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Incorporation of strontium into hydroxyapatite via biomineralization of collagen fibrils

Zhi Huang[a], Fuzhai Cui[b], Qingling Feng[b,*], Xiaodong Guo[c]

[a]*Institute of Biomedical Engineering, School of Geosciences and Info-Physics Engineering, Central South University, Changsha, China*
[b]*State Key Laboratory of New Ceramics and Fine Processing, School of Materials Science and Engineering, Tsinghua University, Beijing 100084, China*
[c]*Department of Orthopedics, Union Hospital, Tongji Medical College, Huazhong University of Science & Technology, 1277 Jiefang Avenue, Wuhan 430022, China*

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Abstract

Mineralized collagen fibrils (nHAC) are the main extracellular matrix composites of hard tissues, such as bone and teeth. nHAC formed in vivo contains trace of foreign ions, such as HPO_4^{2-}, Cl^-, Na^+, and Sr^{2+}. The role of Sr^{2+} on the composition and microstructure of nHAC is still unknown. In this study, we prepared Sr^{2+} incorporated nHAC via biomineralization of collagen fibrils. We evaluated the effects of Sr^{2+} on mineralization of collagen by using FTIR, XRD, SEM and TEM. The FTIR results revealed that HPO_4^{2-}, CO_3^{2-}, and OH^- functional groups were all incorporated into nHAC. The FTIR, XRD, SEM and TEM results revealed that the incorporation of Sr^{2+} into nHAC did not influence the organization of apatite crystals and collagen fibrils. The inorganic phase was determined as apatite and the variation of lattice constants of apatite crystals gave evidence that the incorporation of Sr^{2+} produced distortions in the apatite crystal structure at PO_4^{3-} sites. More CO_3^{2-} ions were incorporated in the Sr-doped nHAC. Thus, the incorporation of Sr^{2+} into nHAC changed the composition and crystal structure of apatite crystals which grew on the surface of collagen fibrils.

Keywords: Biomineralization; Biomimetic; Strontium; Carbonated; Microstructure

1. Introduction

Collagen and biological apatites are the main extracellular matrix compositions of hard tissues, such as bone and teeth. Collagen and biological apatites are assembled into mineralized collagen fibrils (natural nHAC) in vivo [1]. Natural nHAC exhibits special mechanical property due to its complex hierarchical structure. Many scientists have investigated and tried to illustrate the mechanisms of the self-assembling process of collagens and biological apatites in vivo [2,3]. So far, knowledge of the mechanisms of the self-assembling process of collagen and biological apatites is still very limited due to the complexity of the biological system [1].

As we know, natural nHAC is different from the artificially synthesized hydroxyapatite (HA) in some respects [4,5]. Apatites in natural nHAC are poor in crystal structure and non-stoichiometric. The size of crystals is in the nano-scale. Moreover, natural nHAC contains 3–8 wt% of CO_3^{2-} ions [6], trace of foreign ions [7], such as HPO_4^{2-}, Cl^-, Na^+, and Sr^{2+} [8]. Among the foreign ions in natural nHAC, Sr^{2+} is a key factor in the bone metabolism. Sr^{2+} is a trace element in skeleton and 98% of Sr^{2+} in human body is deposited in skeleton [9]. Sr^{2+} can diffuse into the Haversian capillaries walls [10], replace Ca^{2+} [10] and enhance bone volume [11]. Sr^{2+} has been used to prevent and treat osteoporosis [12]. Capuccini [13] found that 3–7 at% Sr-doped HA nanocrystals promoted pre-osteoblast activity and differentiation, while inhibited osteoclast activity and differentiation [13,14]. Furthermore, it is found that Sr^{2+} could enhance cells secreting collagen [15]. However, the role of Sr^{2+} incorporation on the composition and microstructure of nHAC is still unknown.

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*Corresponding author. Tel.: +86 10 62782770; fax: +86 10 62771160.
E-mail address: biomater@mail.tsinghua.edu.cn (Q. Feng).

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In our previous work [16–18], we have synthesized nHAC in vitro by mimicking the biomineralization process of collagen in vivo. In the present study, we hypothesize that Sr^{2+} can be incorporated into nHAC via biomineralization of collagen fibrils. The purpose of this work was to investigate the effects of Sr^{2+} incorporation on chemical composition and crystal structure of nHAC via biomineralization of collagen fibrils. We analyzed the Sr^{2+} incorporated nHAC by a combination of methods: Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), scanning electron microscopy (SEM), transmission electron microscopy (TEM) and energy dispersive X-ray analysis (EDAX).

2. Materials and methods

2.1. Materials

Type I collagen was provided by HWRK Chem. (China). Other reagents, such as NaOH, HCl, $CaCl_2$, $SrCl_2$ and Na_2HPO_4 were analytical pure and provided by Sinopharm Chemical Reagent Limited Corporation (China). Distilled water was used.

2.2. Synthesis of Sr^{2+} incorporated nHAC

$CaCl_2$ was mixed with $SrCl_2$ to prepare (Ca+Sr)-containing solutions. The total concentration of $CaCl_2$ and $SrCl_2$ was 82.4 mM. The Sr/(Ca+Sr) molar ratios in the (Ca+Sr)-containing solutions were set as 0%, 5%, and 10%, respectively. NaH_2PO_4 solution was prepared at a concentration of 82.4 mM and used as a source of phosphate ions. 494 mg collagen was added to 10 mM HCl, then 140 mL of (Ca+Sr)-containing solutions and 84 mL of NaH_2PO_4 solution were gradually added into collagen solution. Then the pH of the solutions was adjusted to 7.0 by adding NaOH solution (100 mM). The mixed solutions were aged for 12 h. The precipitates were separated with centrifugation. The final products were freeze-dried for 24 h and grounded into powders for characterization. The samples were labeled as Sr*x*–nHAC, *x* being Sr/(Ca+Sr) molar ratio in the (Ca+Sr)-containing solutions.

2.3. Methods of measurement

The FTIR spectra were measured with Nicolet 6700 (Thermo Electron Scientific Instruments, U.S.A) using 1.0 wt% Sr*x*–nHAC powders in KBr pellet. Each spectrum was obtained at a 2 cm^{-1} resolution through 256 scans. The second derivative spectra were obtained through Origin 8.0 software.

The phases of Sr*x*–nHAC powders were detected using Rigaku D/max-2550 with Cu Kα radiation. The wavelength λ was 0.15406 nm. The crystal size d in the c-direction was calculated using Debye–Scherrer's relation

$$d = \frac{k\lambda}{T_{1/2}\cos\theta} \tag{1}$$

where k is 0.9. $T_{1/2}$ is half-peak width of the 002 *hkl* reflection.

The lattice parameters were calculated using Bragg's law [19]

$$\lambda = 2d_{hkl}\sin\theta_{hkl} \tag{2}$$

$$\frac{1}{d_{hkl}^2} = \frac{4}{3}\left(\frac{h^2+hk+l^2}{a^2}\right) + \frac{l^2}{c^2} \tag{3}$$

The Sr*x*–nHAC powders were observed on a JEM-2100F (JEOL, Japan) at 200 kV. EDAX was combined with TEM to determine the elemental composition of Sr*x*–nHAC. The Sr/(Ca+Sr) atom ratios in the final products were analyzed by quantitative EDAX.

SEM analyses of Sr*x*–nHAC powders were performed on an environmental electron microscope (Quantan 200, JEOL, Japan).

3. Results

3.1. EDAX

The compositions of the powders examined by EDAX are shown in Fig. 1. These spectra showed the detected elements. The spectra of Sr5–nHAC and Sr10–nHAC revealed the presence of Sr, P and Ca. The Sr/(Ca+Sr) atom ratio was about 3.7 at% in Sr5–nHAC sample and about 7.2 at% in Sr10–nHAC sample.

3.2. FTIR

Fig. 2 shows the normal transmittance-FTIR spectra of Sr0–nHAC (a), Sr5–nHAC (b) and Sr10–nHAC (c). The main peaks are pertaining to C=O, PO_4^{3-}, HPO_4^{2-}, CO_3^{2-} and OH^- groups. The C=O peak at 1645 cm^{-1} originated from amide I peak of collagen fibrils. The characteristic peak of HPO_4^{2-} was detected at 875 cm^{-1}. The most intense peaks of the FTIR spectra between 950 cm^{-1} and 1150 cm^{-1} originated from stretching vibration of $PO_4^{3-}\,\nu_1$ [20]. To analyze the changes of position of characteristic peaks of PO_4^{3-}, we examined the second derivative spectra. Fig. 3 shows the second derivative spectra of $PO_4^{3-}\,\nu_1$ derived from Fig. 2. As shown in Fig. 3, a downshift of the peaks of $PO_4^{3-}\,\nu_1$ was seen. Fig. 4 shows the normalized transmittance-FTIR spectra between 1350 cm^{-1} and 1500 cm^{-1}, which is originated from symmetric stretching of CO_3^{2-}. In Fig. 4, the CO_3^{2-} peaks in the Sr5–nHAC and Sr10–nHAC were more prominent than those in Sr0–nHAC.

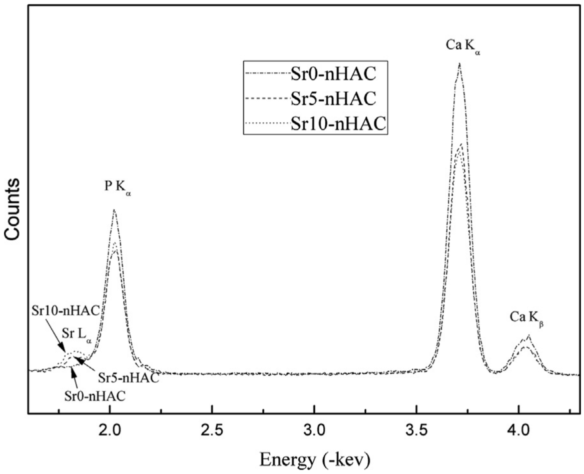

Fig. 1. Measurement of Sr incorporation by EDAX.

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