BULLETIN OF STOMATOLOGY AND MAXILLOFACIAL SURGERY
Volume 21, Issue 9

DOI:10.58240/1829006X-2025.21.9-280

ORIGINAL RESEARCH

SYNTHESIS OF PRASEODYMIUM-DOPED HYDROXYAPATITE (HYDROXYAPATITE) AND IT'S STRUCTURAL,MORPHOLOGICAL, AND TOXICOLOGICAL ANALYSIS: AN EXPERIMENTAL STUDY.

Jennifer Jeyaruby Joyson[1], Kaarthikeyan Gurumoorthy*[2]

[1] Postgraduate student, Department of Periodontology, Saveetha Dental College, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, India. E-mail : jennifer.ruby91@gmail.com ORCID No : 0000-0003-3211-7097 Mobil Phone : 919940371881 Correspondence: 162, Poonamallee High Rd, Velappanchavadi, Chennai, Tamil Nadu 600077.

[2] Professor and Head of the Department of Periodontology, Saveetha Dental College, Saveetha Institute of Medical and Technical Sciences, Saveetha University, , Chennai, India. 162, Poonamallee High Rd, Velappanchavadi, Chennai, Tamil Nadu 600077. ORCID No : 0000-0002-5521-7157

***Corresponding Author:** Kaarthikeyan Gurumoorthy[2] Professor and Head of the Department of Periodontology, Saveetha Dental College, Saveetha Institute of Medical and Technical Sciences, Saveetha University, , Chennai, India E-mail : kaarthikeyan@saveetha.com Mobil Phone : 919841222027

Received: *Sep.22 2025;* ***Accepted:*** *Oct. 29, 2025;* ***Published:*** *Nov 15,2025*

ABSTRACT

Background: Periodontal disease, which is characterized by inflammation and the breakdown of periodontal tissues, requires effective regeneration treatments. A bioceramic alloplast called hydroxyapatite (hydroxyapatite), which resembles natural bone chemically and structurally, has potential for bone regeneration. However, its applicability is hampered by its limitations in resorption rate and mechanical strength. The structural and biological performance of hydroxyapatite may be improved by doping it with rare earth elements like praseodymium (Pr), which are recognized for their osteogenic qualities and biocompatibility.

Aims and Objectives: This study aimed to synthesize praseodymium-doped hydroxyapatite (Pr-hydroxyapatite) using a wet chemical precipitation method and evaluate its structural, morphological, and toxicological properties to assess its potential for periodontal regeneration.

Materials and Methods: Pr-hydroxyapatite was synthesized by incorporating praseodymium ions into the hydroxyapatite lattice through a wet chemical precipitation process. Characterization was performed using Scanning Electron Microscopy (SEM), Energy Dispersive X-ray (EDX) analysis, Attenuated Total Reflectance Infrared Spectroscopy (ATR-IR), and X-ray Diffraction (XRD). Toxicological analysis was conducted using a zebrafish embryo toxicity test at various Pr-hydroxyapatite concentrations (4–200 µg/ml).

Results: SEM revealed well-formed crystals with rough, layered morphologies, while EDX confirmed the presence of praseodymium at 2.5% incorporation. ATR-IR and XRD demonstrated successful integration of Pr^{3+} ions into the hydroxyapatite lattice without compromising crystallinity or phase purity. Toxicity studies indicated dose-dependent effects, with 4 µg/ml identified as a safe concentration for biological applications.

Conclusion: Pr-hydroxyapatite exhibits improved structural and physicochemical properties, supporting its potential in promoting osteogenesis, angiogenesis, and antibacterial activity. Its optical properties further enable bioimaging and antimicrobial photodynamic therapy applications. Despite its promise, additional in vitro and in vivo studies are required to optimize dosage, evaluate resorption rates, and confirm long-term safety and efficacy in clinical applications.

***Keywords*:** Periodontal disease, Periodontal regeneration, Hydroxyapatite, Lanthanides, Praseodymium, Bio-ceramics, Alloplasts

INTRODUCTION

Periodontal disease, a commonly encountered issue in oral health, manifests through inflammation and infection in the tissues surrounding the teeth, such as the gums, periodontal ligament (PDL), and alveolar bone. Its main etiology is the buildup of bacterial plaque and tartar, which gradually erodes the supportive structures of the teeth. Untreated periodontal disease can have serious repercussions, from tooth loss to potential systemic health issues, underscoring the importance of managing it promptly and effectively [1-4]. The existing treatment modalities focus on removing the prime etiology by scaling and root planing, antimicrobial therapy, host modulation followed by regeneration of the lost structures through guided bone and tissue regeneration [3,5-7]. Although regeneration could be achieved through autografts, allografts and xenografts, the invasive surgical procedure involved in procurement of autografts necessitating an additional surgical site, the sensitive manufacturing process and potential immune or graft rejection reactions of allografts and xenografts are certain constraints faced during regeneration procedures. In order to overcome these limitations, synthetic alloplasts that closely resemble human bone in both composition and biology have been developed [8.9].

Hydroxyapatite (hydroxyapatite) is the most commonly utilized bioceramic material for bone grafting. Their structural and chemical similarity to natural bone makes them biocompatible and elicits no immune reaction. Their osteoconductive qualities, permits osteoblast apposition and migration to the defective site. Direct bonding is established between HA and bone surface [7,10-13]. However, the hydroxyapatite also has disadvantages with its slower rate of resorption and inferior mechanical strength which can hinder bone remodeling [14].

To overcome this disadvantage, doping of various elements into hydroxyapatite had been tested without causing a major modification in hydroxyapatite lattice. Rare earth materials due to it's optical, physicochemical, and biocompatible qualities, have been explored for its strong therapeutic potential [15]. Lanthanide ions' ability to incorporate into the crystal matrix of hydroxyapatite have been shown to improve mechanical properties of hydroxyapatite. It also improves biocompatibility and cellular proliferation and adhesion [16]. Also its stimulatory action on bone-building osteoblasts and its lowering effect on osteoclast production have made them a preferred substitute in hydroxyapatite lattice [17]. Praseodymium, third light group member of the lanthanides has the ability to replace calcium without causing a functional substitution in proteins and cell membranes. The similar ionic radii between praseodymium and calcium ions has caused increased affinity [16]. Thus considering advantages brought about by mild structural and chemical alteration of hydroxyapatite with praseodymium, this study aimed at synthesising hydroxyapatite doped with praseodymium and assessment of its physical and biological properties.

MATERIALS AND METHODS:

Preparation of praseodymium doped hydroxyapatite:

Using calcium nitrate tetrahydrate (Ca(NO3)2 4H2O, Merck), diammonium hydrogen phosphate ((NH4)2HPO4, Merck), praseodymium nitrate, and analytical-grade ammonia solution, the hydroxyapatite and praseodymium co-doped hydroxyapatite powder was created via a wet-chemical precipitation method. Triple-distilled water was used to create diammonium hydrogen phosphate at zero point six molar mass. Praseodymium nitrate was combined in different molar concentrations (0.05 M) with one point zero molar of calcium nitrate. Using a magnetic stirrer, the produced reaction mixtures (Ca:Pr) were vigorously stirred for three hours as they were added dropwise to 0.6 M of (NH4)2HPO4 in a 1:1 volume ratio. The aqueous ammonia solution kept the pH of the mixture at 10. The solution was then subjected to ultrasonication for 1 h, using a Sonics-Vibra-Cell VCX 750 (750 W) probe ultrasonicator. The slurry of the mixture was washed with deionized water and dried at 801C in air. Pellets of the samples with 8 mm diameter and 1 mm thickness were prepared by applying a pressure of 2 t using a hydraulic press.

Scanning electron microscope:

SEM was employed for analysis. The samples were placed and assessed using the FEI Quanta FEG 650 SEM (JSM IT-800, JEOL Ltd., Akishima, Tokyo, Japan), operating at an accelerating voltage of 2,000 kV.

Photographs were captured of both the membrane's surface and cross-sections, at magnifications of 500x. It can be employed for imaging samples with a resolution of up to 0.5 nm. Additionally, it comes equipped with an assortment of detectors suitable for tasks such as elemental mapping, crystallographic analysis, and more. EDS analysis on the selected areas was done to obtain the elemental composition of the sample.

ATR-IR:

Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-IR) (Bruker Alpha II, Bruker, Billerica, MA, United States) was used to characterize praseodymium-doped hydroxyapatite. The samples of praseodymium doped hydroxyapatite are introduced onto the ATR crystal . An infrared beam is focused onto the crystal during the ATR-IR examination, and it enters the sample a short distance before undergoing total internal reflection. An absorption spectrum that reveals details about the molecular vibrations and chemical bonds found in the praseodymium-doped hydroxyapatite is produced when the evanescent wave produced at the crystal-sample interface interacts with the sample.

X-RAY DIFFRACTION:

After being properly placed on a sample holder, the powdered samples are put through an X-ray diffraction analysis. The sample is exposed to an X-ray beam during the XRD analysis, causing the atoms in the sample to diffract the X-rays. The crystal structure, phase composition, and crystallite size of the praseodymium-doped hydroxyapatite are ascertained by recording and analyzing the ensuing diffraction pattern. Shifts in the diffraction peaks caused by the presence of praseodymium ions can reveal information on the incorporation of praseodymium into the hydroxyapatite lattice and any ensuing modifications to the crystal structure.

TOXICITY:

Fish embryo acute toxicity test:

The in vitro toxicity study was carried out against zebrafish embryos by using the obtained praseodymium doped hydroxyapatite . On the basis of the OECD-203 protocol, the toxicity was assessed using 25 marked zebrafish eggs at different ratios (4 µg/ml and 8 µg/ml) of the synthesized praseodymium doped hydroxyapatite in Hank's solution. The eggs were then moved into individual wells for further development, such as tails , head, and eyes, which were clearly identified using a 40 × microscope every 24 h. While performing the assay, the water was constantly maintained at room temperature and the dead and live fish were monitored for every 24 h to restrict any contamination in the solution. Every 24 h, the hatching and mortality rates of embryos were calculated.

RESULTS

SEM:

Figure 1: SEM analysis of the praseodymium doped hydroxyapatite

The 5,000x magnification SEM image captured the micrometer-scale intricacies of the crystal structure with exceptional contrast, resolution, and edge definition displays well-formed hydroxyapatite crystals with a rough surface, distinct grain boundaries, and a layered, plate-like shydroxyapatitee (Figure 1).

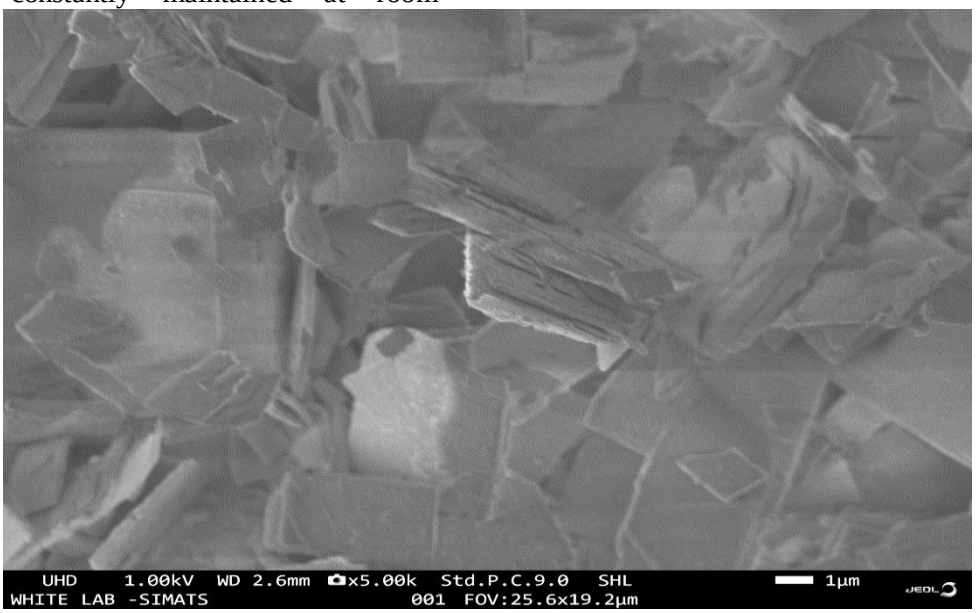


Figure 1. SEM analysis of the praseodymium doped hydroxyapatite

EDX:

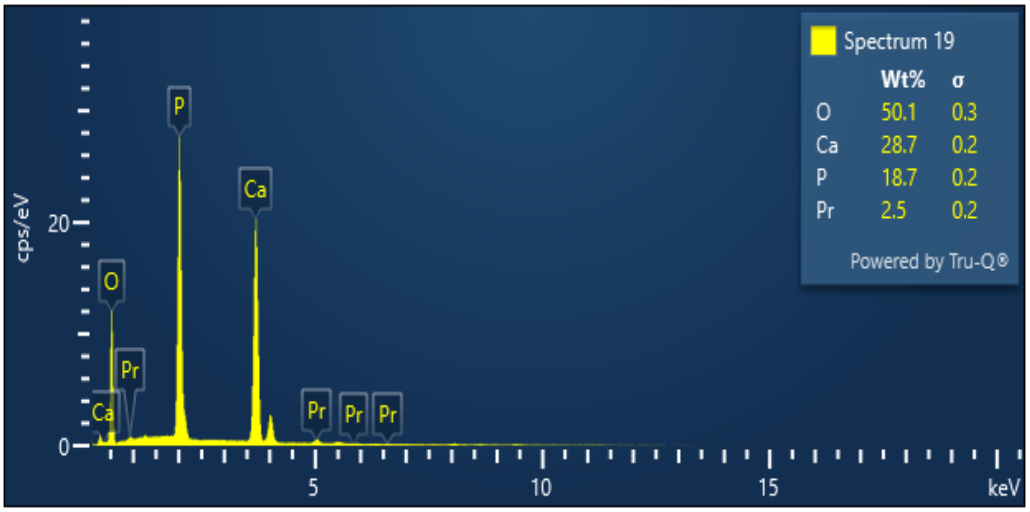
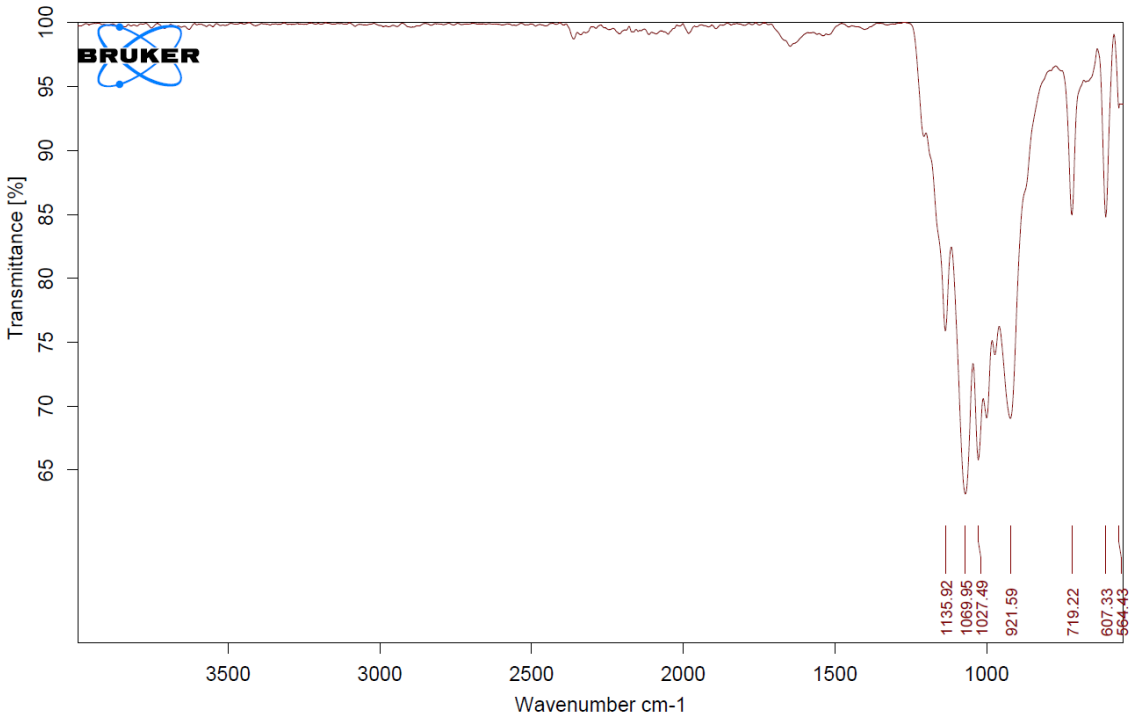


Figure 2. EDX quantitative analysis of elements in the praseodymium doped hydroxyapatite

The elemental composition of praseodymium-doped hydroxyapatite is confirmed by the EDS spectrum image (Figure 2), which shows prominent peaks for praseodymium (2.5%), calcium (28.7%), phosphorus (18.7%), and oxygen (50.1%). These components align with the anticipated elements of hydroxyapatite, where praseodymium doping could improve characteristics of hydroxyapatite.

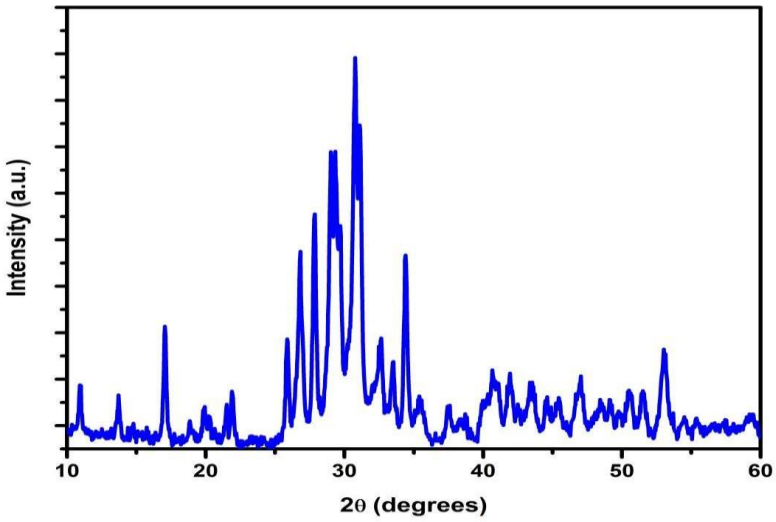


ATR-IR

Figure 3. ATR-IR spectrum of praseodymium doped hydroxyapatite Vibration peaks were observed at various wave numbers

A well-crystallized structure is indicated by the praseodymium-doped hydroxyapatite spectrum, which displays distinctive bands for the phosphate ($PO4^{3-}$) and carbonate ($CO3^{2-}$) groups. These bands include peaks at 1135 cm^{-1} ($HPO4^{2-}$), 1069 cm^{-1} ($CO3^{2-}$ substitution), and additional phosphate peaks at 1027, 921, 719, 607, and 564 cm^{-1} (Figure 3).

XRD:



XRD

Figure 4. XRD analysis of praseodymium doped hydroxyapatite
Diffraction patterns obtained in the 2θ range from 10 to 75 degrees

The XRD pattern displays distinctive hydroxyapatite peaks, with the greatest peak at approximately 32° and the predominant peaks around 2θ = 30-35°, suggesting excellent crystallinity (Figure 4).

TOXICITY TEST

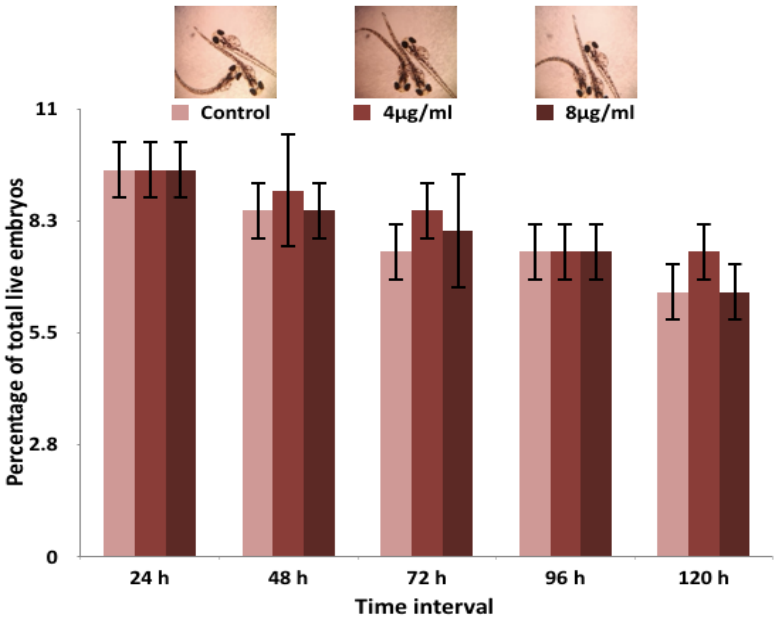


Figure 5. Graphic illustration of embryo viability of praseodymium-doped hydroxyapatite in a dose-dependent manner.

Figure 5 illustrates the viaility of the tested groups - Control (Water), 4 µg/ml and 8 µg/ml of prepared praseodymium-doped hydroxyapatite samples. All groups are very viable at 24 hours, but the control group stays higher the entire time. The 8 µg/ml group has the lowest survivability at 96 and 120 hours, indicating increased toxicity. The viability of 8 µg/ml is less when compared to the 4 µg/ml group except at 96th hour. The viability of the 4 µg/ml group gradually decreased and remained stable after 96th hour. Variability with some group overlap is shown by error bars.

DISCUSSION

Periodontitis which causes attachment loss eventually induces resorption of the periodontal ligament (PDL) and bundle bone. The goal of periodontal regeneration is development of supporting structures , such as alveolar bone, PDL, and cementum. Open flap debridement combined with physiologically active biomaterials which can promote bone regeneration and prevent long junctional epithelial repair can accomplish the goal of periodontal regeneration . This resulted in development of biomaterials that can promote osteogenesis by stabilizing the clot and keep the soft tissues from collapsing into the defect thus accelerating the restoration of destroyed structures, hence improving the function of tooth supporting structures. The quest for search of a biomaterial which provides a less morbid approach unlike autografts or has less immunogenicity and least potential risks for infectious diseases than allografts and xenografts had led to synthesis of artificial bioceramics or alloplasts which resembles natural human bone in its components and structure had been developed and been in use.

Hydroxyapatite is one of the bioceramic alloplastic bone graft materials which possess a similar chemical composition to natural bone and has a Ca:P ratio of 1.67 which imparts a good stability and biocompatibility thus rendering it a most suitable material for bone regeneration [(14)]. However its slower rate of resorption and inferior mechanical strength can hinder bone remodeling. This major setback could be overcome by structural alteration through doping with ionic substitutes. Extensive research has been undertaken to incorporate lanthanide ions into hydroxyapatite. Lanthanides are biocompatible with good optical and absorption properties. Its affinity towards calcium ions and its ability to cause no functional changes to the incorporated material have made lanthanides an ideal candidate for doping with hydroxyapatite [(14,16)].

This study focused on doping praseodymium into hydroxyapatite using a wet chemical precipitation method. The prepared material was subjected to physical characterisation tests and toxicity was tested. SEM analysis revealed the crystals' angular and uneven pattern with interconnected shydroxyapatitee and mixed orientations which indicated a successful incorporation of praseodymium. The praseodymium doping affected crystal formation, resulting in asymmetrical sizes and forms. EDX showed alignment with the anticipated elements of hydroxyapatite, where praseodymium doping (2.5%) could improve characteristics of hydroxyapatite with only minor chemical modification. ATIR-IR showed apatite phase development with intact phosphate structure which is confirmed by modest peak shifts (1027, 921, 719, 607, and 564 cm^{-1}) that imply Pr^{3+} integration, while carbonate and hydrogen phosphate bands at 1135 cm^{-1} and 1069 cm^{-1} indicate structural replacements suggesting successful incorporation of praseodymium into the hydroxyapatite lattice and potentially changing the characteristics of hydroxyapatite. XRD results revealed consistent peak locations with the hexagonal hydroxyapatite structure and the absence of secondary phases which verify praseodymium doping. Phase-pure Pr-doped hydroxyapatite is confirmed by peaks that range 10–60° with a stable baseline and no impurities, matching standard hydroxyapatite. The toxicity analysis inferred decreased viability with increasing concentrations over time, indicating dose-dependent toxicity. The viability at a dosage of 4 µg/ml could be used safely as the embryo survivability was better than the control group and was also stable after 96th hour. However further cytotoxicity and drug release pattern analysis are required to determine the safety dosage.

Praseodymium doped hydroxyapatite had been proved to promote osteogenesis as well as enhance tissue response through its cellular and immunomodulatory actions. Also it possess antibacterial properties [(18)]. It's angiogenesis activity is stimulated by endothelial cell proliferation which can promote wound healing post periodontal surgery [(19)]. It could be further incorporated into scaffolds for prompting regeneration in periodontal Intra bony defects and for arresting the periodontal disease progression and restoring the subgingival micro iota balance [(15,16)]. Additionally the optical property of praseodymium helps in bio imaging which could help study the resorption rate of various bone graft materials thus determining the prognosis of periodontal disease [(16)]. It could also be used for diagnosis by determining the pocket depth and also locating and measuring depth of bony defects [(16)]. Moreover, the capacity to transform visible emission light from near-infrared (NIR) excitation light could enable the material to be used for antimicrobial photodynamic therapy (aPDT) [(20)].

Despite the promising potential, additional studies are warranted in gingival fibroblast cell line and osteoblast cell line for cytotoxicity and regeneration potential. Resorption rate should also be evaluated. Optimal dosage should be determined to avoid adverse tissue response. The therapeutic and diagnostic potential should be substantiated by further animal studies and large scale clinical trials.

CONCLUSION

The synthesis of praseodymium-doped hydroxyapatite (Pr-hydroxyapatite) was accomplished, indicating the promise of this material for bone regeneration, especially in periodontal therapy. Characterization verified that the praseodymium was successfully incorporated, improving the physicochemical and structural characteristics. According to toxicological investigation, a safe dose of 4 µg/ml was found, and Pr-hydroxyapatite showed promising osteogenesis,

angiogenesis, and antimicrobial activities. Its optical characteristics make it a promising candidate for antimicrobial photodynamic treatment and bioimaging.To assess resorption rates, improve dosage, and confirm therapeutic efficacy through extensive clinical trials, more research is required.

DECLARATIONS

Conflict of interests

There are no conflicts of interests

Funding

This study did not receive any funding

Ethical Approval

Not obtained as no human or animal subjects were involved.

Informed Consent

No human subjects were involved in this study.

Author Contributions:

Concept - Jennifer Jeyaruby J ; Design - Jennifer Jeyaruby J & Kaarthikeyan Gurumoorthy ;

Supervision - Kaarthikeyan Gurumoorthy; Resources - NA; Materials - Jennifer Jeyaruby J;

Data Collection and/or Processing - Jennifer Jeyaruby J & Kaarthikeyan Gurumoorthy ;

Analysis and/or Interpretation - Jennifer Jeyaruby J & Kaarthikeyan Gurumoorthy;

Literature Search - Jennifer Jeyaruby J; Writing Manuscript - Jennifer Jeyaruby J;

Critical Review - NA; Other – NA

REFERENCES

1) Bhuyan R, Bhuyan S, Mohanty J, Das S, Juliana N, Abu I. Periodontitis and its inflammatory changes linked to various systemic diseases: a review of its underlying mechanisms. *Biomedicines*. 2022;10(10):2659.
2) Ng H, Kin L, Dashper S, Slakeski N, Butler C, Reynolds E. Bacterial interactions in pathogenic subgingival plaque. *Microbial Pathogenesis*. 2016;94:60-69.
3) Bajpai D, Rajasekar A. Development and Characterization of Gadolinium and Copper Reinforced Bioactive Glass: An In Vitro Study. *Cureus*. 2024;16(2).
4) Chatterjee S, Kaarthikeyan G. A Novel Candidate for Guided Tissue Regeneration: Chitosan and Eggshell Membrane. *Cureus*. 2023;15(11).
5) Cobb C. Clinical significance of non-surgical periodontal therapy: an evidence-based perspective of scaling and root planing. *Journal of clinical periodontology*. 2002;29:22-32.
6) Ilyes I, Boariu M, Rusu D, Iorio-Siciliano V, Vela O, Boia S, Radulescu V, Șurlin P, Jentsch H, Lodin A, et al. Comparative Study of Systemic vs. Local Antibiotics with Subgingival Instrumentation in Stage III–IV Periodontitis: A Retrospective Analysis. Antibiotics. 2024; 13(5):430. https://doi.org/10.3390/antibiotics13050430
7) Hirshasri M, Sangeetha S, Prathydroxyapatite L. Development of nanohydroxyapatite and curcumin based bilayer scaffold for periodontitis treatment. *J Periodontol*. 2024;95(1):123-130.
8) Zhao R, Yang R, Cooper PR, et al. Bone grafts and substitutes in dentistry: A review of current trends and developments. *Molecules*. 2021;26(10):3007.
9) Sri H, Ganapathy D, Venugopalan S, Deshmukh M. Current trends in bone grafts: A review. *J Pharm Negative Results*. 2022;13(10):474-481.
10) Sheikh Z, Hamdan N, Ikeda Y, et al. Natural graft tissues and synthetic biomaterials for periodontal and alveolar bone reconstructive applications: a review. *Biomater Res*. 2017;21(1):9.
11) Dewi AH, Ana ID. The use of hydroxyapatite bone substitute grafting for alveolar ridge preservation, sinus augmentation, and periodontal bone defect: A systematic review. *Heliyon*. 2018;4(10):e00915.
12) Kishen A, Cecil A, Chitra S. Fabrication of hydroxyapatite reinforced polymeric hydrogel membrane for regeneration. *Saudi Dent J*. 2023;35(6):678-683.
13) Shankar P, Arumugam P, Kannan S. Development, characterisation and biocompatibility analysis of a collagen-gelatin-hydroxyapatite scaffold for guided bone regeneration. *Odovtos Int J Dent Sci*. 2024;26(4):235-248.
14) Zhao R, Yang R, Cooper PR, et al. Bone grafts and substitutes in dentistry: A review of current trends and developments. *Molecules*. 2021;26(10):3007.
15) Natarajan D, Ye Z, Wang L, Ge L, Pathak JL. Rare earth smart nanomaterials for bone tissue engineering and implantology: Advances, challenges, and prospects. *Bioeng Transl Med*. 2022;7(1):e10262.
16) Agid RS, Kaygili O, Bulut N, et al. Investigation of the effects of Pr doping on the structural properties of hydroxyapatite: an experimental and theoretical study. *J Aust Ceram Soc*. 2020;56:1501-1513.
17) Wieszczycka K, Staszak K, Woźniak-Budych MJ, Jurga S. Lanthanides and tissue engineering strategies for bone regeneration. *Coord Chem Rev*. 2019;388:248-267.
18) Shanmugapriya P, Veerasamy R, Bhuvaneshwari N, Brindha T. Multifaceted properties of Pr-Ha/Pla co-doped nanohydroxyapatite composite: Insights into thermal and antioxidant activity. *J Nanomater*. 2024;2024:123456.
19) Zrieq R, Alzain MA, Haouas N, et al. Surface modification of praseodymium oxide/hematite doped into polycaprolactone for enhanced wound management demands. *J Saudi Chem Soc*. 2023;27(5):101708.
20) Zhang Y, Huang P, Wang D, et al. Near-infrared-triggered antibacterial and antifungal photodynamic therapy based on lanthanide-doped upconversion nanoparticles. *Nanoscale*. 2018;10(33):15485-15495.

Jennifer Jeyaruby Joyson, Kaarthikeyan Gurumoorthy. Synthesis of Praseodymium-Doped Hydroxyapatite (hydroxyapatite) and it's Structural, Morphological, and Toxicological Analysis: An experimental study. Bulletin of Stomatology and Maxillofacial Surgery.2025;21(9)280-286 doi:10.58240/1829006X-2025.21.9-280