BULLETIN OF STOMATOLOGY AND MAXILLOFACIAL SURGERY
Volume 21, Issue 2

DOI: 10.58240/1829006X-2025.2-239

RESEARCH ARTICLE

IN VITRO STUDY ON THE SYNTHESIS AND CHARACTERIZATION OF ERBIUM-DOPED HYDROXYAPATITE/BIOGLASS-POLYVINYL ALCOHOL SCAFFOLD FOR PERIODONTAL BONE REGENERATION

Maria Sharon V[1],Nidhita Suresh M.D.S[2],Kaarthikeyan Gurumoorthy M.D.S,Phd[3] ,Chitra Shivalingam[4]

[1.]Postgraduate Student, Department of Periodontics,Saveetha Dental college,SaveethaInstitute of Medical and Technical Sciences,Saveetha University 162,Poonamallee High Road, Chennai, India

[2.]Assistant Professor, Department Of Periodontics, Saveetha dental college, Saveetha Institute Of Medical And Technical Sciences, Saveetha University 162,Poonamallee High Road, Chennai, India, Email ID: drnidhitasuresh@gmail.com

[3.]Department Of Periodontics, Saveetha dental college, Saveetha Institute Of Medical And Technical Sciences, Saveetha University 162,Poonamallee High Road, Chennai, India, Email ID: kaarthikeyan@saveetha.com.

[4.]Professor, Department Of Periodontics Saveetha dental college, Saveetha Institute Of Medical And Technical Sciences, Saveetha University 162,Poonamallee High Road, Chennai, India. Email ID: Chitras.sdc@saveetha.com

Corresponding author: Dr Nidhita Suresh , Assistant Professor, Department Of Periodontics, Saveetha dental college, Saveetha Institute Of Medical And Technical Sciences, Saveetha University 162,Poonamallee High Road, Chennai, India, Email ID: drnidhitasuresh@gmail.com

Received: *Feb. 27, 2025;* ***Accepted:*** *Mar4, 2025;* ***Published:*** *Mar. 20, 2025*

Abstract

Background:Periodontal disease leads to alveolar bone loss, necessitating bone grafting for regeneration. Traditional grafts, including autografts and allografts, have limitations such as donor site morbidity and immune rejection. Bone tissue engineering (BTE) offers an alternative approach using biocompatible scaffolds. This study investigates the potential of an erbium-doped hydroxyapatite (HA)-bioglass-polyvinyl alcohol (PVA) composite scaffold for bone regeneration.

Materials and Methods: The study synthesized an erbium-doped HA, bioglass, and PVA composite scaffold. The scaffold was characterized morphologically using Fourier Transform Infrared Spectroscopy (FTIR) and Scanning Electron Microscopy (SEM). Biological evaluations included hemostatic behavior, anti-inflammatory response, and biocompatibility assessments using an MTT assay and live/dead cell analysis.

Results: FTIR confirmed the structural integrity of the scaffold with characteristic peaks of HA, bioglass, and PVA. SEM revealed a porous, flower-like morphology, promoting cell attachment and nutrient exchange. Hemostatic analysis demonstrated enhanced clot formation, with SEM micrographs showing aggregated RBCs on the scaffold surface, indicating strong hemostatic efficiency.Anti-inflammatory studies indicated a dose-dependent effect, with the scaffold exhibiting significant protein denaturation inhibition at 50 µg. MTT and live/dead assays confirmed high biocompatibility, with over 90% cell viability across tested concentrations.

Conclusion: The erbium-doped HA/bioglass-PVA scaffold demonstrated excellent biocompatibility, hemostatic potential, and anti-inflammatory properties. These findings suggest its suitability for periodontal bone regeneration. Further in vivo studies are required to validate its clinical applicability.

Keywords: **Rare earth element, Periodontal Regeneration, Erbium, Scaffold, Bioglass**

Maria Sharon V, Nidhita Suresh, Kaarthikeyan Gurumoorthy,Chitra Shivalingam.In Vitro Study on the Synthesis and Characterization of Erbium-Doped Hydroxyapatite/Bioglass-Polyvinyl Alcohol Scaffolds for Periodontal Bone Regeneration. Bulletin of Stomatology and Maxillofacial Surgery. 2025;21(2).239-249 DOI:10.58240/1829006X-2025.-2-239

INTRODUCTION

Periodontal disease, a chronic inflammatory condition affecting the supporting structures of the teeth, ranks among the most prevalent oral health problems worldwide.[1] Characterized by the progressive destruction of the periodontal ligament and alveolar bone, it ultimately leads to tooth mobility and potential tooth loss if left untreated.[1] A significant consequence of periodontal disease, is the formation of bone defects. Insufficient alveolar bone necessitates bone grafting or augmentation procedures to provide adequate support for the tooth to restore proper function and aesthetics.[2]

Traditional bone grafting methods, primarily relying on autografts harvested from the patient's own body, have long been considered the gold standard.[3]Autografts possess inherent osteogenic potential, containing viable bone cells that can directly contribute to new bone formation.[3] However, the limitations associated with autografts are significant. Donor site morbidity, including pain, infection, and prolonged healing time, is a major drawback. Furthermore, the availability of autogenous bone is limited, particularly in cases of extensive bone loss.[4] Allografts, derived from cadaveric bone, and xenografts, sourced from animals, offer alternative options but carry the risk of disease transmission and immune rejection, respectively.[5] These limitations have spurred intense research and development in the field of bone tissue engineering (BTE), aiming to create biocompatible and osteoinductive substitutes for traditional bone grafts.[6]

BTE has emerged as a multidisciplinary field that seeks to regenerate damaged bone tissue by combining osteogenic cells, biocompatible scaffolds, appropriate vascularization, and controlled culture conditions to mimic natural bone regeneration.[7] This approach leverages biochemical and physical stimuli to promote bone ingrowth, restore physiological function, and facilitate healing at the defect site. The core of BTE lies in the utilization of 3D scaffolds that serve as temporary templates for tissue regeneration.[7]

An ideal bone scaffold should fulfill several essential design requirements: (1) biocompatibility, ensuring cells can attach, proliferate, and function without triggering toxicity or immune reactions;(2) biodegradability, allowing the scaffold to break down gradually as it is replaced by newly formed bone; (3) mechanical stability, providing adequate strength to support physiological loads during the healing period;(4)structural design, incorporating appropriate porosity and pore size to enable cell migration, nutrient diffusion, and vascularization; (5) stabilizability, ensuring that the scaffold remains free from contaminants while preserving its bioactivity; and (6) controlled release of bioactive compounds, enabling the gradual delivery of growth factors or therapeutic agents to enhance bone regeneration.

Numerous materials have been explored for the fabrication of bone scaffolds, each with its own strengths and weaknesses. Ceramic scaffolds, particularly those based on calcium phosphate materials such as hydroxyapatite (HA) and tricalcium phosphate (TCP), are widely utilized due to their biocompatibility and resemblance to the mineral composition of bone.[11]

HA, with its chemical similarity to the mineral component of bone has exceptional biocompatibility and osteoconductivity that makes it an ideal choice for promoting bone apposition. Their bioactivity allows for direct bonding with native bone tissue, making them a preferred choice in BTE applications. Moreover, bioactive glasses, capable of bonding to bone and stimulating bone cell activity, have garnered increasing attention in bone tissue engineering.[13] These glasses release bioactive ions such as silicon, calcium, and phosphate upon implantation, that promote osteoblast differentiation and angiogenesis, accelerating bone formation.[13] In the periodontal environment, bioglass can effectively stimulate the recruitment and differentiation of bone-forming cells from the adjacent alveolar bone, accelerating the healing process and promoting periodontal tissue regeneration.[14] However, their intrinsic brittleness and low fracture toughness pose significant challenges, particularly in load-bearing applications where mechanical stability is critical.

To improve the mechanical properties of ceramic scaffolds**,** composite scaffolds, which integrate ceramics with polymers or other biomaterials, have been developed. By combining the osteoconductive properties of ceramics with the flexibility and degradability of polymers, these scaffolds offer improved mechanical strength and controlled degradation. Polyvinyl alcohol (PVA), a water-

Maria Sharon V, Nidhita Suresh, Kaarthikeyan Gurumoorthy,Chitra Shivalingam.In Vitro Study on the Synthesis and Characterization of Erbium-Doped Hydroxyapatite/Bioglass-Polyvinyl Alcohol Scaffolds for Periodontal Bone Regeneration. Bulletin of Stomatology and Maxillofacial Surgery. 2025;21(2).231-249
DOI:10.58240/1829006X-2025.-2-231

soluble, non-toxic, biocompatible, and biodegradable polymer, is frequently incorporated into HA-based scaffolds to improve their flexibility and processability.[12] PVA's ability to form hydrogels allows for efficient cell encapsulation and nutrient diffusion, creating a conducive microenvironment for tissue growth.[12] PVA's biocompatibility and biodegradability ensure that it does not interfere with the bone regeneration. Despite these advantages, polymer-based composites still fall short in load-bearing capacity, often necessitating additional reinforcement.

Hybrid scaffolds that integrate ceramics, polymers, and metals offer a promising approach to overcoming the limitations of individual materials. By combining the osteoconductive properties of HA and bioactive glass with the flexibility of polymers and the mechanical strength of metals, these composite scaffolds provide a multifunctional solution for bone regeneration

The incorporation of erbium (Er) is a key innovation of this study. Erbium is a rare earth metal that has demonstrated potential for stimulating osteogenesis. Known for its unique physicochemical properties, erbium offers several advantages when incorporated into ceramic-polymer-metal composites for bone regeneration. Its biocompatibility, antimicrobial effects, and role in enhancing osteogenesis make it a promising candidate for improving scaffold performance. Moreover, erbium has been shown to promote cell proliferation and differentiation in vitro.[15] The rationale for doping the HA-bioglass-PVA scaffold with erbium is to further enhance its osteoinductive properties and improve the mechanical properties of scaffolds.

This study takes a novel approach by exploring a composite scaffold composed of HA, bioglass, and PVA, further enhanced by the incorporation of erbium (Er). The rationale behind this specific combination of materials lies in their complementary properties, aimed at overcoming the limitations of each individual component and creating a synergistic effect that promotes enhanced bone regeneration.

MATERIALS AND METHODS

The study protocol approval was obtained from the Scientific Review Board (SRB) of Saveetha Dental College and Hospitals(SRB/SDC/PERIO-2302-24-428).

Materials

PVA(Polyvinylalcohol-341584-25G)and Cetyltriammonium bromide (Hexadecyltrimethylammonium bromide CTAB H5882-100G) were purchased from Sigma-Aldrich® US, Merck. Calcium nitrate $Ca(NO_3)_2$, Erbium chloride $ErCl_3$, Diammonium hydrogen phosphate $(NH_4)_2HPO_4$, Acetone C_3H_6O, Ethanol C_2H_5OH, Tetraethyl orthosilicate (TeOS) $Si(OC_2H_5)_4$, Phosphorus pentoxide P_2O_5, Calcium oxide CaO, Sodium oxide Na_2O, Ammonia solution $–NH_4OH$ and Tris-HCl buffer $C_4H_{11}NO_3·HCl$ used were of analytical grade.

Preparation of Erbium-Doped Hydroxyapatite (HA)

The synthesis of erbium-doped hydroxyapatite (Er-HA) begins with the dissolution of 2.1 g of cetyl triammonium bromide (CTAB), a surfactant that helps control particle morphology, in an aqueous solution. Next, 0.099 mol of calcium nitrate($Ca(NO_3)_2$), a calcium source, and 0.01 g of erbium chloride (ErCl), the dopant, are introduced into the solution. To facilitate the formation of hydroxyapatite, 0.67 g of diammonium hydrogen phosphate($(NH_4)_2(HPO_4)$) is added, ensuring a controlled Ca/P ratio. The reaction mixture is maintained at 90°C under reflux conditions overnight, promoting homogeneity and allowing complete reaction. The resultant precipitate is thoroughly washed with acetone and ethanol to remove impurities and excess reactants, yielding purified erbium-doped HA nanoparticles.

Preparation of Erbium-Doped Bioglass

For the synthesis of erbium-doped bio glass, 2 g of cetyl triammonium bromide (CTAB) is dissolved in a solvent mixture of 55 ml of water and 45 ml of ethanol. The solution is stirred for 2 hours to ensure complete dissolution of CTAB, which aids in the formation of a mesoporous structure in the bio glass. The bio glass composition consists of TeOs (0.45% silica diatomaceous), 6% phosphorus pentoxide (P_2O_5), 22.5% calcium oxide (CaO), 24.5% sodium oxide (Na_2O), and 2% erbium. To maintain uniformity in composition, tetraethyl orthosilicate (TeOs) and phosphorus pentoxide (P_2O_5) are incrementally added every 30 minutes over the course of the reaction. This step ensures controlled

Maria Sharon V, Nidhita Suresh, Kaarthikeyan Gurumoorthy,Chitra Shivalingam.In Vitro Study on the Synthesis and Characterization of Erbium-Doped Hydroxyapatite/Bioglass-Polyvinyl Alcohol Scaffolds for Periodontal Bone Regeneration. Bulletin of Stomatology and Maxillofacial Surgery. 2025;21(2).231-249
DOI:10.58240/1829006X-2025.-2-231

deposition and incorporation of these components into the bio glass matrix.

Preparation of PVA Scaffold

The fabrication of the polyvinyl alcohol (PVA) scaffold begins by adding 3 mL of ammonia solution to a precursor mixture to initiate the precipitation process. The mixture is allowed to react overnight, ensuring thorough gelation and the formation of a uniform polymeric network. The precipitate is then sintered and subjected to washing at 40°C for 3 hours to remove residual byproducts and improve material purity. After this treatment, a 10% PVA solution is incorporated into the scaffold .

Lyophilization (Freeze-Drying) Process

In the final step, the prepared materials are combined to create a composite scaffold. A mixture containing 1% erbium-doped HA, 1% erbium-doped bio glass, and 10% PVA solution is homogenized to achieve a uniform distribution of all components. This mixture is then subjected to lyophilization (freeze-drying) using a lyophilizer (Lyoxl Pilot range **,** LSI).

MORPHOLOGICAL CHARACTERIZATION

Fourier Transform Infrared (FTIR) Spectroscopy

FTIR spectroscopy was performed on erbium-Doped HA/Bioglass-PVA Scaffold to characterize the chemical changes. The spectra were recorded in the range of 4000-1000 cm^{-1} using Bruker's Alpha II FTIR Spectrometer [Thermo Scientific Nicolet 6700]. The obtained spectra were analysed to identify any functional group changes present in the scaffold.

Scanning Electron Microscopy (SEM) Analysis

The surface morphology of the Erbium-Doped HA/Bioglass-PVA Scaffolds was examined using SEM. The samples were sputter coated with gold and then observed under a Scanning Electron Microscope [ZEISS Gemini SEM] at an accelerating voltage of 1kV. The obtained images were analysed to visualize the morphology of the scaffold.

BIOLOGICAL CHARACTERIZATION

Hemostatic Behavior Analysis

The hemostatic potential of the scaffold was evaluated by analyzing its blood clotting efficiency and interaction with blood components. To assess clot formation time, 100 µL of fresh human blood was applied directly onto the scaffold surface, and the clotting time was measured under controlled conditions at 37°C.

For a detailed investigation of scaffold-blood interactions, Scanning Electron Microscopy (SEM) was employed. The scaffold was immersed in fresh human blood to observe red blood cell (RBC) adhesion, platelet aggregation, and fibrin network formation. To preserve cellular morphology, the samples were fixed using 2.5% glutaraldehyde and incubated at 37°C. Following incubation, excess blood was removed with phosphate-buffered saline (PBS), and the samples underwent sequential dehydration using graded ethanol solutions (30%, 50%, 70%, 90%, and 100%) before being vacuum-dried.

To enhance conductivity, the dried samples were coated with a thin layer of gold and examined under SEM, focusing on RBC adhesion, platelet aggregation, and fibrin deposition, which contribute to clot formation and overall hemostatic performance.

Anti-inflammatory Analysis

The anti-inflammatory potential of the prepared erbium-doped HA/Bioglass-PVA scaffold, different concentrations (25% and 50%) were added to a 2% bovine serum albumin (BSA) solution. The prepared solutions were then adjusted to a final volume of 2 mL using Tris-HCl buffer. The samples were incubated at room temperature for 30 minutes, followed by heating in a water bath at 75°C for 10 minutes. After heating, the samples were allowed to cool for 20 minutes. Their optical density was then measured using a UV-Visible spectrophotometer at 660 nm to assess the scaffold's anti-inflammatory properties and was compared with untreated sample(Control). The anti-inflammatory potential was calculated using a standard formula,

BIOCOMPATIBILITY TEST

MTT Assay

Human gingival fibroblast Cells were divided into

96-well microplates as much as 100 µl with density 3-5x10 to which different concentrations of the scaffold (12.5µg/ml, 25µg/ml,50µg/ml, 75µg/ml, and 100 µg/ml) were added and incubated for 24 hours at 37°C to evaluate the cytotoxicity. They were compared with untreated culture. After adding 25 µl of MTT solution, a microplate reader was used to measure the absorbance at 570 nm. The data was statistically analysed using One-way ANOVA with Tukey's multiple comparisons.

Live/Dead Cell Assay

The biocompatibility of the prepared erbium-doped HA/Bioglass-PVA scaffold up treated human gingival fibroblast cells and the control group (untreated culture) was evaluated by morphological assessment using Phase contrast microscopy .

RESULT

MORPHOLOGICAL CHARACTERIZATION

FTIR analysis

The FTIR spectrum (Figure 1,2) of the erbium-doped HA/Bioglass-PVA scaffold exhibits characteristic absorption peaks corresponding to functional groups from hydroxyapatite (HA), bioglass, and PVA, confirming the scaffold's structural integrity. The broad peak at 3289 cm^{-1} indicates O-H stretching, confirming the presence of hydroxyl (-OH) groups and hydration in the scaffold. The absorption band between 3000–2900 cm^{-1} corresponds to C-H stretching vibrations, primarily from PVA. A distinct peak at 1660 cm^{-1} is attributed to C-O stretching, suggesting the presence of organic acetate residues. The CH_2 bending vibrations observed at 1420 cm^{-1} contribute to the organic framework of PVA, while the peak at 1327 cm^{-1} corresponds to OH rocking and CH wagging, indicating polymeric network interactions. The strong absorption band at 1065 cm^{-1} represents C-O and OH stretching vibrations, further confirming the structural integrity of the scaffold. The presence of hydroxyapatite is validated by characteristic phosphate group (PO_4^{3-}) peaks at 1012, 710, and 536 cm^{-1}, indicating effective HA formation. Additionally, a peak at 909 cm^{-1} corresponds to CH_2 rocking vibrations, contributing to the scaffold's stability, while the band at 836 cm^{-1} represents C=C stretching, further supporting polymeric interactions. These spectral features collectively confirm the successful incorporation of HA, bioglass, and PVA into the scaffold, ensuring its suitability for bone tissue engineering.

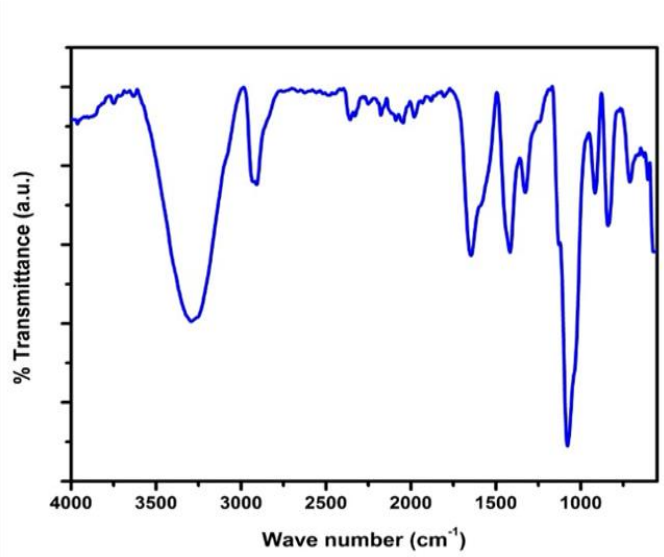

Figure 1. FTIR spectra of Erbium-doped HA/Bioglass-PVA scaffold

Wave number (cm^{-1})	Interpretation
3289	stretching O-H
3000-2900	C-H from alkyl groups
1660	C-O from acetate group
1420	CH2 bending
1327	δ (OH), rocking with CH wagging
1065	stretching of C—O and bending of OH
1012	Phosphate group
909	CH_2 rocking
836	C—C stretching
710	Phosphate group
536	Phosphate group

Figure 2. FTIR Peak Interpretation for Erbium-Doped HA/Bioglass-PVA Scaffold

SEM Analysis

The SEM analysis of the erbium-doped HA/Bioglass-PVA scaffold reveals a distinct flower-like morphology (Figure 3) with a highly porous and interconnected structure, essential for promoting cell adhesion, proliferation, and nutrient exchange.. The observed flower-like formation indicates successful incorporation of hydroxyapatite and bioglass within the PVA matrix, ensuring mechanical stability and

biomimetic properties. Additionally, the presence of micropores and nanostructured features supports the scaffold's potential for bone tissue regeneration, making it a promising material for applications in bone grafting and periodontal repair.

Figure 3. SEM analysis of erbium-doped HA/Bioglass-PVA scaffold under 500X magnification

BIOLOGICAL CHARACTERIZATION

Hemostatic Behaviour Using SEM Analysis

The SEM analysis of the erbium-doped HA/Bioglass-PVA scaffold revealed significant blood cell adhesion and interaction. The micrographs (Figure 4) displayed aggregated red blood cells (RBCs) on the scaffold surface, demonstrating its effectiveness in promoting blood clotting. The presence of platelet clusters and fibrin networks further confirmed its hemostatic potential.

The blood clotting time (BCT) for the scaffold was recorded at 4 minutes 35 seconds, significantly shorter than the average human blood clotting time of 7 minutes 10 seconds. This reduction indicates that the scaffold enhances coagulation efficiency and accelerates hemostasis.

Additionally, the rough and porous nature of the scaffold facilitated rapid blood absorption, promoting clot stabilization. The uniform dispersion of blood cells across the scaffold suggests strong scaffold-blood interactions, which play a crucial role in improving hemorrhage control and tissue regeneration.

The SEM analysis of the hemostatic behaviour of the erbium-doped HA/Bioglass-PVA scaffold reveals notable blood cell adhesion and interaction. The micrograph (Figure 4) shows aggregated red blood cells (RBCs) on the scaffold surface, indicating its effectiveness in promoting blood clotting which was found to be 4 mins 35 secs while the normal range of human blood clotting time has proven to be on an average of 7 mins 10 secs . The uniform dispersion of cells suggests strong scaffold-blood interactions, which play a crucial role in accelerating hemostasis. Additionally, the rough and porous nature of the scaffold enhances blood absorption and clot stabilization, highlighting its potential for hemorrhage control and tissue regeneration.

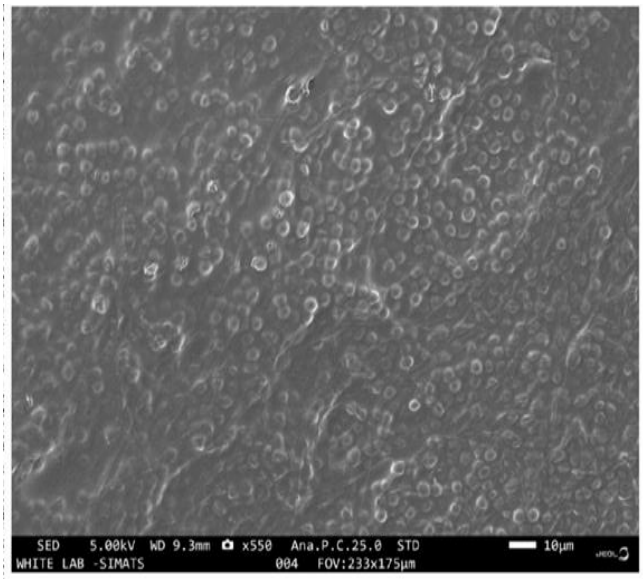

Figure 4. SEM analysis of the hemostatic behaviour of the erbium-doped HA/Bioglass-PVA scaffold under 550X magnification

Anti-inflammatory Analysis

The anti-inflammatory analysis of the Erbium-doped HA/Bioglass-PVA scaffold shows a dose-dependent effect, with the control group exhibiting the highest anti-inflammatory response (100%). At 25 µg, the scaffold significantly reduces anti-inflammatory activity to approximately 25%, increasing the scaffold weight to 50 µg results in a moderate recovery (around 50%), indicating improved but not fully restored anti-inflammatory properties (Figure

5). These findings suggest that scaffold exhibits a positive correlation with its anti-inflammatory potential and increasing concentration.

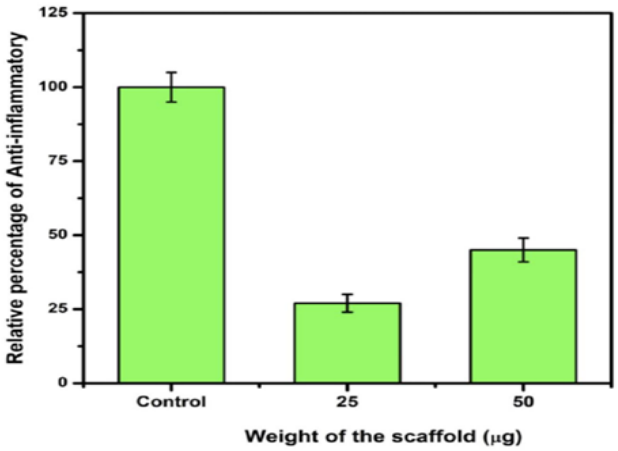

Figure 5. The bar graph represents the anti-inflammatory response of an Erbium-doped HA/Bioglass-PVA scaffold at different weights (25 µg and 50 µg) compared to a control group.

BIOCOMPATIBILITY TEST

MTT Assay

The MTT assay results indicate high cell viability across all tested concentrations (12.5–100 µg) of the PVA scaffold, comparable to the control group. At each concentration, the PVA scaffold (blue bars) maintains cell viability above 90%, with no significant cytotoxic effects observed (Figure 6). The slight variations between the scaffold and control groups are minimal, suggesting that the PVA scaffold supports cell proliferation and does not induce toxicity. These findings confirm the biocompatibility of the PVA scaffold, making it a promising material for bone tissue engineering.

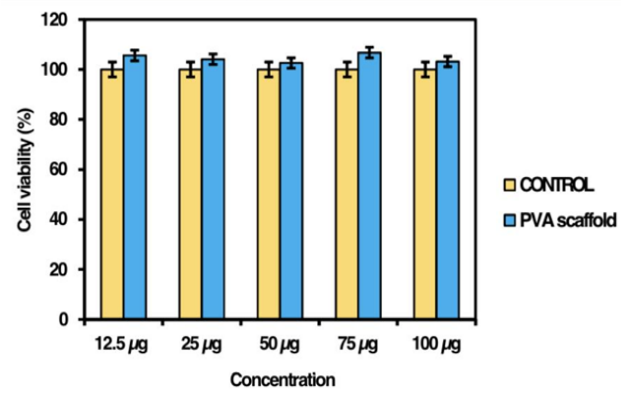

Figure 6. The bar graph represents the Biocompatibility of an Erbium-doped HA/Bioglass-PVA scaffold at different concentrations compared to control group.

Live and Dead cell assay

The live and dead cell assay image (Figure 7) of the Erbium-doped HA/Bioglass-PVA scaffold using Phase contrast microscopy shows a high density of adherent cells with an elongated and spindle-like morphology, indicating good cell viability and proliferation. The absence of significant dead cells suggests minimal cytotoxic effects, further supporting the biocompatibility of the scaffold.

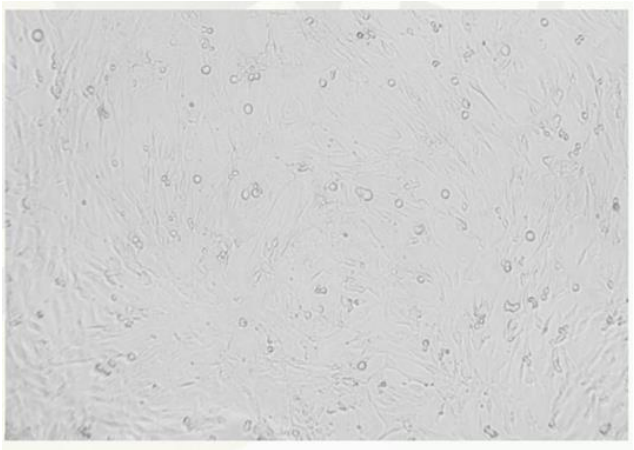

Figure 7. Live and dead cell assay of the Erbium-doped HA/Bioglass-PVA scaffold using Phase contrast microscopy

DISCUSSION

This study comprehensively examined the structural, chemical, and biological properties of Erbium-doped Hydroxyapatite (HA)-Bioglass-Polyvinyl Alcohol (PVA) scaffolds, with a primary focus on their cytocompatibility and potential application in bone tissue engineering. Fourier-transform infrared spectroscopy (FTIR) confirmed the presence of characteristic functional groups, such as hydroxyl stretching at 3289 cm^{-1} and phosphate group absorption at 536 cm^{-1}, which verified the successful integration of HA and Bioglass within the polymeric matrix. Scanning Electron Microscopy (SEM) revealed a distinct flower-like morphology, indicative of an increased surface area that enhances cellular interactions and adhesion. The consistency between our FTIR results and standard HA

crystalline structures suggests that the addition of Erbium does not disrupt the scaffold's structural integrity, reinforcing its potential for bone regeneration applications. The scaffold's hemostatic behavior and anti-inflammatory effects were assessed using SEM and bovine serum albumin (BSA) denaturation assays, respectively. Hemostatic scaffolds play a crucial role in tissue engineering by facilitating blood clotting and promoting wound healing. Prior studies, such as that of Rahman M S .,[16] have demonstrated that bioactive glass materials enhance platelet adhesion, leading to fibrin clot formation—a vital step in wound healing. Similarly, in this study, SEM analysis of the Erbium-doped HA/Bioglass-PVA scaffold revealed substantial platelet aggregation and fibrin network formation, highlighting its strong hemostatic potential. These findings correlate with the work of Lee J et al.,[17] who reported that platelet-rich plasma (PRP)-incorporated HA scaffolds significantly improved bone regeneration by stimulating the release of growth factors. Furthermore, in comparison to conventional hydroxyapatite-based scaffolds studied by Fendi et al.,[18] which primarily support osteogenesis but exhibit limited hemostatic properties, the incorporation of Bioglass and Erbium doping in the present scaffold formulation significantly improved clot formation and cellular adhesion.

The scaffold's anti-inflammatory potential was assessed using a BSA denaturation assay, a well-established method for determining protein denaturation inhibition—an essential marker of inflammation. The current study demonstrated that the Erbium-doped HA/Bioglass-PVA scaffold effectively inhibited protein denaturation, particularly at a 50% concentration, indicating significant anti-inflammatory potential. This observation aligns with research by Rahaman et al.,[16] which highlighted the intrinsic anti-inflammatory nature of Bioglass-based materials due to the controlled release of bioactive ions such as Si^{4+}, Ca^{2+}, and PO_4^{3-}, which modulate macrophage activity. The present study supports these findings while also demonstrating that Erbium doping further amplifies these effects, likely due to its established anti-inflammatory and antibacterial properties.

Comparisons with existing literature indicate that Erbium-doped Bioglass scaffolds exhibit superior hemostatic and anti-inflammatory characteristics compared to conventional biomaterials. For instance, Tian Y et al.,[19] examined chitosan-based hemostatic scaffolds, which, although effective in clot formation, lacked osteoconductive properties necessary for bone integration. Similarly, Ndlovu SP et al.,[20] explored gelatin-based scaffolds, which, despite their bioactivity and wound dressing ability , were prone to rapid degradation, thereby limiting their long-term functionality in bone regeneration. By contrast, the Erbium-doped HA/Bioglass-PVA scaffold in this study demonstrated a balanced combination of clot formation, cellular adhesion, and controlled degradation, making it an ideal candidate for bone tissue engineering applications.

A further comparison with Sr- and Co-doped bioactive glass scaffolds studied by Kermani F et al.,[21] revealed that these materials enhance osteogenesis and angiogenesis through ionic dissolution, akin to the effects observed in the current Erbium-doped scaffold. However, while strontium-based scaffolds are primarily designed to stimulate osteoinductive responses, Erbium-doped scaffolds, as demonstrated by Sepahvandi A et al.,[22] offer additional benefits such as photoluminescence, which could facilitate bio-imaging and real-time monitoring of scaffold integration in vivo. Although this study did not investigate the photoluminescent properties of the Erbium-doped HA/Bioglass-PVA scaffold, future research could explore this aspect to further expand its potential biomedical applications.

Additionally, titanium-based scaffolds, widely regarded as a gold standard in bone tissue engineering due to their mechanical strength and osseointegration capabilities , yet Topuz M et al.,[23] showed that they lack inherent bioactivity and require surface modifications to enhance cellular responses. In contrast, the Erbium-doped HA/Bioglass-PVA scaffold demonstrated in this study offers a combination of bioactivity and structural stability within a polymeric matrix, providing a significant advantage over traditional metal implants.

The biocompatibility of the scaffold was confirmed through MTT assays and live/dead cell staining, both of which demonstrated excellent cytocompatibility. These results are consistent with prior studies on composite scaffolds by Oryan et al.,[24] such as gelatin/nano-hydroxyapatite with Bioglass and strontium (G/nHAp/BG/Sr), which have also shown

enhanced biocompatibility. Furthermore, Mohan AS et al.,[25] reported that Sr-doped boron-based bioactive glass scaffolds minimized cytotoxicity while promoting controlled ion release, supporting the notion that metal-doped bioactive glasses can improve scaffold functionality while maintaining biocompatibility.

Despite the promising findings, previous research has highlighted that some metal-doped bioactive glass materials may induce mild cytotoxicity at higher concentrations. For instance , Awais M et al.,[26] observed that selenium-, silver-, and zinc-doped HA scaffolds exhibited strong antibacterial properties but induced cytotoxic effects at elevated doses. In contrast, the current study confirmed that the Erbium-doped HA/Bioglass-PVA scaffold maintains high cell viability across all tested concentrations, underscoring its superior biocompatibility with minimal adverse effects.

Overall, this study contributes to the field of bone tissue engineering by validating the cytocompatibility, hemostatic efficiency, and anti-inflammatory potential of the Erbium-doped HA/Bioglass-PVA scaffold. By integrating these findings with existing literature, it is evident that this scaffold exhibits superior multifunctional properties compared to conventional biomaterials. However, further investigations, including in vivo studies, mechanical testing, and antibacterial assessments, are necessary to fully establish its clinical relevance and therapeutic potential.

CONCLUSION

The synthesized Erbium-doped HA/Bioglass-PVA scaffold exhibited excellent biocompatibility and structural stability, making it a promising candidate for periodontal bone regeneration. Its non-cytotoxic nature supports cell adhesion and proliferation. Further in vivo studies are needed to confirm its efficacy and potential clinical applications in regenerative dentistry.

DECLARATIONS

Acknowledgments

Nil

Funding

Self Funded

Conflict of Interest

All the authors declare that there was no conflict of interest in the present study

Ethical Approval

Not Applicable

Informed Consent

Not Applicable

CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

Dr.Maria Sharon V: Formal analysis, Investigation, Data curation, Writing - original draft, Visualization Dr.**Nidhita Suresh:** Conceptualization, Methodology, Validation, Writing - review & editing, Supervision. Dr.**Kaarthikeyan Gurumoorthy** - Conceptualization, Methodology, Validation, Writing - review & editing, Supervision. **Dr.Chitra Shivalingam-** Methodology, Validation, Writing - review & editing, Supervision

REFERENCES

1. Di Stefano, M., Polizzi, A., Santonocito, S., Romano, A., Lombardi, T., and Isola, G. Impact of oral microbiome in periodontal health and periodontitis: a critical review on prevention and treatment. Int J Mol Sci. 2022; 23(9): 5142. https://doi.org/10.3390/ijms23095142.

2. Ray, R.R. Periodontitis: an oral disease with severe consequences. Appl Biochem Biotechnol. 2023; 195(1): 17-32. https://doi.org/10.1007/s12010-022-04127-9.

3. Robinson, P.G., Abrams, G.D., Sherman, S.L., Safran, M.R., and Murray, I.R. Autologous bone grafting. Oper Tech Sports Med. 2020; 28(4): 150780. https://doi.org/10.1016/j.otsm.2020.150780.

4. Betz, R.R. Limitations of autograft and allograft: new synthetic solutions. Orthopedics. 2002; 25(5): S561-70.https://doi.org/10.3928/0147-7447-20020502-04.

5. Ferraz, M.P. Bone grafts in dental medicine: an overview of autografts, allografts and synthetic

materials.Materials.2023;16(11):4117. https://doi.org/10.3390/ma16114117.

6. Percival, K.M., Paul, V., and Husseini, G.A. Recent advancements in bone tissue engineering: integrating smart scaffold technologies and bio-responsive systems for enhanced regeneration. Int J MolSci.2024;25(11):6012. https://doi.org/10.3390/ijms25116012.

7. Roseti, L., Parisi, V., Petretta, M., Cavallo, C., Desando, G., Bartolotti, I., and Grigolo, B. Scaffolds for bone tissue engineering: state of the art and new perspectives. Mater Sci Eng C. 2017; 78: 1246-62. https://doi.org/10.1016/j.msec.2017.05.017.

8. Collins, M.N., Ren, G., Young, K., Pina, S., Reis, R.L., and Oliveira, J.M. Scaffold fabrication technologies and structure/function properties in bone tissue engineering. Adv Funct Mater. 2021; 31(21):2010609. https://doi.org/10.1002/adfm.202010609.

9. Prasadh, S., and Wong, R.C. Unraveling the mechanical strength of biomaterials used as a bone scaffold in oral and maxillofacial defects. Oral Sci Int.2018;15(2): 48-55. https://doi.org/10.1016/S1348-8643(18)30005-3.

10. Kumar, A., Nune, K.C., Murr, L.E., and Misra, R.D. Biocompatibility and mechanical behaviour of three-dimensional scaffolds for biomedical devices: process–structure–property paradigm. Int Mater Rev. 2016;61(1):20-45. https://doi.org/10.1080/09506608.2015.1128310.

11. Kubiak-Mihkelsoo, Z., Kostrzębska, A., Błaszczyszyn, A., Pitułaj, A., Dominiak, M., Gedrange, T., Nawrot-Hadzik, I., Matys, J., and Hadzik, J. Ionic doping of hydroxyapatite for bone regeneration: advances in structure and properties over two decades—a narrative review.Appl Sci. 2025;15(3):1108.doi.org/10.3390/app15031108.

12. Teixeira, M.A., Amorim, M.T., and Felgueiras, H.P. Poly (vinyl alcohol)-based nanofibrous electrospun scaffolds for tissue engineering applications.Polymers.2019;12(1):7. https://doi.org/10.3390/polym12010007.

13. Fernandes, H.R., Gaddam, A., Rebelo, A., Brazete, D., Stan, G.E., and Ferreira, J.M. Bioactive glasses and glass-ceramics for healthcare applications in bone regeneration and tissue engineering. Materials. 2018; 11(12): 2530. https://doi.org/10.3390/ma11122530.

14. Chen, H., Song, G., Xu, T., Meng, C., Zhang, Y., Xin, T., Yu, T., Lin, Y., and Han, B. Biomaterial scaffolds for periodontal tissue engineering. J Funct Biomater.2024;15(8):233. https://doi.org/10.3390/jfb15080233.

15. Huang, Y., Zhai, X., Ma, T., Zhang, M., Pan, H., Lu, W.W., Zhao, X., Sun, T., Li, Y., Shen, J., and Yan, C. Rare earth-based materials for bone regeneration: breakthroughs and advantages. Coord ChemRev.2022;450:214236. https://doi.org/10.1016/j.ccr.2021.214236.

16. Rahman, M.S. Preliminary investigation of the potential of mesoporous bioactive glasses to treat hemorrhage. (Doctoral dissertation, Toronto MetropolitanUniversity). https://doi.org/10.32920/ryerson.14657013.v1.

17. Lee, J., and Kim, G. Calcium-deficient hydroxyapatite/collagen/platelet-rich plasma scaffold with controlled release function for hard tissue regeneration. ACS Biomater Sci Eng. 2018; 4(1): 278-89. https://doi.org/10.1021/acsbiomaterials.7b00640.

18. Fendi, F., Abdullah, B., Suryani, S., Usman, A.N., and Tahir, D. Development and application of hydroxyapatite-based scaffolds for bone tissue regeneration: a systematic literature review. Bone. 2024;117075. https://doi.org/10.1016/j.bone.2024.117075.

19. Tian, Y., Wu, D., Wu, D., Cui, Y., Ren, G., Wang, Y., Wang, J., and Peng, C. Chitosan-based biomaterial scaffolds for the repair of infected bone defects. Front Bioeng Biotechnol. 2022; 10: 899760. https://doi.org/10.3389/fbioe.2022.899760.

20. Ndlovu, S.P., Ngece, K., Alven, S., and Aderibigbe, B.A. Gelatin-based hybrid scaffolds: promising wound dressings. Polymers. 2021; 13(17): 2959. https://doi.org/10.3390/polym13172959.

21. Kermani, F., Mollazadeh Beidokhti, S., Baino, F., Gholamzadeh-Virany, Z., Mozafari, M., and Kargozar, S. Strontium- and cobalt-doped

multicomponent mesoporous bioactive glasses for bone tissue engineering applications. Materials. 2020;13(6):1348.
https://doi.org/10.3390/ma13061348.

22. Sepahvandi, A., Eskandari, M., & Moztarzadeh, F. Fabrication and characterization of SrAl2O4: Eu2+ Dy3+/CS-PCL electrospun nanocomposite scaffold for retinal tissue regeneration. Materials Science and Engineering: C, 2016; 66: 306-314. https://doi.org/10.1016/j.msec.2016.03.028

23. Topuz, M., Dikici, B., & Gavgali, M. Titanium-based composite scaffolds reinforced with hydroxyapatite-zirconia: Production, mechanical and in-vitro characterization. Journal of the Mechanical Behavior of Biomedical Materials, 2021; 118: 104480.https://doi.org/10.1016/j.jmbbm.2021.10448.

24. Oryan, A., Baghaban Eslaminejad, M., Kamali, A., Hosseini, S., Sayahpour, F.A., & Baharvand, H. Synergistic effect of strontium, bioactive glass and nano-hydroxyapatite promotes bone regeneration of critical-sized radial bone defects. Journal of Biomedical Materials Research Part B: Applied Biomaterials.2019;107(1):50-64.
https://doi.org/10.1002/jbm.b.34094

25. Mohan, A.S., Ravindran, D.R., Marudhamuthu, M., & Rajan, M. Investigation of Osteomyelitis Inducing Methicillin-Resistant Staphylococcus aureus Inhibition Effect by Strontium-Substituted Borate Bioactive Glasses. ACS Applied Bio Materials,2024./doi.org/10.1021/acsabm.4c00219.

26. Awais, M., Aizaz, A., Nazneen, A., Bhatti, Q.U., Akhtar, M., Wadood, A., & Atiq Ur Rehman, M. A review on the recent advancements on Therapeutic effects of ions in the physiological environments. Prosthesis,2022;4(2):263-316.
doi.org/10.3390/prosthesis4020026