Int.J.Nano.Dim 1(1): 57-63, Summer 2010
ISSN: 2008-8868

Contents list available at **IJND**

International Journal of Nano Dimension

Journal homepage : **www.IJND.ir**

Hydroxyapatite - starch nano biocomposites synthesis and characterization

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Received: 6 April 2010; Accepted: 18 July 2010

Abstract

Bone like hydroxyapatite (nHAp) was synthesized via an in situ biomimetic process in presence of wheat starch. The effect of polymer concentration alteration on the final structure of nHAp was investigated. Formation of the nHAp at room temperature was confirmed by X-ray diffraction (XRD) and Fourier transform infrared spectroscopy (FT-IR). Size and morphology of the nHAp samples were characterized using scanning and transmission electron microscopy (SEM and TEM). Starch as a template agent has influence on shape of the HAp precipitated in its aqueous solution. It leads to formation of rode like nHAp which is similar to this inorganic component in natural bone.

Keywords: *Nanostructures, hydroxyapatite, Starch, Biomaterial, Template.*

1. Introduction

Chemical formula of Hydroxyapatite (HAp) is $Ca_{10}(PO_4)_6(OH)_2$ which is very similar to the materials forming the bones in the human body [1]. The HAp crystals in natural bone are needle-like or rod-like in shape 40–60 nm in length, 10–20 nm in width, and 1–3 nm in thickness. The synthesized hydroxyapatite with bone-bonding properties is widely used in hard tissue replacement due to their biocompatibility and osteoconductive properties [2-5]. Many characteristics of HAp, such as surface characteristics and bioactivity can be affected by The shape of HAp crystal. Therefore, the applications of the HAp can be expanded by controlling the crystal shape of the nanometer HAp, such as needle-like, spherical, plate-like shape and so on. At present many studies have reported to synthesize the nanometer HAp with different shapes. For example, the needle-like HAp has been synthesized by different processing methods including organic gel systems, homogeneous precipitation or hydrothermal technology [6,7]. The rod-like HAp has been synthesized by precipitating calcium nitrate tetra hydrate and ammonium dibasic phosphate in the presence of polyacrylic acid followed by hydrothermal treatment [8]. Brittleness

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of HAp limits its use. One of the methods to solve the problem is combination of it with polymer. Surfaces of organic materials can be tailored to achieve different properties, such as the capability of carrying functional groups, chelate to metal ions by their functional groups and hydrophilicity[9]. For instance, major approach in the development of materials for bone regeneration and replacement is the use of degradable polymers as matrices. Biodegradable materials have to degrade without an unresolved inflammatory response or an extreme immunogenicity or cytotoxicity [10,11]. Some attempts via in situ mineralization technique have been done using polymeric additives such as poly (vinyl alcohol) poly (lactic acid) (PLA), poly (acrylic acid) (PAAc), collagen, and due to their calcium binding properties [5,12,13]. Biopolymers are an important source of materials for biomedical applications. One of the biopolymers with biomedical applications is starch. This natural polymer is biodegradable, biocompatible, water soluble and inexpensive in comparison with other biodegradable polymers [14,15]. The polar nature of starch facilitates strong adhesion between the HAp and starch. Here the synthesis of bone like nano HAp in starch matrix, their characterization and investigation of the effect of polymer concentration alteration on the final structure will be reported.

2. Materials and methods

Extra pure water soluble Wheat starch was obtained from Merck Inc. For the synthesis of hydroxyapatite analytical grade $CaCl_2.2H_2O$, NaH_2PO_4 and NH_4OH were procured from Merck Inc. All chemicals were used without any further purification.

In situ synthesis of hydroxyapatite rods was carried out in the presence of wheat starch. HAp particles were also prepared in the absence of starch for comparison. The effect of biopolymer concentration on the final structure was studied by using different concentration of starch (0.2, 0.5 and 0.8 g of starch in 70ml water). At first, water based solution of starch, 0.5M solution of calcium chloride and 0.3M solution of sodium dihydrogen phosphate, using double-distilled water were prepared separately. Calcium chloride solution was slowly added to biopolymer solution for preparation of HAp in the presence of starch. The calcium chloride-starch solution was stirred slowly for 3h, and then proper amount of sodium dihydrogen phosphate solution was added little by little to above mixture. The quantities of reactants were selected to provide a Ca/P molar ratio of 1.66. The mixture became opaque after addition of sodium dihydrogen phosphate. NH_4OH solution was added to maintain and control pH at 10-10.5 in the solution. A milky white coloration was observed immediately after the addition of ammonia. HAp nucleation is promoted by High values of pH in the solution.

Obtained solutions via this manner (with and without biopolymer) were aged overnight at room temperature, then centrifuged, decanted, washed with deionized water and dried at $60^{\circ C}$ for 6h in vacuum oven. Dried products were characterized by (XRD; Philips expert pro with Cu-Ka radiation (λ=0.154 nm)), scanning electron microscope (SEM; Philips XL30), transmission electron microscope (TEM; Philips) and Fourier-transform infrared spectroscopy (FT-IR; Thermo Nicolet Nexus 870.

3. Results and discussion

In this study we tried to synthesis hydroxyapatite via biomimetic method. Biocompatible and biodegradable wheat starch has been chosen as a templating agent for in situ precipitation of nano HAp. Starch is polar, water soluble and consists of a mixture of the linear and helical amylose and the branched amylopectin. There is OH in its formula. Binding sites for the Ca ions in the solvent can be provided by the negatively charged surface groups upon ionization. The attraction of PO_4 anions towards uniformly distributed Ca sites will be followed and the crystallization process of hydroxyapatite initiated. Such an electrostatic model for the hydroxyapatite formation has been indicated in earlier studies [5,16,17]. As mentioned before, we want to investigate the effect of polymer amount alteration on HAp crystals. Functional group of prepared hydroxyapatite in starch matrix was investigated by FT-IR. The FT-IR spectra of synthesized nano HAp in the absence of starch biopolymer (a), prepared HAp in the presence of 0.2g starch (b), prepared HAp in the presence of 0.5 g starch(c) and prepared HAp in the presence of 0.8 g starch (d) are presented in Figure 1.

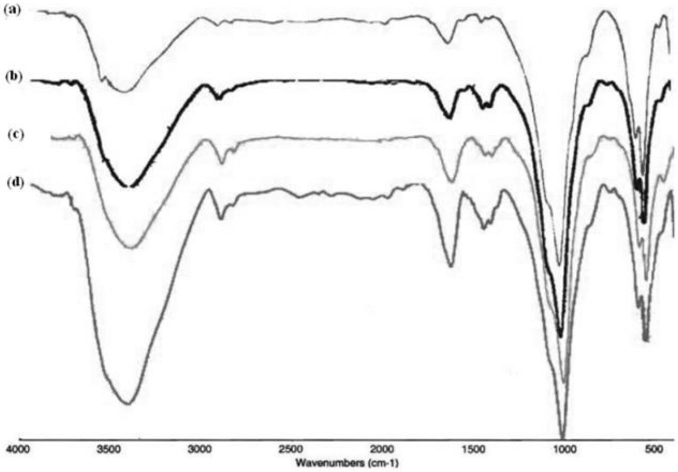

Fig.1. IR spectra of synthesized HAp , **a.** in the absence of polymer, **b.** in the presence of 0.2g starch, **c.** in the presence of 0.5g starch, and **d.** in the presence of 0.8g starch

The peaks from all samples match well with the standard HAp patterns and starch patterns. All samples revealed almost similar FT-IR spectrum, except that a small increase in the absorbance of starch specific bands(at about 3500cm^{-1}) could be seen for prepared nano HAp in 0.8g starch. FT-IR study of samples confirmed the formation of nanocomposite structures. The 470 cm^{-1} band results from the õ $_2$ phosphate mode. The bands at 560 and 600 cm^{-1} are derived from the õ4 phosphate modes. The peak at 960 cm^{-1} is assigned to õ1 phosphate mode. The bands at 1039 and 1086 cm^{-1} stand for the õ$_3$ phosphate mode. The absorption band at 1639 cm^{-1} reflects H_2O bending mode. The band at 3439 cm^{-1} band may come from lattice H_2O because this band exists in the range of 3200–3550 cm^{-1}. The stretching vibration and bending modes of the OH^- appeared at 3569 and 631 cm^{-1}, respectively. Absorbance band obtained at 3425 cm^{-1} corresponds to hydrogen bonded OH^- stretching band. Weaken bands at 2926.87 and 2850.11 cm^{-1} may be assigned with asymmetric stretching of starch C-H. Absorbance bands in the range of 1458.10 – 1401.15 cm^{-1} represent both phosphate and CH_2 bonds [18,19]. The slight shift in the position of absorption bands for the HAp prepared in the presence of polymer is indicative of dissociation and interaction of polymer with the nucleating crystals 5. Figure 2 shows the X-ray diffraction patterns of prepared nano HAp in presence and absence of starch. As shown in this figure, HAp and HAp/starch composite have similar XRD patterns. All the diffraction peaks can be assigned to monophase low crystalline HAp. It indicates that using starch has no effect on changing the crystallographic structure of HAp in the composite. Broadening the peaks in XRD pattern implys small size and low crystallinity of HAp similar to natural bone mineral. Using low temperature procedure may be the cause of poor crystalline nature of the prepared HAp. The peaks in HAp/Starch composite in comparison to pure HAp are slightly broader which can be a sign for decreasing the HAp crystallinity in the presence of matrix 20.

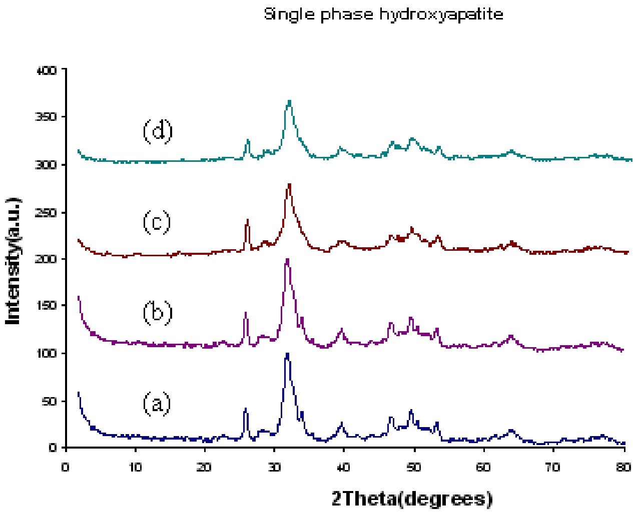

Fig.2. XRD patterns of synthesized Hap, **a.** in the absence of polymer, **b.** in the presence of 0.2g starch, **c.** in the presence of 0.5g starch, and **d.** in the presence of 0.8g starch

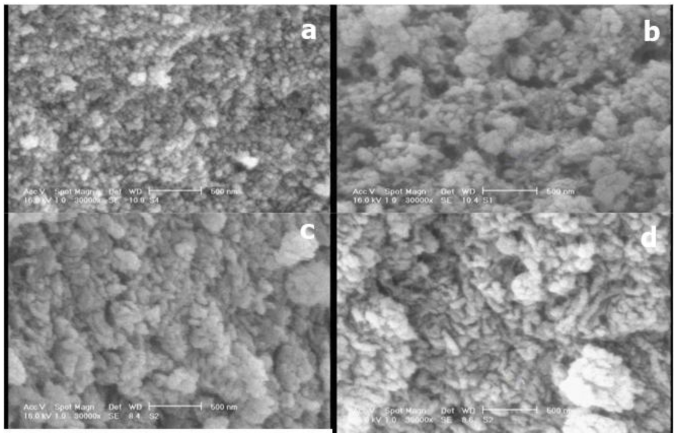

Fig.3. SEM micrographs of synthesized Hap, **a.** in the absence of polymer, **b.** in the presence of 0.2g starch, **c.** in the presence of 0.5g starch, and **d.** in the presence of 0.8g starch

SEM micrographs of the HAp and HAp in starch matrix are shown in Figures 3a-d. It seems that the overall morphology of the obtained powders in absence and presence of starch matrix is rod-like and angular, respectively. This suggests that the presence of starch have great influence on the morphology of the product due to interaction between the OH groups of starch and the Ca^{2+} ions in the solution and on the surface of HAP particles. Micrographs reveal that increasing the starch content can be the cause of uniformly distribution of the rode like particles. Figures 4a and 4b shows the transmission electron micrographs of HAp prepared in the absence and presence of 0.8 g starch. Primary particles are difficult to identify but mostly particles with angular and irregular morphology can be seen in the sample prepared in the absence of polymer (Figure 4a). The particle sizes are about 15-20 nm which is obtained from TEM image. Using the starch as a template has an influence on the morphology of prepared HAp. Figure 4b shows that the morphology of HAp particles which are prepared in the presence of starch similar to bone is in rod shape. The ultimate particle size of rod like HAp which is revealed by TEM, is about 6-12 nm in width and 45-85 nm in length.

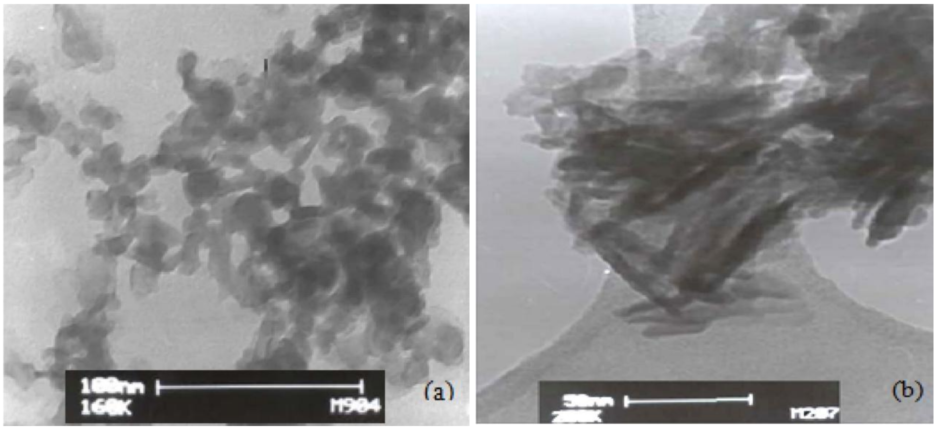

Fig.4. TEM micrographs of synthesized Hap, **a.** in the absence of polymer, and **b.** in the presence of 0.8 g starch

4. Conclusion

In summary, HAp particles are synthesized at room temperature by using starch as a templating agent via biomimetic route. XRD patterns and absorption bands in the IR spectra confirm the formation of the HAp. The presence of starch has influence on prepared HAp morphology. Experimental results show that the templating biopolymer act as an important factor in determining the morphology of final HAp. Less agglomeration can be seen in synthesized particles via in situ method because of HAp crystallites nucleation on the regularly arranged side groups of biopolymer chains. Using this biopolymer and applying condition of synthesis leads to product of bone like HAp. Different morphology by using starch has been shown by TEM micrographs. It is expected that prepared nanocomposite via in situ synthesis method are comparable to the natural bone from mechanical and biological properties point of view.

Acknowledgements

The financial and encouragement support provided by the Research vice Presidency of Science and research campus, Islamic Azad University and Executive Director of Iran-Nanotechnology Organization (Govt. of Iran).

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