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Synthesis and Characterization of Surface Functionalized Nanobiocomposite by Nano Hydroxyapatite

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***Abstract*—**In this study, synthesis of biomemitic patterned nano hydroxyapatite-starch biocomposites using different concentration of starch to evaluate effect of polymer alteration on biocomposites structural properties has been reported. Formation of hydroxyapatite nano particles was confirmed by X-ray diffraction (XRD) and Fourier transform infrared spectroscopy (FT-IR). Size and morphology of the samples were characterized using scanning and transmission electron microscopy (SEM and TEM). It seems that by increasing starch content, the more active site of polymer (oxygen atoms) can be provided for interaction with Ca^{2+} followed by phosphate and hydroxyl group.

***Keywords*—**Biocomposite, Biomimetic, Nano hydroxyapatite, Starch

I. Introduction

TISSUE engineering is often defined as application of engineering and medical science for design, synthesis, modifying, growth and regeneration of living tissues [1]. Development of three- dimensional (3D) porous scaffolds that can serve as a support, reinforce and organize the tissue regeneration or replacement in a natural way has been focused by recent researches in tissue engineering [2]. The 3D macro porous scaffolds play an important role in the formation of new tissues and provide a temporary scaffold to guide new tissue in-growth and regeneration. So, the scaffold is a key component of tissue engineering [3].

Important factor for the success of tissue engineering is ability of developing materials which can interface with tissues structurally, mechanically and biofunctionally [4]. Many biomaterials lack of desired functional properties to interface with biological systems in spite of numerous use of materials in tissue engineering. Thus, developing new materials to reach these issues is necessary. Composites of hydrophilic polymers and inorganic minerals like hydroxyapatite can be a good candidate for biomedical application. Bone apposition and differentiation of mesenchymal cells to osteoblasts can be promoted by attachment of hydroxyl apatite nanoparticles to polymer surface [5].

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One of the biopolymers with biomedical application is starch. This natural polymer is biodegradable, biocompatible, water soluble and inexpensive in comparison to other biodegradable polymers. The nature of starch facilitates strong adhesion between the HAp and starch [6],[7]. Based on this we have tried to develop in situ mineralization of starch scaffold by nano hydroxyapatite. We report the synthesis and characterization of nanocomposites which have surfaces patterned by nano hydroxyapatite.

II. Material and Methods

A. Material

Water soluble wheat starch was obtained from Merck. All chemicals needed for synthesis of hydroxy apatite $Ca(NO_3)_2.4H_2O$, K_2HPO_4, and NH_4OH were also supplied from Merck and used without any further purification

B. Method

In this research, synthesis of porous starch patterned by hydroxyapatite nano particles has been carried out via biomimetic method. Pure nano hydroxylapatite particles were also prepared in the absence of biopolymers for comparison At first, Different concentration of starch (1, 2 and 3 g in 50 ml double-distilled water) were prepared separately for studying the effect of polymer alteration on biocomposites structural properties. 75 ml aqueous solution of 0.8 M calcium nitrate tetrahydrate was made alkaline using ammonia solution to reach pH about 11. Resultant alkialine solution was added to starch solution and incubated at 30 º C for 3h. Then, 175 ml solution of 0.3 M dipotassium hydrogen phosphate was alkaline by ammonia solution up to pH = 11 and added to incubated starch-calcium salt solution. System was aged for 3 days at room temperature. After this period, obtained slurry was incubated at -4 º C for 3 days. Frozen slurry were thawed at room temperature for one day and again incubated at -4 º C for 3 days. This temperature cycle was repeated 5 times for all samples. Finally, white spongy shape composites will be obtained. Sample was washed by double distilled water for several times to remove contaminants and then freez dried at -35 º C. This process has been done for two remained starch solutions. The obtained three samples have bee named S-1, S-2 and S-3, for 1, 2 and 3 g starch solutions, respectively.

Resultant products were characterized using a Fourier Infrared spectroscopy (FT-IR) Thermo Nicolet Nexus 870, X-ray Powder diffraction (XRD) Seisert Argon 3003 PTC using nickel-filtered XD-3a Cu Kα radiations (λ=0.154 nm), Scanning Electron Microscopy (SEM) Philips, and Transmission electron microscope (TEM) Phliips operated at 100 kV.

III. Result and Discussions

A. X-ray Diffractio (XRD)

X-ray diffraction patterns of the synthesized nano hydroxyapatite in the presence and absence of biopolymer has been shown in Fig. 2. As it can be seen, hydroxyapatite and biocomposites have similar XRD patterns which the diffraction peaks can be assigned to monophase low crystalline hydroxyapatite. Broadening the peaks in XRD pattern of hydroxyapatite, imply to small size and low crystallinity of hydroxyapatite which is similar to natural bone mineral. Slightly broadening of nanobiocomposite peaks compare to pure hydroxyapatite can be sign of decreasing the hydroxyapatite size and crystallinity in the presence of biopolymers matrix [8].

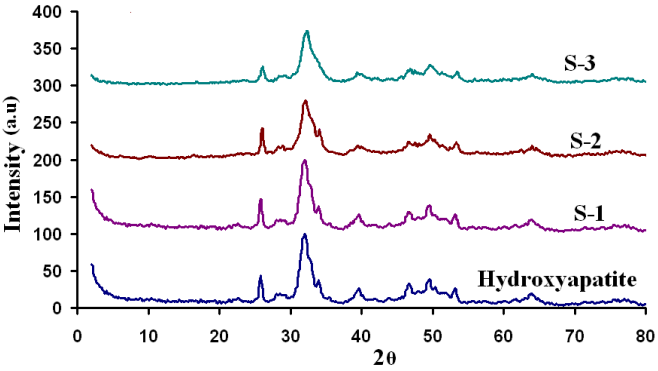


Fig. 1 XRD patterns of S-3,S-2,S-1 and synthesized hydroxyapatite in the absence of polymer

B. Fourier Transform Infrared Spectroscopy (FT-IR)

Fig.1 presents FT-IR spectra of amylopectin-rich starch, synthesized hydroxyapatite at the absence of biopolymer, S-1, S-2 and S-3, respectively from top to bottom. In the starch spectrum, the wide band observed at 3348 cm^{-1} is assigned to the O-H stretching of the amylopectin and its width ascribed to the formation of inter and intra molecular hydrogen bonds. The bands at 2935 and 2887 cm^{-1} are due to the asymmetric stretching of C-H, while the band at 1656 cm^{-1} is attributed to the adsorbed water. The band at 1015 cm^{-1} is assigned to the C-O alcohol bond and the bands at 1421 and 1357 cm^{-1} may concern to the angular deformation of C-H bonds in starch molecule [7],[9]-[11].

In the spectrum of hydoxyapatite, the band at 560-604 cm^{-1} is due to characteristic bands of v_4 (PO_4^{3-}). The υ_2 of the phosphate is observed at 470 cm^{-1} as weak band. v_1 (PO_4^{3-}) can be seen at 960 cm^{-1} and the bands at 1030-1100 cm^{-1} assigned to the v_3 (PO_4^{3-}). In phosphate network, bending and stretching modes of P-O vibrations are present as bands around 600 cm^{-1} and 1049 cm^{-1}, respectively. A broad band concerning to the main vibration of v OH^- at 3566 cm^{-1}, joined with the bands at 3400 and 1629 cm^{-1} (H-O-H) of water absorption in the products can be observed [12],[13]. Remained spectra are matched well with the pure synthesized Hydroxyapatite as well as starch spectra. There are the slight shifts in the position of absorption bands for the hydroxyapatite prepared in the presence of polymer is indicative of dissociation and interaction of polymer with the nucleating crystals [14].

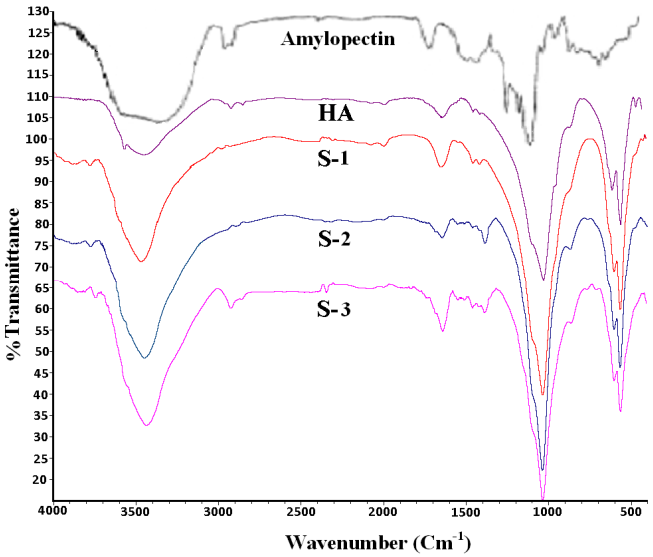


Fig. 1 FT- IR spectra of Amylopectin, synthesized hydroxyapatite in the absence of polymer, S-1, S-2 and S-3

C. Scanning Electron Microscopy (SEM)

Fig. 3a-c show the SEM micrographs of S-1,S-2 and S-3. As it can be seen, starch scaffolds have been mineralized via in situ nucleation of hydroxyapatite nanoparticles. Obtained composites have irregular pores in the size range of 1- 5 μm. It seems that porous starch scaffold surfaces have been patterned by spherical hydroxyapatite nanoparticles and by increasing the starch concentration in composite, the quantity of nano hydroxyaptite grows so that in third sample all the surface region has been covered by hydroxyapatite nanoparticles.

D. Transmission Electron Microscopy (TEM)

Fig 4a-b presents the TEM image of S-3 by 2 different magnifications. Particles have been located near each other regularly like chain rings by size about 15 nm.

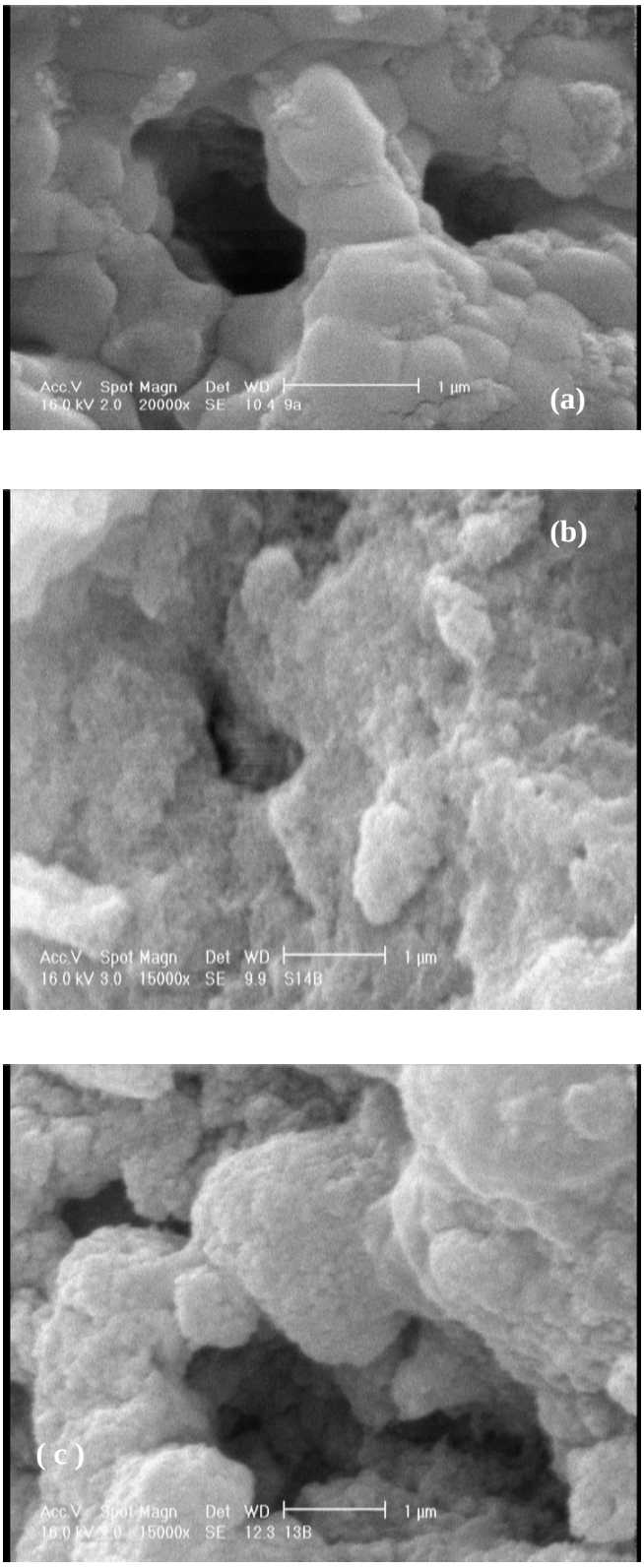
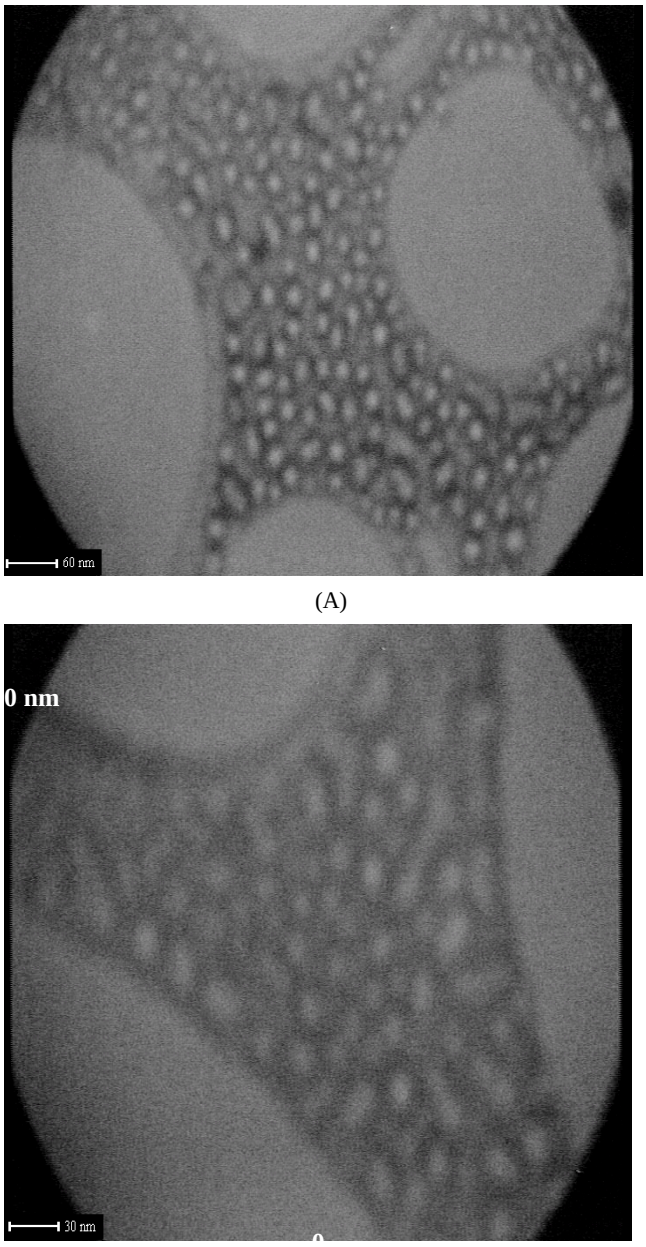


Fig. 3 SEM micrographs (a) S-1 (b) S-2 and (c) S-3



(A)

(B)

Fig. 4 TEM micrographs of S-3 by magnification (a) 60 nm and (b) 30 nm

IV. CONCLUSION

The present study is focused on producing surface functionalized nanobiocomposite via biomimetic method. These kinds of scaffolds may be suitable for bone/cartilage tissue engineering. Functional group of starch (hydroxyl) can be cause of nucleation and growth of hydroxyapatite nanoparticles. Frequent freez thawing cycle can induce hydrogen bond which aids formation of three dimensional structures of nanocomposite scaffolds. Increasing the polymer concentration in composite, can be cause of nano hydroxyaptite growth so that in third sample which starch content is maximum, all the surface region is covered by hydroxyapatite nanoparticles.

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