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Synthesis of strontium substituted hydroxyapatite whiskers used as bioactive and mechanical reinforcement material

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ABSTRACT

The strontium substituted hydroxyapatite (SrHA) whiskers with 6.6 mol% of Ca replaced by Sr were successfully synthesized via homogeneous precipitation method. The product had a width of 0.2–8 μm and length up to 155 μm. The human osteoblast-like cell (MG-63) culture showed that Sr substitution could stimulate the proliferation of MG-63 at certain extracted concentrations. Furthermore, the addition of SrHA whiskers could significantly improve the mechanical properties of calcium phosphate cement (CPC). The 5 wt.% SrHA whisker reinforced CPC revealed the highest compressive strength of 2.92 MPa, which was almost 2-times higher than that of the pure CPC. Our study indicated that SrHA whisker might be a potential candidate as a new bioactive and mechanical reinforcement material for hard tissue regeneration applications.

1. Introduction

The ideal hard-tissue regeneration materials should be biocompatible and bioactive, and possess excellent mechanical properties. Calcium phosphate (Ca-P) materials, such as hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$, HA], β-tricalcium phosphate and calcium phosphate cement (CPC), etc., are widely used in biomedical fields due to their excellent biocompatibility, osteo-conductive properties and similarity to the inorganic component of human beings [1]. Unfortunately, the mechanical property of Ca-P materials is low, which has severely hindered their clinical applications [1,2]. Several attempts are used to solve this problem, such as using ZrO_2, metals and carbon nanotubes etc. as mechanical reinforcement [1,3]. However, most of these reinforcements are bioinert and/or non-biocompatible, which remarkably decreases the bioactivity and biocompatibility of materials. HA whiskers have greatly aroused interests because of their excellent biocompatibility. However, the traditional Ca-P based materials, including the HA whiskers, lack the ability to stimulate the formation of new bone. Recently, strontium (Sr) ranelate, a newly developed drug treating osteoporosis, has been shown to have dual effects of stimulating osteoblast differentiation and inhibiting osteoclast activity and bone resorption and could reduce the incidence of fractures in osteoporotic patients [4]. In addition, the partial substitution of Ca by Sr can apparently improve the biological properties of Ca-P materials [5]. Thus, comparing with traditional whiskers, the Sr-substituted HA whiskers (SrHA) might possess excellent mechanical and biological properties. However, to the best of our knowledge, no related studies have been reported up to now.

Herein, the SrHA whiskers with 6.6 mol% of Ca substituted by Sr were synthetized via homogeneous precipitation method. Then the effect of Sr substitution on osteoblast proliferation of the whisker, and the effect of the whisker additive on the compressive strength of CPC were preliminarily studied.

2. Materials and methods

2.1. Synthesis and characterization of SrHA whiskers

The SrHA whiskers designed Sr/(Ca + Sr) molar ratios of 0.1 were hydrothermally synthesized using acetamide as homogeneous precipitation reagent. Aqueous solutions containing 40 mmol Ca^{2+}, 10 mmol Sr^{2+} and 29.94 mmol HPO_4^{2-} were prepared by dissolving analytical grade reagents (Wako, Japan) of $Ca(NO_3)_2 \cdot 4H_2O$, $Sr(NO_3)_2 \cdot 4H_2O$ and $(NH_4)_2HPO_4$ in distilled water with 1 mol/L acetamide, and 0.1 mol/L HNO_3 solution was used to adjust the pH to 2.75 to obtain clear solutions. Then 85 mL of the obtained solution was transferred into 100 mL Teflon autoclaves and heated at 180 °C for 10 h, followed by cooling to room temperature naturally. Pure HA whiskers without Sr substitution were synthesized as the control sample through the similar method. After hydrothermal reaction, the obtained suspensions were filtrated and washed with distilled water and anhydrous ethanol, respectively, and then dried at 60 °C for 24 h. The products were characterized by XRD and FTIR. The morphology and size of

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the products were characterized by SEM. The Sr content of the samples was quantified by X-ray fluorescence (XRF).

2.2. Effect of ionic product from SrHA whiskers on MG-63 proliferation

The method was carried out with a series of concentrations of whisker extract in contact with MG-63 cell (ATCC, Manassas, VA) according to ISO 10993–5. To prepare the extracts, a stock solution of 100 mg/mL was first prepared by adding the whiskers into DMEM culture medium. After incubation at 37 °C for 24 h, the mixture was centrifuged and the supernatant was collected. The serial diluted extracts (100, 50, 25, 12.5 mg/mL) were prepared by diluting the stock solution with serum-free DMEM. Subsequently, these extracts were sterilized by filtration through 0.2 μm filter membranes for cell culture experiments. The ion concentrations of the extracts were measured by ICP-OES (710-ES, Varian, USA).

The MG-63 was seeded in 96-well plates at 5×10^3 cell/well and incubated for 24 h at 37 °C with 5% CO_2, 95% air at 100% RH. The medium in the well was then replaced by extracts prepared. After 3 d culture, 10 μL (5 mg/mL) of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) plus 100 μL of DMEM were added into each well. After additional incubation for 4 h, the MTT solution was removed and replaced with 100 μL of dimethylsulfoxide (DMSO). After 10 min of slow shaking (Vibramax 100, Metrohm, USA), the absorbance was read at 570 nm against the reference value at 630 nm, and the results were expressed as optical density (OD).

2.3. Fabrication and characterization of the SrHA whiskers reinforced CPC

The CPC powder consisted of a mixture of β-tetracalcium phosphate [β-$Ca_4(PO_4)_2O$, β-TTCP] and dicalcium phosphate anhydrous ($CaHPO_4$, DCPA) (Wako, Japan) with mean particle size about 10 μm at weight ratio of 1:1 was applied. The CPC and SrHA whiskers with SrHA whiskers varying in content from 0 to 10 wt.% were mixed. The mixtures were ultrasonically dispersed in ethanol for 10 min before drying in oven at 70 °C for 12 h to remove the ethanol. Then the citric acid (15 wt.%) solution was used to set the CPC, and the weight ratio of mixtures (CPC and SrHA whiskers) to citric acid was set at 2:1. The mixtures were stirred to form homogeneous pastes within 60 s, transferred into Teflon moulds to form cylinders with 6 mm in diameter and 12 mm in height without any compression and finally cured in a 100% humidity incubator at 37 °C for 3 d. Five pieces from each group were measured to obtain compressive strength using a mechanical testing machine (MTS 858 Bionix, USA) at a speed of 0.1 mm/s. Data were analyzed for statistical significance using an analysis of variance. Differences at p values of <0.05 were considered significant.

3. Results and discussion

3.1. Characterization of SrHA whiskers

Fig. 1 shows the SEM morphologies, XRD patterns and FTIR spectra of the products. The SEM results revealed that both of the products were whisker-like morphology with width of 0.2–8 μm and length up to 155 μm. The sharp and strong XRD peaks indicate the samples are well crystallized. All peaks matched the standard values of HA (JCPDS: No. 09–0432) both in terms of d-spacings and 2θ-positions, suggesting the powders were pure HA. However, the peak intensities of the (211) and (300) reflections were different from the standard values, which might be attributed to the preferential orientation growth of the HA powders. However, the detailed mechanism needs to be further investigated. In FTIR spectra, the peaks at 3435 and 1639 cm^{-1} were the bending mode of the adsorbed water, while the peaks at around 3572 and 630 cm^{-1} were assigned to the stretching vibration of the lattice OH group of HA. The characteristic bands for PO_4^{3-} appeared at 563, 602, 1034 and 1092 cm^{-1} [6]. FTIR results further confirmed that the whiskers were pure hydroxyapatite. XRF

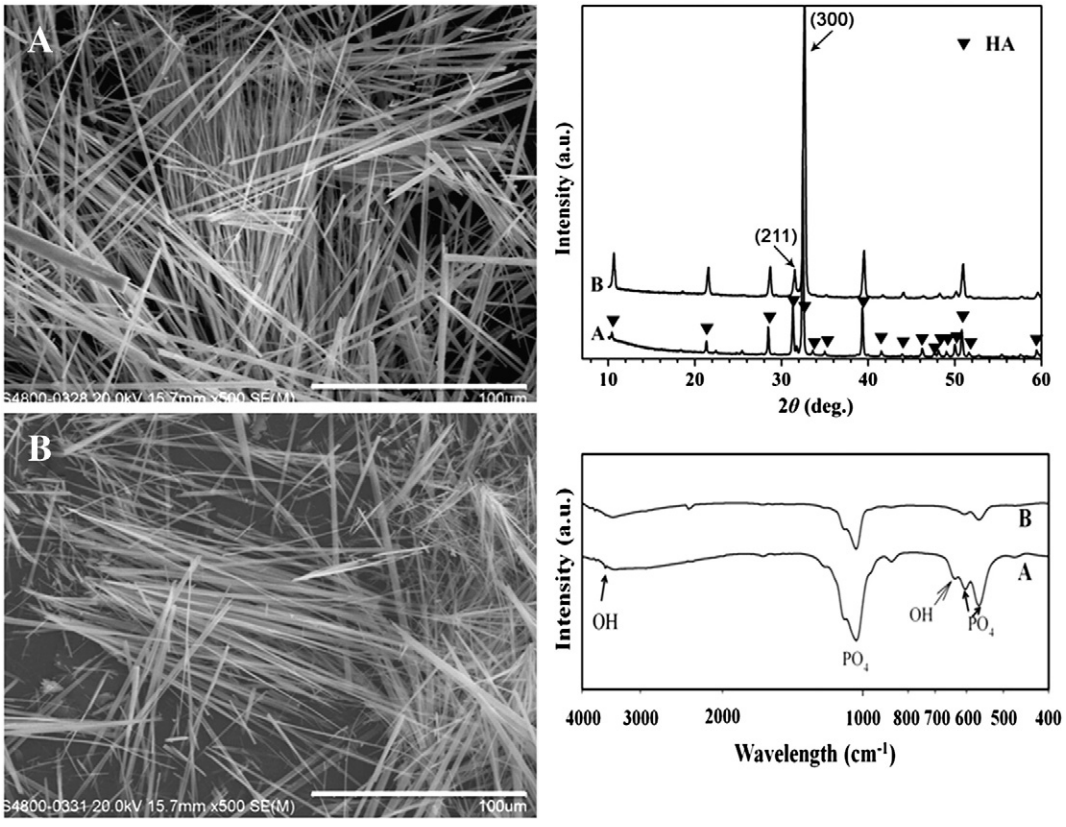

Fig. 1. The SEM morphologies, XRD patterns and FTIR spectra of the obtained HA (A) and SrHA (B) whiskers, bar = 100 μm.