Trends Biomater. Artif. Organs, Vol 20(1), pp 20-23 (2006) *http://www.sbaoi.org*

Development of Hydroxyapatite from Natural Fish Bone Through Heat Treatment

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The objective of the present study is to develop hydroxyapatite powder from fishbone through heat treatment method. Fourier transform-infrared spectroscopy (FT-IR) and x-ray diffraction (XRD) techniques were employed to investigate the proof of formation of HAP phase. Presence of characteristic peaks for hydroxyl and phosphate groups were identified by FT-IR studies. XRD analysis reveals the formation phase pure HAP at 900°C. Electrochemical study involving cyclic polarization experiment was carried out to assess the corrosion resistance behaviour of HAP coated 316L SS in Ringer's solution. The results have indicated the efficiency of fishbone-originated HAP coatings on 316L SS surface.

Introduction

Hydroxyapatite (HAP) with the chemical formula $Ca_{10}(PO_4)_6(OH)_2$ is one of the inorganic components of the hard tissues of living tissues of living bodies such as bones, teeth, etc. [1] HAP is a calcium phosphate-based bioceramic, which has been used in medicine and dentistry for over 20 years because of its excellent biocompatibility with human tissue [2]. Thus, HAP has been widely used in dental implants [3], alveolar bridge augmentation, orthopaedics [4], maxillofacial surgery [5] etc. It is also a promising material as reinforcing filler for composites, insulating agents and chromatomedium for simple and rapid fractionation of proteins and nucleic acids. Moreover, HAP is known to be bioactive, osteoconductive, non-toxic, non-inflammatory and non-immunogenic agent [6-9]. Considering the numerous application of HAP in biomedical fields, various synthesis techniques have been developed. In the present investigation, an attempt was made to develop HAP powder from fishbone, a natural apatite rich substance [10]through heat treatment method. Fishbone, a cheaper source of HAP was generated as waste material during fish processing. HAP developed from fishbone was electro- phoretically deposited on type 316L SS and tested for its corrosion resistance behaviour through electrochemical study involving cyclic polarization in Ringer's solution.

Materials and Methods

Development and characterization of HAP powders

Fish bones were obtained from fish [Commercial name: Sier fish, species name: *Thynnus thynnus*] caught in the coastal area of Chennai. The fish bones were boiled in distilled water for 1 h and washed using a strong water jet to eliminate the fish meat. The washed fish bones were then dried and heated in an air oven for 100°C for 3 h. It was then crushed and calcined at various temperatures, say, 400°C, 700°C, 900°C and 1200°C for 2 h. The sintered powders were characterized by FT-IR and XRD techniques for HAP formation and crystallographic identification of the phases, respectively. Infrared spectra of the crushed samples were obtained using a fourier-transform infrared spectrometer (Perkin Elmer model Paragon 1000) at 4000-400 cm^{-1} region by using KBr pellet technique (0.1wt. %). X-ray diffraction analysis of all the heated samples were performed by RICHSEIFERT

model 3000 diffraction system, Germany, using CuKα (λ = 1.5405Å) radiation. Samples were analyzed over a 2θ range of 10°-70° with a sampling interval of 0.02°. Crystallographic identification of the phases was accomplished by comparing the experimental XRD patterns to standards compiled by the Joint Committee on Powder Diffraction and Standards (JCPDS), which were card #09-432 for HAP, #09-0169 for β-$Ca_3(PO_4)_2$ and #09-0348 α-$Ca_3(PO_4)_2$.

Substrate preparation and electrophoretic deposition

Type 316L stainless steel was used as the metal substrate and the elemental composition is given in Table 1. The 316L SS specimens used in the present study were cut into 10mm x 10mm x 2mm pieces. Prior to the study, the metal specimens were mechanically polished using silicon carbide papers of 120-600 grit. Final polishing was done using coarse (5mm) and fine (1mm) diamond pastes in order to produce scratch-free mirror finish surface. The polished specimens were washed with distilled water and then ultrasonically degreased with acetone.

Table 1 Chemical composition of type 316L SS

Element	Wt%
Cr	18
Ni	12
Mo	2.5
Mn	1.7
P	0.04
C	0.02
S	0.01
Si	0.15
Fe	Balance

Fishbone sintered at 900°C was ground to fine powder and sieved to a particle size of 100 mesh. It was then electrophoretically deposited onto the surface of 316L SS substrate from a 3% suspension in isopropanol. The deposition conditions were done at various potentials and optimized. The optimized potential was found to be 60 V for 3 minutes. The coated specimens were then dried in air oven at 90°C for 1h, cooled and corrosion studies were made.

Electrochemical measurements

All HAP coated 316L SS specimens were subjected to cyclic polarisation experiment in Ringer's solution. The electrochemical cell of 500 ml capacity fitted with saturated calomel electrode (SCE) as reference electrode, platinum foil as auxillary electrode and 316L SS as working electrode was used for all measurements. The working electrode having a test surface area of 1 cm^2 was used for all experiments. The pH of the electrolyte was adjusted to 7.4 and maintained at 37°C. All the electrochemical tests were carried out using Solatron SI 1287 Potentiostat/Galvanostat electrochemical interface controlled by commercial software. The cyclic polarization study was carried out by increasing the potential in noble direction at a preselected scan rate of 1mV/sec until the pitting potential was observed. After reaching the specific current density of 3mA/cm^2 the sweep direction was then reversed.

Results and Discussion

FT-IR analysis

FT-IR spectral analysis of fishbone heated at 100°C, 400°C, 700°C and 900°C were shown in Fig. 1a-d. The spectrum of fishbone heated at 100 °C shows a broad band between 2800 and 3600 cm^{-1}. It includes O-H stretch of HPO_4^{2-}, adsorbed water and $\nu_1(a_1)$ vibrations of the former. The peak due to $\nu_1(a_1)$ vibration of HPO_4^{2-} appears as a strong peak at around 2900 cm^{-1}.

The $\nu_2(a_1)$ vibration of HPO_4^{2-} occurs at 988 cm^{-1}, $\nu_3(a_1)$ at 872 cm^{-1}, $\nu_4(a_1)$ at 524 cm^{-1}, $\nu_5(a_1)$ at 1212 cm^{-1} and $\nu_6(a_1)$ at 1054 cm^{-1}. The bending mode of H_2O is positioned at 1650 cm^{-1} is very intense and narrow, which suggests that the intermolecular hydrogen bonding of water molecules is not proper. This is also evident form the resolved peaks due to O-H stretch of water in the high-energy region.

In addition to the above peaks, the absorption peaks appeared around 1400-1600 cm^{-1} indicates carbonate ion substitution. However, the intensity of adsorbed water and carbonate ion peaks got decreased with increase in temperature (Fig. 1b-d). The characteristic structural OH peaks appeared at 3570 and 633 cm^{-1} for the sample heated 900°C indicates the formation of HAP (Fig. 1d).

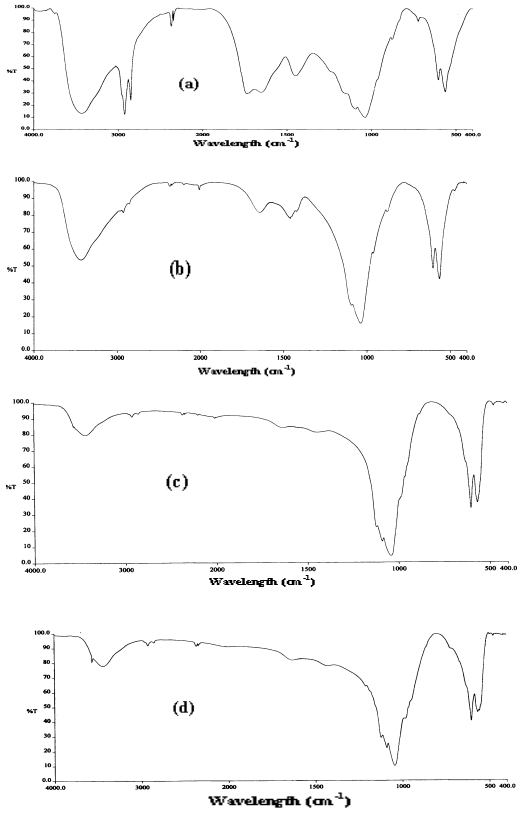

Fig. 1 FT-IR spectra of fishbone heated at (a) 100°C (b) 400°C (c) 700°C (d) 900°C

XRD analysis

Fig. 2 elicits the XRD patterns of fishbone heated at various temperatures, say, 100°C, 400°C, 700°C, 900°C and 1200°C. Fishbone heated at 100 °C show broad and merged peaks (Fig. 2a) indicating the amorphous nature i.e. low crystallinity of the biologically mineralized hydroxyapatite. Under heat treatment at 400-900°C, the bone initially transformed to a well-crystallized hydroxyapatite crystal (Figs. 2b-2d). The presence of tricalcium phosphate (TCP) phases in the sample sintered 1200 °C indicates the decomposition of HAP. This phenomenon is consistent with a previous report on the phase transformation of synthetic HAP to TCP at 1250°C (11). Thus, it is possible to develop phase pure HAP at temperature below 1200°C and a sintered composite of TCP/HAP with heating to 1200°C.

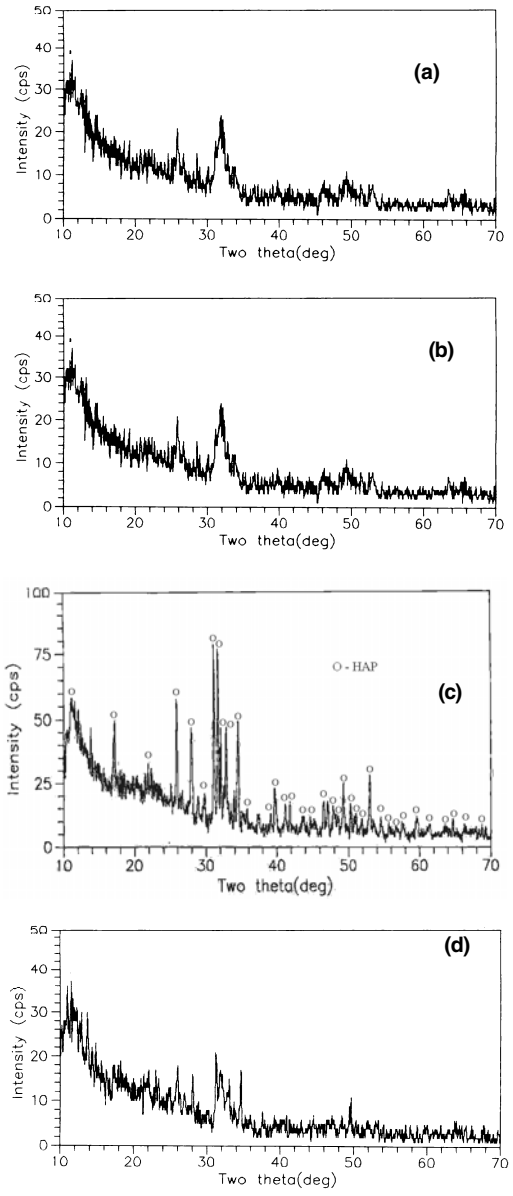

Fig. 2 : XRD patterns for fishbone heated at 100°C (b) 400°C (c) 700°C (d) 900°C (e) 1200°C

Cyclic polarization studies

Fig. 3 depicts the cyclic polarization curves of pristine 316L SS and fishbone originated-HAP coated 316L SS.

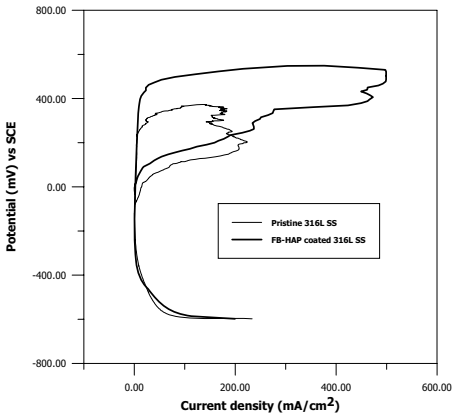

Fig. 3 Cyclic polarisation curves of pristine and fishbone-originated HAP coated 316L SS in Ringer's solution

Table 2 Corrosion kinetic parameters for pristine and HAP coated 316L SS in Ringer's solution

Sl No	Materials and Condition	Kinetic Parameter	
		E_b, mV	E_p, mV
1	Pristine 316L SS in Ringer's solution	320	-80
2	Fish bone originated HAP coated 316L SS in Ringer's solution	460	20

The observed corrosion kinetic parameters were illustrated in Table 2. The figure clearly elicits that the type 316L SS pristine material registered a maximum breakdown potential (E_b) value of +320 mV and a repassivation potential (E_p) of -80 mV. However, in the case of fishbone originated-HAP coated 316L SS an increased E_b value (+460 mV) was observed, when compared to the pristine 316L SS. The E_p value (+20 mV) was also found to increase in this case. This indicates that fishbone originated-HAP coated 316L SS is nobler than pristine 316L SS and hence it would provide better protection over the surface of pristine 316L SS.

Conclusion

The above-proposed work leads to a simple method to develop phase pure HAP powder from fishbone waste. Both FT-IR and XRD results revealed that the fish bone heated at 900°C produces a major HAP phase. Cyclic polarization studies indicated that the corrosion resistance behaviour of fishbone originated-HAP coated 316L SS is nobler than the pristine 316L SS. Thus, the present study suggests fish bone as a useful inexpensive ceramic material to develop phase pure hydroxyapatite crystals, which can be applied as a coating on 316L SS to prevent the release of metal ions and to improve the corrosion resistance of the metal.

Acknowledgement

The authors are thankful to the UGC programme on "University with Potential for Excellence (UWPFE)" for rendering financial support to carry out the work.

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