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Next-Generation Dental Bone Implants – Sustainable 3D Nanostructures Derived from Animal By-Products: A Preliminary Study

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ABSTRACT

OBJECTIVE The development of next-generation dental bone implants focuses on sustainable, bioactive materials derived from animal by products, offering a cost-effective and eco-friendly alternative for bone regeneration. This study explores the fabrication of a 3D nano-structured dental implant (3D-DI) using fish bone collagen (FBC) and fibrin nanoparticles (FN), chosen for their excellent biocompatibility, bioactivity, and availability.

METHODS FBC and FN scaffolds were synthesized and incorporated into an implant framework. Fourier-transform infrared spectroscopy (FTIR) analysis confirmed the presence of bioactive functional groups. High-resolution scanning electron microscopy (HRSEM) characterized the porous microstructure (100–500 nm) as conducive to cell adhesion and proliferation. *In-vitro* cytotoxicity (MTT assay) and cell viability (live cell staining) were evaluated using MG63 osteoblast-like cells.

RESULTS The 3D-DI demonstrated significant bioactivity after 14 days in simulated body fluid (SBF), with a mineralization rate of 68% ($p < 0.01$). MG63 cell viability increased to 85% after 72 hours ($p < 0.05$) (ANOVA using Microsoft Excel Office 2013), indicating excellent biocompatibility. Mechanical testing showed compressive strength of 83.41 ± 0.56 MPa and tensile strength of 76.83 ± 0.81 MPa suitable for load-bearing dental and orthopedic applications ($p < 0.05$).

CONCLUSIONS This study presents a novel approach for transforming animal waste into sustainable, bioactive dental bone implants, offering an eco-friendly alternative to conventional graft materials. Unlike previous reports that primarily focus on single-component or synthetic scaffolds, 3D-DI exhibits superior mechanical strength, enhanced mineralization, and excellent biocompatibility. These unique properties highlight its potential for load-bearing dental and orthopedic applications, addressing the current gap in development of cost-effective, high-performance biomaterials from bio-waste sources.

KEYWORDS dental implant, 3D structure, bioactive, biocompatibility, animal by-Producs

INTRODUCTION

When a person with good general oral health loses one or more teeth due to periodontal disease, trauma, or other causes, dental implants are a good solution. Surgically placed in the jaw bone (also known as surgically traumatized bone) beneath the gums, dental implants—sometimes referred to as artificial tooth roots—can support an artificial crown in the absence of natural teeth (1).

Used to support an artificial crown in the absence of natural teeth, dental implants—also referred to as artificial tooth roots—are biocompatible metal anchors that are surgically placed in the jaw bone or surgically damaged bone behind the gums. Failures of implant integration continue to occur and are challenging to predict even with their widespread clinical use. Because of the fierce and intense competition between dental surgeons and implant manufacturers, it is challenging to estimate dental implant success rates with any degree of accuracy (2).

The mechanical environment is changed when one or more implants are surgically inserted into the mandible. In reaction to implant implantation and loading, bone remodels internally, rearranging its structure as described by Wolff's law (3). Most current biomechanical research assumes 100% bone–implant contact, which overlooks the possibility of full contact between the implant and the bone. Implant surface topography and bone quality which are routinely observed in clinical settings are just two of the variables that affect the relationship between the implant and the surrounding bone (4-6).

The marine food product industry uses its waste to create higher-value biomaterials. Numerous products derived from processed fish waste have been used in diverse applications, including animal feed, collagen, chitosan, natural colors, biodiesel/biogas, and bone implants. Collagen makes up the majority of the protein in bones, while hydroxyapatite bone minerals are also abundant. Plate-like tiny crystals of hydroxyapatite (HA) are implanted in collagen, which serves as a structural framework, strengthening the bone (7, 8). Muntean et al. (9) present a comprehensive review of the production and characteristics of mollusk shell–derived hydroxyapatite, highlighting its bioactivity, osteoconductivity, sustainability, and potential for dental implant coatings and bone graft substitutes.

The aim of this investigation was to synthesize a 3D nano-structured dental implant (3D-DI) from biowaste. Using fourier-transform infrared spectroscopy (FTIR), thermogravimetric analysis (TGA), and high resolution scanning electron microscopy (HRSEM), the BI produced was evaluated for its physicochemical properties. An *in-vitro* study of 3D-DI was conducted using MG63 osteoblast-like cell lines.

METHODS

Preparation of fish bone collagen (FBC)

Fish bone waste was obtained from a local fish market (Vanagaram, Chennai, Tamil Nadu 600095) for use in the present study. The bone portion was then removed and cleaned. Later, the fishbone was defatted by treating it with acetone and ethanol. The bones were cut into small-sized pieces and then demineralized using 2N HCI followed by H_2O_2 treatment. The samples were washed thoroughly with cold distilled water and ground to form a paste. The resultant fine paste was subjected to centrifugation (8,000-10,000 rpm). The supernatant was collected and its pH was adjusted to the required level. The supernatant was then further centrifuged at 12,000 rpm.

Preparation of fibrin nanoparticles (FN)

Bovine blood collected from a municipal slaughterhouse was stirred well using a glass rod to obtain fibrin which was then washed with distilled water and incubated in 0.5M sodium acetate and 30% hydrogen peroxide. The fibrin was then washed with distilled water, ground using a mortar and pestle (mechanical homogenizer), and stored at −20 °C. The fibrin was then treated with NaOH, to make a clear, yellow-colored solution which was treated with HCL under vigorous stirring at the required pH and the resultant formation of fibrin nanoparticles.

Preparation of 3D dental implant (3D-DI)

3D-DI was prepared by mixing FBC, FN, calcium carbonate and biopolymer gelatin in a ratio of 5:1:8:0.5 by weight. The resultant mixture was packed into a glass tube and extruded with a suitable glass rod. The cylindrical implant formed was cut into the required length and cured at 30°C for 12 h then at 100°C for 4-5 h. Computer-aided design (CAD) software was used with the dried material to model the 3D structure of the implant.

RESULTS

Physicochemical properties

UV-Vis spectroscopy is a valuable method for determining the existence and optical characteristics of FN (Figure 1A). FN typically shows a characteristic absorption peak in the UV area, indicating its proteinaceous nature. This peak often appears between 270 nm and 290 nm, demonstrating the absorption of aromatic amino acids such as tyrosine, tryptophan, and phenylalanine, which are important components of fibrin proteins. The strength and position of the peak can confirm the effective creation of fibrin nanoparticles while also providing information on their concentration, purity, and potential for aggregation. Furthermore, any shifts in the peak position could suggest changes in the structural conformation or interactions with other components in a composite system. The FTIR spectrum was used to examine and identify the functional groups in the samples, as illustrated in Figure 1B and C. The FN exhibited a peak of around 2,600 cm^{-1}, showing the presence of particular linkages associated to protein structures, likely linked to N-H stretching vibrations. This indicates the fibrin's contribution to the total bioactivity of the composite material. These peaks reflect each component's own chemical signatures, providing information on their composition and functional properties, which are critical for their roles in bioactive bone implants. Furthermore, collagen sample FTIR spectra showed a peak of about 1,300 cm^{-1}, which corresponds to the bending vibrations of C-H bonds and amide III bands, confirming the existence of collagen-specific functional groups.

Figure 2A shows the FTIR analysis of 3D-DI. The 3D-DI exhibited a strong absorption band at 2,200 cm^{-1}, indicating stretching vibrations

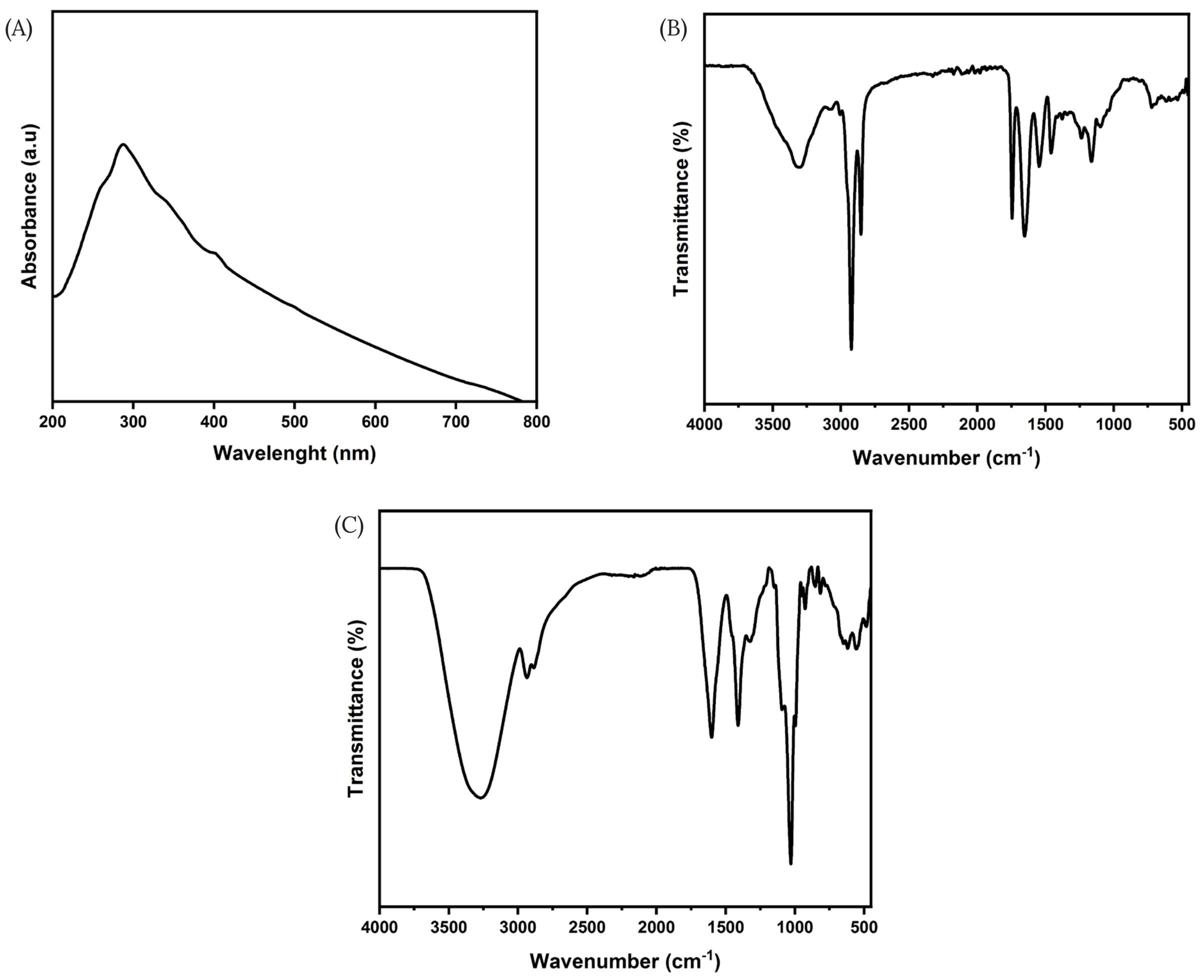

Figure 1. (A) UV-Vis spectroscopy analysis of fibrin nanoparticles, (B) Fourier transform infrared spectroscopy of fibrin nanoparticles, (C) Fourier transform infrared spectroscopy analysis of fish bone collagen

of carbonyl (C = O) or other organic functional groups. These vibrations are critical for the implant's structural stability. Figure 2B shows the thermal stability of a 3D-DI made of collagen, fibrin nanoparticles, and calcium carbonate. TGA was utilized to determine the material's stability. Moisture evaporation causes initial weight loss at lower temperatures, whereas collagen and fibrin degradation causes significant weight loss between 250°C and 350°C. The final degradation happens above 500°C, which corresponds to the breakdown of calcium carbonate. The 3D-DI has exceptional temperature stability, making it appropriate for biomedical applications. Figure 2C depicts the surface morphology of a 3D-DI made of collagen, fibrin nano-particles, and calcium carbonate. This material was thoroughly examined using HRSEM to gain a better understanding of its microstructure. The HRSEM images show a highly porous architecture with linked nano-sized pores that promote cell adhesion and nutrient exchange, both of which are important for bone tissue regeneration. The collagen component adds flexibility, and the fibrin nanoparticles improve bioactivity and cell interactions. The addition of calcium carbonate increases the scaffold's osteoconductivity, which promotes bone mineralization. The homogeneous distribution of these components inside the 3D matrix maintains structural integrity, allowing the implant to replicate natural bone tissue, encouraging faster integration and healing in the dental setting.

The mechanical properties of a 3D-DI made of FBC, FC, and calcium carbonate were assessed in terms of tensile strength, compressive strength, and water permeability. The collagen-based implant's tensile strength was 76.83 ± 0.81 MPa, demonstrating that it can sustain stretching pressures. The 3D-DI compressive strength (83.41 ± 0.56 MPa) indicates its resistance to compres-

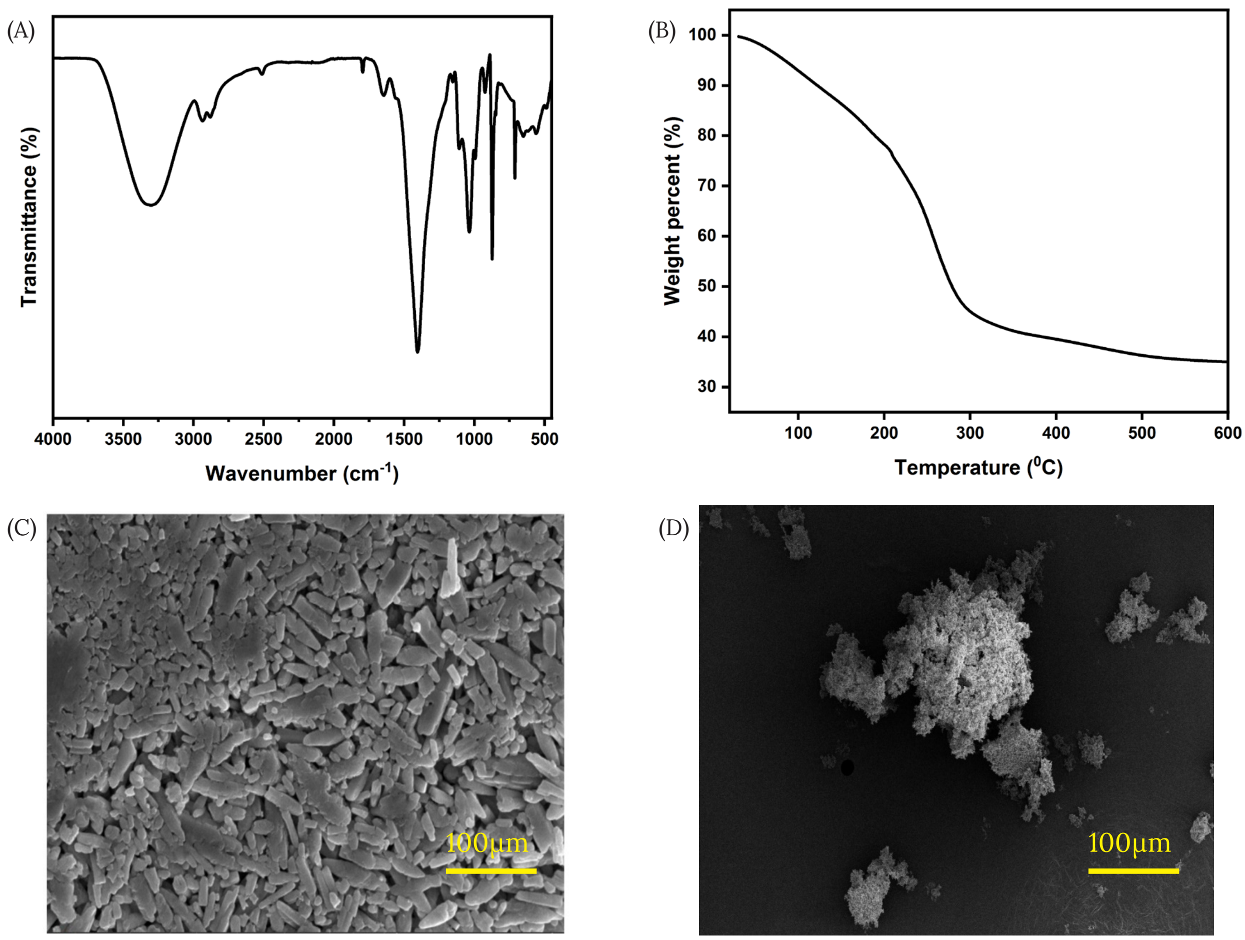

Figure 2. (A) Fourier transform infrared spectrum of 3D-DI, (B) thermogravimetric analysis curve of 3D-DI, (C) high resolution scanning electron microscope image of 3D-DI, (D) high resolution scanning electron microscope picture of 3D-DI after simulated bodily fluid treatment

sion, which is important for bone growth. The water absorption capacity (27.34 ± 1.65%) shows its ability to retain moisture, which is crucial for hydration and biocompatibility.

Bioactivity test

The bioactivity of the 3D-DI, composed of FBC, FN, and calcium carbonate as demonstrated in HRSEM is shown in Figure 2D. The implant was tested in phosphate buffer solution (PBS) to evaluate its ability to support mineral deposition, which is crucial for bone regeneration. During the test, the material was immersed in PBS to simulate physiological conditions, and its interaction with the solution was monitored over time. The formation of hydroxyapatite-like layers on the surface of the implant, indicated by mineral deposits, confirms its bioactivity. This suggests that the collagen and fibrin nanoparticles promote cell interaction, while the calcium carbonate enhances osteoconductivity, encouraging bone-like mineral formation. The bioactivity test highlights the material's potential for effective bone regeneration in dental applications.

***In-vitro* analysis**

Figure 3A shows the *in-vitro* evaluation of the control and 3D-DI sample. The 3D-DI was evaluated on the MG63 osteoblast-like cell line to determine its biocompatibility and ability to promote cell growth. The cells were cultivated on both the control and 3D-DI surfaces to determine how they affected cell attachment, growth, and viability at 48 and 78 h. The results show that the 3D-DI improved cell adhesion and proliferation, indicating that it has bioactive qualities and is suitable for bone tissue engineering. The combination of collagen and fibrin created a favorable environment for cell contact, whereas calcium carbonate increased osteoconductivity, which is required for bone repair. This *in-vitro* investigation verifies the implant's suitability for dental bone restoration. The fluorescence surface morphology images show in 3D-DI and control interacts with cells (Figure 3B). Fluorescence microscopy was utilized to investigate the adhesion and dispersion of MG63 cells on the 3D-DI surface. The fluorescently marked cells enabled easy identification of cell shape, spreading, and density across the implant. The image shows evenly distributed cells adhering to the porous

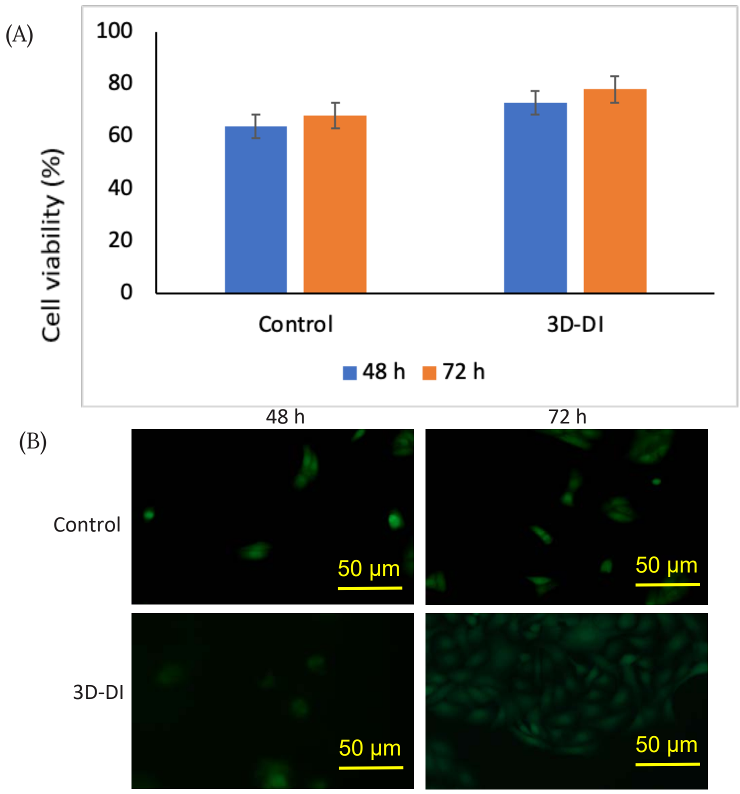

Figure 3. (A) MTT assay showing the viability of MG-63 cells for the control and 3D-DI groups. (BS) Fluorescence micrographs (20X) of MG-63 cells cultured for 48 and 72 hours.

structure, demonstrating efficient cell infiltration and interaction with the FBC, FN, and calcium carbonate components. The fluorescent signal shows cytoskeletal structure, implying that the implant facilitates cell adhesion and proliferation. These findings confirm the 3D-DI potential for bone tissue regeneration by demonstrating its biocompatibility and ability to enable cellular integration.

DISCUSSION

The complete assessment of the 3D-DI demonstrates its remarkable potential for bone regeneration. The UV-Vis spectroscopy study of FN proved its proteinaceous composition by revealing typical absorption peaks, indicating effective nanoparticle production (10). FTIR spectra revealed information about the chemical structures of collagen, fibrin, and calcium carbonate, indicating the presence of functional groups required for bioactivity and stability. The characteristic peaks of each material component, particularly the amide III bands in collagen and the N-H stretching in fibrin, contribute to the composite implant's overall structural integrity and biofunctionality (11, 12). HRSEM and fluorescent microscopy revealed the implant's highly porous design, which promotes effective cell attachment and interaction. This porosity is vital for cell infiltration and nutrition exchange, both of which are necessary for bone tissue repair. The equally distributed cells seen in fluorescence pictures indicate that the implant's material composition, notably the fibrin nanoparticles and collagen, supports cellular proliferation and integration (13, 14).

The bioactivity and thermal stability tests confirmed the implant's ability to enable bone-like mineral formation and to endure physiological circumstances. The creation of hydroxyapatite-like layers on the implant's surface following immersion in PBS proved its osteoconductivity, which was caused by calcium carbonate's contribution to mineralization (15). TGA revealed that the implant can maintain structural integrity at a variety of temperatures, assuring its suitability for biological purposes. *In-vitro* testing employing the MG63 cell line confirmed the implant's biocompatibility, with results indicating increased cell growth and adherence compared to control samples. These findings indicate that the combination of collagen, fibrin nanoparticles, and calcium carbonate provides an optimal environment for osteoblast function, making this 3D-DI a promising candidate for clinical usage in bone tissue engineering and dental repair.

The remarkable performance of the 3D-DI composite aligns well with prior research showing that fibrin-based scaffolds—especially when combined with osteoconductive bioceramics—enhance osteoblast adhesion, proliferation, and differentiation, acting as both a provisional matrix and a delivery platform for bioactive molecules (16). Likewise, nanostructured collagen scaffolds with high porosity and inorganic components have been shown to mimic native bone architecture, facilitating cell migration, mineral deposition, and mechanical integration *in-vivo* (17). These outcomes—demonstrating hydroxyapatite-like mineral formation, robust structural stability, and favorable MG63 osteoblast responses—further substantiate the applicability of the 3D-DI as a biomimetic and efficient scaffold for bone regeneration.

This discussion highlights the promising potential of the 3D-DI synthesized using FBC, FN, and calcium carbonate. The physicochemical characterization confirmed the retention of critical functional groups, thermal stability, and a highly porous architecture that enhances cell adhesion, nutrient exchange, and osteoconductivity. The excellent mechanical properties, including tensile and compressive strength, make the implant suitable for load-bearing dental applications, mimicking the natural strength and flexibility of bone tissue. Bioactivity analysis demonstrated the formation of hydroxyapatite-like deposits, indicating the implant's capability to support mineralization, which is essential for bone regeneration. *In-vitro* studies using the MG63 osteoblast-like cell line confirmed enhanced cell adhesion, proliferation, and biocompatibility, underscoring the scaffold's suitability for bone tissue engineering. The combination of FBC and FN created a bioactive environment conducive to cellular interactions, while calcium carbonate promoted osteogenic responses. These results confirm 3D-DI as an eco-friendly, cost-effective, and biocompatible alternative to conventional implants, addressing critical clinical needs in bone repair and paving the way for sustainable regenerative dentistry solutions.

CONCLUSIONS

The 3D-DI, made of FBC, FN, and calcium carbonate, shows great promise for bone tissue regeneration. The material's bioactivity was demonstrated by mineral deposition in simulated bodily fluid, and its biocompatibility and ability to induce cell proliferation were established using MG63 cell cultures. Structural study with FTIR, HRSEM, and fluorescence microscopy revealed a highly porous, stable implant that promotes cell adhesion and proliferation. The implant's heat stability enhances its usefulness for biomedical applications. Overall, the study demonstrates the implant's potential for dental bone regeneration and regenerative medicine.

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CONFLICT OF INTEREST

The authors have no conflicts of interest to report.

AUTHOR CONTRIBUTION

V.K.: methodology, software; R.S.: conceptualization, methodology, software, supervision , writing – review & editing.

All authors have read and agreed to the published version of the manuscript."

DATA AVAILABILITY STATEMENT

All data generated or analyzed during this study are included in this published article

INSTITUTIONAL REVIEW BOARD STATEMENT

This study did not involve any experiments on human participants or animals, and therefore, Institutional Review Board approval was not required. All experimental procedures were performed using in vitro and material-based methods.

INFORMED CONSENT STATEMENT

Not under consideration for publication elsewhere

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