Dielectric and photoluminescence properties as the tools to assess bioactivity of nano-hydroxyapatite biomaterial

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ABSTRACT

Nano hydroxyapatite (nano-HAp) is synthesized via wet chemical precipitation route and *in vitro* bioactivity study is carried out using modified simulated body fluid (m-SBF) as a function of time. The pristine and incubated nano-HAp pellet samples are characterized by XRD, FTIR and SEM/EDAX for structural analysis, functional group identification, surface morphology and elemental analysis respectively. Dielectric measurements of pristine and incubated nano-HAp pellet samples are carried out as a function of frequency (10Hz–1MHz) at room temperature in order to describe interaction of biomaterial with the electric field. In order to see effect of incubation, PL spectra are also recorded as a function of soaking time. XRD shows the hexagonal phase of HAp which remains stable throughout incubation periods. FTIR confirms the apatite growth on nano-HAp pellet surface. SEM shows the tiny agglomerated apatite particles formed on the nano-HAp surface after incubation for various durations while EDAX data also confirms the formation of apatite particles upon incubation. Dielectric study reveals that dielectric constant of nano–HAp varies with incubation period and frequency of applied ac field. Intensities of photoluminescence spectra show incubation period dependent behaviour. The change in intensities of blue emission in PL and the dielectric properties of HAp reveals their close relation with the tissue growth. The study indicates the future potential application of nano-HAp in dielectric coatings and bio-electronic imaging devices.

Keywords: nano-hydroxyapatite, bioactivity, dielectric properties, photoluminescence, XRD, FTIR, SEM and EDAX

INTRODUCTION

From the past few decades, progress in hydroxyapatite (HAp) biomaterial shows extremely substantial impact on biomedical and tissue engineering field. HAp shows good bioactivity, biocompatibility and non toxic behavior *in vivo* [1]. The interaction of HAp with soft and hard tissue determines its biocompatibility. The ability to actively and physiologically stimulate the surrounding tissues is known as bioactivity. The progress in bioactivity study of HAp both *in vitro* and *in vivo* has been largely associated with elaboration of biocompatible bone substitute [2]. Simulated body fluid (SBF) is an appropriate medium for the evaluation of *in vitro* bioactivity of biomaterials by forming apatite layer on their surfaces [3,4]. Nowadays, the dielectric properties of HAp have special relevance in tissue engineering field. The effect of electric field (polarization) on bone healing process and bioactivity has already been reported [5,6]. These studies reveal that electric properties of HAp play an important role to understand the response of a tissue to electric stimulation. Therefore, this paper deals with *in-vitro* bioactivity study and its relation with PL and

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dielectric properties of HAp upon incubation. The change in electrical properties due to formation of apatite layer on the surface during incubation is reported.

MATERIALS AND METHODS

Nano-HAp is synthesized via wet chemical method wherein the chemical proportions of calcium nitrate tetrahydrate ($Ca(NO_3)_2.4H_2O$) and di-ammonium hydrogen orthophosphate ($(NH_4)_2HPO_4$) are selected so as to have Ca/P ratio 1.67. The preparation of m-SBF is carried out by dissolving, various precursors, viz. NaCl, $NaHCO_3$, KCl, $Na_2HPO_4.2H_2O$, $MgCl_2.6\ H_2O$, $CaCl_2.2H_2O$, Na_2SO_4 in one litre double distilled water in order to maintain the molar concentration of 142, 5, 1.5, 2.5, 125, 27, 1, 0.5 for Na^+/ K^+/ Mg^{2+}/ Ca^{2+}/Cl^-/HCO_3^-/HPO_4^{2-}/ SO_4^{2-} ions respectively. The solution, thus formed, is then buffered at a pH 7.4 with the help of 1M HCl. Initially the compatibility test for m-SBF is carried out followed by *in-vitro* bioactivity studies. The *in-vitro* bioactivity is evaluated by incubating HAp pellets in m-SBF as described earlier [7]. HAp pellets, pre-treated at 500°C temperature, are immersed in 15 ml freshly prepared m-SBF maintained at 37°C in an incubator for variable durations of 2, 4, 8,16, 24 and 32 days. At the end of each immersion time period, the pellets are removed from the m-SBF solution and rinsed with deionized water. Further, these pellets are dried at room temperature and used for surface analysis. The pellets, dried at 100°C for two hours, are used for dielectric measurements and photoluminescence studies.

RESULTS AND DISCUSSION

Figure 1 shows the XRD patterns for HAp pellets before and after soaking in m-SBF for various time durations. The XRD analysis, carried out using JCPDS data for hydroxyapatite, reveals the development of hexagonal phase which remains intact after incubation for various durations. The average grain size of HAp samples are found to be in the range of 36-57 nm. It is interesting to note that no other structure or phase appears in the deposited apatite layer. Figure 2 represents the FTIR spectra of the HAp pellet before and after incubation in m-SBF. The asymmetric bending motions of phosphate group in hydroxyapatite are found to be present at 540 and 607 cm^{-1} respectively. The absorption peak occurring at 632cm^{-1} is due to bending mode of $(OH)^-$ group. The absorption peaks appearing at 940, 967, 1030 and 1090cm^{-1} are attributed to fundamental mode of $(PO4)^{3-}$ group. A new phosphate peak in the range of 970-944 cm^{-1} is observed for all incubated samples and the appearance of absorption peak at 1129cm^{-1} for HPO_4^{2+} confirms the apatite growth.

The SEM micrographs of HAp sample are depicted in figure 3. Microstructure of the sample incubated for two days shows tiny agglomerated apatite particles formed on the surface of the HAp pellet. It is observed that longer the incubation duration, more is the number of particulates and larger is the size of grown apatite particles and the surface is found to be fully covered with apatite agglomerates and the apatite layer becomes denser after eight days of incubation. The Ca/P atomic ratios for HAp samples are determined From EDAX graph and are observed to be changing with incubation period.

Dielectric behaviour of HAp as a function of frequency applied field is presented in figure 4A-D. Dielectric constant of HAp, prior to incubation, is observed to be 40 and in case of incubated samples, the maximum value of dielectric constant (120) is observed for two days incubation. It is found to be decreasing with increase in incubation period, as depicted in figure 4(A). It is concluded from figure 4(B) that the dielectric loss also decreases with increase in frequency with a hump in the lower frequency region. The AC conductivity remains constant in the lower frequency region and it increases for further increase in frequency.

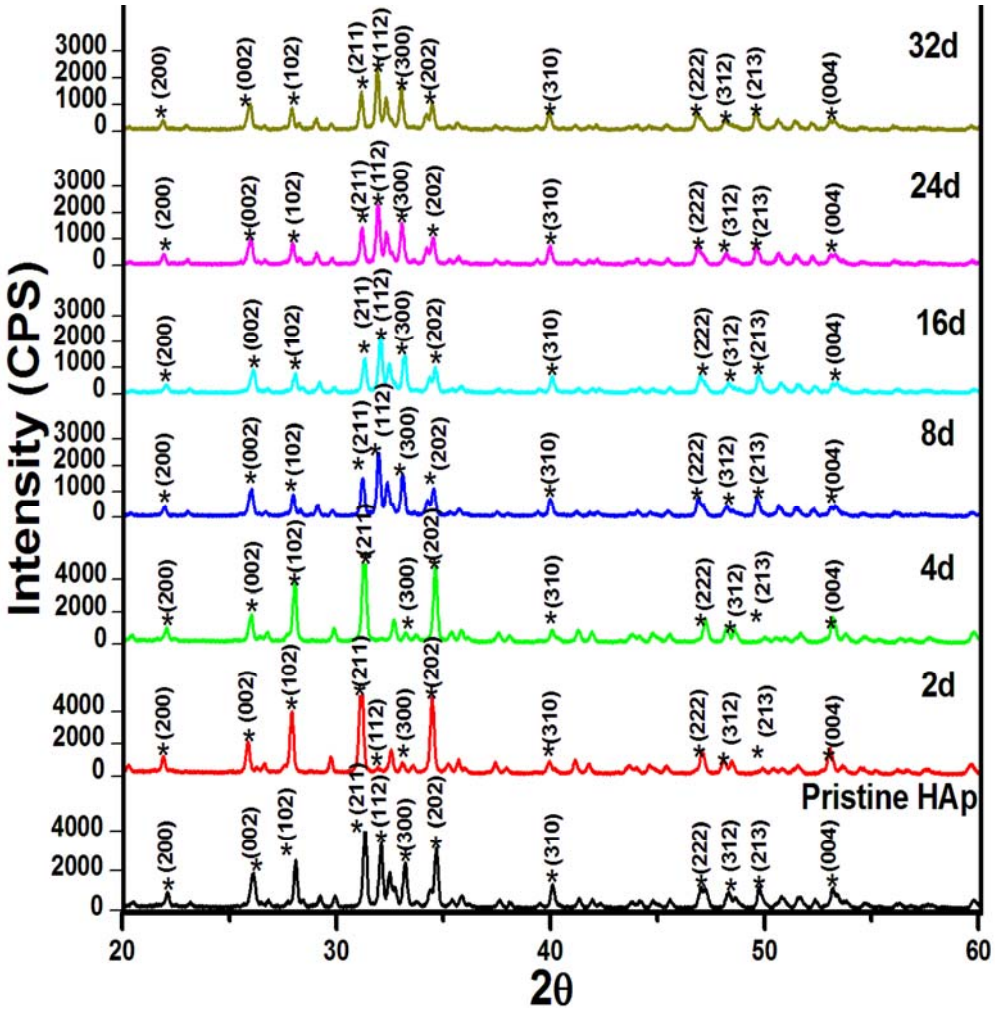


Figure 1. XRD patterns for HAp pellets before and after incubation showing the development of HAp phase.

The dependence of AC conductivity on incubation can be seen in figure 4(C). Figure 4(D) shows the complex impedance profiles for variable incubation durations. The two semicircles at higher and lower frequency region depict the relaxation mechanisms. The bulk resistances (R_b), determined by extrapolating the bulk impedance arc, increase with increase in incubation period and bulk capacitances are found to be 13.8 pF, 12.2 pF, 12.7 pF, 13.78 pF, 21.88 pF, 26.98 pF, 36.26 pF for pure HAp , 2 days, 4 days, 8 days, 16days, 24days and 32 days of incubation respectively. Relaxation time for pristine sample is observed to be 1.3 msec which suddenly changes to 1.7 msec from 16 to 32 days of incubation, showing the remarkable effect of long term incubation. The photoluminescence spectra show blue luminescence wherein the intensity of absorption peak near 468 nm increases with increase in incubation time. Pure HAp exhibits excellent bioactivity. The growth of calcium and phosphate layers on HAp surface is confirmed from XRD, FTIR, SEM and EDAX. Dielectric constant values and photoluminescence are found to be dependent on incubation period.

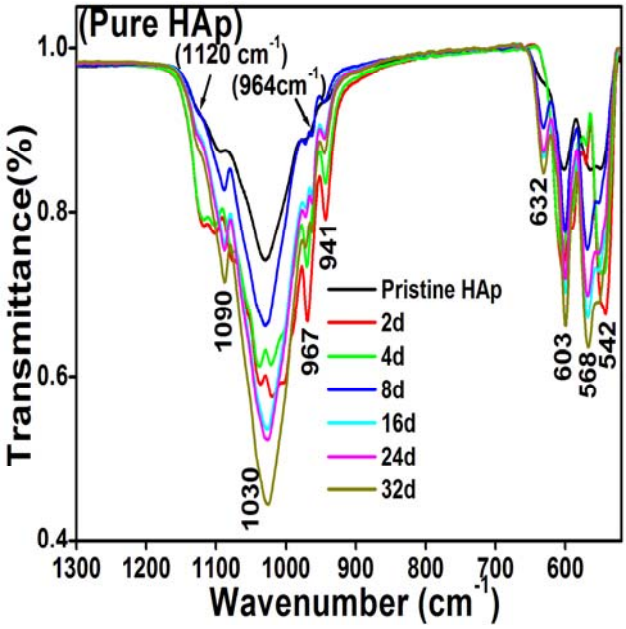
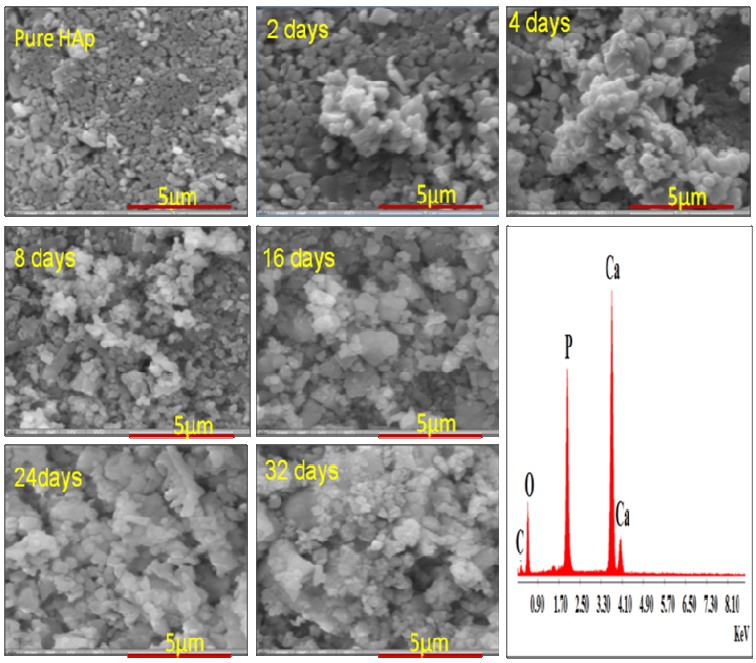


Figure 2. FTIR spectra of HAp pellets, before and after soaking in m-SBF, for various time durations displaying development of peculiar HAp peaks.



Sample	Ca/P ratio
Pristine	1.60
2d incubated sample	1.62
4d incubated sample	1.65
8d incubated sample	1.48
16d incubated sample	1.45
24d incubated sample	1.43
32d incubated sample	1.42

Figure 3. Scanning electron micrographs of HAp samples, before and after incubation in m-SBF, revealing the formation of hydroxyapatite layer. The adjacent table provides the Ca/P ratio calculated as per EDAX data.

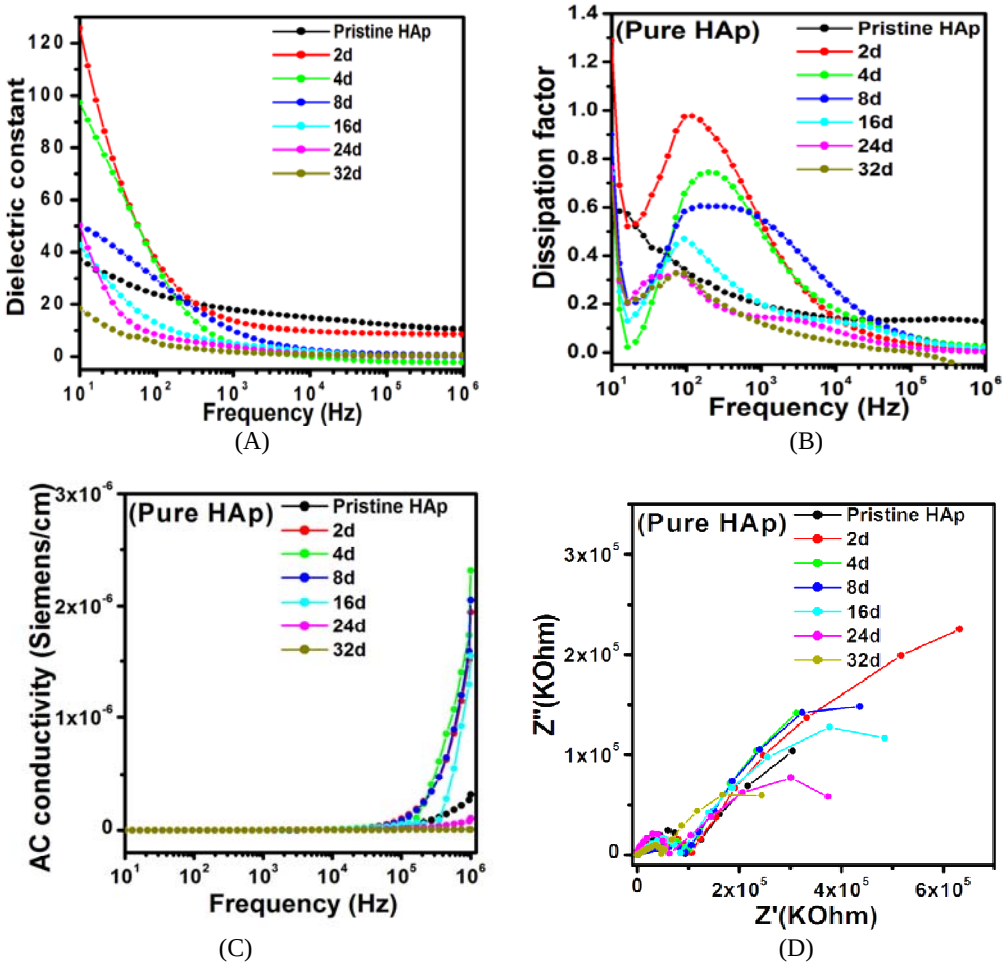


Figure 4. Frequency dependent behaviour of pristine and incubated Hap: A) Dielectric constant, B) Dielectric loss, C) AC Conductivity and D) Cole-Cole plots.

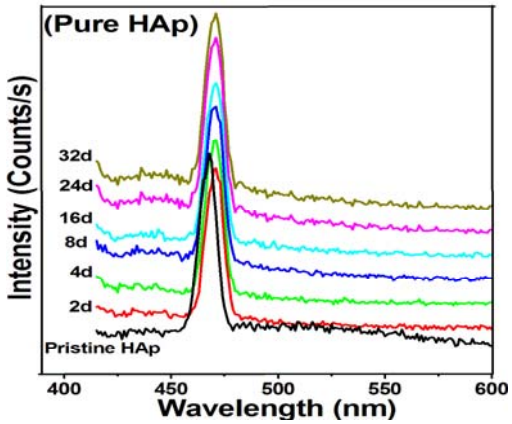


Figure 5. Photoluminescence of pristine and incubated HAp pellets as a function of incubation period.

Two distinct relaxation mechanisms are observed for incubated samples associated with (i) grain and (ii) grain boundary layer. The change in intensities of blue emission in PL and the dielectric properties of HAp reveals their close relation with the tissue growth. Therefore, these techniques can be used as tools to assess the bioactivity.

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