International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 21s, 2025
https://theaspd.com/index.php

Advanced Fabrication and Characterization of Biphasic Calcium Phosphate Scaffolds for Bone Tissue Engineering

Shubhangee Tomar[1], Zil Patel[2], Payal S Makwana[3], Het P Solanki[4], Mehak Bhat[5], Divyang Dave[6]
[1]Assistant Professor, Department of Pharmaceutics, KB Institute of Pharmaceutical Education and Research, KSV, Gandhinagar, Gujarat, India,
[2]Professor, Department of Pharmaceutics, Arihant School of Pharmacy and BRI, SSIU, Gandhinagar, Gujarat, India, zilaspbri@gmail.com
[3]Assistant Professor, Department of Pharmacology and Pharmacy Practice, KB Institute of Pharmaceutical Education and Research, KSV, Gandhinagar, Gujarat, India, payal.makwana@kbiper.ac.in
[4]Assistant Professor, Department of Pharmaceutics, KB Institute of Pharmaceutical Education and Research, KSV, Gandhinagar, Gujarat, India, het.solanki@kbiper.ac.in
[5]Assistant Professor, Department of Pharmaceutics, Arihant School of Pharmacy and BRI, SSIU, Gandhinagar, Gujarat, India, mehakbhatofficial@gmail.com
[6]Professor, Department of Pharmaceutics, KB Institute of Pharmaceutical Education and Research, KSV, Gandhinagar, Gujarat, India, divyang@kbiper.ac.in
Corresponding author: Shubhangee Tomar
shubhangee.tomar@kbiper.ac.in

Abstract:
Purpose: *Bone tissue engineering (BTE) faces significant challenges in replicating the natural bone environment and ensuring long-term viability of engineered tissues. This study investigated the potential of biphasic calcium phosphate (BCP) scaffolds integrated with collagen (Col), chitosan (Ch), and hyaluronic acid (HyA) to enhance bone regeneration.*
Methods: *BCP scaffolds were prepared using the sponge replica method and functionalized with Col, Ch, and HyA to mimic the extracellular matrix. Fourier transform infrared (FTIR) spectroscopy was used to confirm the incorporation of biomaterials. Scanning electron microscopy (SEM) was employed to examine the scaffold morphology. In vitro bioactivity assays were performed using simulated body fluids. MTT assays using MRC-5 cells were performed to assess cytocompatibility. Swelling and degradation studies were performed to evaluate hydrophilicity and biodegradability.*
Results: *FTIR confirmed the successful incorporation of Col, Ch, and HyA into the BCP scaffolds. SEM revealed a highly porous, interconnected structure with rough surfaces. In vitro bioactivity assays showed bone-like apatite formation after 14 days. MTT assays demonstrated enhanced cytocompatibility of Col-Ch-HyA-BCP scaffolds compared to that of plain BCP scaffolds. The composite scaffolds exhibited higher water uptake and faster degradation rates than BCP, indicating improved hydrophilicity and biodegradability.*
Conclusion: *BCP scaffolds functionalized with Col, Ch, and HyA provide a promising platform for bone tissue engineering, offering a conducive microenvironment for cell attachment, proliferation, and differentiation. Further in vivo studies are required to validate the efficacy of these scaffolds in promoting bone regeneration.*
Key words: *Bone tissue engineering, BCP scaffold, Collagen, Hyaluronic acid, Chitosan*

INTRODUCTION:

Bone tissue engineering (BTE) encounters numerous obstacles that have impeded its progression from research to broad clinical use. These obstacles mainly stem from the difficulty of mimicking the natural bone environment and ensuring the long-term survival of engineered bone tissues. A major hurdle in BTE is achieving adequate vascularization at the site of the defect. Without proper formation of blood vessels, or angiogenesis, the delivery of nutrients and oxygen to the newly developed tissue is compromised, which can hinder bone regeneration and its integration into the host tissue[1]. This insufficient vascularization can result in the failure of engineered tissues to survive and function properly. An associated concern is the mechanical strength and stability of the biomaterials employed in scaffold construction. Numerous existing materials either degrade too rapidly or lack the necessary strength to adequately support the formation of new tissue, which hinders the provision of mechanical signals essential for successful bone regeneration[2]. Interactions between scaffolds and host cells present a significant challenge due to their complexity. The material used for scaffolds must encourage cells to

adhere, multiply, and transform into osteogenic cells to support the formation of new bone[3]. Nevertheless, the variability in how cells interact with and respond to scaffolds has complicated the ability to reliably direct tissue-engineering processes [4]. Furthermore, selecting the appropriate cells for seeding onto scaffolds is crucial. Although stem cells hold significant promise due to their capacity to transform into different cell types, there are still considerable obstacles concerning the sources of stem cells, their delivery, and ensuring their regulated differentiation within a living organism[5]. Challenges related to materials also involve managing the rates at which they degrade and the byproducts they produce. For instance, magnesium-based biomaterials tend to break down quickly, releasing a large amount of ions that can negatively impact bone healing[6].

To tackle these challenges, a multidisciplinary strategy is essential, integrating progress in biomaterials, scaffold fabrication techniques like 3D printing, and a more comprehensive understanding of bone biology and vascularization. Future studies should aim to create intelligent and adaptive biomaterials that more accurately replicate the dynamic nature of natural bone tissue, facilitating the effective transition of BTE from the lab to clinical settings[7].

Biphasic calcium phosphate scaffolds are highly valued in bone tissue engineering due to their natural biocompatibility, osteoconductive properties, and capacity to facilitate stem cell differentiation and bone regeneration. These scaffolds are generally composed of a combination of hydroxyapatite and either β-tricalcium phosphate (β-TCP) or α-tricalcium phosphate (α-TCP)[8]. Recent progress in BCP scaffolds has concentrated on improving both their mechanical characteristics and their capacity to deliver signalling molecules essential for bone development. This enhancement replicates the natural bone extracellular matrix, creating a favorable environment for cell adhesion and growth[9][10].

Furthermore, advancements in scaffold manufacturing methods, like 3D printing, have facilitated the development of BCP scaffolds with precise control over porosity and structural characteristics[11][12]. Nevertheless, it cannot preserve the physicochemical properties of BCP, which leads to a decrease in osteogenic differentiation and, consequently, bone regeneration. An exciting advancement is the incorporation of scaffolds with vehicles that release growth factors. This approach can greatly improve vascularization, a crucial element for bone regeneration. These bioactive scaffolds facilitate the adhesion, proliferation, and formation of blood vessel-like structures by endothelial cells, thereby aiding bone healing processes in vivo[13][14].Nevertheless, when cells are integrated into the BCP scaffold, its mechanical properties are compromised, and only a small number of cells manage to survive until the procedure is completed.

BCP scaffolds serve as a flexible foundation for bone tissue engineering, creating a distinct microenvironment that supports bone regeneration. They also present opportunities for further advancements through innovations in material science and biological enhancements[15][16][17]. BCP scaffolds are customized with bioactive elements like chitosan, collagen, hyaluronic acid, and gelatine to enhance both their mechanical and biological properties[18][19][20].By integrating Collagen, HyA, and chitosan, the distinct characteristics of each polymer are harnessed to form a composite that significantly promotes bone tissue regeneration[21][22]. Chitosan offers structural support and osteoconductive properties, which enhance HyA's capacity to replicate the ECM and Col's role in osteochondral regeneration by aiding the migration of bone marrow stem cells[23]. These materials can collectively create scaffolds that not only aid in cell attachment and growth but also promote the integration of newly developed bone tissue with existing bone structures, both structurally and functionally. In this research, Ch, HyA, and Col were combined with BCP scaffolds to enhance bone regeneration.

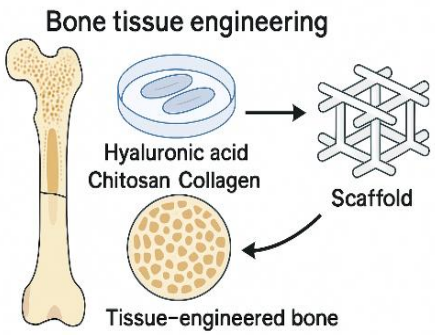

Figure 1 Bone tissue engineering by combining Collagen, hyaluronic acid and Chitosan with BCP scaffold

MATERIALS AND METHODS:

Materials: BCP and Collagen were sourced from Nano Research Lab, Jharkhand. Chitosan with a deacetylation level greater than 88% was procured from Bangalore Fine Chemicals. Sodium salt of hyaluronic acid was supplied by R P Chemicals (Maharashtra). PVB was provided as a free sample by Siva Chemical Industries, Maharashtra. MRC-5 Cells were procured from the National Centre for Cell Science (NCCS), Pune.

Formulation of BCP scaffolds integrated with Col, Ch, and HyA: Various techniques were explored to fabricate BCP scaffolds, but the Sponge Replica method yielded the most favorable outcomes. This approach creates macro- and microporous structures that facilitate cell passage and support degradation and resorption. To prepare the BCP slurry[2, a mixture of 10% BCP and 5% polyvinyl butyral was used to saturate a polyurethane sponge, specifically an HD sponge with a density of 60 ppi. The scaffolds underwent sintering in a Microwave Furnace at 1200°C for 10 minutes, with a heating rate of 100°C/min. To remove impurities from the sponge scaffolds, they were ultrasonically cleaned with distilled water and then dried. A 10% w/w Col/Ch solution was dissolved in 0.01 N HCl and combined with sufficient HyA (0.5% by weight) to create a slurry. This mixture was poured over the BCP scaffold and stored at -20°C overnight. The scaffolds were then frozen at −80°C for 8 hours and freeze-dried for 48 hours at −80°C. Ultimately, the Col-Ch-HyA-BCP Scaffold was obtained through lyophilization.

Chemical analysis by FTIR Fourier transform infrared (FT-IR) spectroscopy was utilized to determine the chemical characteristics of the scaffold using transmittance mode, concentrating on the mid-infrared spectrum ranging from 4000 to 500 cm^{-1}. For the FTIR analysis, the sample was cut into squares measuring 2 × 2 cm^2.

Morphology and in vitro bioactivity: To prepare the samples, Col-Ch-HyA-BCP scaffolds were cut into squares measuring 1 × 1 cm^2 and placed in polyethylene tubes filled with 30 mL of freshly made Simulated Body Fluid (SBF), as prepared by Kokubo et al[25]. After a 14-day immersion period, the samples were removed. They were then placed in a shaking incubator set to 37 °C with a shaking speed of 90 rpm, and the SBF was refreshed every 48 hours. Following this, the samples were coated with gold and examined using SEM. The Electron High Tension (EHT) was set at 20.00 kV, Signal A = SE1 was used for secondary electron imaging to achieve topographical contrast, and a Working Distance (WD) of 8.5 mm was maintained for high-resolution imaging. Finally, the results were compared with the plain BCP Scaffold.

Cell cytotoxicity using The MTT Assay: The MTT assay is a technique employed to assess cell viability or proliferation by measuring optical density (OD). It is frequently utilized to assess the cytocompatibility of different biomaterials. In the MTT assay procedure, samples were cut into 1 × 1 cm^2 pieces and sterilized using UV light for 2 hours on each side. MRC-5 cells, which are normal human lung fibroblasts, were used 24 hours post-seeding. The microtiter plates were incubated in a humidified environment at 37 °C with 5% CO2 for 48 hours. Three wells were allocated for the test sample, while control cells were incubated without it. After a seven-day incubation period, the MTT assay was conducted to quantify the number of viable cells.

Evaluation of scaffold swelling and degradation rates: To evaluate the swelling rates of different scaffolds, the initial weights of the samples were noted before immersion in SBF. The samples were then removed from the SBF after 2, 7, and 14 days, rinsed with distilled water, and dried using filter paper to remove any excess water. The samples were then weighed and the swelling ratio (S) was calculated using the following formula

$S(\%) = W_S - W_0 \ / \ W_0 * 100$

where W_0 is the weight of the sample (in grams) before immersion in SBF and W_S is the weight of the swollen sample (in grams).

To assess the rate of degradation after the samples were taken out of the SBF, they were washed with distilled water, patted dry with filter paper, and then placed in an incubator until their weight stabilized. After they were fully dried, the samples' weights were measured. Weight loss (WL) was determined using the following formula:

$W_L(\%) = (W_0 - W_D) / W_0 * 100$

where W_0 is the weight (in grams) of the sample before immersion and W_D is the weight (in grams) of the sample after removal from the SBF and complete drying.

RESULTS AND DISCUSSION:

Chemical analysis: FTIR is an effective technique for analysing the organic makeup of various compounds. Figure 2 presents the FTIR findings for Col-Ch-HyA-BCP scaffolds, verifying the success of all interaction processes. The Fingerprint Region at 419.25 cm^{-1}, 1033.87 cm^{-1}, and 1050.98 cm^{-1} highlights the distinct structural characteristics of hyaluronic acid. Peaks at 1507.31 cm^{-1}, 1521.57 cm^{-1}, and 1540.11 cm^{-1} indicate aromatic or C=C stretching vibrations. The peaks at 1652.76 cm^{-1} and 1684.14 cm^{-1} may suggest C=O stretching (carbonyl). Peaks at 3567.91 cm^{-1}, 3587.88 cm^{-1}, 3629.23 cm^{-1}, and 3670.59 cm^{-1} imply O-H or N-H stretching (hydroxyl or amine groups). The presence of peaks in the 1700-1750 cm^{-1} range suggests carbonyl groups (C=O), while those beyond 3500 cm-1 indicate hydroxyl (-OH) or amine (-NH) groups, confirming the presence of Collagen, BCP, and chitosan.

FTIR

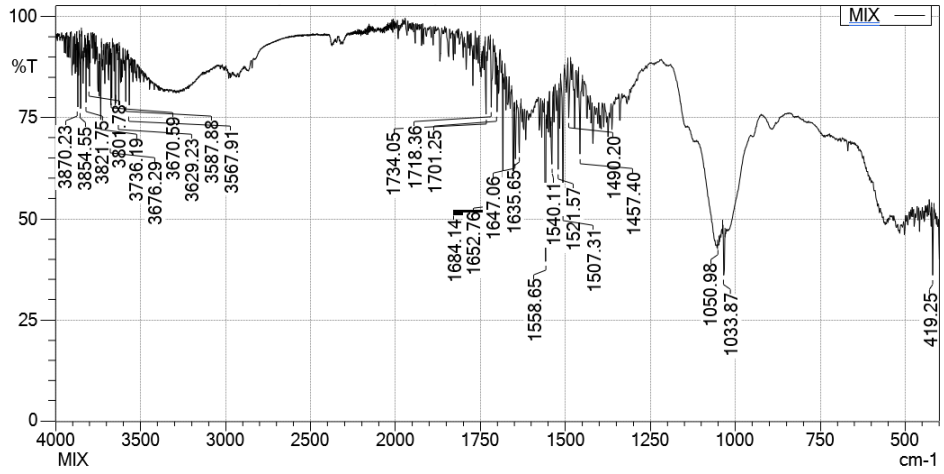

Figure 2 FTIR spectra for Col-HyA-Ch-BCP Scaffold, indicates presence of chitosan, collagen, hyaluronic acid and BCP

Morphology and in vitro bioactivity: The BCP scaffold displayed a morphology characterized by a highly porous, interconnected, and rough surface at both macro and micro levels. These attributes are ideal for bone regeneration and tissue engineering applications, as they facilitate cell migration, nutrient diffusion, and mechanical stability. The macroscopic morphology shows a highly porous surface, indicating a structure designed to support cell infiltration and nutrient flow, which are crucial in tissue engineering. The porosity increased throughout the scaffold's thickness, indicating a 3D interconnected pore network. This is advantageous for vascularization and integration with host tissue. SEM images (figure 7 (a) and (b)) reveal a fine, interconnected porous structure at the microscale. The pores vary in size and shape, which can enhance mechanical interlocking with surrounding tissues. The rough surface texture is beneficial for cell attachment and proliferation. The scale bar (200 µm) helps estimate pore sizes, which appear to be within the range suitable for bone tissue engineering (typically 100–500 µm).

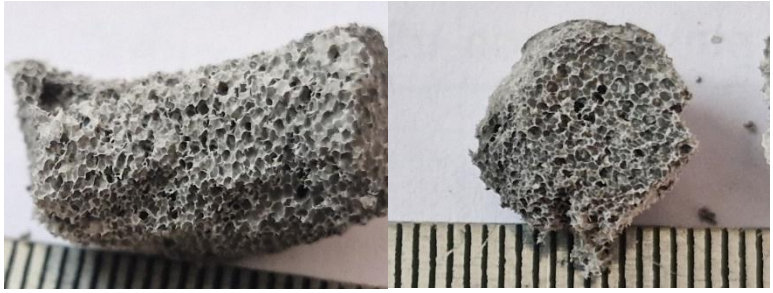

Figure 3 Sponge Replica method but Sintering was done for 1 hour, as a result scaffold with poor mechanical strength was developed

Figure 4 shows the Preparation of BCP sheet and its subsequent rolling down to the cylindrical shape of the scaffold, followed by lyophilization. Mechanical Strength was good but Pores were not present

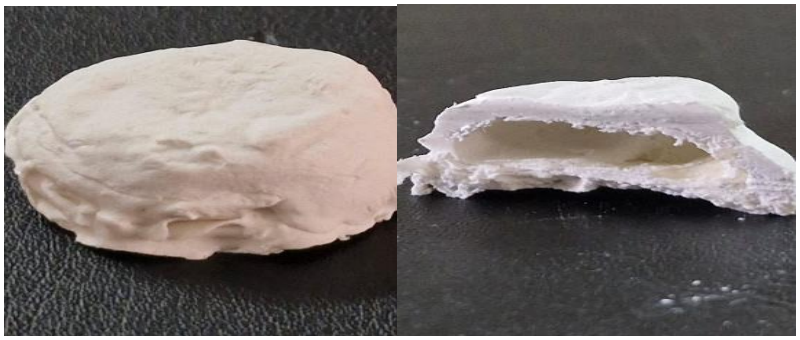

Figure 5 represents preparation of BCP Sheets and then placing them on top of another. almost 15 sheets were used, but after lyophilization core got emptied and again mechanical strength was found to be poor

Figure 6 BCP scaffold prepared by the Sponge replica method. Micro and macro pores were present and interconnected with each other

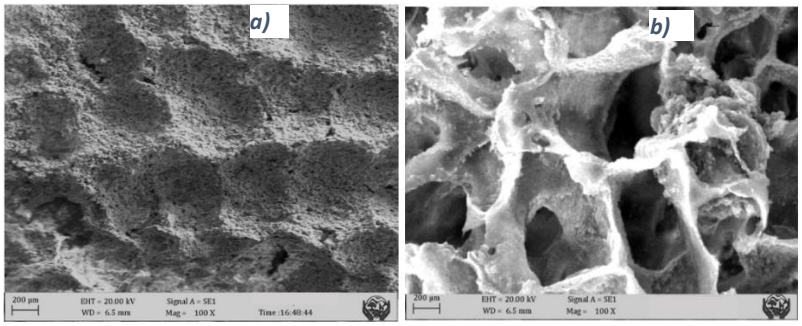

Figure 7 SEM images of Col-Ch-HyA-BCP scaffolds a) Day 1 of in vitro assay b) Day 14 of In vitro assay

Cell cytotoxicity using the MTT assay:

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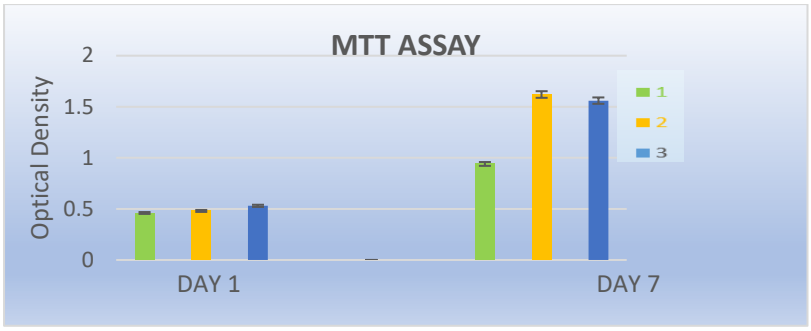

Figure 8 Cytocompatibility results for 1-BCP, 2-Col-HyA-Ch-BCP Scaffold, 3- Control

On the initial day, all groups, including BCP, Col-Ch-HyA-BCP scaffold, and the control, displayed low and similar OD values, ranging from about 0.4 to 0.5. By the seventh day, a significant rise in OD values was observed across all groups, indicating cell growth or increased metabolic activity over time. The Col-Ch-HyA-BCP scaffold group achieved the highest OD values, approximately 1.6 to 1.65. In contrast, the Control and BCP alone exhibited lower OD values, around 1 to 1.5. The Col-Ch-HyA-BCP scaffold group demonstrated the most substantial cell viability or proliferation over time, suggesting enhanced biocompatibility or superior cell growth compared to BCP alone or the control. The increase from day 1 to day 7 illustrated a time-dependent rise in cell proliferation, as expected in healthy, biocompatible environments.

Swelling and Degradation rates: The initial graph illustrates that Col-Ch-HyA-BCP scaffolds exhibit greater water absorption compared to BCP, demonstrating their superior hydrophilicity. The subsequent graph reveals that Col-Ch-HyA-BCP scaffolds experience more significant weight reduction over time, indicating a quicker degradation rate. Collectively, these findings emphasize the improved water uptake and biodegradability of the composite scaffolds, which are advantageous for applications in bone tissue engineering.

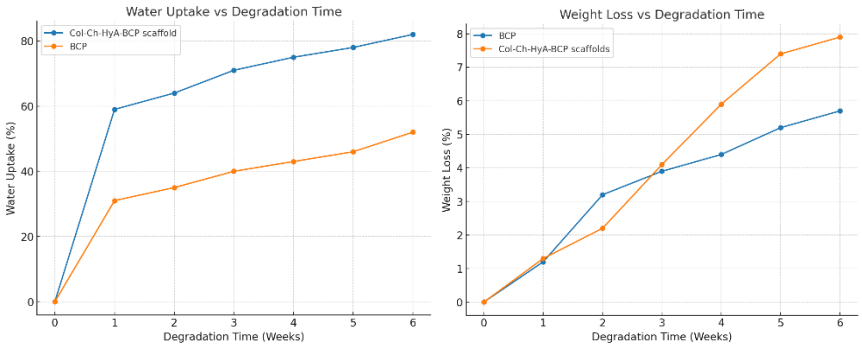

Figure 9 swelling and degradation properties of BCP scaffold and COL-HyA-Ch-BCP scaffold

CONCLUSION:

Integration of collagen, hyaluronic acid, and chitosan with biphasic calcium phosphate (BCP) scaffolds represents a significant advancement in bone tissue engineering. This composite scaffold demonstrated several key improvements over traditional BCP scaffolds, such as enhanced biocompatibility and cell viability, as evidenced by the MTT assay results showing higher optical density values for the composite scaffold compared to BCP alone; improved hydrophilicity and degradation characteristics, indicated by higher water uptake and weight loss rates in the composite scaffold; preservation of the desirable porous structure of BCP scaffolds, crucial for cell infiltration and nutrient flow, as observed in SEM images; and successful incorporation of organic components (collagen, hyaluronic acid, and chitosan) into the inorganic BCP matrix, as confirmed by FTIR analysis. These findings suggest that the Col-Ch-HyA-BCP scaffold provides a favorable microenvironment for cell attachment, proliferation, and osteogenic differentiation. The enhanced degradation rate may allow for better scaffold resorption and replacement by the newly formed bone tissue. However, further research is needed to evaluate the mechanical properties of the composite scaffold under physiological conditions, to assess long-term in vivo performance and bone regeneration capacity, and to optimize the ratios of organic components for ideal

biological and mechanical properties. This composite scaffold shows promise in addressing some of the key challenges in bone tissue engineering, particularly in terms of improving the biological performance of BCP scaffolds. This represents a step forward in developing more effective biomaterials for bone-regeneration applications.

Abbreviations:
Ch- Chitosan
HyA- Hyaluronic acid
NP- Nanoparticle
BCP- Biphasic Calcium Phosphate
Col-Ch-HyA-BCP scaffold- Collagen, chitosan, hyaluronic acid integrated BCP scaffold
ALP Staining- Alkaline phosphatase staining
OPN- Osteopontin
OCN- Osteocalcin
WA study- water absorption study, WL study- weight loss study
BMSCs- Bone marrow mesenchymal stromata cells
MTT- (3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide)
SEM-scanning electron microscopy

Acknowledgement:
The authors acknowledge the support provided by the Biochemistry Department MSU Baroda, National centre for Cell Science (NCCS), Gujarat Biotechnology Research Centre (GBRC), and the GEER Foundation.

Statements and declarations

Authors Contribution:
ST was in charge of the research's conception and design, collected the data, contributed to the data or analysis tools, implemented the methods, and drafted the initial version of the manuscript. DD offered guidance on the work's design and helped interpret the results. ZP and PSM conducted study for cell lines and cytotoxicity. MB was responsible for acquiring the materials, took part in morphological characterization, and helped draft the manuscript. HP conducted a thorough literature review and carried out preformulation studies. All authors reviewed and approved the final manuscript.

Competing Interest
The Authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article

Ethics Statement
This study does not involve any living invertebrate, human participants or patients' data and does not require ethical approvals

Availability of Supporting data
The datasets used and/or analysed during the current study available from the corresponding author on reasonable request

Funding
This study was not financially supported by any organization

REFERENCES:

1. Bai, Y., Chen, S., Wang, Z., Zhu, Y., Xu, X., Mei, G., Zhu, R., He, X., Chen, S., Zhu, R., Yang, H., & Ma, B. (2024). Application of bioactive materials for osteogenic functions in bone tissue engineering. Small Methods, 8(8). https://doi.org/10.1002/smtd.202301283
2. Farjaminejad, S., Farjaminejad, R., Hasani, M., Garcia-Godoy, F., Abdouss, M., Marya, A., Harsoputranto, A., & Jamilian, A. (2024). Advances and Challenges in Polymer-Based Scaffolds for Bone Tissue Engineering: A Path Towards Personalized Regenerative Medicine. Polymers, 16(23), 3303. https://doi.org/10.3390/polym16233303
3. Wu, J., Pei, X., Cheng, X., Wu, J., & Chen, J. (2023). The development of magnesium-based biomaterials in bone tissue engineering: A review. Journal of Biomedical Materials Research Part B: Applied Biomaterials, 112(1). https://doi.org/10.1002/jbm.b.35326
4. Ercal, P., Kose, G. T., & Pekozer, G. G. (2018). Dental Stem Cells in Bone Tissue Engineering: Current Overview and Challenges. Advances in Experimental Medicine and Biology, 1107, 113–127. https://doi.org/10.1007/5584_2018_171
5. Gao, X., Huard, J., Layne, J. E., Ruzbarsky, J. J., & Xiao, X. (2024). Stem Cells and Bone Tissue Engineering. Life, 14(3), 287. https://doi.org/10.3390/life14030287

6. Augustine, R., Gezek, M., Nikolopoulos, V. K., Buck, P. L., Bostanci, N. S., & Camci-Unal, G. (2024). Stem Cells in Bone Tissue Engineering: Progress, Promises and Challenges. Stem Cell Reviews and Reports, 20(7), 1692–1731. https://doi.org/10.1007/s12015-024-10738-y
7. Cheng, J., Liu, J., Wu, B., Liu, Z., Li, M., Wang, X., Tang, P., & Wang, Z. (2021). Graphene and its Derivatives for Bone Tissue Engineering: In Vitro and In Vivo Evaluation of Graphene-Based Scaffolds, Membranes and Coatings. Frontiers in Bioengineering and Biotechnology, 9(3). https://doi.org/10.3389/fbioe.2021.734688
8. Fan, J., Jiang, B., Cai, Y., Huang, C., Luo, Z., Ding, Z., Lai, Y., & Zhou, Z. (2025). Revolutionizing Bone Regeneration: Vascularized Bone Tissue Engineering with Advanced 3D Printing Technology. Aggregate, 6(4). https://doi.org/10.1002/agt2.731
9. Fourie, J., Taute, F., Du Preez, L., & De Beer, D. (2020). Chitosan Composite Biomaterials for Bone Tissue Engineering—a Review. Regenerative Engineering and Translational Medicine, 8(1), 1–21. https://doi.org/10.1007/s40883-020-00187-7
10. Gan, D., Liu, M., Wang, K., Tan, H., Lu, X., & Xu, T. (2018). Chitosan/biphasic calcium phosphate scaffolds functionalized with BMP-2-encapsulated nanoparticles and RGD for bone regeneration. Journal of Biomedical Materials Research Part A, 106(10), 2613–2624. https://doi.org/10.1002/jbm.a.36453
11. Zeng, H., Liang, L., Ran, J., Pathak, J. L., Fan, Q., Li, M., Yan, Q., Bai, Y., Shi, Y., & Wu, T. (2020). Indirect selective laser sintering-printed microporous biphasic calcium phosphate scaffold promotes endogenous bone regeneration via activation of ERK1/2 signaling. Biofabrication, 12(2), 025032. https://doi.org/10.1088/1758-5090/ab78ed
12. Hwang, H. S., & Lee, C.-S. (2023). Recent Progress in Hyaluronic-Acid-Based Hydrogels for Bone Tissue Engineering. Gels, 9(7), 588. https://doi.org/10.3390/gels9070588
13. Liu, G., Wan, T., Jing, W., Zhang, B., Tian, W., Fan, Y., & Zhou, C. (2022). A 3D-printed biphasic calcium phosphate scaffold loaded with platelet lysate/gelatin methacrylate to promote vascularization. Journal of Materials Chemistry B, 10(16), 3138–3151. https://doi.org/10.1039/d2tb00006g
14. Liu, G., Wu, S., Sun, J., Xiang, Z., Gong, M., & Xing, F. (2021). Urine-derived stem cells loaded onto a chitosan-optimized biphasic calcium-phosphate scaffold for repairing large segmental bone defects in rabbits. Journal of Biomedical Materials Research Part B: Applied Biomaterials, 109(12), 2014–2029. https://doi.org/10.1002/jbm.b.34850
15. Lobo, S. E., & Livingston Arinzeh, T. (2010). Biphasic Calcium Phosphate Ceramics for Bone Regeneration and Tissue Engineering Applications. Materials, 3(2), 815–826. https://doi.org/10.3390/ma3020815
16. Zhu, Y., Zhang, Y., & Zhou, Y. (2022). Application Progress of Modified Chitosan and Its Composite Biomaterials for Bone Tissue Engineering. International Journal of Molecular Sciences, 23(12), 6574. https://doi.org/10.3390/ijms23126574
17. Nie, L., Wu, Q., Long, H., Hu, K., Li, P., Wang, C., Sun, M., Dong, J., Wei, X., Suo, J., Hua, D., Liu, S., Yuan, H., & Yang, S. (2019). Development of chitosan/gelatin hydrogels incorporation of biphasic calcium phosphate nanoparticles for bone tissue engineering. Journal of Biomaterials Science, Polymer Edition, 30(17), 1636–1657. https://doi.org/10.1080/09205063.2019.1654210
18. Ressler, A. (2022). Chitosan-Based Biomaterials for Bone Tissue Engineering Applications: A Short Review. Polymers, 14(16), 3430. https://doi.org/10.3390/polym14163430
19. Signorini, L., Marano, R. M., Pacifici, L., Valletta, A., Marrelli, B., Severino, M., Sammartino, G., Gasparro, R., Marenzi, G., & Facente, A. (2023). Critical Overview on Pure Chitosan-based Scaffolds for Bone Tissue Engineering: Clinical insights in Dentistry. International Journal of Medical Sciences, 20(12), 1527–1534. https://doi.org/10.7150/ijms.87978
20. Xing, F., Wang, W., Zhang, X., Gu, L., Hui, D., Wang, L., Zhou, C., Wu, L., Pu, X., Xiang, Z., Liu, L., & Du, C. (2020). Hyaluronic acid as a bioactive component for bone tissue regeneration: Fabrication, modification, properties, and biological functions. Nanotechnology Reviews, 9(1), 1059–1079. https://doi.org/10.1515/ntrev-2020-0084
21. Xu, J., Brama, P. A. J., Locs, J., Kops, N., Kok, J., Filardo, G., Misciagna, M. M., Labberté, M., Vecstaudza, J., Van Rietbergen, B., Dolcini, L., Salerno, M., Farrell, E., Wesdorp, M. A., Harmand, M.-F., Vancíková, K., Van Osch, G. J. V. M., & Simon, M. (2024). Incorporating strontium enriched amorphous calcium phosphate granules in collagen/collagen-magnesium-hydroxyapatite osteochondral scaffolds improves subchondral bone repair. Materials Today Bio, 25, 100959. https://doi.org/10.1016/j.mtbio.2024.100959
22. Zhou, C., Fan, Y., Zhang, X., Tan, Y., Qing, F., Ye, X., & Ma, L. (2014). Biomimetic fabrication of a three-level hierarchical calcium phosphate/collagen/hydroxyapatite scaffold for bone tissue engineering. Biofabrication, 6(3), 035013. https://doi.org/10.1088/1758-5082/6/3/035013
23. Sarita, S., D, P. M., Singh, B., Rai, A. K., Tewari, R. P., & Dutta, P. K. (2023). An injectable blend hydrogel for bone tissue engineering application: synthesis and characterization. Journal of Macromolecular Science, Part A, 61(1), 2–10. https://doi.org/10.1080/10601325.2023.2277211
24. Nguyen, T. B., & Lee, B. T. (2014). A combination of biphasic calcium phosphate scaffold with hyaluronic acid-gelatin hydrogel as a new tool for bone regeneration. Tissue engineering. Part A, 20(13-14), 1993–2004. https://doi.org/10.1089/ten.TEA.2013.0352
25. Kokubo T, Takadama H (2006) How useful is SBF in predicting in vivo bone bioactivity? Biomaterials 27:2907–2915 http://dx.doi.org/10.1016/j.biomaterials.2006.01.017