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Dextrin-Assisted Hydroxyapatite Nanopowders Using the Hydrothermal Method for Biomedical Applications

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Abstract

In the present work, the hydroxyapatite nanopowders were successfully synthesized by hydrothermal method using calcium nitrate tetrahydrate and diammonium hydrogen phosphate as precursor materials with addition of dextrin. Concentrations of 1% and 1.5% of dextrin were selected, and its effect on the structural, morphological and biological characteristics of HAP nanoparticles was investigated. The synthesized samples were analyzed by Fourier Transform Infrared Spectroscopy (FTIR), X-ray Diffraction (XRD) and Scanning Electron Microscopy (SEM). FTIR analysis showed the existence of characteristic functional groups of phosphate and hydroxyl of hydroxyapatite and dextrin related functional groups, thus demonstrating dextrin interaction with HAP. The XRD results showed that the phase-pure hexagonal hydroxyapatite with higher crystallinity was obtained with the increase of dextrin concentration. SEM images showed that dextrin substantially decreased the particle agglomeration and enhanced the dispersion of particles, leading to an even more homogeneous structure of the nanostructure. The cytotoxicity assays conducted on the MG-63 osteoblast-like cell lines determined a high degree of biocompatibility, with a maximum cell viability of 98.24% after 24 hours for Dx–1% at 0.5 µg/ml. The obtained results indicate that the hydroxyapatite nanopowders prepared by the addition of dextrin have superior physicochemical and biological characteristics, which makes them very appealing for biomedical applications.

Keywords: Hydroxyapatite nanoparticles, Dextrin, Hydrothermal synthesis, Biomedical applications.

1. Introduction

Hydroxyapatite (HAP), with the chemical formula $Ca_{10}(PO_4)_6(OH)_2$, is a calcium phosphate bioceramic widely recognised for its excellent biocompatibility, bioactivity, and osteoconductivity. Owing to its chemical similarity to the mineral phase of natural bone, HAP has been extensively utilised in biomedical applications such as bone tissue engineering, dental implants, drug delivery systems, and orthopaedic coatings [1, 2]. In recent years, nanoscale hydroxyapatite (nHAp) has gained significant attention because of its higher surface area, enhanced reactivity, and superior biological performance compared to bulk materials [3]. Despite these advantages, the practical application of hydroxyapatite nanopowder is often limited by issues such as particle agglomeration, poor dispersion, and lack of control over morphology and crystallinity [4]. Therefore, considerable research has focused on developing

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modified synthesis strategies to tailor the physicochemical properties of nHAP [5]. One promising approach involves the use of natural polymers and polysaccharides as structure-directing agents. Polysaccharides such as alginate, chitosan, and dextrin have been widely explored due to their biodegradability, non-toxicity, and functional versatility [6]. Among these, owing to its molecular weight (< 2800 g/mol), it can minimize the risk of tissue accumulation, is biodegradable, has low viscosity, and is tunable, thereby enhancing its suitability as a shape- filling material [7]. Dextrin is available in medical grade, has high solubility in water and DMSO, and holds hydroxyl groups suitable for bioconjugation, which has prompted this glucose polymer for several biomedical applications [8]. Dextrin-assisted synthesis plays a crucial role in controlling nucleation and crystal growth of hydroxyapatite nanoparticles. The abundant hydroxyl functional groups in dextrin interact with calcium and phosphate ions, thereby regulating particle size, morphology, and dispersion [9]. Additionally, dextrin improves colloidal stability and prevents nanoparticle aggregation. Recent advancements in dextrin-based nanocarriers also demonstrate their ability to enhance drug loading capacity and controlled release behaviour, making them highly suitable for biomedical applications [10]. Furthermore, hybrid systems combining hydroxyapatite with biopolymers such as alginate or cellulose derivatives have demonstrated improved osteogenic activity, mechanical strength, and bifunctionality [11]. These findings indicate that integrating hydroxyapatite with biocompatible polymers like dextrin can effectively mimic the natural extracellular matrix, thereby improving biological performance [12]. The incorporation of dextrin further enhances these functionalities by providing a biodegradable matrix and functional surface for drug conjugation. Overall, dextrin-assisted hydroxyapatite nanopowders represent a promising class of hybrid biomaterials with tunable physicochemical and biological properties

2. Experimental selections

2.1 Chemicals and Reagents

All chemicals used in this study were analytical grade and the chemicals used to synthesize hydroxyapatite nanopowder were purchased from Sigma-Aldrich. Calcium nitrate tetrahydrate, Diammonium hydrogen phosphate, Dextrin, ammonia and ethanol were used without purified. All the synthesis was carried out in deionized water.

2.2 Synthesis of HAP nanopowders

Calcium nitrate tetrahydrate ($Ca(NO_3)_2 \bullet 4H_2O$) and diammonium hydrogen phosphate ($(NH_4)_2HPO_4$) were employed as the calcium and phosphate sources respectively, for the synthesis of hydroxyapatite by the hydrothermal method. Both precursors were dissolved in deionized water. Solutions of 0.04 M $Ca(NO_3)_2 \bullet 4H_2O$ and 0.024 M $(NH_4)_2HPO_4$ were prepared to give a 1.67 molar ratio of Ca/P. The calcium solution was added drop wise to the phosphate solution with constant stirring. The precursor mixture was prepared in 100 mL of deionized water and then 50 mL of the precursor was dissolved in 50 mL of deionized water, which was a dextrin additive, and 50 mL of alumina precursor solution, which was a

growth regulating agent, was added drop wise. The pH was raised to 11 by adding ammonia solution. The mixture was stirred for 1 hour on a magnetic stirrer with 600 rpm. The whole solution was then transferred to an autoclave (Teflon-lined), and the solution underwent a hydrothermal treatment for 3 hours in an oven at 120 °C. The precipitate was washed with deionized water and ethanol several times and dried at 100 °C for 3 hours. The dried powder was additionally calcined at 600 °C and sintered at 800 °C to produce phase-pure hydroxyapatite. The experiment was repeated for the addition of additives like dextrin using 1 % and 1.5 % concentration. Various analysis techniques were used to characterise the final product

2.3 Characterization

A Bruker D2 Phaser X-ray diffractometer was used for powder X-ray diffraction (XRD) analysis with Cu-Kα radiation (k = 1.5418 Å). The FT-IR spectra of the synthesized samples were also taken with a Perkin Elmer spectrum two FTIR spectrometer in the range of 4000 to 350 cm−1 and with S/N = 2000:1 ppm, for 1 minute scan. FESEM investigations were performed by using a thermo Fisher Scientific QUANTAA 250 FEG microscope with a field-emission gun (FEG). The MG63, human osteosarcoma cell line was obtained from the National Centre for Cell Science (NCCS), Pune, India. Cells were grown in Minimum Essential Medium (MEM) containing 1% antibiotic-antimycotic and 10% fetal bovine serum (FBS). The cultures were grown in a humidified incubator at 5% CO_2 and 18-20% O_2 at 37 °C. Sub-culturing was performed every 3 days to ensure optimal growth and viability of cells.

3. Results and Discussions

3.1. Fourier Transform Spectroscopic Studies (FTIR)

The blank HAP, Dx–1% and Dx–1.5% show the formation of hydroxyapatite and the interaction between dextrin and the HAP matrix in the FTIR spectra. Hydroxyapatite shows the characteristic bands of hydroxyapatite (a) in the spectrum. The large broad band between 3400 and 3500 cm-1 is associated with the stretching vibration of hydroxyl groups (–OH) and adsorbed water molecules. The weak band around 1630–1650 cm^{-1} is assigned to the bending vibration of H–O–H due to the presence of absorbed water. The bands at 1030-1100 cm^{-1} and 960 cm^{-1} are considered to be the asymmetric and symmetric stretching bands of phosphate (PO_4^{3-}) groups, respectively. A further set of bending vibrations of the phosphate group are seen around 560 cm^{-1} and 600 cm^{-1}, indicating the formation of crystalline hydroxyapatite. With dextrin incorporation, all the characteristic phosphate and hydroxyl bands of HAP are preserved for the Dx–1% sample (b), suggesting that the hydroxyapatite structure is not changed by dextrin incorporation. Besides, the slight broadening and enhancement of the bands in the region of 3300–3500 cm^{-1} are also noted, which can be attributed to the hydroxyl groups of dextrin. The bands around 2920 cm^{-1} might be associated with the polysaccharide backbone C–H stretching modes. There is a band around 1400 – 1450 cm-1 and 1000 – 1150 cm-1

indicating C–O and C–O–C vibrations that are related to the dextrin molecules. When the concentration of dextrin is increased in the sample (c) the bands related to dextrin become more prominent. There is an enhancement of bands and slight shifts of the phosphate peaks, which may suggest intermolecular interactions (hydrogen bonding) between the dextrin and hydroxyapatite particles. The characteristic phosphate bands are, on the other hand, not affected, however, confirming that the basic HAP structure is not modified by dextrin addition.

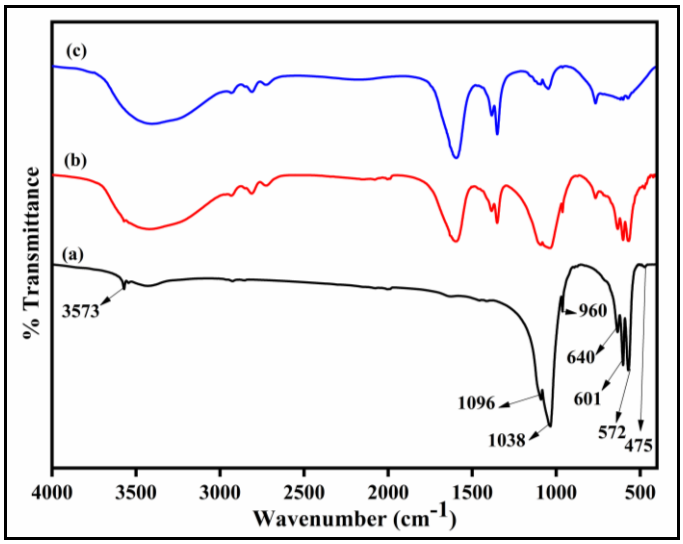

Fig. 1 FTIR spectrum of (a) Blank HAP, (b) Dx-1% and (c) Dx -1.5%

3.2 X-Ray Diffraction Studies (XRD)

The XRD patterns for Blank HAP, Dx–1%, and Dx–1.5% indicate the formation of hydroxyapatite (HAP) crystals to varying extents of crystallinity by increasing the amount of dextrin (Dx) added. The broad and low intensity diffraction peaks of the Blank HAP sample (a) suggest the presence of semi-crystalline hydroxyapatite nanoparticles. The presence of the broad hump around 2θ = 25–35° indicates that the synthesised HAP is partially amorphous and has a small crystallite size.

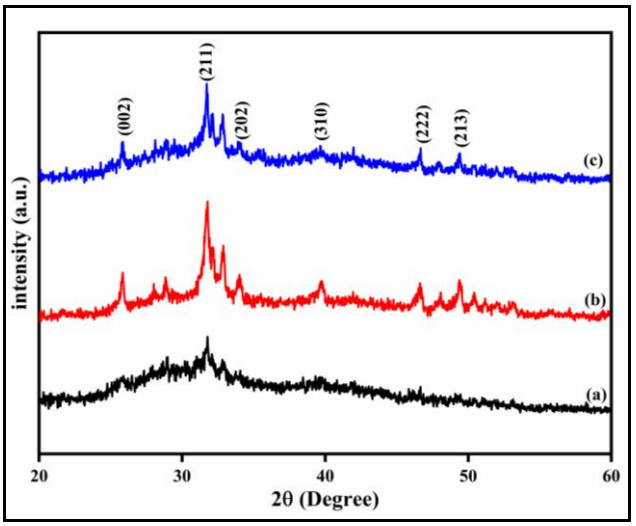

Fig. 2 XRD spectrum of (a) Blank HAP, (b) Dx-1% and (c) Dx –1.5%

The diffraction peaks are sharper and more intense in the Dx–1% sample (b) than in blank HAP, indicating that the existence of 1% dextrin has beneficial effects on the crystallinity of hydroxyapatite. The characteristic peaks, which are observed at about 2θ = 25.9°, 31.8°, 32.9°, 39.8°, 46.7°, and 49.5°, respectively, correspond to the crystal planes (002), (211), (202), (310), (222), and (213), which are typical of hexagonal hydroxyapatite structure according to the JCPDS standard. The improvement in the peak intensity suggests improved crystal growth and ordering. The diffraction peaks become even clearer and sharper for the Dx—1.5% sample (c), especially the strong (211) peak at 31–32°, which suggests that further improvement of the crystallinity has been achieved. The greater intensity along with decreased peak broadening

indicates that dextrin is a stabilizer and templating agent for the formation of HAP, favouring nucleation and crystal growth. All these samples contain no extra impurity peaks, which means that the hydroxyapatite is a phase pure.

3.3 Scanning Electron Microscopic Analysis (SEM)

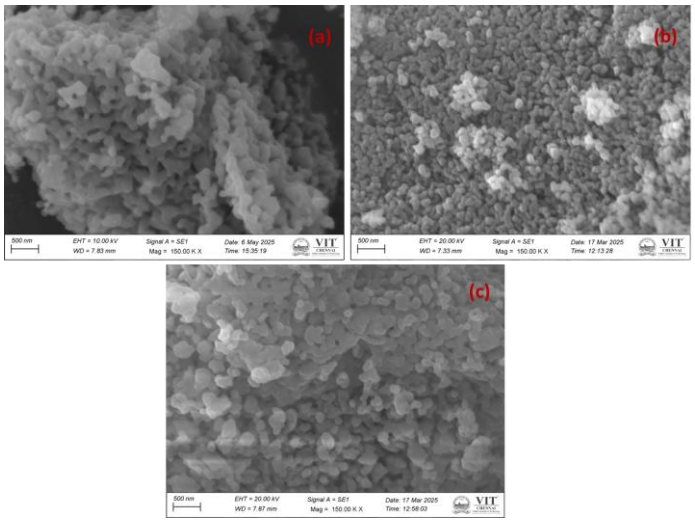

Fig. 3 SEM images of (a) Blank HAP, (b) Dx-1% and (c) Dx -1.5%

The SEM images of (a) Blank HAP, (b) Dx–1%, and (c) Dx–1.5% clearly show that the surface morphology and particle distribution of hydroxyapatite changes significantly after the addition of dextrin (Dx). As seen in the Blank HAP sample (a), the particles are irregularly shaped, and there is some significant agglomeration. Particles are found to be clustered due to the 'high surface energy' of hydroxyapatite nanoparticles. The surface is relatively rough and non-uniform, which means that the crystal growth has not been controlled, and the dispersion of particles is not good in the process of synthesis. For the Dx–1% sample (b), the morphology is more uniform than that of the other samples, with less agglomeration. The particles are also more finely divided and dispersed than blank HAP. Adding dextrin (1% with the weight of the feed) has a stabilizing and capping effect that helps to control particle growth and prevent unwanted clumping. Surface texture is also more apparent, which is an improvement in the homogeneity of the particles. The SEM image is taken from the sample with a Dx of 1.5% (c), and it appears that the morphology and dispersion have improved further. The particles have a more uniform distribution and a more dense and compact microstructure. Agglomeration is greatly reduced and the particles have a less coarse or lump-like appearance at the nanoscale. This means that as the dextrin concentration increases the nucleation process becomes more favorable and the growth of the particles is suppressed, resulting in an increase in the size of the crystallite and an improvement in structural organization.

3.4 Cyctotoxic Analysis

These images of MG-63 cell line after 24 hours of treatment with various concentrations of Dx–1% show the cytocompatible and biological responses of dextran–hydroxyapatite to osteoblast-like cells. The morphology and density of the MG-63 cells are significant evidence of the cell adhesion, proliferation and toxicity behavior of the material. The cells, at the lower concentration of 0.5µg/ml, show normal morphology with well-spread and elongated structures, which are due to excellent cell attachment and proliferation. These healthy appearing cells are also densely populated with cells with a cell viability value of

98.24%. This indicates that Dx–1% has a high level of biocompatibility at lower concentrations and acts as a growth medium for osteoblastic cells.

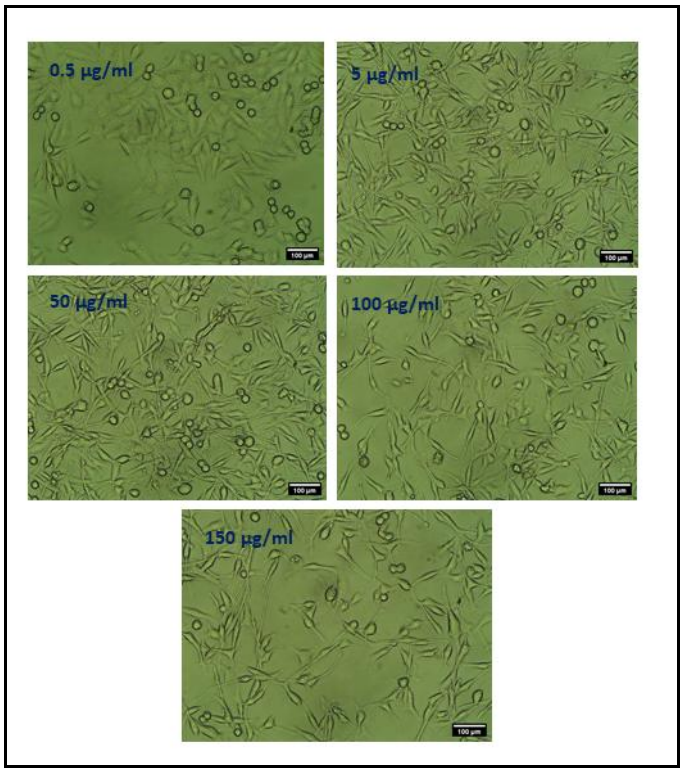

Fig. 4 Microscopic images of Dx-1% for 24 hours on MG 63 cell line

The cells are still in their normal spindle shape and have only a little decrease in cell density at 5 µg/ml. Good compatibility with the material, most cells are still attached to the surface. The observed viability of 89.97% indicates that the sample is not toxic and is good for cellular activity. A moderate reduction in cell population and spreading can be seen at the concentration of 50 µg/ml. There may be a few rounded cells as a result of low stress on the cells, but most of the cells are viable and attached. However, the viability of the cells (84.23%) shows good biocompatibility at this concentration.

5. CONCLUSION

Dextrin-assisted hydroxyapatite nanopowders were successfully prepared by a hydrothermal technique, and their structural, morphological and biological characteristics were investigated in this study. The hydroxyapatite formation and successful incorporation of dextrin were confirmed by FTIR analysis by observing the presence of phosphate, hydroxyl and dextrin related functional groups. XRD analysis showed the production of phase-pure hexagonal hydroxyapatite and that the amount of dextrin led to an increase in crystallinity and crystal growth without the appearance of impurity phases. SEM observations indicated that dextrin was able to effectively decrease the agglomeration of particles and increase the uniformity and dispersion of them, forming a more compact and uniform nanostructure. The MG-63 osteoblast-like cell lines cytotoxicity evaluation proved excellent biocompatibility of dextrin-assisted hydroxyapatite, particularly at low concentrations where high cell viability and good cell morphology was detected. The better physicochemical and biological properties show dextrin's ability as a stabilizing and template agent in the synthesis of hydroxyapatite. In conclusion, the hydroxyapatite nanopowders synthesized with dextrin have shown great promise for biomedical applications, such as bone tissue engineering, orthopedic implants, and regenerative medicine.

Conflict of Interest: Nil

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