

Original Article

HAp/TiO₂ Composite Coatings and its Effective Use in Biomedical Applications

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Hydroxyapatite and Titania being able to exhibit a plethora of distinct applications related to bone-tissue regeneration and orthopaedic implantations, we consider them as our essential materials under concern. The present investigation deals with HAp/TiO₂ composites and its preliminary characterizations based on the results from XRD, FTIR, SEM and further extending the studies to electrophoretic deposition of the composite coating. The results from XRD and FTIR gave a confirmation on the phase transformation studies of TiO₂ from anatase to rutile. HAp inherently exhibits adequate biocompatibility and osteoregenerative applications and hence used extensively for coatings over implants. Composite was made using HAp and TiO₂ to provide a better preferable coating for the orthopaedic implant through electrophoretic deposition technique by varying voltage parameter. From our current study with preliminary outcomes, we confirmed that the HAp/TiO₂ composite is a promising coating and could be opted for biomedical applications.

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Introduction

The class of materials which are engineered with mere intentions of improving the healthy living of human race, aiming to aid human health by interactions with cells associated with the injured system are termed biomaterials. In other words, biomaterials are accounted as materials that exhibits likeness to human tissues and aids in any diagnostic or therapeutic procedures [1]. Biomaterials majorly includes metals, ceramics, polymers and composites which employs applications in biomedical fields including dental, and orthopaedic applications accompanied with other applications in making sutures, pacemakers drug delivery and much more. An ideal biomaterial is obliged to be biocompatible, non-cytotoxic, impart adequate mechanical strength, corrosion resistance, adequate tribological properties, non-carcinogenic, anti-inflammation and least side effects including barely any metal leaching [2-4]. Compatibility with tissues or cells is a principal characteristic of the biomaterial. This interaction with the host cells is the rationale for appropriate selection of certain materials for their specific applications. The crucial downside of

this compatibility is that occasional degradation of materials happens which leads to the local deleterious changes and harmful systemic effects.

Our material of concern in this article is hydroxyapatite (HAp) and titania (TiO₂) which falls under the category of advanced ceramics. Hydroxyapatite is a bioactive ceramic, coupled with biodegradability to certain variable extent [3] and Titania is bioinert by nature. Combining them together is anticipated to deliver a synergistic effect of its attributes and thereby exhibit bioactivity. Hydroxyapatite [(Ca)₁₀(PO₄)₆(OH)₂] was investigated to be highly effective for apatite formation and inducing bone cell proliferation and osseointegration capabilities along with osteoblastic gene expression. They are also evidenced for osteoconductive properties and inherent osteoinductive properties and a great amount of bioactivity and biodegradability also helping with protein adhesion [5,6]. They behold the ability to form a direct active chemical bond with the host bone and hence is accounted as bioactive. They show great similitude with natural bones which is a composite by nature, which made it an efficacious alternative to opt as a bone substitute. They had been successfully extracted from bones of bovine by Barakat et. al, once again proving that they exist naturally. They used different methods to finally obtain crystalline hydroxyapatites of varying structures including nanorods and

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nanoflakes [7]. The shortcoming of this material is that they display low mechanical properties which hinders them to be used as load bearing implants despite being exceptionally biocompatible and get integrated with the associated tissues easily and adeptly. Discerning the mediocre mechanical characteristics such as low fracture toughness, lesser tensile strength, brittleness etc. remains the rationale behind the inability for using HAp for load bearing applications. Reason for deterioration of mechanical properties of HAp is due to the decomposition of hydroxyapatite to certain calcium phosphate phases including Tricalcium phosphate (TCP). Hydroxyapatite coatings over metallic implants are usually carried out to improve their surface properties by aiding in the osteogenic abilities and exhibit potential activity in drug delivery. Kobayashi et.al, has already confirmed the bone bonding ability of HAp through his studies of apatite formations by aiding faster bone apposition from the O, P and N surfaces [8].

Titania or titanium dioxide (TiO_2) is naturally occurring oxide of Titanium which is a bioinert material. It has different phases majorly anatase, brookite and rutile among which rutile seems to be the thermodynamically stable form and is more prevalent in biomedical applications rather than anatase which shows better photocatalytic activity [9]. The material is said to be non-toxic and hence found to be safer for medical use. While manufacturing alloys Titania is also used as a coating material and since a layer of oxide protects the internal implants. It provides assistance as a chemical barrier against the *in vivo* release of metal ions in the human system from the metallic implants. They display superior corrosion resistance and optical properties including photoluminescence which allows them to excel as a relevant material for biomedical domain. The interaction between titania and bone-associated tissues promotes the apatite formation which accelerates the bone regeneration process. They also possess good osseointegration character and has a great role in drug delivery system as carriers. TiO_2 derived by sol-gel method has been shown to display bone bonding capability when synthesized through sol-gel method since they are acidic in nature and hence have Ti-OH groups which helps the effective osseointegration [10-12]. Hydroxyapatite is one such bone alternative which is comparatively more biocompatible and meets the requirements of bone tissue regeneration, but lacks mechanical strength. Hence, we combine hydroxyapatite along with titania to obtain a composite to exhibit synergistic properties of both. Composites are combined that they are distinguishable rather than merging with each other and displays an interface since the structural units are formed at the macroscopic level. They demonstrate greater attributes than the individual components. They could be fabricated by either combining the desired precursors during the synthesis or by mixing them post-synthesizing individually. In our study, we prefer mixing them after individual synthesis by the process of ball-milling. Titanium alloy (Ti-6Al-4V) is broadly used as orthopaedic implants and in dental applications. They also show great biocompatible properties by preventing corrosion accounting to the protective layer of TiO_2 formed over the surface of the implant when exposed to Oxygen containing environment including body fluid. Apart from being stable in physiological environment, they can also increase the calcium ion interactions and thereby enhance the osteoblast adhesion which is of paramount importance in bone regeneration. Ti-6Al-4V metal alloy has characteristics like longer fatigue life, enhanced corrosion resistance and superior durability; which increases the aptness of the same in load bearing applications [13]. To suppress the degradation property of hydroxyapatite, it is combined with Titania which can withstand corrosion to a certain extent and ability to reveal favorable mechanical properties.

In the present study, we deal with preparation of hydroxyapatite - titania composites and a minor probe into the phase transition studies on different sintering temperatures. The transformation of phase in titania and the surface morphology of the composites was analysed. Further coating by EPD over Ti-6Al-4V and optimizing the coating at varying voltage at defined time intervals was carried out to minimize the drawback of the coating with respect to uniformity.

Materials and Methods

Major precursors used for the synthesis of HAp and TiO_2 are Calcium nitrate tetrahydrate, Diammonium hydrogen orthophosphate and Titanium (IV) isopropoxide respectively. Calcium Nitrate Tetrahydrate [$\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$] was procured from SD Fine Chemicals Limited with an assay of 98%. Diammonium hydrogen ortho phosphate [$(\text{NH}_4)_2\text{HPO}_4$] was obtained from Merck Life Science Private Limited with a purity of 98%. Extrapure Titanium (IV) Isopropoxide [$\text{Ti}(\text{OCH}(\text{CH}_3)_2)_4$] was obtained from Sisco Research Laboratories Private Limited. Solvents include ethanol and glacial acetic acid which are obtained from Changshu Song Shey Fine Chemicals and Avra Synthesis Private Limited respectively. Chemicals required for Electrophoretic Deposition process are Isopropyl alcohol, Polyvinyl pyrrolidone and Iodine crystals which are procured from SD Fine Chemical Limited. Analytical grade Titanium metal alloy (Ti-6Al-4V) is cut into 10mm x 10mm and polished using abrasive SiC grid sheets of 400, 600, 800, 1000, 1200 and finally mirror polished using diamond paste in BAINPOL-ET polishing machine at a fixed rpm.

Synthesis of Hydroxyapatite via co-precipitation

Effective co-precipitation method is used for synthesis of hydroxyapatite (HAp) [14]. 0.6M of diammonium hydrogen phosphate [$(\text{NH}_4)_2\text{HPO}_4$] is dissolved in 100ml of double distilled water. Alongside, 1M calcium nitrate tetrahydrate [$\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$] is also dissolved in an equal amount of double distilled water. To the solution of calcium nitrate hexahydrate, diammonium hydrogen orthophosphate is added dropwise under constant stirring for 2 hours. To the solution ammonia is added slowly to maintain the pH at 9. Solution is stirred for overnight and the precipitate obtained is aged, filtered, dried and then further calcination process is carried out at different temperatures of 300°C, 600°C, 900°C and 1000°C with a holding time of 2 hours. Figure 1 illustrates the schematic representation of synthesis methods of hydroxyapatite and titania powders.

Synthesis of titania via sol-gel process

Sol-gel method is used for the synthesis of TiO_2 . 0.02 M titanium (IV) isopropoxide [$\text{Ti}(\text{OCH}(\text{CH}_3)_2)_4$] is the precursor used for which the solvent is glacial acetic acid [CH_3COOH] mixed with stoichiometric amount of double distilled water to obtain the ratio of [$\text{Ti}(\text{OCH}(\text{CH}_3)_2)_4$] : [CH_3COOH] : [H_2O] as 1:30:300. The mixture is stirred for 2 hours and the refluxed in oil bath for 16 hours. The solution obtained is evaporated and gel is obtained which is dried and further calcinated at different temperatures of 300°C, 600°C, 900°C and 1000°C with a holding time of 2 hours [15].

Electrophoretic deposition technique

Hydroxyapatite and titania powders which are calcined at optimum temperature is separately selected for the required amount in a ratio of 70:30 wt% for HAp: TiO_2 [16]. They are further ball-milled using

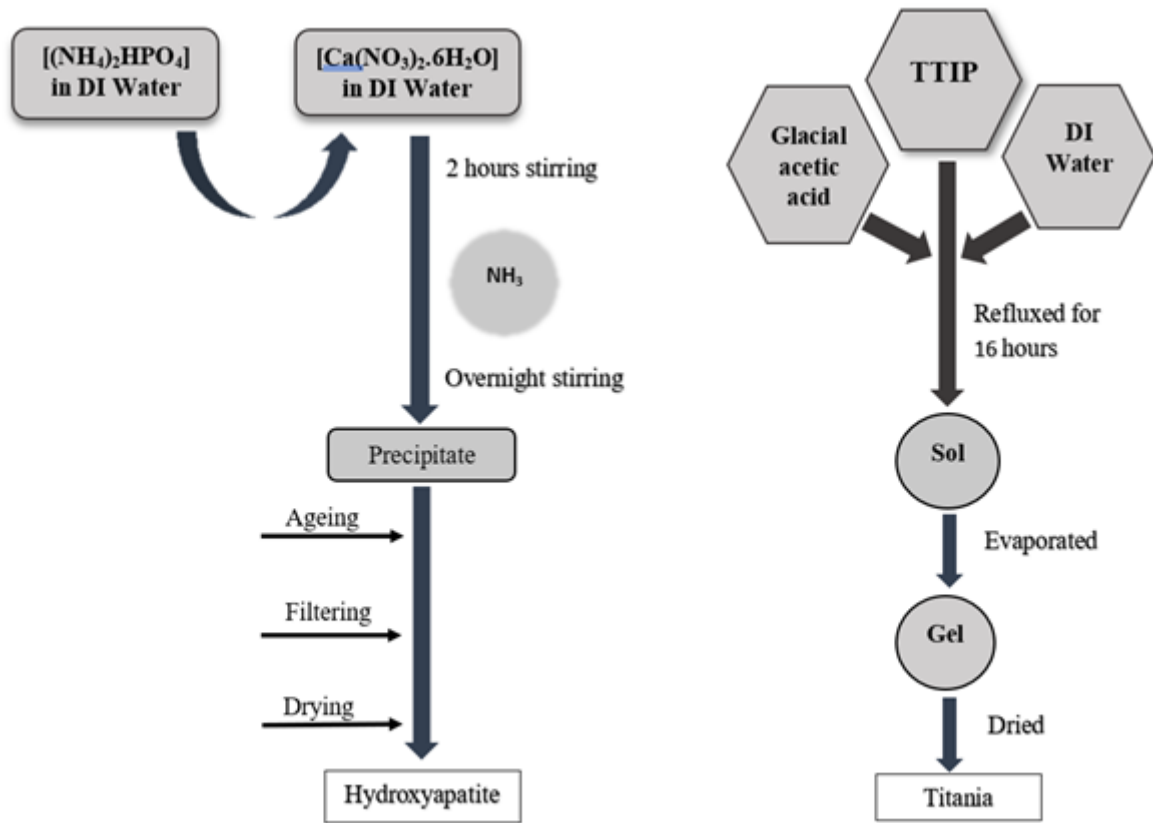


Figure 1: Schematic representation of hydroxyapatite and titania synthesis

a Fritsch Planetary Mill - Pulverisette 5 to receive a very fine powder. They are dissolved in 75ml of isopropyl alcohol. PVP is the polymeric binder and stoichiometric amount of iodine which acts as a surfactant is added. They are stirred for 2 hours to obtain a homogenous suspension. Titanium metal which is mirror polished is ultrasonicated in acetone for the purpose of degreasing. They are arranged in electrophoretic deposition instrument with the substrate as the cathode and stainless steel as anode. Voltage is applied from 40V to 80V at defined time interval of 180 seconds. Figure 2 illustrates the schematic representation of electrophoretic deposition.

Characterizations

Characterizations of the samples are carried out using X-Ray Diffraction Spectroscopy (XRD) which is of Bruker DS Advance within a 2θ range of $10^\circ - 90^\circ$ using a Cu-K α radiation of 1.5406 \AA with a scanning speed of $2^\circ/\text{min}$, for revealing the phase transitions. Fourier Transformation Infra-Red spectroscopy (FTIR) was done by Thermo Nicolet iS50 with ATR (Thermo Fisher Scientific, USA) in a spectral range of $4000 \text{ cm}^{-1} - 400 \text{ cm}^{-1}$ for identification of phases and Scanning Electron Microscopy (SEM-EDX) instrument used for characterization using Carl Zeiss model (ZEISS, India) and Atomic Force Microscopy (AFM) was carried out after gold sputtering over the samples, surface morphology is observed with varying scales of $200\text{nm} - 1\mu\text{m}$ with a resolution of $0.25 - 32 \text{ cm}^{-1}$ and a signal to noise ratio of 55,000:1. AFM model is NaioAFM (Nanosurf AG, Switzerland) with contact mode of

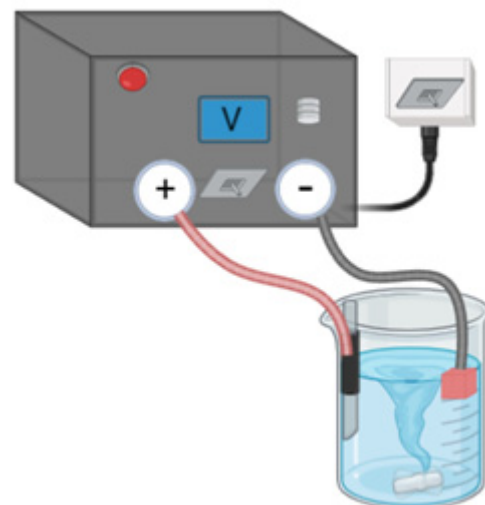


Figure 2: Schematic representation of electrophoretic deposition

operation for observing the topography, roughness and 3D image of the coating implant.

Results and Discussion

X-Ray Diffraction

Figure 3 represents the XRD spectrum of TiO_2 . A major peak for anatase phase is observed for the raw TiO_2 and TiO_2 sintered till a temperature of 800°C . The greater number of peaks for raw TiO_2 is indicating amorphous nature which is subsiding with temperature rise and takes crystalline nature with increased temperature. The phase transformation from anatase to rutile is clearly visible from the spectrum at the sintering temperature of 900°C . The major peak is observed at $2\theta=24.98^\circ$, $2\theta=25.06^\circ$, $2\theta=25.36^\circ$, $2\theta=24.94^\circ$ for non-sintered and sintered TiO_2 at 300°C , 600°C , and 800°C respectively, which denotes the prevalence of anatase phase. They are matched with the anatase phase JCPDS card number 89-4921. Similarly, an observation can be made for major peak of sintered TiO_2 at 900°C and 1000°C for $2\theta=27.53^\circ$ and $2\theta=27.20^\circ$ which denotes rutile phase; agreeing with the standard data of JCPDS card number 89-4920. At 1000°C a very minute peak of anatase is observed at 25.06° which indicates a mixture of anatase and rutile phases after 1000°C . An increase in crystallinity could be viewed from observing the increasing peak intensity. From the XRD Spectrum of TiO_2 , we confirm that the phase transformation from anatase to rutile occurs at 900°C . This is in agreement to the studies observed by Chellappa et.al, for the phase changes of TiO_2 noticed with annealing [17].

Figure 4 represents the XRD spectrum of hydroxyapatite sintered at different temperatures. A slight variation from amorphous nature to crystallinity is observed on sintering HAp till 1000°C . Raw HAp has a peak at plane (211) which corresponds to the minute presence of calcium nitrate precursor. The hydroxyapatite formation is confirmed with the major peak formation at $2\theta = 32.20^\circ$, $2\theta = 31.84^\circ$, $2\theta = 31.89^\circ$, $2\theta = 32.02^\circ$ and $2\theta = 31.84^\circ$ for HAp sintered at 300°C , 600°C , 900°C and 1000°C respectively; which matches with the JCPDS card number 09-0432. More defined peaks arise from sintering starting at a temperature of 600°C . At 1000°C , well-defined peaks with more crystallinity are observed. The XRD peaks of raw HAp and sintered at 300°C have certain extra peaks at $2\theta = 29.51^\circ$ and $2\theta = 33.50^\circ$. This corresponds to the major peaks of calcium pyrophosphate and β -TCP correspondingly in accordance with the JCPDS card number of 09-0436 for calcium pyrophosphate and 09-0169 for β -TCP [18]. With increased temperature, the other peaks of hydroxyapatite with well-defined crystallinity are observed. There is removal of all extra peaks noted at a sintering temperature starting from 600°C and gives distinct HAp peaks.

Figure 5 represents the coating mixture of HAp/ TiO_2 at a sintering temperature of 900°C . The peaks of HAp and TiO_2 are distinctly present and the 2θ values corresponds to the JCPDS card number of 09-432 and 89-4920 with respect to HAp and Rutile phase of TiO_2 respectively are clearly present in the XRD spectra without any other secondary phases. Hence, the coating mixture contains 70:30 %wt. with prominent peaks of HAp and TiO_2 are selected for EPD coating.

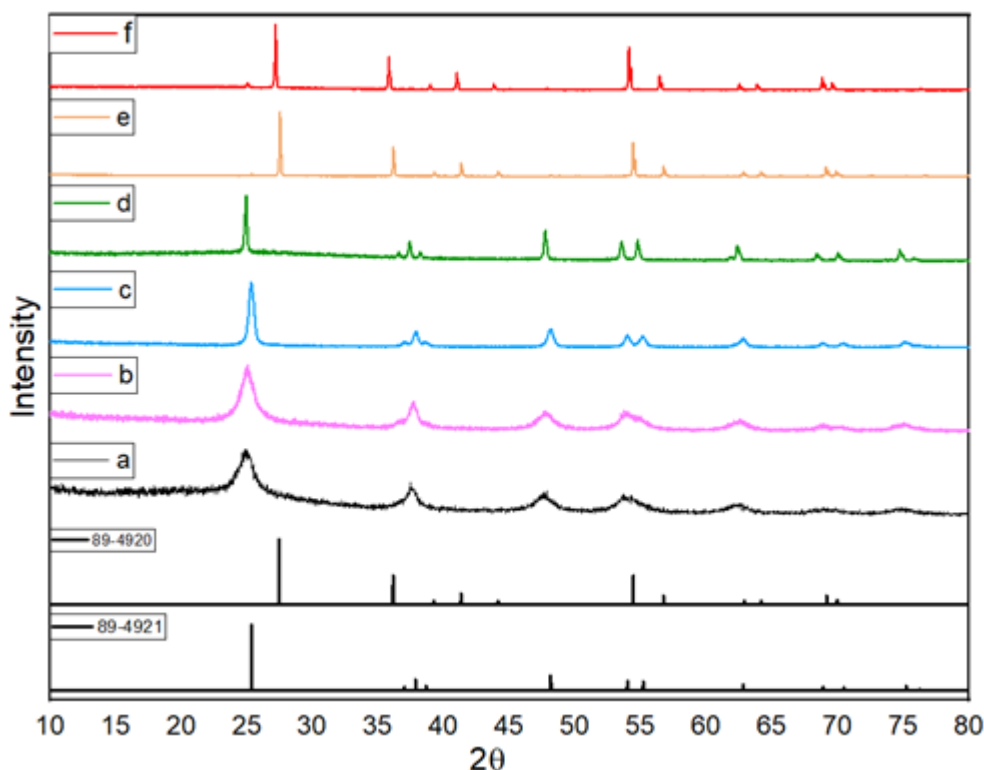


Figure 3: XRD Image of (a) Raw TiO_2 (b) TiO_2 sintered at 300°C (c) TiO_2 sintered at 600°C (d) TiO_2 sintered at 800°C (e) TiO_2 sintered at 900°C (f) TiO_2 sintered at 1000°C

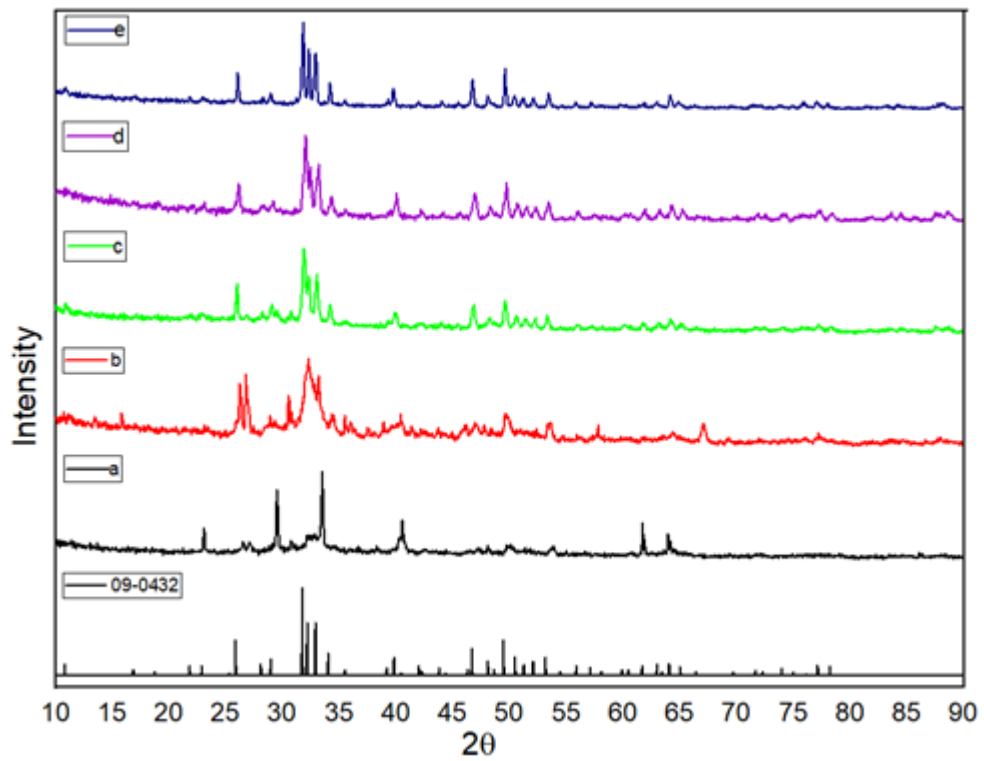


Figure 4: XRD Image of (a) Raw HAp (b) HAp sintered at 300°C (c) HAp sintered at 600°C (d) HAp sintered at 900°C (e) HAp sintered at 1000°C

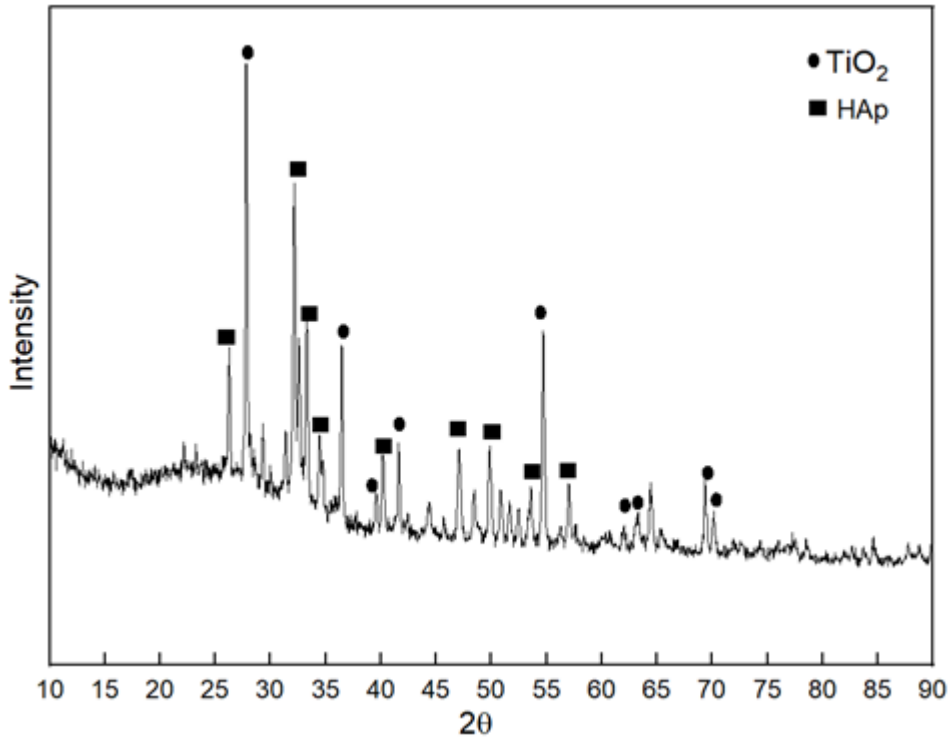


Figure 5: XRD pattern for HAp/TiO₂ composite selected for the EPD coating

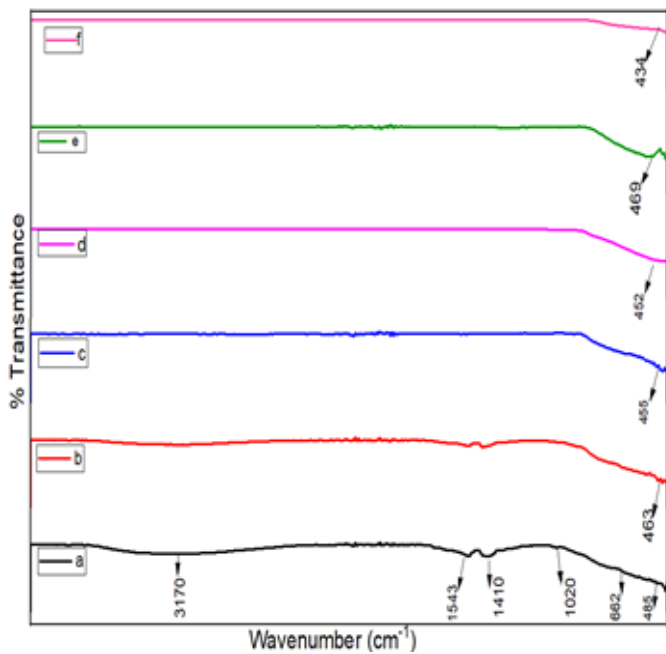


Figure 6: FTIR spectra of (a) Raw TiO_2 (b) TiO_2 sintered at 300°C (c) TiO_2 sintered at 600°C (d) TiO_2 sintered at 800°C (e) TiO_2 sintered at 900°C (f) TiO_2 sintered at 1000°C

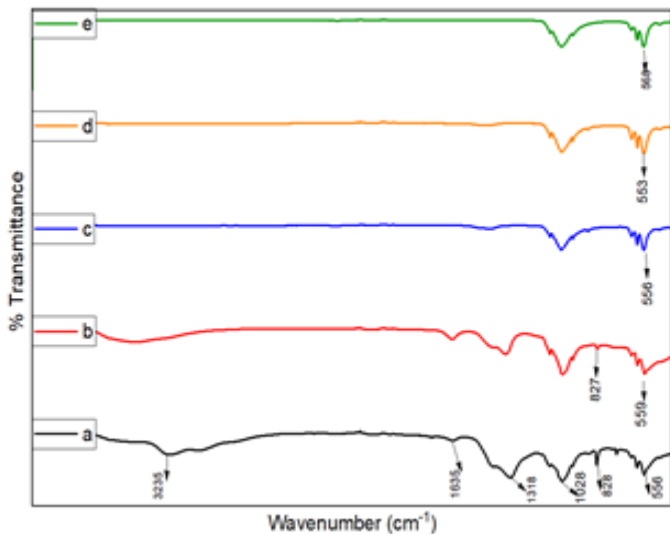


Figure 7: FTIR spectra of (a) Raw hydroxyapatite (b) HAp sintered at 300°C (c) HAp sintered at 600°C (d) HAp sintered at 900°C (e) HAp sintered at 1000°C

FTIR

FTIR characterizations are carried out for the samples to observe difference according to the sintering temperatures. Figure 6 shows the spectrum of TiO_2 . A broad peak at 3170cm^{-1} is due to the presence of hydroxyl group (O-H). The peak at 1543cm^{-1} corresponds to the presence of C-C aromatic stretching and peak at 1410cm^{-1} corresponds to the symmetric and asymmetric stretching of C=O group. Another peak at around 1020cm^{-1} shows presence

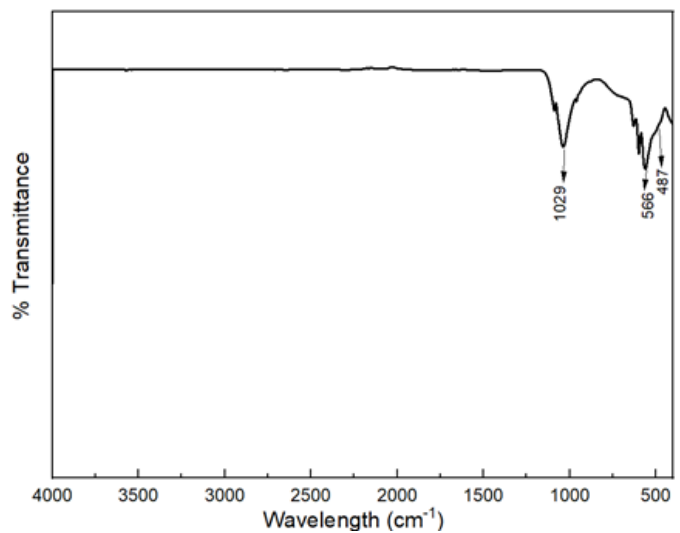


Figure 8: FTIR spectra of HAp/ TiO_2 composite

of C-O stretching of alcohols/carboxylic acid groups. Peaks around $450 - 650\text{cm}^{-1}$ confirms the presence of Ti-O bonding of the TiO_2 . With an increase in sintering temperature the hydroxyl group diminishes as the moisture content is disappearing with heating. Also, the peaks at $490 - 550\text{cm}^{-1}$ shows the presence of TiO_2 . Peaks observed at 1020cm^{-1} confirms the Ti-O-Ti vibrations. The peaks were in good agreement with studies by Haider et.al [19].

Similarly, the FTIR spectrum recorded for the synthesized hydroxyapatite and the presence of functional groups are shown in figure 7. A peak is observed for raw HAp around 3235cm^{-1} which confirms the presence of hydroxyl group. Carbonate (CO_3^{2-}) presence is confirmed by the peaks obtained at 1318 to 1635cm^{-1} . This might be an indication for the presence of carbonated apatite which is absorbed from atmosphere [20]. At 1028cm^{-1} asymmetric stretching of phosphate (PO_4^{2-}) group is observed and at 556cm^{-1} asymmetric bending vibration of O-P-O is seen. At increasing sintering temperatures, hydroxyl and carbonate peaks vanishes and the peaks at 1422cm^{-1} , 828cm^{-1} are due to CO_3^{2-} bending and stretching vibrations respectively, confirming the presence of carbonated hydroxyapatite. Figure 8 is the FTIR spectra of HAp and TiO_2 composite having the composition of 70% HAp and 30% TiO_2 for the EPD coating. The major peaks observed is of HAp and Titania also having the vanishing of OH peaks since they are calcinated at a temperature of 900°C .

Scanning electron microscopy

Scanning electron microscopy picturize the morphology and surface topography of the materials of our concern consisting of TiO_2 and HAp. They seek attention towards the crystals of biological apatite from its original form while exposed to different sintering temperature conditions. Structural changes are observed at 600 to 800°C indicating greater crystallinity. Slightly irregular shaped morphology with a smooth surface was observed for HAp, displaying a significant increase in the size and shape of the particles with increasing the sintering temperature. At the sintering temperature of 900°C , clear and evident image of shape formation and clustering is observed with increased particle size. Oval shaped morphology is observed with slight aggregation. This is similar to the morphology observed earlier by Aarthy et.al. [21].

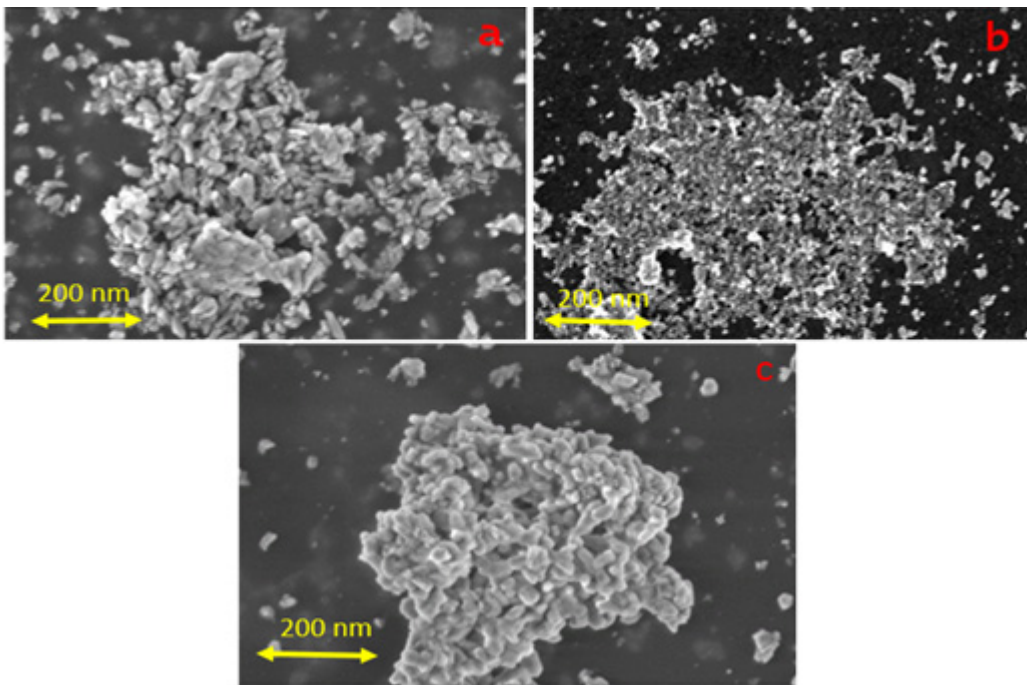


Figure 9: SEM images of HAP (a) Raw HAP (b) HAp sintered at 600°C (c) HAp sintered at 900°C

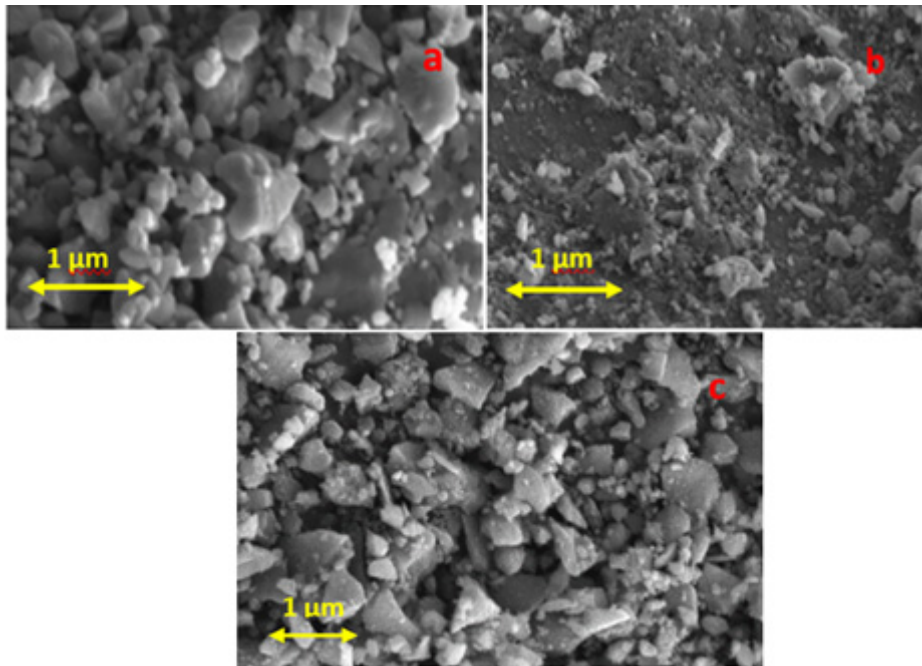


Figure 10: SEM images of TiO₂ (a) Raw TiO₂ (b) TiO₂ sintered at 600°C (c) TiO₂ sintered at 900°C

For TiO₂, there is smaller microstructure or grain size obtained for samples having a calcination temperature of 300°C. At lower sintering temperatures, uniformity in grain size is noted. The particle size is becoming bigger with 900°C which indicates the effect of heat treatment over TiO₂ particles. They are almost spherical in shape with higher crystallinity. The SEM obtained are well in

agreement with TiO₂ nanoparticles synthesized through sol gel methods in prior [22]. Figure 9 and Figure 10 shows the SEM morphology of HAp and TiO₂ sintered at different temperatures. Sintered and further ball-milled samples show uniformity and similar structural aspects [23]. Figure 11 is the SEM image of HAp/TiO₂ composite combined with a ratio of 70:30%wt. The

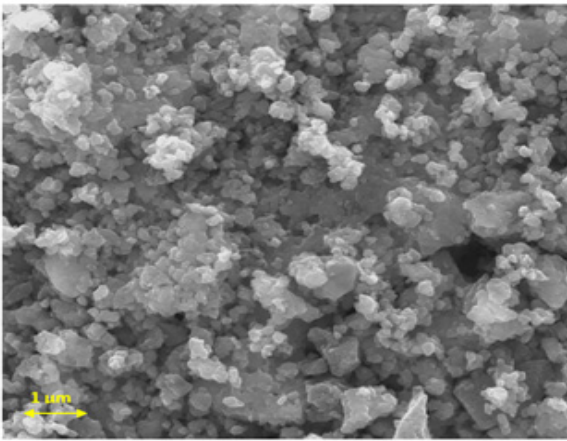


Figure 11: SEM image of HAp/TiO₂ composite sintered at 900°C

morphology shows monodispersity when sintered at 900°C. They exhibit a nearly spherical structure with more uniformity. With this the observation of morphology we reckon that combining the two ceramic materials at nanoscale provides a better morphology with more uniformity which in turn is beneficial for biomedical applications.

Atomic force microscopy

The atomic force microscopy images of EPD coated HAp/TiO₂ composites over Ti-6Al-4V substrate are given in figure 12. The images are captured after varying the voltage of the EPD process from 40 V to 60V and 80 V. The time interval for the process is kept constant in our study, as 180 seconds. From AFM results, it is observed that the uniformity is better in coating at which the applied voltage was 60V.

Conclusion

Our work elaborates about HAp/TiO₂ composite and the influence it can pronounce in biomedical field. The sintering temperature of 900°C is favoured throughout our study for TiO₂ as well as HAp. In addition, rutile phase of titania is well-suited for the biomedical field rather than its counterpart which is anatase. Since we have focused into orthopaedic implant coatings in biomedical domain, we prefer the sintering temperature of 900°C for both TiO₂ and HAp. The FTIR spectrum agrees the presence of TiO₂ and HAp accounting to their bending and stretching vibrations. The SEM results also provides the morphology identification of nanoparticle ranged powders of TiO₂ and HAp. Seeking an advancement in coatings, we have prepared the composite consisting of TiO₂ and HAp at a fixed proportion of weight and coated them using EPD method. The preliminary studies suggest that as a composite, they exhibit good morphology to be used as a coating. The composite coating by EPD by varying voltage alone and keeping the time as constant. The AFM morphology images have proved the uniformity of the coating at the optimum voltage of 60V at 180 seconds. Further studies will proceed to varying other parameters of the EPD coating technique.

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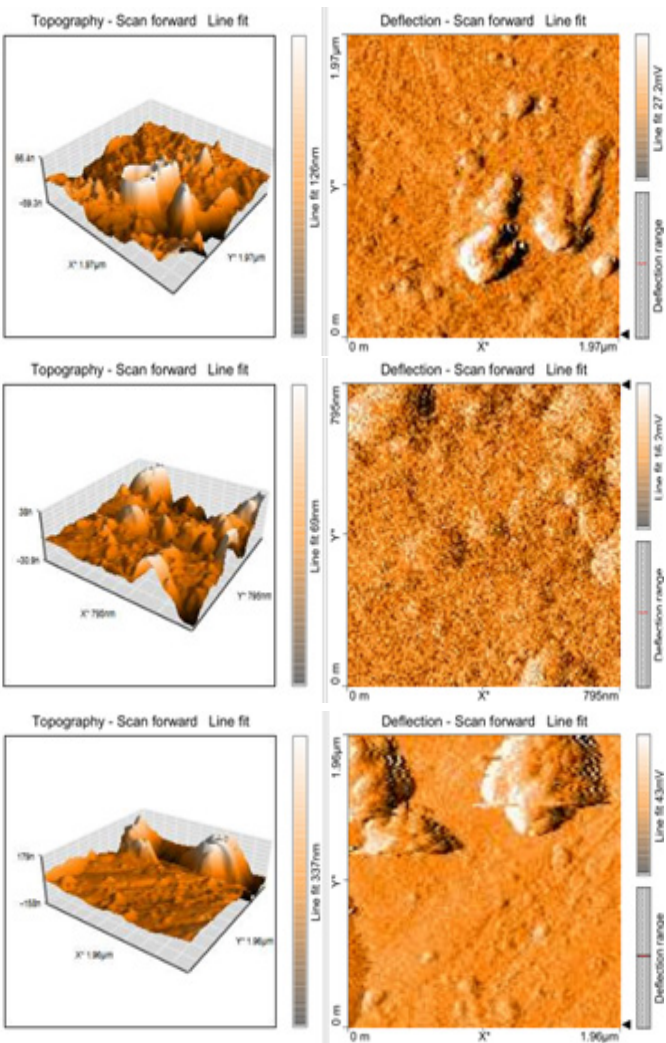


Figure 12: AFM Images of EPD Coated over Ti-6Al-4V substrate (a) 40V – 3min (b) 60 V – 3 min (c) 80V-3 min

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