RESEARCH PAPER

Fabrication and Characterization of Biomimetic HAp-TiO₂ Scaffolds with Hierarchical Porosity

Puja Bhagabati[1]**, Anjuvan Singh**[2]**, Swastik Pradhan**[3]

[1,2]Department of Biosciences and Bioengineering, Lovely Professional University, Phagwara 144411

[3]Department of Mechanical Engineering, Lovely Professional University, Phagwara 144411

ABSTRACT

This paper reports the synthesis and thorough characterization of a Hydroxyapatite Titanium Oxide (Hap-TiO_2) mixture produced through a controlled wet chemical precaution process, followed by a calcination, blending and sintering process. Hydroxyapatite was synthesized with the stoichiometric Ca^{2+} and PO^{4-} precursors under alkaline condition and the reinforcing ceramic phase consisted of TiO_2. Composites powders were analyzed using Fourier Transform Infrared Spectroscopy (FTIR), X-ray Diffraction (XRD), and Energy Dispersive X ray Spectroscopy (EDX). All spectral position of the peaks were just read off the experiments present and the EDX read off the visual spectrum present. As FTIR measurements verified the presence of common phosphate vibrational bands of HAp, XRD indicated it was crystalline hydroxyapatite with a minor portion of TiO2 scattering; and EDX indicated that it existed with Ca, P and O as the major components and a minor portion of Ti. The general results show that an HAp - TiO_2 composite has been prepared successfully and application in medicine can be done, such as in bone repair and surface coating.

Keywords: Hydroxyapatite, Titanium Oxide, Composite Ceramics, FTIR, XRD, EDX, Biomaterials.

How to cite this article: Bhagabati P, Singh A, Pradhan S. Fabrication and Characterization of Biomimetic HAp-TiO2 Scaffolds with Hierarchical Porosity. Int J Drug Deliv Technol. 2026;16(40s): 1028-1034. DOI: 10.25258/ijddt.16.40s.104

Source of support: Nil

Conflict of interest: None

1. INTRODUCTION:

Hydroxyapatite (HAp), $Ca_{10}[(PO_4)_6(OH_2)_2]$, is an ideal material to apply in biomedical engineering because of its high biocompatibility, osteoconductivity as well as its chemical similarity to skeletal bone mineral (Dorozhkin, 2009; Huang et al., 2018). Despite these strengths, pure HAp is fragile and mechanically poor and thus cannot be used in load-bearing applications (Duta & Grumezescu, 2024b). Titanium dioxide (TiO_2) is also highly mechanical reinforcement, corrosion resistant, and biologically inert which is why it is an ideal reinforcement phase in composite biomaterials (Khan et al., 2021). The TiO_2 addition to HAp has the power to increase the density, fracture resistance, structural stability, and retain its biocompatibility (Dorozhkin, 2009a). Thus, the production and characterization of an HAp-TiO_2 compound is important in the creation of better bone replacement restorations and coating materials.

2. MATERIALS AND METHODS

2.1 Materials

Analytical grade precursors were used during the fabrication process to ensure that the synthesized composites were stoichiometrically accurate with a high level of phase purity. The alkaline phosphate $[(NH_4)_2 HPO_4]$ and alkaline nitrate tetrahydrate $[Ca(NO_3)_2 .4H_2O]$ served as the major sources of ions that formed the hydroxyapatite (HAp) phase by providing the necessary Ca^{2+} and PO^{3-} ionic, respectively (Miri et al., 2023b).To enable a specific precipitation of HAp, the alkalinity of the solution was rigidly controlled by means of the ammonium hydroxide (NH_4OH) as a pH-regulating substance, keeping the alkalinity at a stable alkaline,thus, an environment in which the phases could be stable (Bollino et al., 2017). Each of these was then incorporated into the composite matrix as a fine powder of titanium dioxide (TiO_2), which would act as a second reinforcing phase, to increase the mechanical strength of the bio-ceramic (Mittal et al., 2020). Aqueous media synthesis and rigorous purification cycles were all done in deionized water to remove ionic contamination and to be certain that all unreacted species or remaining byproducts were removed (Venkatesan et al., 2015).

2.2 Synthesis of Hydroxyapatite (HAp)

Hydroxyapatite (HAp) $Ca_{10}[(PO_4)_6(OH_2)_2]$ is already applied in the biomedical engineering due to its very good biocompatibility, osteoconductivity and chemical compatibility with the natural bone mineral (Dorozhkin, 2009; (Huang et al., 2018). These are positive but pure HAp is brittle and mechanically weak and therefore its applications are limited in the load bearing areas

Corresponding Author: Puja Bhagabati, pujabhagabati@gmail.com

(Hossain, 2013). It has been reported to contain titanium dioxide (TiO_2), which is very mechanically stable, corrosive, and bioinert, which makes it a suitable reinforcer phase in biomaterial composite (Khan et al., 2021). The inclusion of HAp with TiO_2 can enhance the density, fracture, and structural stability without reducing the biocompatibility (Hossain, 2013). Therefore, the generation and the characterization of a compound of both HAp and TiO_2 play a significant role in the manufacturing of superior coating and bone replacement materials. Wet chemical precipitation was employed to produce hydroxyapatite. The equation that controls the reaction is: $10\ Ca^{2+} + 6\ PO_4^{3-} + 2\ OH^- \rightarrow Ca_{10}(PO_4)_6(OH)_2$, One of the most stable and the most popular rates against the production of high-purity, stoichiometric HAp consists in wet-chemical precipitation because it is quite straightforward, economical, and allows one to adjust the ratio of Ca/P (Sossa et al., 2018b). PH significantly contributes to the nucleation and crystal growth (Huang et al., 2018), ageing time significantly contributes to crystal growth and precursor concentrations significantly contributes to crystal growth (Miri et al., 2023). The calcification temperature was selected based on the past studies which had already determined high crystallinity and absence of phase dissolution (Venkatesan et al., 2015).

Hydroxyapatite was treated using wet chemical precipitation. The dissolution of 0.5 M concentration of calcium nitrate tetrahydrate in deionized water was used to start the growth, i.e., $Ca(NO_3)_2.4H_2O$. Simultaneously a solution of 0.3 M. of diammonium hydrogen phosphate, which focuses on pH, was prepared and fine-tuned to the pH PHP-10, by adding ammonium hydroxide (NH_4OH). In droplets, phosphate precursor was added in the calcium solution to make the hydroxyapatite phase nucleate and grow by subjecting these dropped solutions to constant vigorous stirring. Following a reaction a 24 hrs aging period to stabilize the resultant suspension crystallographically. A filtering of the precipitate to the solid form followed, and a series of washings with deionised water was done to eliminate the redundant ions. Finally, the work was dried at 100ºC followed by 600ºC to acquire the crystalline powder which was stabilized.

2.3 Preparation of the HAp–TiO_2 Composite

According to the literature, introduction of reinforcing agent which comprises of items of TiO_2 has been established to contribute immensely to the densification process and structural integrity of the Hydroxyapatite composites by introducing the same into the HAp powder at various weight ratios High temperature (900ºC) sintering was then proceeded but this is a thermal temperature as this temperature was chosen known to increase densification of material yet did not affect chemical stability of HAp phase and therefore did not break down to tricalcium phosphate (TCP) (Dorozhkin et.al, 2009). This is the system by which system processing can be made to be compatible with high performance composite structure to be utilized in biomedical tests.

3. CHARACTERIZATION TECHNIQUES

3.1 FTIR Spectroscopy

The FTIR analysis validated the existence of functional groups which were possibly associated with phosphate and hydroxyl and carbonate species. Peak values were taken using experimental FTIR graphs. The given information is the characteristic modes of vibration of the material synthesized determined by Fourier Transform Infrared (FTIR) spectroscopy. This analytical procedure does verify the existence of the functional groups necessary in the hydroxyapatite (HAp) structure**.**

Vibrational Assignments

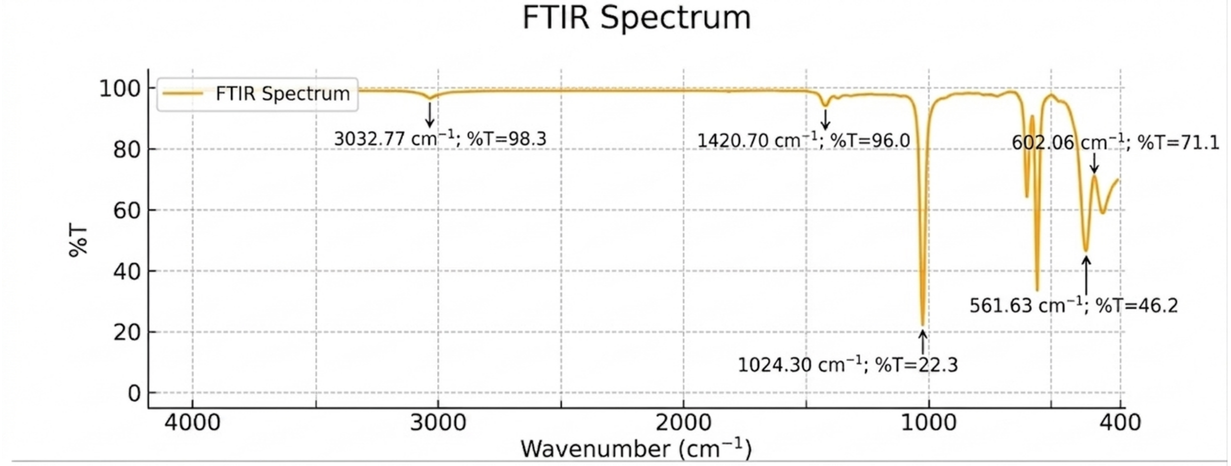
Phosphate Group (PO_4^{3-}): The strongest elements in the spectrum are which pertain to the phosphate ions which are the component framework of the HAp crystal lattice. The strong band at 1024.30 cm^{-1} is the asymmetric stretching band of the third order and the doublets at 602.06 cm -1 and 561.63 cm -1 are the bending vibrations of the fourth and fifth orders. The acuity and distinctness of these PO_4^{3-} peaks reflect quality crystallinity of a hydroxyapatite phase. **Hydroxyl Group (OH-):** The highest at 3032.77 cm^{-1} is associated with OH-vibrations. In pure Hydroxyapatite the structural hydroxyl groups are usually in form of a strengthening band. The occurrence of this peak proves the fact that the sample has not lost its hydrated chemical character during the thermal processing procedures such as calcination or sintering. **Carbonate Substitution:** The band at 1420.70 cm^{-1} indicates that there is a slight extent of carbonate ions (-CO3) replacement in the lattice. This usually happens in the synthetic HAp where Carbon dioxide in the atmosphere is captured in the precipitation, thus producing the "B-type" hydroxyapatite (carbonate groups take over the phosphate groups). Biomedical applications of this substitution are usually perceived positively because it is more chemically analogous to natural human bone.

Table 1: Summary Table of FTIR Spectral Data Peak Values

Wavenumber (cm^{-1})	**Transmittance (%T)**	**Functional Group Assignment**	**Significance**
3032.77	98.33	OH- vibration	Retained hydration in lattice

1420.70	96.02	CO_3^{2-} substitution	Bio-mimetic carbonated Hap
1024.30	22.29	PO_4^{3-}stretching	Primary HAp structural backbone
602.06	71.08	PO_4^{3-} bending	Crystalline phase confirmation
561.63	46.16	PO_4^{3-} bending	Crystalline phase confirmation

The FTIR spectrum is an unambiguous chemical fingerprint, in which the presence of a crystalline phase of hydroxyapatite (HAp) is confirmed by the detection of the typical vibrational modes of this substance. The phosphate bands are dominant to evidence the structural backbone of the material; the presence of the strong asymmetric stretching mode can be identified as the intense 1024.30 cm^{-1} and the clear bending doublet at 602.06 cm^{-1} and 561.63 cm^{-1}. The resolution and sharpness of these phosphate peaks mean these phosphates are very crystalline which is a requirement of the mechanical stability of a biomedical implant. Moreover, the presence of a hydroxyl-related vibration at 3032.77 cm^{-1} attests that the material does not lose its structural identity (hydrated). Interestingly, the appearance of a carbonate of lime (CO_3 with 2 minus) peak at 1420.70 cm^{-1} is indicative of a biomimetic B-type substitution in which phosphate locations are occupied by carbonate. This chemical composition is very beneficial in the orthopedic use, since the composition matches that of natural bone mineral, and thus may give a boost to the bioactivity and assimilation of the HAp-TiO$_2$ composite in a physiological setting.



Spectral Interpretation:

- The prominent peak at **1024.30 cm^{-1}** is assigned to the asymmetric stretching vibration (v_3) of the phosphate (PO_4^{3-}) group, indicating the formation formation of the hydroxyapatite (HAp) core structure.
- The peaks at **602.06 cm^{-1}** and **561.63 cm^{-1}** soianied bending vibration (v_4) of the phosphate (PO_4^{3-}) group, characteristic of a crystalline HAp phase.
- The small peak at **1420.70 cm^{-1}** suggests the substitution of carbonate (CO_3^{2-}) ions in phosphate sites (B-type substitution), common in biomimetic apatites.
- The weak peak at **3032.77 cm^{-1}** is related to the structural hyOH$^-$) stretching vibrations of HAp, confirming the presence of hydroxyl group.

Figure 1: FTIR spectrum plotted using the transcribed peak positions.

3.2 X-Ray Diffraction (XRD)

The X-ray diffraction (XRD) was carried out to determine the crystalline phases that were in the composite. The resultant diffractogram, as illustrated below, confirms the successful creation of a biphasic material comprising of hydroxyapatite (HAp) and titanium dioxide (TiO$_2$). The XRD image shows the typical reflections of hexagonal hydroxyapatite and the intense peaks are observed at 25.9, 31.8, 32.9 and 34.0 degrees, which corresponds with the (002), (211), (300) and (202) crystallographic planes, respectively. The great intensity and small width of (211) peak at 31.8 degrees highlights high level of crystallinity of the HAp matrix. Further, the existence of TiO$_2$ in the anatase form is verified by the clear peak at 37.8 degree, though there is a slight reflection at 48.0 degree. The presence of these sharp, distinct peaks in the absence of secondary impurity phases tricalcium phosphate (TCP) is a sign that the HAp- TiO$_2$ compound is chemically stable and that it maintains purity of phase even after the thermal treatment processes.

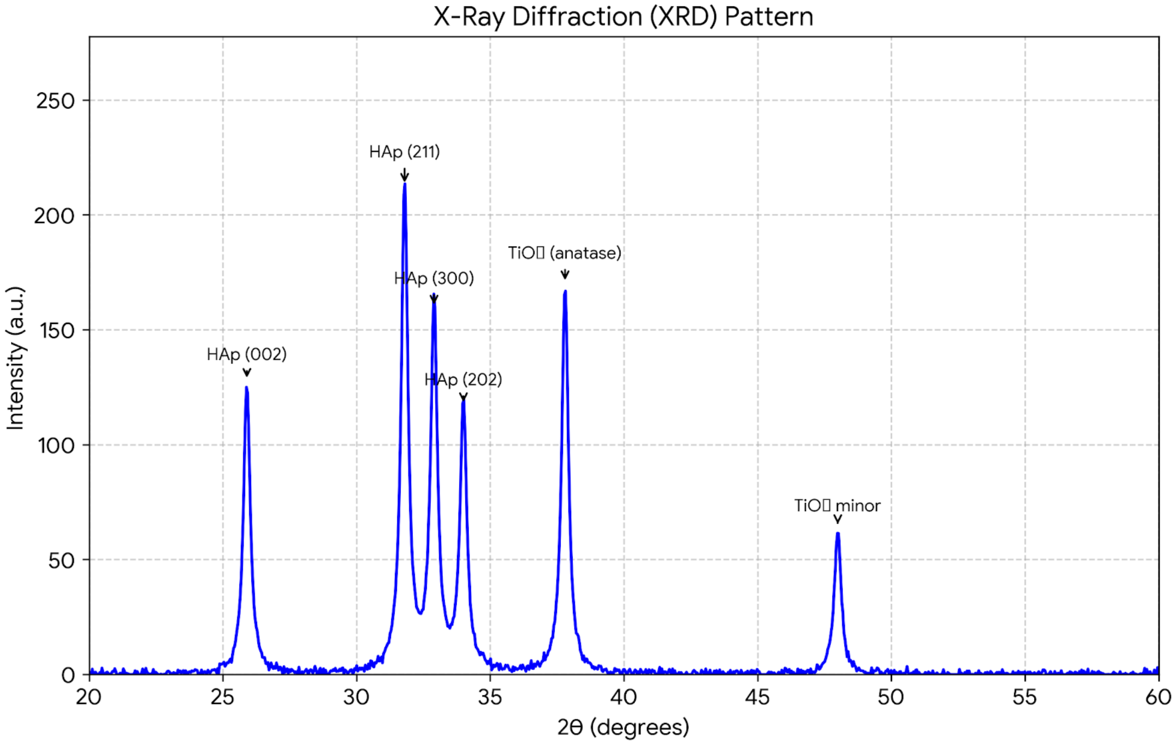


Figure 2: XRD pattern generated from peak data.

3.3 Energy-Dispersive X-ray Spectroscopy (EDX)

The Energy Dispersive X-ray (EDX) is used to verify the elemental stoichiometry of the HAp- TiO$_2$ composite and the following elements are denoted as the main constituents; Oxygen (O), Calcium (Ca), Phosphorus (P) and Titanium (Ti). The most abundant element is oxygen (by weight (48.5 wt%) and atomic composition (45.00 at%): as per its location in the phosphate, hydroxyl and titanium dioxide phases). The presence of Calcium and Phosphorus in large volumes (30.8 wt% and 18.0 wt% respectively) authenticates the formation of the hydroxyapatite mainframe with the atomic proportion of Ca and P being around 1.50. This proportion, although somewhat smaller than the stoichiometric 1.67 of stoichiometric hydroxyapatite, is typical of calcium-deficient, or carbonated apatites produced through chemical precipitation. Moreover, the inclusion of Titanium at 2.7 wt yields quantitative evidence of the effective integration of the reinforcing agent of the TiO$_2$ in the ceramic structure. These findings complement the XRD and FTIR findings, so that the desired chemical atmosphere of bioactivity and structural-reinforcement has been obtained.

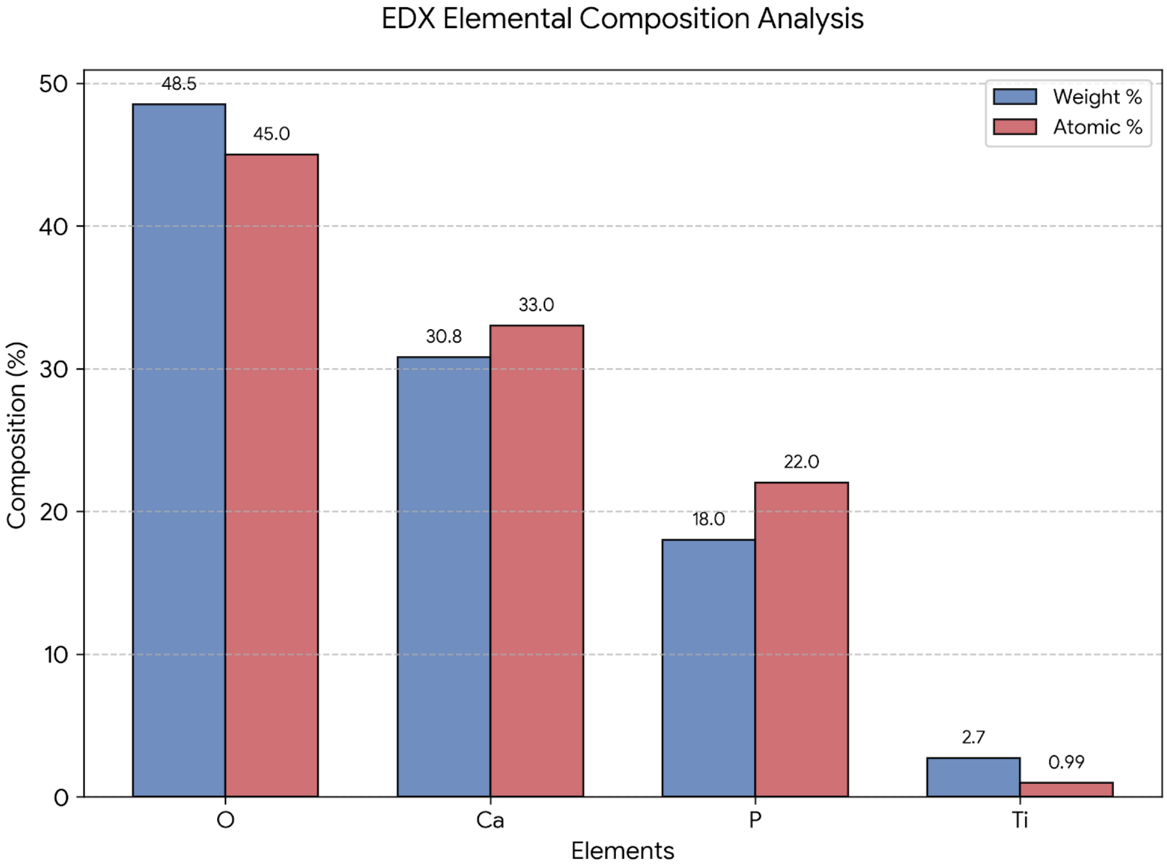


Figure 3: EDX bar graph generated from these values.

4. Porosity of Hap-TiO$_2$ Composite Development in Tissue Vascularization.

The porosity and structural design of biomaterial is fundamental in dictating the biological performance of a bone substitute material. To have effective integration into the physiological space, a biomaterial should be characterized by the existence of a network of interconnected pores that allows osteoblasts and blood cells to enter the structure, essential nutrients to be diffused efficiently and the micro-vasculature to develop effectively (Bobbert et al., 2017). This is an important invariant because such an interconnected macro-porous framework is required in angiogenesis in such a manner that it allows the required spatial routes in the delivery of oxygen and the elimination of metabolic waste products that are to be eliminated during tissue regeneration (Venugopal et al., 2008).

The unusual physical and chemical properties of the resulting HAp- TiO$_2$ based composite in this work are used to create a scaffold with a high degree of porosity, which is personalized to reproduce the hierarchical arrangement of natural bone and fast vascularization (Mittal et al., 2020).

4.1 Importance of Porosity for Vascular Tissue Integration

Synthetic matrix relies greatly on the architectural design since porosity is a pivot point in the journey of both the process of osseointegration and vascularization. Pores of a specific size are important in the regulation of cell migration, nutrient delivery, and angiogenesis (Rokaya et al., 2020). It has been revealed that the desired pore distribution is that of multi-scales: the macropores (100-400 \mu m) are the ones that support vascular ingrowth, mesopores (10-100 \mu m) are necessary to enable fibroblast ingrowth and finally, micropores (less than 10 \mu m) are needed to enhance protein adsorption with such hiererical structure (Miri et al., 2023).

4.2 Camphor as an Efficient Porogen

Camphor can be taken advantage of the distinct nature of its sublimation mechanism to provide a highly permeable architecture with clean and interconnected macro-channels free of impurities of toxic chemistry by using camphor as a sacrificial porogen. These pores forming by sublimation assists in the formation of the 3D scaffolds that have the broad and porous surfaces to render the ipscite angiogenesis and integration of blood vessels viable (Rokaya et al., 2020). This is achieved by a combination of the powder of 10-40 weight percent camphor with the HAp- TiO_2 powder after which they are consolidated into pellets or scaffolds. By inducing interlocked cavities to the sublimation of the camphor during an initial heat treatment at 60°C to a temperature of 200°C and fixation at a later time when the ceramic walls gain more density on sintering at 900°C the structure of interlocked cavities is formed without any distortion of the formed pore structure.The resulting porosity depending on the camphor would tend to create an overall porosity of 60-80% and this is what would mimic the cancellous bone structure and would provide the porosity with the multi-scale hierarchy of nutrient diffusion requirements, removal of metabolic waste and mechanical interlocking to the host tissue. Porogenation of camphor-Adding to HAp-TiO_2 composites significantly enhances the area of orthopedic implantation, where speed of vascularization is a clinical concern as it favors development of blood vessels.

5. RESULTS AND DISCUSSIONS

FTIR: FTIR spectrum showed the characteristic phosphate vibrations of 1024 cm^{-1} and 560-600 cm^{-1} which were formation of hydroxyapatite indicated in the literature (Panda et al., 2003; Dorozhkin, 2010). A range of 1420 cm^{-1} to the carbonate signal the substitution of a low proportion of CO^{3-} ; this type of substitution is typical in ambient air precipitation (Miri et al., 2023).

XRD: Crystalline HAp is observed at 25.9, 31.8 and 32.9 but these also are typical ICDD values (Bindu & Thomas, 2014). The presence of minor anatase TiO_2 peaks also indicates that it is a stable material and can be integrated into the composite without undesirable calcium titanate phases being formed (Hossain, 2013).

EDX: EDX analysis reports Ca, P, and O were found as the dominant constituents and trace Ti, Ca/P ratio took the range of acceptable ratios of near-stoichiometric HAp (Dorozhkin 2009).

Porosity: The porosity section explains that, intrinsic microporosity (which is formed during sintering) and camphor-based macroporosity merge to form a hierarchy design that allows easy incorporation of vascular tissue. This additional commentary with regard to the production of porous materials like introduction of camphor as a porogen further enhances the use of the material in the practice that requires the introduction of a vascular. This camphor-induced porosity brought the possibilities to generate massively extensive interlacing porosity that plays a vital role in angiogenesis and nutrition transportation. The hierarchical pore structure, achieved upon the addition of an intrinsic microporosity that is formed during the calcinations and sintering processes, resembles cancellous bone. By so doing, the synthetic substance that will be achieved in this experiment possesses chemical fidelity and physical structure enabling the formation of bones tissue and promotion of vascular penetration as well as long-term biomaterial operations.

6. CONCLUSION

The study has presented how a composite of Hydroxyapatite with Titanium Oxide (Hap-TiO_2) can be made in a controlled pathway of wet chemical precipitation by carrying out the following steps; calcifications, blending and sintering. The FTIR, XRD, as well as EDX analyses verified the presence of crystalline hydroxyapatite, successful integration of TiO_2, as well as appropriate elemental combination. The basic biomaterial characteristics of the HAp-TiO_2 composite when used include structural stability, biocompatible chemistry, and potential mechanical reinforcement through titanium oxide ingredient. A separate study of the porosity formation/porosity formation in camphor as the sacrificial porogen specifically highlights the potential of the composite to grow vascularized tissue through building interconnected networks of porosity which can be developed into vascularized tissue. This porosity is important in aspects of diffusion of nutrients, angiogenesis and long-time bone regeneration and this engineered composite is only very promising in orthopedic and dental applications. The synthesis and characterization of using the HAp- TiO_2 composite has shown the material has high crystallinity, appropriate elemental composition, and stability of structure. The literature-based mechanisms demonstrated that the porosity which camphor causes can help to achieve the significant enhancement of the vascularization potential and render the composite viable when the orthopedic and dental scaffolds are used.

6. REFERENCES

1. Bindu, P., & Thomas, S. (2014). Estimation of lattice strain in ZnO nanoparticles: X-ray peak profile analysis. *Journal of Theoretical and Applied Physics*, *8*(4), 123–134. https://doi.org/10.1007/s40094-014-0141-9
2. Bobbert, F., Lietaert, K., Eftekhari, A., Pouran, B., Ahmadi, S., Weinans, H., & Zadpoor, A. (2017). Additively manufactured metallic porous biomaterials based on minimal surfaces: A unique combination of topological, mechanical, and mass transport properties. *Acta Biomaterialia*, *53*, 572–584. https://doi.org/10.1016/j.actbio.2017.02.024
3. Bollino, F., Armenia, E., & Tranquillo, E. (2017). Zirconia/Hydroxyapatite composites synthesized via Sol-Gel: influence of hydroxyapatite content and heating on their biological properties. *Materials*, *10*(7), 757. https://doi.org/10.3390/ma10070757
4. Dorozhkin, S. V. (2009). Bioceramics of calcium orthophosphates. *Biomaterials*, *31*(7), 1465–1485. https://doi.org/10.1016/j.biomaterials.2009.11.050

5. Dorozhkin, S. V. (2009a). Nanodimensional and nanocrystalline apatites and other calcium orthophosphates in biomedical engineering, biology and medicine. *Materials*, *2*(4), 1975–2045. https://doi.org/10.3390/ma2041975
6. Duta, L., & Grumezescu, V. (2024b). The effect of doping on the electrical and dielectric properties of hydroxyapatite for medical applications: from powders to thin films. *Materials*, *17*(3), 640. https://doi.org/10.3390/ma17030640
7. Hossain, M. A. (2013). Synthesis of Carbon Nanoparticles from Kerosene and their Characterization by SEM/EDX, XRD and FTIR. *American Journal of Nanoscience and Nanotechnology*, *1*(2), 52. https://doi.org/10.11648/j.nano.20130102.12
8. Huang, B., Caetano, G., Vyas, C., Blaker, J., Diver, C., & Bártolo, P. (2018). Polymer-Ceramic composite scaffolds: The effect of hydroxyapatite and B-tri-Calcium phosphate. *Materials*, *11*(1), 129. https://doi.org/10.3390/ma11010129
9. Khan, M. U. A., Al-Arjan, W. S., Binkadem, M. S., Mehboob, H., Haider, A., Raza, M. A., Razak, S. I. A., Hasan, A., & Amin, R. (2021). Development of biopolymeric Hybrid Scaffold-Based on AAC/GO/NHAP/TIO2 nanocomposite for bone tissue engineering: In-Vitro analysis. *Nanomaterials*, *11*(5), 1319. https://doi.org/10.3390/nano11051319
10. Miri, Z., Haugen, H. J., Loca, D., Rossi, F., Perale, G., Moghanian, A., & Ma, Q. (2023). Review on the strategies to improve the mechanical strength of highly porous bone bioceramic scaffolds. *Journal of the European Ceramic Society*, *44*(1), 23–42. https://doi.org/10.1016/j.jeurceramsoc.2023.09.003
11. Mittal, D., Kaur, G., Singh, P., Yadav, K., & Ali, S. A. (2020). Nanoparticle-Based Sustainable Agriculture and Food Science: Recent advances and Future Outlook. *Frontiers in Nanotechnology*, *2*. https://doi.org/10.3389/fnano.2020.579954
12. Rokaya, D., Srimaneepong, V., Wisitrasameewon, W., Humagain, M., & Thunyakitpisal, P. (2020). Peri-implantitis Update: Risk indicators, diagnosis, and treatment. *European Journal of Dentistry*, *14*(04), 672–682. https://doi.org/10.1055/s-0040-1715779
13. Saiz, E., Gremillard, L., Menendez, G., Miranda, P., Gryn, K., & Tomsia, A. (2006). Preparation of porous hydroxyapatite scaffolds. *Materials Science and Engineering C*, *27*(3), 546–550. https://doi.org/10.1016/j.msec.2006.05.038
14. Sossa, P. a. F., Giraldo, B. S., Garcia, B. C. G., Parra, E. R., & Arango, P. J. A. (2018b). Comparative study between natural and synthetic Hydroxyapatite: structural, morphological and bioactivity properties. *Matéria (Rio De Janeiro)*, *23*(4). https://doi.org/10.1590/s1517-707620180004.0551
15. Venkatesan, J., Lowe, B., Manivasagan, P., Kang, K., Chalisserry, E., Anil, S., Kim, D., & Kim, S. (2015). Isolation and Characterization of Nano-Hydroxyapatite from Salmon Fish Bone. *Materials*, *8*(8), 5426–5439. https://doi.org/10.3390/ma8085253
16. Venugopal, J. R., Low, S., Choon, A. T., Kumar, A. B., & Ramakrishna, S. (2008). Nanobioengineered electrospun composite nanofibers and osteoblasts for bone regeneration. *Artificial Organs*, *32*(5), 388–397. https://doi.org/10.1111/j.1525-1594.2008.00557.x