6.1 software [24]. To evaluate the crystallinity degree, the reflection (2 1 1) of HAp powders at around $2\theta = 31.8°$ (JCPDS No. 09-0432) was selected as the diffraction plane for crystallinity measurement to calculate the relative index of crystallinity (IOC, %) defined as follows [25]:

$$IOC = (I_c/I_o) \times 100\%$$

where I_c and I_o are the peak intensity of the selected diffraction plane in the obtained products and the control sample, respectively. In present study, the fully crystallized HAp structure prepared by wet chemical precipitation method and then calcined in furnace at 900 °C for 1.5 h was used as the control sample [26]. The morphology and size of the obtained powders were characterized by scanning electron microscopy (SEM: JSM-6700F, JEOL, Japan) and transmission electron microscopy (TEM: JEM-2100F, JEOL, Japan).