

Synthesis and Characterization of Nano Hydroxyapatite with Poly Propylene Fumarate Nanocomposite for Bone Tissue Engineering

A. Ragu¹, P. Sakthivel², K. Senthilarasan³

^{1,2,3}Department of Physics Urumu Dhanalakshmi College, Kattur, Tiruchirappalli, India

Abstract: Hydroxyapatite (HAp) is one of the most attractive materials for bone tissue engineering. HAp with polymer nano composites are currently being intensively investigated as materials for bone tissue regeneration and reconstruction. It can be obtained in a nano structured form, which facilitates its fine dispersion in the polymer as well as producing advantageous interactions with bioactive polymer and tissue. This paper reviews recent advantages in HAp with polymer nano composite for bone tissue regenerativity with polymer emphasis on the material characterization. In this work, hydroxyapatite with poly propylene fumarate (PPF) nanocomposite were analyzed and confirmed by Fourier Transform Infrared Spectroscopy (FTIR), X-ray diffraction (XRD), Transmission Electron Microscopy (TEM), thermal stability analysis of TG/DTA.

Keywords: FTIR, XRD, TEM, TGA/DTA

1. Introduction

Hydroxyapatite ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$) is a well-known calcium phosphate ceramic found as a major component of bone and tooth [1, 2]. When a bone fractures (or) a cavity is formed in the tooth, synthesized HAp powder is used as a substitute material. This HAp powder alleviates the pain through the growth of bone like an apatite layer on its surface and gets integrated with the surrounding bone [3, 4]. It has been reported that porous HAp samples when implanted in bone defects show osteoconductivity, osteoinductivity and directly bond to living [5, 6]. In some cases, composite materials such as synthesized HAp ceramic mixed with polymer are used for better mechanical interaction as well as bioactivity when implanted in the body and provide an excellent platform for the existing bone to grow [8, 12]. In order for HAp with polymer bio ceramic materials used for medical applications to be biocompatible, non-cytotoxic and biodegradable. Since those, which are chemically similar to calcified biological tissue, they exhibit remarkable biocompatibility and are widely used in orthopedics and dentistry as both prostheses coating and bone fillers [5]. Currently several calcium phosphates are being often used, such as hydroxyapatite (HAp), tricalcium phosphate (α and β -TCP) and dicalcium phosphate (BCP). However, since HAp is the only one stable at physiological conditions, it is thus the most commonly used for medical applications [13].

Hydroxyapatite nanocomposites have been used in several application fields especially in bone tissue engineering [14]. Hydroxyapatite is a biocompatible and bioactive material for the construction of bone composition and it is osteoconductive [15]. Many studies demonstrated hydroxyapatite – polymer nanocomposites can stimulate osteoblast growth and proliferate [16]; however, there are also some studies regarding cartilage tissue engineering and cartilage replacement [17, 18]. The synthetic polymeric materials have several important uses in addition to tissue engineering. Examples include poly (methyl methacrylate) bone cement, polyoxymethylene bone tissue replacement, poly vinyl pyrrolidone bone tissue regeneration and poly (vinyl siloxane) dental impression

materials. A variety of materials have been used for replacement and repair of damaged bone tissues. These materials include metals; ceramics, polymers (natural and synthetic) and their combinations. Metals and ceramics have two major disadvantages for tissue engineering applications: they are lack of degradability in a biological environment and their processability is very limited. In contrast, polymers have great design flexibility because the combination and structure can be tailored to the specific needs.

2. Materials and Methods

2.1 Materials

Nano hydroxyapatite has been synthesized through a microwave accelerated wet chemical method. The raw materials required to start the processing of the composite were: analytical grade calcium hydroxide ($\text{Ca}(\text{OH})_2$) and ammonium dihydrogen phosphate ($\text{NH}_4\text{H}_2(\text{PO}_4)$) obtained from Merck (India) and Poly propylene fumarate [PPF] was purchased from Alfa Aesar. Doubly distilled water with ethanol was used as the solvent.

2.2 Methods

Synthesis of HAp and HAp with PPF composites

The first calcium hydroxide was slowly added to a solution of ammonium dihydrogen phosphate and after proper mixing, the HAp was precipitated by adding ammonia and the pH of the solution was maintained from 9 to 11. The solution was constantly stirred for 24h by a magnetic stirrer, allowing the reaction to complete. The resultant precipitate was separated and ammonia was removed by repeated washing. The precipitate was allowed to dry in an oven at 90 °C subsequently; aggregates formed were crushed into fine powder. In a separate study as - synthesized HAp powder (80) was mixed with polymer solution (20) where the number denotes the wt% and the resultant HAp with PPF nano composite powder was thoroughly mixed using a mortar and pestle for 30 min.

3. Results and Discussion

FTIR

The prepared samples were studied by Fourier Transform Infrared Spectroscopy (FTIR) using Perkin Elmer spectrometer in the range of 400 cm^{-1} to 4000 cm^{-1} . The FTIR spectra of pure HAp with PPF nano composites are shown in fig.1. The peak observed at 3242.07 cm^{-1} is due to stretching vibration of the HAp (hydroxyl group). The characteristic band of PPF absorption of C=O, -CH₂- appear at 1727.47 , 1644.50 , 1588.18 , 1304.60 and 126.11 cm^{-1} respectively. The strong peaks at 1452.80 cm^{-1} and 1405.59 cm^{-1} corresponds to the stretching vibration of CO₃²⁻ groups. The characteristic peaks at 1035.75 cm^{-1} is attributed to the PO₄³⁻ group. The phosphate stretching mode appeared at 566.31 cm^{-1} and 602.85 cm^{-1} corresponding to PO₄³⁻ groups.

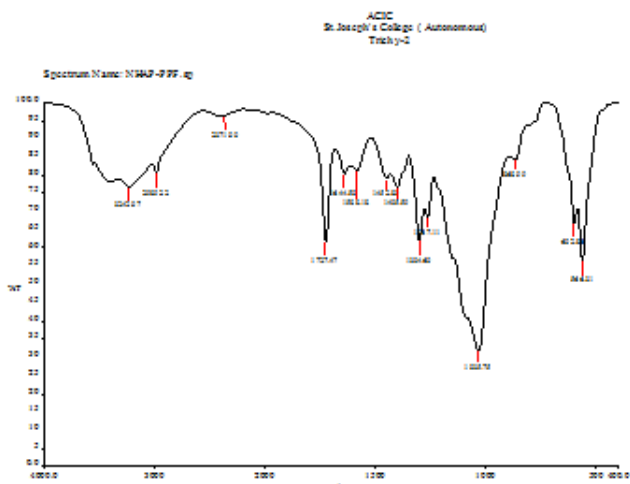


Figure 1: FTIR spectrum of nHAp with PPF nano composite

XRD

Structural analysis of the hydroxyapatite with polymer nano composite was conducted using XRD fig.2. The XRD pattern of nano hydroxyapatite with polymer shows sharper peaks which indicate better crystalline. The peak positions

are in good agreement with the JCPDS 09-0432. As can be seen, HAp with polymer XRD patterns, with diffraction peaks, obtained with value of (210), (211), (202) and (213) and the polymer value of (310), (331) and (215) match exactly with the hexagonal system with primitive lattice. The results of XRD analysis obtained in the present investigation are in good agreement with the reported results (Bouyer et al., 2000).

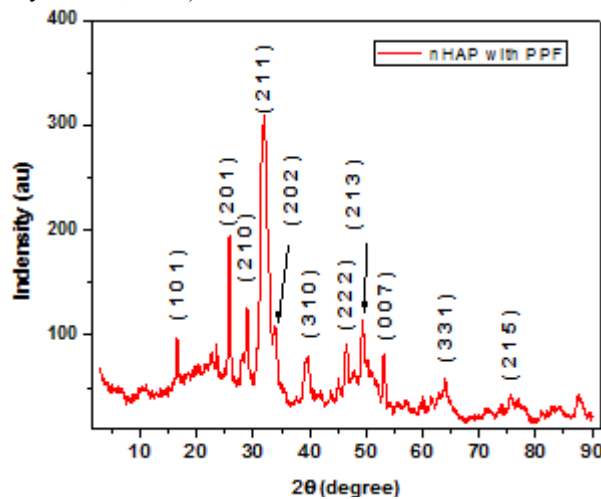


Figure 2: XRD spectrum of HAp with PPF Nanocomposite

TEM

The structure and morphology of the sample were further confirmed by the TEM and TEM images of the prepared nano hydroxyapatite with polymer as shown in figure 3. The Transmission Electron Microscopic analysis confirmed the presence of the rod - like morphology of the prepared hydroxyapatite with polymer nanocomposite with the particle size of the nanometer range length 50 nm and width is 3 to 5 nm. In addition the selected area electron diffraction (SAED) of the precipitates shows diffraction ring of patterns, this implies that the precipitates are crystalline nature. This is agreed with XRD results

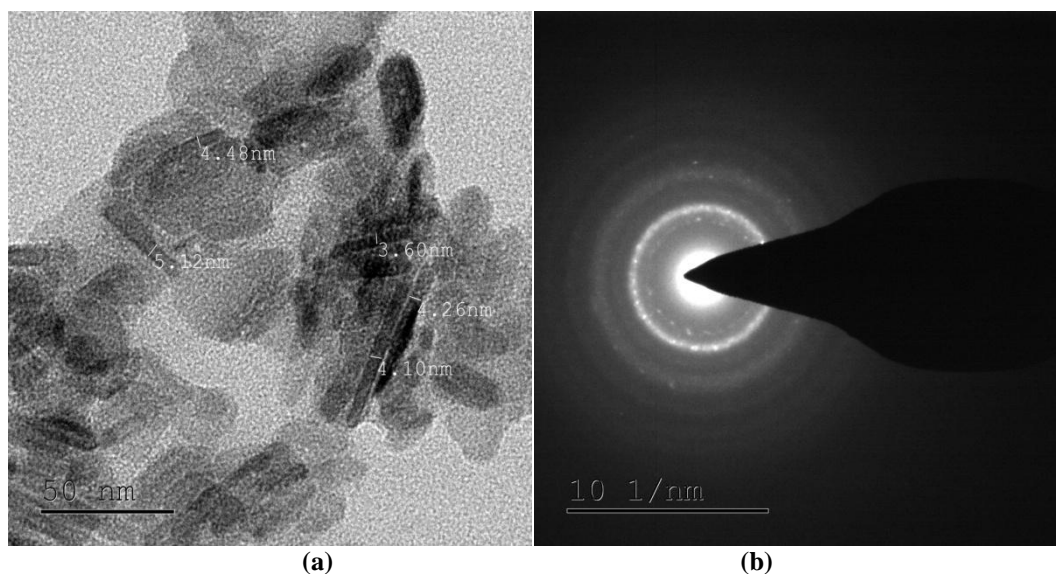


Figure 3: (a) TEM image of HAp with PPF and (b) Selected Area Electron Diffraction (SAED) image of HAp with PPF.

TGA/DTA

The Thermal gravimetric (TG) analysis is performed in an atmosphere (i.e.) air (or) oxygen with a linear ramp temperature. Thermal stability of synthesized nanoHAp/PPF was analyzed using Perkin Elemer experiment for TG-DTA. The TGA of the HAp/PPF nano composites powder was carried out between 30 °C to 800 °C in air at a heating rate 25 °C /min. The decomposition behaviour of HAp/PPF nano composite is as shown in fig.4. In the TG curves several steps are observed (Rajendran et al. 2002; Singh et al. 2008;

Wang et al. 2007). The first step, the temperature at the maximum weight loss rate is 170 °C for the 21.24 % of HAp with PPF composite. The second step, from 280 °C for the 7.03 % of HAp with PPF composite. In the DTA curve as sharp exothermic peak is located was a weight loss indicated by the endothermic peaks at 720 °C respectively. The transition may be due to the HAp with polymer interaction.

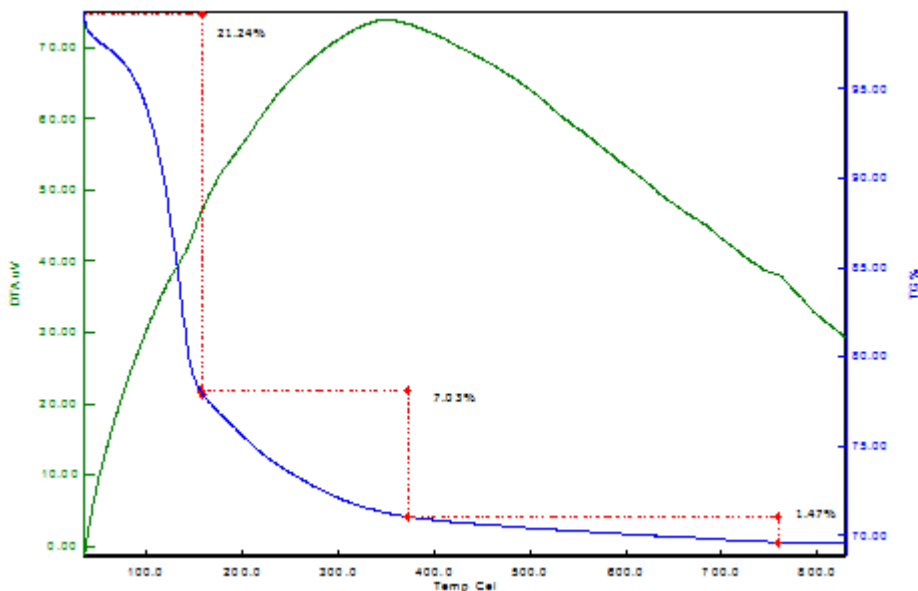


Figure 4: is HAp with PPF nanocomposite

4. Conclusion

Recent developments in biomaterials have provided many biodegradable polymers having interesting properties for varied biomedical applications. However, this variety remains limited in view of the wide range of material properties required to fulfill the almost unlimited requirements in these potential medical applications. Another important aspect of research on biodegradable biomaterials is that few of the biodegradable polymers proposed for specific medical applications. In this work, hydroxyapatite with poly propylene fumarate (PPF) nanocomposite were analyzed and confirmed by Fourier Transform Infrared Spectroscopy (FTIR), X-ray diffraction (XRD), Transmission Electron Microscopy (TEM), thermal analysis of TG/DTA and the HAp with PPF can be used to antibacterial activity. Therefore, we concluded that the Poly propylene fumarate with hydroxyapatite nanocomposite could be used as an appropriate alternative for bone tissue engineering applications.

5. Acknowledgements

Authors thanks to the management of Urumu Dhanalakshmi College, Tiruchirappalli for providing research facilities in the campus.

References

- [1] M. Jarcho, Calcium phosphate ceramics as hard tissue prosthetics, Clin. Orthop. Rel. Res. 157 (1981) 259-278.
- [2] N. Tamai, A. Myoui, T. Tomita, T. Nakase, J. Tanaka, T. Ochi, H. Yoshikawa, novel hydroxyapatite ceramics with an inter connective porous structure exhibit superior osteoconduction in vivo, J. Biomed. Mater. Res. 59A (2002) 110-117
- [3] H. M. Kim, T. Himeno, T. Kokubo, T. Nakamura, Process and kinetics of bonelike apatite formation on sintered hydroxyapatite in a simulated body fluid, Biomaterials 26 (2005) 4366-4373
- [4] A. Nakahira, M. Tamai, H. Aritani, S. Nakamura, K. Yamashita, Biocompatibility of dense hydroxyapatite prepared using an SPS process, J. Biomed. Mater. Res. 62 (2002) 550-557.
- [5] J. R. Woodward, A.J. Hildore, S.K. Lan, C.J. Park, A. W. Morgen, J. A.C. Eurell, S.G. Clark, M.B. Wheeler, R.D. Jamison, A.J.W. Johnson, The mechanical properties and osteoconductivity of hydroxyapatite bone scaffolds with multi-scale porosity, Biomaterials 28 (2007) 45-54.
- [6] L. Lin., K.L. Chow, Y. Leng, study of hydroxyapatite osteoinductivity with an osteogenic differentiation of mesenchymal stem cells, J. Biomed. Mater. Res. A 89 (2009) 326 - 335
- [7] U. Ripamonti, osteoconduction in porous hydroxyapatite implanted in heteropic sites of different animal models Biomaterials, 17 (1996) 31-35

- [8] C.Q. Ning, y. Zhou, In vitro bioactivity of a biocomposite fabricated from HA and Ti powders metallurgy method, *Biomaterials* 23 (2002) 2909 - 2915
- [9] S. Ramakrishna, J. Mayer, E. Wintermantel, K.W. Leong, Biomedical application of polymer composite materials: a review, *Compos. Sci. Technol.* 61 (2001) 1189 - 1224
- [10] D.K. Pattanayak, Apatite wollastonite - poly methyl methacrylate biocomposites, *J. Mater. Sci. Eng. C* 29 (2009) 709-714.
- [11] C.M. Agrawal, R.B. Ray, Biodegradable polymeric scaffold for musculoskeletal tissue engineering, *J. Biomed. Mater. Res.* 55 (2001) 141 – 150.
- [12] D. K. Pattanayak, B.T. Rao, T.R. Rama Mohan, Calcium phosphate bioceramics and bioceramic composites, *J. Sol-gel Sci. Technol.* 59 (2011) 432 - 447
- [13] T. Hara, K. Hayashi, Y. Nakashima, T. Kamemaru, Y. Iwamoto, The effect of hydroxyapatite coating on the bonding of the bone to titanium implants in the femora of ovariectomised rats. *J. Bone. Joint surg. (Br)* 81 (1999) 705-709.
- [14] E.S. Ahn; N.J. Gleason; A. Nakahira; J.Y. Ying. Nanostructure processing of hydroxyapatite – based bioceramics. *Nano Lett.* 2001, 1, 149 – 153.
- [15] L. Lin; K.L. Chow; Y. Leng; study of hydroxyapatite osteoconductivity with an osteogenic differentiation of mesenchymal stem cells. *J. Biomed. Mater. Res. A* 2009, 89, 326 -335.
- [16] P. Ducheyne; Q. Qiu, Bioactive ceramics; The effect of surface reactivity on bone formation and bone cell function. *Biomaterials* 1999, 20, 2287 – 2303.
- [17] C.-Y. Kuo; S. -N. Leou, Chondrogenesis of articular chondrocytes in hydroxyapatite chitin / chitosan scaffolds supplemented with pituitary extract. *Eng. Life Sci.* 2010, 10, 65 – 74.
- [18] C. Spadaccio, A. Rainer; M. Trombetta; G. Vadala; M. Chello; E. Covino; V. Denaro; Y. Toyoda; J.A. Genovese. Poly – L – Lactic acid /Hydroxyapatite electrospun nanocomposites induce chondrogenic differentiation of human. *M.Sc. Ann. Biomed. Eng.* 2009, 37, 1376 – 1389.