



Effect of bovine hydroxyapatite–gelatin–vitamin D supplementation on bone regeneration in a rabbit femoral defect model

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Article information

Article history:

Received January 7, 2026

Accepted March 19, 2026

Published April 6, 2026

Keywords:

ALP

Osteocalcin

Bovine Hydroxyapatite

Gelatin

Bone

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Abstract

Bone defects resulting from fractures remain a major clinical challenge due to impaired bone continuity and limited regenerative capacity, particularly in critical-sized defects. Biomimetic oral supplementation combining mineral components, gelatin-derived organic support, and osteogenic regulators may enhance bone regeneration by accelerating osteogenic maturation. This study aimed to evaluate the effectiveness of a capsule formulation containing bovine hydroxyapatite (BHA), gelatin, and vitamin D in promoting bone regeneration in a rabbit femoral bone defect model using osteogenic markers Runx2, alkaline phosphatase (ALP), and osteocalcin (OCN). Fifteen healthy male New Zealand white rabbits were divided into three groups (n = 5): bone defect (control), commercialized calcium supplementation, and BHA–gelatin–vitamin D supplementation. A standardized femoral bone defect (4 mm diameter × 6 mm depth) was created surgically. Supplements were administered orally for 28 days. Femoral bone samples were collected and analyzed using immunohistochemistry for Runx2, ALP, and OCN expression. Immunoreactive scores (IRS) were assessed semi-quantitatively. Data were analyzed using the Kruskal–Wallis test (P<0.05). The BHA–gelatin–vitamin D group showed significantly higher IRS values for Runx2, ALP, and OCN than the control group (P<0.05). No significant differences were observed between the BHA-based formulation and commercial calcium supplementation, indicating comparable osteogenic efficacy. The combination of BHA, gelatin, and vitamin D effectively accelerates bone regeneration by enhancing osteogenic differentiation, matrix formation, and osteoblast maturation. This formulation shows potential as an alternative strategy for promoting bone healing with efficacy comparable to commercially available calcium supplements.

DOI: [10.33899/ijvs.2026.168417.4668](https://doi.org/10.33899/ijvs.2026.168417.4668), ©Authors, 2026, College of Veterinary Medicine, University of Mosul.

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Introduction

Bone defects may arise from various pathological and traumatic conditions, with fractures representing one of the most clinically significant skeletal injuries. A fracture is defined as a partial or complete disruption of bone continuity

caused by trauma, excessive mechanical stress, or pathological weakening of bone tissue (1). Globally, fractures constitute a major health burden, with approximately 178 million new fracture cases reported in 2019, representing a 33.4% increase since 1990. In the same year, fractures accounted for 455 million prevalent cases and

25.8 million years lived with disability worldwide (2,3). In elderly populations, hip fractures are associated with substantial morbidity and elevated mortality, with approximately 20–30% of patients dying within one year after injury, mainly due to complications related to immobility and systemic deterioration (4). Fractures are commonly caused by traumatic events such as traffic accidents, falls, and sports-related injuries, as well as by osteoporosis, which compromises bone density and increases fracture susceptibility (5,6).

One therapeutic approach to manage bone defects is the development of biomimetic matrices that closely resemble the composition of native bone tissue (7-9). Bovine hydroxyapatite (BHA) is an inorganic component capable of inducing bone regeneration due to its biocompatible, bioactive, non-toxic, non-inflammatory, and non-immunogenic properties (10,11). Compared with human-derived hydroxyapatite, BHA offers several advantages, including abundant availability and lower cost, given the limited supply of human bone donors. Furthermore, BHA exhibits osteoconductive properties, high biocompatibility, and high porosity, which facilitate osteoblast colonization and provide an optimal surface for cell attachment (12,13). Although BHA is widely used as a scaffold material, its performance in drug delivery is restricted by superficial adsorption of bioactive compounds and their rapid desorption, which can be improved through polymer coating (14,15). In an *in vivo* study, BHA demonstrated a bioavailability of up to 42.5%, which was higher than that of calcium lactate (approximately 25%) (16).

Gelatin plays a crucial role in the formation of soft callus during fracture healing, rapidly promoting trabecular bridging between defect sites and facilitating bone continuity (11). Gelatin offers several advantages, including excellent biocompatibility and biodegradability. Due to the brittle nature of BHA as a bone-forming material, gelatin is incorporated as a binding and smoothing agent to enhance mechanical integrity and biological performance (12). In addition, vitamin D plays a pivotal role in optimizing bone regeneration by enhancing intestinal calcium absorption and maintaining calcium homeostasis. Optimal bone quality requires sufficient vitamin D levels, as only 10–15% of dietary calcium can be absorbed in the absence of vitamin D (17).

Bone regeneration is a highly regulated biological process involving the differentiation of mesenchymal stem cells (MSCs) into osteoblasts through the coordinated regulation of transcription factors and osteogenic enzymes (18). Runt-related transcription factor 2 (Runx2) serves as a master regulator during the early stage of osteogenesis by directing MSC commitment toward the osteoblastic lineage. Runx2 activation is stimulated by osteogenic signaling pathways, including bone morphogenetic proteins (BMPs) and transforming growth factor- β (TGF- β) (11,19). Elevated

Runx2 expression indicates early osteogenic differentiation and pre-osteoblast formation and also induces expression of early osteogenic genes, such as alkaline phosphatase (ALP) and collagen type I. Consequently, Runx2 is widely used as a marker of early osteogenesis in studies of bone regeneration (20,21).

As osteogenic differentiation progresses, increased ALP expression characterizes the matrix formation phase, during which immature osteoblasts actively prepare the microenvironment for mineralization by increasing phosphate availability and reducing pyrophosphate levels (22-24). In the final stage, mature osteoblasts express osteocalcin (OCN). This bone-specific non-collagenous matrix protein binds calcium and hydroxyapatite, playing a critical role in mineralization and bone tissue maturation. Elevated OCN expression reflects the formation of mineralized bone and the differentiation of osteoblasts into osteocytes (1,25). Therefore, the sequential expression of Runx2–ALP–OCN is widely used to represent the progression of osteogenesis from early differentiation to late-stage maturation in various bone regeneration models (23,26).

This study aimed to evaluate the efficacy of an oral capsule formulation containing bovine hydroxyapatite (BHA), gelatin, and vitamin D in enhancing bone regeneration in a rabbit femoral defect model, as assessed by immunohistochemical markers of osteogenic differentiation and maturation (Runx2, ALP, and OCN). The novelty of this work lies in the development of an integrated combination formulation that delivers complementary osteogenic effects within a single dosage form, offering a more comprehensive approach than single-component supplementation.

Materials and methods

Ethical Issues

All protocols in this study complied with the Guidelines for the Care and Use of Laboratory Animals issued by the National Institutes of Health, revised in 1985, and were approved by the Research Ethics Committee of the Faculty of Veterinary Medicine, Universitas Airlangga (Approval No. 2.KEH.97.06.2025).

Animal

This study was conducted in accordance with the ARRIVE guidelines for the reporting of animal research. Fifteen healthy male New Zealand White rabbits aged 6–8 months and weighing 3.5 – 4.5 kg were included in the experiment. The animals were housed individually under controlled laboratory conditions with a temperature of 22.5 ± 2.5 °C, a 12 h light–dark cycle, and *ad libitum* access to standard chow and water. Prior to the experimental procedures, all rabbits were acclimatized to the housing environment and diet for 7 days. Animal welfare was

monitored daily throughout the study period to minimize stress and discomfort, and all experimental procedures were performed by trained personnel.

Materials

Bovine hydroxyapatite (BHA) was obtained from the Faculty of Pharmacy, Universitas Airlangga (Surabaya, Indonesia), gelatin (St. Louis, MO, USA), vitamin D₃ (750 IU) (Kimia Farma, Jakarta, Indonesia), ketamine and xylazine (Interchemie, Venray, The Netherlands), povidone-iodine (Mahakam Beta Pharma, Jakarta, Indonesia), ampicillin antibiotic (Kalbe Farma, Jakarta, Indonesia), normal saline solution (Widatra, Pasuruan, Indonesia), 10% buffered formalin (Merck, Darmstadt, Germany), ethanol 70% (Chemical Indonesia Multi Sentosa, Surabaya, Indonesia), distilled water (prepared in-house), gentamicin ointment (First Medipharma, Sidoarjo, Indonesia), xylene was obtained from (Merck, Darmstadt, Germany), water for injection (Widatra, Pasuruan, Indonesia), ethylenediaminetetraacetic acid (EDTA) (Merck, Darmstadt, Germany).

Methods

The rabbits were randomly allocated to three experimental groups (n = 5 per group): a control group, a commercially available calcium-supplementation group, and a bovine hydroxyapatite–gelatin–vitamin D supplementation group. The sample size was determined using the Lemeshow formula for minimum sample estimation (27). This calculation yielded a minimum sample size of 4.8 animals per group, which was rounded to 5 rabbits per group. Therefore, a total of 15 rabbits were included in this study, distributed equally into three experimental groups. Following general anesthesia, a standardized bone defect model was created by surgically drilling the femoral bone using a 4-mm diameter drill bit to a depth of 6 mm. The defect was irrigated using normal saline to prevent thermal necrosis (9). Rabbits were anesthetized using ketamine 50 mg/kg BW and xylazine 5 mg/kg BW intramuscularly, while pain was minimized using metamizole at a dose of 45 mg/kg BW every 12 hours for 2 days. For 7 consecutive days after surgery, each rabbit received intramuscular ampicillin (25 mg/kg body weight) to prevent infection. All animals received oral supplementation according to their assigned groups for 28 consecutive days. At the end of the experimental period (day 28), the rabbits were humanely euthanized, and femoral bone samples were harvested for further analysis.

Dose Calculation and Administration

The dose of bovine hydroxyapatite–gelatin–vitamin D was determined based on human daily intake and converted to rabbits using body surface area (BSA) normalization (Km factor: human = 37; rabbit = 12) (28). Based on this

approach, the administered dose corresponded to approximately 26–28 mg/kg body weight. Accordingly, rabbits weighing 3.5–4.5 kg received approximately 92–118 mg/day of bovine hydroxyapatite–gelatin–vitamin D (29). The commercial calcium supplement was administered at an equivalent dose of approximately 29 mg/kg body weight, corresponding to 102–131 mg/day (16). All formulations were prepared as oral suspensions by gavage and administered once daily at a volume of 2 mL per rabbit.

Immunohistochemistry Analysis

Harvested femoral bone specimens were decalcified using 10% ethylenediaminetetraacetic acid (EDTA) with a pH 7.3 – 7.5 at room temperature for 30 days, with regular solution replacement for each 2 days. Following complete decalcification, the samples were processed for paraffin embedding. Serial sections were prepared and immunohistochemically stained with primary antibodies against Runx2, osteocalcin (OCN), and alkaline phosphatase (ALP). Five representative high-power fields were evaluated under 400x magnification using the IRS method under blinded conditions (30), and the IRS score was determined as shown in Table 1.

Table 1: Semi-quantitative Immunoreactive Score (31)

Percentage of Positive Cells (A)	Intensity (B)	A x B
0: No positive cells	0: No staining	0-1: Negative
1: < 10% positive cells	1: Weak	2-3: Weak
2: 11-50% positive cells	2: Moderate	4-8: Moderate
3: 51-79% positive cells	3: Strong	9-12: Strong
4: > 80% positive cells		

Statistical Analysis

Data analysis and visualization were performed using GraphPad Prism version 9.0.2 (GraphPad Software, San Diego, CA, USA). Data normality was assessed using the Shapiro–Wilk test. Because the sample size was small and the data were not normally distributed, differences among groups were analyzed using the Kruskal–Wallis test, followed by Dunn's test for pairwise comparisons. A *p*-value < 0.05 was considered statistically significant.

Results

Immunohistochemical Expression of Runx2

Immunohistochemical analysis of Runx2 expression (Figures 1 and 2) demonstrated that the highest mean immunoreactive score (IRS) was observed in the group receiving BHA–gelatin–vitamin D supplementation. Statistical analysis revealed that IRS values in the BHA supplementation group were significantly higher than those in the control group (*P* < 0.05), but did not differ significantly

from those in the commercially available calcium supplementation group ($P>0.05$). These findings indicate that the BHA–gelatin–vitamin D combination effectively

enhances early osteogenic differentiation and osteoblast commitment, with an efficacy comparable to that of existing calcium supplements.

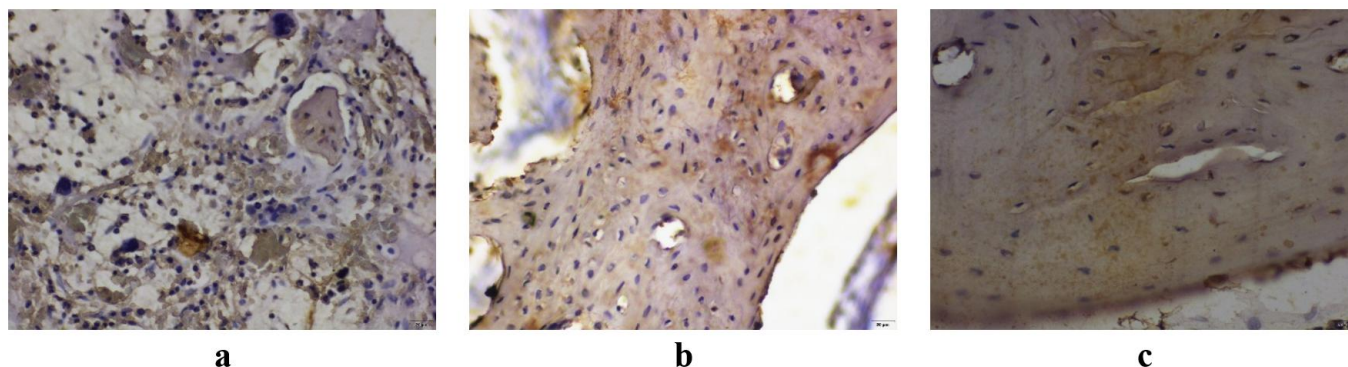


Figure 1. The expression of Runx2 in (a) bone defect group, (b) commercialized calcium supplement, and (c) BHA supplement. Data were taken at 400x magnification.

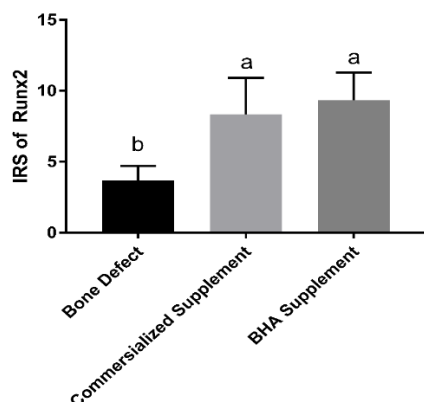


Figure 2: IR score of the expression of Runx2. Data were represented by mean $\pm$ SD. Different letters indicate different groups of significance, whereas $P<0.05$ indicates a significant result.

Immunohistochemical Expression of ALP

Evaluation of ALP expression (Figures 3 and 4) showed a similar pattern, with the highest mean IRS observed in the BHA–gelatin–vitamin D supplementation group. The ALP IRS in this group was significantly higher than in the control group ($P<0.05$) and was not statistically different from the commercial calcium supplementation group ($P>0.05$). Given that ALP is a marker of matrix maturation and early mineralization, these results suggest that the BHA-based formulation effectively promotes osteoblast functional activity during the intermediate phase of bone regeneration.

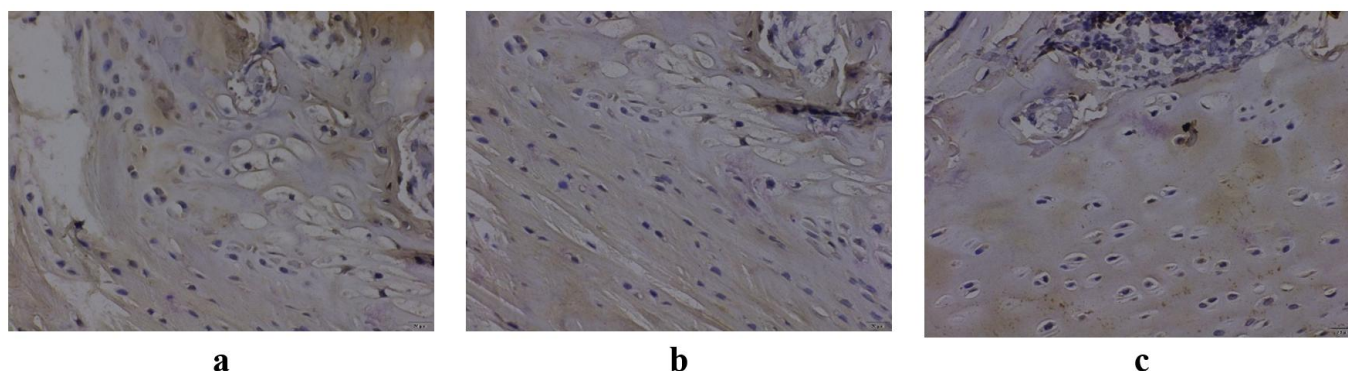


Figure 3. The expression of ALP in (a) bone defect group, (b) commercialized calcium supplement, and (c) BHA supplement. Data were taken at 400x magnification.

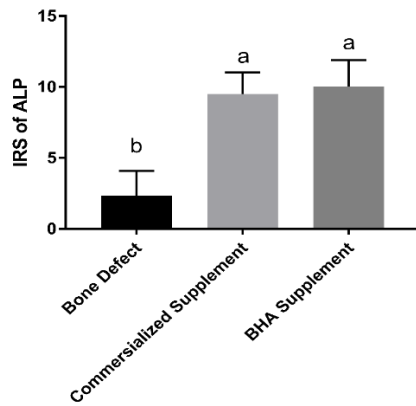


Figure 4: IR score of the expression of ALP. Data were represented by mean $\pm$ SD. Different letters indicate different groups of significance, whereas $P < 0.05$ indicates a significant result.

Immunohistochemical Expression of OCN

Analysis of OCN expression (Figures 5 and 6) revealed that the BHA–gelatin–vitamin D supplementation group had the highest mean IRS. Similar to the findings for Runx2 and ALP, no significant difference was observed between the BHA supplementation group and the commercial calcium group ($P > 0.05$), whereas both groups showed significantly higher IRS values than the control group ($P < 0.05$). As OCN is a marker of mature osteoblasts and mineralized bone matrix, these results indicate that the BHA–gelatin–vitamin D combination supports late-stage osteogenic maturation and bone mineralization to a degree comparable to that of commercially available calcium supplementation.

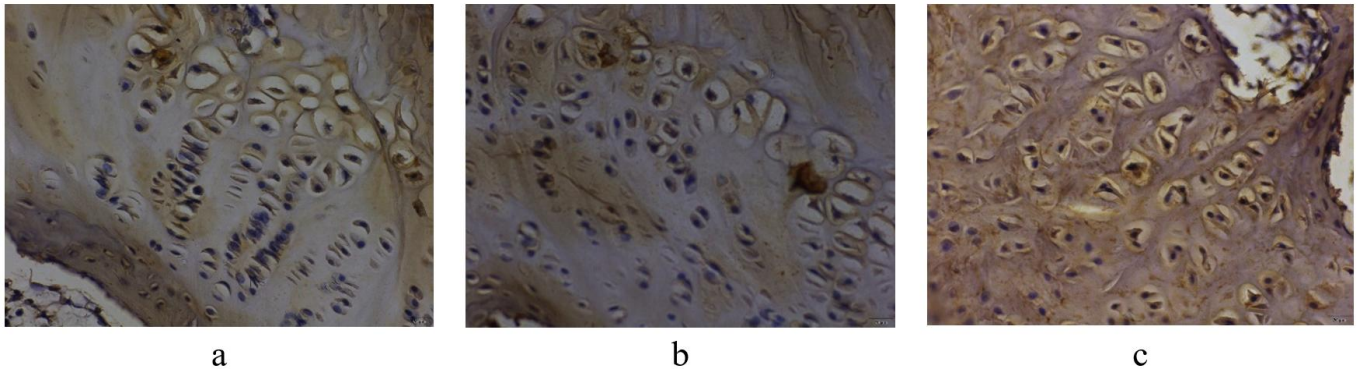


Figure 5: The expression of OCN in (a) bone defect group, (b) commercialized calcium supplement, and (c) BHA supplement. Data were taken at 400x magnification.

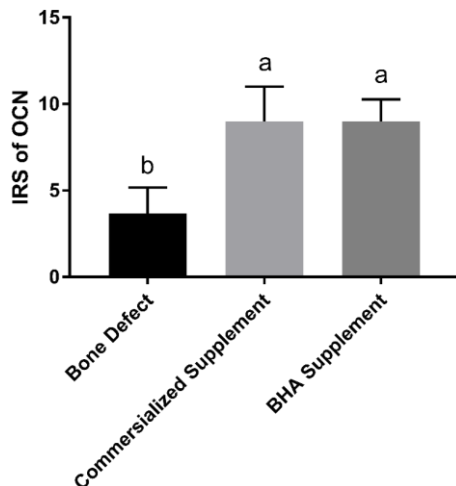


Figure 6: IR score of the expression of OCN. Data were represented by mean $\pm$ SD. Different letters indicate different groups of significance, whereas $P < 0.05$ indicates a significant result.

Discussion

Bone defect healing is a coordinated biological process involving inflammation, cell proliferation, osteogenic differentiation, matrix formation, and mineralization toward mature bone formation (3,32). In critical-sized defects, healing is often impaired by the absence of an adequate scaffold, limited mineral availability, and insufficient osteogenic signaling (33). The present study demonstrates that oral supplementation with a combination of bovine hydroxyapatite (BHA), gelatin, and vitamin D significantly accelerates bone defect healing compared to the control group, as evidenced by the increased expression of key osteogenic markers Runx2, alkaline phosphatase (ALP), and osteocalcin (OCN). From a theoretical perspective, the coordinated upregulation of these markers reflects an enhanced and well-orchestrated progression of osteogenesis from early commitment to late-stage mineralization (34).

During the early phase of bone regeneration, the activation and commitment of mesenchymal stem cells (MSCs) toward the osteoblastic lineage are critical

determinants of successful healing (35). Runx2 acts as a master transcription factor in osteoblast differentiation by regulating osteogenic genes such as ALP, collagen type I, and OCN. Higher Runx2 expression in the BHA–gelatin–vitamin D group suggests that this combination promotes osteogenic commitment of MSCs by providing a biomimetic mineral surface that enhances cell adhesion and activates osteogenic signaling, including BMP/SMAD pathways (36,37). In contrast, the absence of a local scaffold and limited mineral cues in the control group may reduce Runx2 activation and delay osteogenic differentiation (38).

As bone regeneration enters the proliferative and matrix formation phases, elevated ALP expression indicates increased osteoblast activity in preparing the extracellular matrix for mineralization (39). ALP promotes hydroxyapatite formation by hydrolyzing inorganic pyrophosphate and increasing phosphate availability. Gelatin contributes by acting as a collagen-like organic matrix that supports osteoblast adhesion, proliferation, and survival (39,40). Its biodegradable nature also allows gradual calcium–phosphate release from BHA, sustaining ALP activity and matrix maturation, consistent with the role of matrix formation as a key transition toward mineralization (41).

In the late phase of bone regeneration, increased osteocalcin expression in the BHA–gelatin–vitamin D group indicates enhanced osteoblast maturation and matrix mineralization. OCN, a bone-specific protein secreted by mature osteoblasts, binds calcium and hydroxyapatite and contributes to bone strength (42,43). The observed upregulation of OCN suggests that the regenerated tissue in the treatment group had progressed toward a more mature osteogenic phenotype (44). Vitamin D supports this process by regulating calcium–phosphate homeostasis and osteoblast gene expression through (Vitamin D Receptor) VDR activation (45). The synergistic interaction between vitamin D and BHA provides a mechanistic explanation for the OCN expression levels in the treatment group being comparable to those observed with commercial calcium supplementation (18,46).

Notably, the BHA–gelatin–vitamin D combination showed osteogenic efficacy comparable to commercial calcium supplements in terms of Runx2, ALP, and OCN expression. Unlike conventional calcium supplements that mainly increase systemic mineral availability, this formulation may provide mineral components, gelatin-derived nutritional support, and vitamin D-related osteogenic regulation that collectively enhance bone regeneration. These findings support current bone tissue engineering concepts emphasizing the interaction of cells, scaffolds, and osteogenic signaling in effective bone repair.

Conclusion

The combination of bovine hydroxyapatite (BHA), gelatin, and vitamin D accelerated bone regeneration in a rabbit bone defect model over 28 days, as shown by sequential increases in Runx2, ALP, and OCN expression, indicating progression from early osteogenic differentiation to mature bone mineralization. This suggests faster osteogenic maturation through coordinated transcriptional regulation, matrix support, and mineral availability. BHA provides an osteoconductive scaffold and calcium–phosphate source, gelatin supports a collagen-like organic matrix, and vitamin D enhances osteogenic signaling and mineral homeostasis. Its comparable efficacy to commercial calcium supplements indicates that effective bone regeneration depends not only on mineral supply but also on a supportive local microenvironment for osteoblast differentiation and maturation.

Acknowledgement

The authors would like to thank to the Faculty of Pharmacy Universitas Airlangga and Kuliyah of Pharmacy International Islamic University Malaysia.

Funding Statement

This research was funded by Universitas Airlangga through Penelitian Research Group Fakultas Skema B No. 3251/B/UN3.FF/PT.01.03/2025.

Conflict of interest

The authors declare there is no conflict of interest.

References

1. Al-Saiegh AM, Al-Qadhi AS, Ibrahim SM, Al-Hyani OH. Effect of aloe vera gel on articular cartilage regeneration in dogs. *Iraqi J Vet Sci.* 2024;38(2):275–283. DOI: [10.33899/ijvs.2023.142075.3155](https://doi.org/10.33899/ijvs.2023.142075.3155)
2. World Health Organization. Fragility fractures. Switzerland: World Health Organization; 2024.
3. GBD 2019 Fracture Collaborators. Global, regional, and national burden of bone fractures in 204 countries and territories, 1990–2019: a systematic analysis from the Global Burden of Disease Study 2019. *Lancet Healthy Longev.* 2021;2(9):580–592. DOI: [10.1016/S2666-7568\(21\)00172-0](https://doi.org/10.1016/S2666-7568(21)00172-0)
4. Harvey LA, Payne NL, Tan A. Variation in mortality following hip fracture across the Asia Pacific region: systematic review and proportional meta-analysis. *Arch Gerontol Geriatr.* 2024;126:105519. DOI: [10.1016/j.archger.2024.105519](https://doi.org/10.1016/j.archger.2024.105519)
5. Ismail R, Fitriyana DF, Bayuseno AP, Munanda R, Muhamadin RC, Nugraha FW, Rusiyanto, Setiyawan A, Bahatmaka A, Firmansyah HN, Anis S, Irawan AP, Siregar JP, Cionita T. Design and characterization of biodegradable PLA bone screw fabricated by FDM 3D printing. *J Adv Res Fluid Mech Therm Sci.* 2023;103(2):205–215. DOI: [10.37934/arfmts.103.2.205215](https://doi.org/10.37934/arfmts.103.2.205215)

6. Wang Z, Zhang Y, Wu J, Zhang Q. Global burden of patella, tibia, fibula, and ankle fractures. BMC Public Health. 2024;24(1):20675. DOI: [10.1186/s12889-024-20675-7](https://doi.org/10.1186/s12889-024-20675-7)
7. Lee CY, Nedunchezian S, Lin SY, Su YF, Wu CW, Wu SC, Chen CH, Wang CK. Bilayer osteochondral graft using 3D-printed bioceramic and stem cell-laden hydrogel in rabbit model. J Biol Eng. 2023;17(1):74. DOI: [10.1186/s13036-023-00389-x](https://doi.org/10.1186/s13036-023-00389-x)
8. Pratama YA, Ananta IP, Haris MS, Maghfiroh N, Nabila H, Ardianto C, Rahmadi M, Khotib J, Hendradi E, Taher M, Mazidda S, Budiati AS. Accelerated bone defect closure using nano bovine hydroxyapatite scaffold. J Pharm Pharmacogn Res. 2025;13(6):1728–1741. DOI: [10.56499/jppres24.2310_13.6.1728](https://doi.org/10.56499/jppres24.2310_13.6.1728)
9. Suyatno A, Nurfinti WO, Kusuma CP, Pratama YA, Ardianto C, Samirah, Rahadiansyah E, Khotib J, Budiati AS. Effectiveness of bilayer scaffold containing chitosan/gelatin/diclofenac and bovine hydroxyapatite. Adv Pharmacol Pharm Sci. 2024;2024:6987676. DOI: [10.1155/2024/6987676](https://doi.org/10.1155/2024/6987676)
10. Khotib J, Gani MA, Budiati AS, Lestari ML, Rahadiansyah E, Ardianto C. Signaling pathways and transcriptional regulation in osteoblasts during bone healing involving hydroxyapatite biomaterial. Pharmaceuticals. 2021;14(7):615. DOI: [10.3390/ph14070615](https://doi.org/10.3390/ph14070615)
11. Khotib J, Marhaeny HD, Miatmoko A, Budiati AS, Ardianto C, Rahmadi M, Pratama YA, Tahir M. Differentiation of osteoblasts: links between essential transcription factors. J Biomol Struct Dyn. 2022;40:1–20. DOI: [10.1080/07391102.2022.2148749](https://doi.org/10.1080/07391102.2022.2148749)
12. Budiati AS, Samirah, Gani MA, Nilamsari WP, Ardianto C. Characterization of bovine bone-derived hydroxyapatite isolated using a novel non-hazardous method. J Biomim Biomater Biomed Eng. 2020;45:49–56. DOI: [10.4028/www.scientific.net/JBBE.45.49](https://doi.org/10.4028/www.scientific.net/JBBE.45.49)
13. Budiati AS, Su'aida N, Lamakluang AI, Rahma SS, Zulkarnain BS, Isadiartuti D. Effect of glutaraldehyde concentration variation on diclofenac sodium scaffolds as cross-linking agent. Res J Pharm Technol. 2022;15(11):4974–4980. DOI: [10.52711/0974-360X.2022.00836](https://doi.org/10.52711/0974-360X.2022.00836)
14. Iaquina MR, De Pace R, Benkhalqui A, D'Agostino A, Trevisiol L, Finotti A, Breveglieri G, Tognon M, Martini F, Mazzoni E. Secretome release during in vitro osteogenic differentiation induced by Bio-Oss® collagen. Int J Mol Sci. 2025;26(8):3807. DOI: [10.3390/ijms26083807](https://doi.org/10.3390/ijms26083807)
15. Haris MS, Budiati AS, Rahmadi M, Aryani T, Ramadhaniar M. Fabrication of nano bovine hydroxyapatite-gelatin-calcium sulfate bone graft. Res J Pharm Technol. 2025;18(1):326–332. DOI: [10.52711/0974-360X.2025.00051](https://doi.org/10.52711/0974-360X.2025.00051)
16. Budiati AS, Khotib J, Samirah, Ardianto C, Gani MA, Putri BR, Arofik H, Sadiwa RN, Lestari I, Pratama YA, Rahadiansyah E, Susilo I. Acceleration of bone fracture healing using bovine hydroxyapatite or calcium lactate oral and implant bovine hydroxyapatite-gelatin in animal bone defect model. Polymers. 2022;14(22):4812. DOI: [10.3390/polym14224812](https://doi.org/10.3390/polym14224812)
17. Fischer V, Haffner-Luntzer M, Amling M, Ignatius A. Calcium and vitamin D in bone fracture healing and post-traumatic bone turnover. Eur Cells Mater. 2018;35:365–385. DOI: [10.22203/eCM.v035a25](https://doi.org/10.22203/eCM.v035a25)
18. El Moshly S, Radwan IA, Rady D, Abbass MM, El-Rashidy AA, Sadek KM, Dörfer CE, El-Sayed KM. Dental stem cell-derived secretome/conditioned medium for regenerative therapeutic applications. Stem Cells Int. 2020;2020:7593402. DOI: [10.1155/2020/7593402](https://doi.org/10.1155/2020/7593402)
19. Okajima LS, Martinez EF, Pinheiro IF, Silva AS, Demasi AP. Effect of sodium ascorbyl phosphate on osteoblast viability and differentiation. J Periodontol Res. 2020;55(5):660–666. DOI: [10.1111/jre.12752](https://doi.org/10.1111/jre.12752)
20. Nugraha AP, Kamadaja DB, Sumarta NP, Rizqiawan A, Pramono C, Yuliaty A, Hendrianto E, Rahman MZ. Osteoinductive capacity of freeze-dried bovine bone scaffold. Eur J Dent. 2023;17(4):1106–1113. DOI: [10.1055/s-0042-1758786](https://doi.org/10.1055/s-0042-1758786)
21. Zhu S, Chen W, Masson A, Li YP. Cell signaling and transcriptional regulation of osteoblast lineage commitment. Cell Discov. 2024;10(1):89. DOI: [10.1038/s41421-024-00689-6](https://doi.org/10.1038/s41421-024-00689-6)
22. Sekino Y, Imaizumi A, Komune N, Ono M, Sato K, Masuda S, Fujimura A, Koike K, Hongo T, Uchi R, Onishi H, Nakagawa T. Establishment of primary cell culture from external auditory canal squamous cell carcinoma. FEBS Open Bio. 2021;11(8):2211–2224. DOI: [10.1002/2211-5463.13225](https://doi.org/10.1002/2211-5463.13225)
23. Zadeh FB, Zamanian A. Optimum glutaraldehyde cross-linking condition for chitosan/gelatin scaffolds. Int J Eng Trans A. 2022;35(10):1967–1980. DOI: [10.5829/ije.2022.35.10A.15](https://doi.org/10.5829/ije.2022.35.10A.15)
24. Anaraki N, Beyraghi AH, Raisi A, Davoodi F, Farjanikish G, Sadegh AB. Effect of aqueous extract of *Prunus dulcis* on tibial bone healing in rabbits. J Orthop Surg Res. 2021;16(1):1–11. DOI: [10.1186/s13018-021-02498-z](https://doi.org/10.1186/s13018-021-02498-z)
25. Edward M, Dominica H, Mahyudin F, Rantam FA. Differences of bone regeneration using bovine hydroxyapatite and bovine hydroxyapatite with freeze-dried platelet-rich plasma allograft in rabbit femoral bone defect. Joints. 2020;9(2):34–54. DOI: [10.20473/joints.v9i2.2020.34-54](https://doi.org/10.20473/joints.v9i2.2020.34-54)
26. Ma S, Li Y, Yao S, Shang Y, Li R, Ling L, Fu W, Wei P, Zhao B, Zhang X, Deng J. SIS/HA composite hydrogel scaffold promotes alveolar bone regeneration. Bioact Mater. 2025;46:97–117. DOI: [10.1016/j.bioactmat.2024.12.008](https://doi.org/10.1016/j.bioactmat.2024.12.008)
27. Lwanga SK, Lemeshow S. Sample size determination in health studies: a practical manual. Switzerland: World Health Organization; 1991.
28. Jacob S, Nair AB, Morsy MA. Dose conversion between animals and humans: a practical solution. Indian J Pharm Educ Res. 2022;56(3):600–607. DOI: [10.5530/ijper.56.3.108](https://doi.org/10.5530/ijper.56.3.108)
29. Reid IR. Calcium supplementation: efficacy and safety. Curr Osteoporos Rep. 2025;23(1):1–8. DOI: [10.1007/s11914-025-00904-7](https://doi.org/10.1007/s11914-025-00904-7)
30. Raj V, Zaini ZM, Harun N, Zainul Abidin NF. Expression of RANKL, IL-1 α and M-CSF in fibroblast within periapical granulomas, periapical cysts and dentigerous cysts. Teikyo Med J. 2025;48(3):8535–8546. [\[available at\]](#)
31. Rizzardi AE, Johnson AT, Vogel R. Quantitative comparison of immunohistochemical staining measured by digital image analysis versus pathologist visual scoring. Diagn Pathol. 2012;7:42. DOI: [10.1186/1746-1596-7-42](https://doi.org/10.1186/1746-1596-7-42)
32. Schmidt AH. Autologous bone graft: is it still the gold standard?. Injury. 2021;52(2):S18–S22. DOI: [10.1016/j.injury.2021.01.043](https://doi.org/10.1016/j.injury.2021.01.043)
33. Tang G, Zhu L, Wang W, Zuo D, Shi C, Yu X, Chen R. Alendronate-functionalized hydrogel scaffolds for osteogenesis. Front Chem. 2022;10:977419. DOI: [10.3389/fchem.2022.977419](https://doi.org/10.3389/fchem.2022.977419)
34. Parisi L, Buser D, Chappuis V, Asparuhova MB. Cellular responses to deproteinized bovine bone mineral biofunctionalized with conditioned medium. Clin Oral Investig. 2021;25(4):2159–2173. DOI: [10.1007/s00784-020-03528-6](https://doi.org/10.1007/s00784-020-03528-6)
35. Hariawan BS, Cahyani DM, Anjani QK, Annuryanti F, Kamadaja DB, Nurkanto, Purwati, Hariyadi DM, Miatmoko A. Development of liposomal amniotic mesenchymal stem cell metabolite-loaded scaffold for osteogenesis induction. Eur Polym J. 2024;220:113482. DOI: [10.1016/j.eurpolymj.2024.113482](https://doi.org/10.1016/j.eurpolymj.2024.113482)
36. Feng J, Meng Z. Insulin-like growth factor-1 promotes proliferation and osteogenic differentiation of bone marrow mesenchymal stem cells via Wnt/ β -catenin pathway. Exp Ther Med. 2021;22(2):1–8. DOI: [10.3892/etm.2021.10323](https://doi.org/10.3892/etm.2021.10323)
37. Ma'arif B, Sholihah FA, Mahardiani A, Fauziyah B, Mirza DM, Agil M. Runx2 measurement in phytoestrogen-induced bone: comparison of western blot and immunohistochemistry. Biomed Pharmacol J. 2022;15(2):1039–1052. DOI: [10.13005/bpj/2439](https://doi.org/10.13005/bpj/2439)
38. Pratama YA, Marhaeny HD, Deapsari F, Budiati AS, Rahmadi M, Miatmoko A, Taher M, Khotib J. Development of hydroxyapatite as a bone implant biomaterial for triggering osteogenesis. Eur J Dent. 2026;20(1):49–55. DOI: [10.1055/s-0045-1809312](https://doi.org/10.1055/s-0045-1809312)
39. Vimalraj S. Alkaline phosphatase in bone mineralization. Gene. 2020;754:144855. DOI: [10.1016/j.gene.2020.144855](https://doi.org/10.1016/j.gene.2020.144855)
40. Gyanewali S, Kesharwani P, Sheikh A, Ahmad FJ, Trivedi R, Talegaonkar S. Protransfersomal gel formulation for osteoporosis therapy. Int J Pharm. 2021;608:121060. DOI: [10.1016/j.ijpharm.2021.121060](https://doi.org/10.1016/j.ijpharm.2021.121060)

41. Budiatin AS, Gani MA, Samirah, Ardianto C, Raharjanti AM, Septiani I, Putri NP, Khotib J. Bovine hydroxyapatite-based bone scaffold with gentamicin accelerates vascularization and remodeling of bone defect. *Int J Biomater.* 2021;2021:5560891. DOI: [10.1155/2021/5560891](https://doi.org/10.1155/2021/5560891)
42. Komori T. Functions of osteocalcin in bone, pancreas, testis, and muscle. *Int J Mol Sci.* 2020;21(20):7513. DOI: [10.3390/ijms21207513](https://doi.org/10.3390/ijms21207513)
43. Licini C, Vitale-Brovarone C, Mattioli-Belmonte M. Molecular crosstalk of collagen and non-collagenous proteins in osteoporosis. *Cytokine Growth Factor Rev.* 2019;49:59–69. DOI: [10.1016/j.cytogfr.2019.09.001](https://doi.org/10.1016/j.cytogfr.2019.09.001)
44. Meng ZL, Wu ZQ, Shen BX, Li HB, Bian YY, Zeng DL, Fu J, Peng L. Reconstruction of large segmental bone defects using Masquelet technique in rabbits. *J Orthop Surg Res.* 2019;14(1):1–12. DOI: [10.1186/s13018-019-1235-5](https://doi.org/10.1186/s13018-019-1235-5)
45. Föger-Samwald U, Dovjak P, Azizi-Semrad U, Kersch-Schindl K, Pietschmann P. Osteoporosis: pathophysiology and therapeutic options. *EXCLI J.* 2020;19:1017–1037. DOI: [10.17179/excli2020-2591](https://doi.org/10.17179/excli2020-2591)
46. Venkatesan J, Anil S. Marine-derived hydroxyapatite and biomedical applications. *Biotechnol Bioprocess Eng.* 2021;26(3):312–324. DOI: [10.1007/s12257-020-0359-0](https://doi.org/10.1007/s12257-020-0359-0)

تأثير مكملات هيدروكسي أباتيت البقري - الجيلاتين - فيتامين د على تجديد العظام في نموذج فخذ الأرانب المعيوب

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الخلاصة

تُعد العيوب العظمية الناتجة عن الكسور من التحديات السريرية الرئيسة بسبب فقدان استمرارية العظم ومحدودية قدرته على التجدد، ولا سيما في العيوب العظمية ذات الحجم الحرج، وقد يسهم الدعم الحيوي المحاكى للطبيعة، الذي يجمع بين السقالات الموصلة للعظم والمصفوفات العضوية والعوامل المنظمة لتكوين العظم، في تعزيز تجدد النسيج العظمي من خلال تسريع نضج الخلايا المكونة للعظم. هدفت هذه الدراسة إلى تقييم فعالية تركيبة كبسولية تحتوي على الهيدروكسي أباتيت البقري والجيلاتين وفيتامين د في تعزيز تجدد العظام باستخدام نموذج عيب عظمي في عظم الفخذ لدى الأرانب، وذلك بالاعتماد على مؤشرات التكوين العظمي (Runx2) والفسفاتاز القلوية (ALP) والأوستيوكالسين (OCN). شملت الدراسة خمسة عشر أرنبًا ذكرًا سليمًا من السلالة النيوزلندية البيضاء، وجرى تقسيمها إلى ثلاث مجموعات (خمس أرانب في كل مجموعة): مجموعة العيب العظمي (السيطرة)، ومجموعة مكملات الكالسيوم التجارية، ومجموعة الهيدروكسي أباتيت البقري-الجيلاتين-فيتامين د. تم إحداث عيب عظمي قياسي جراحيًا في عظم الفخذ بقطر ٤ ملم وعمق ٦ ملم، وأعطيت المكملات فمويًا لمدة ٢٨ يومًا، ثم جُمعت عينات عظم الفخذ وُحُللت باستخدام تقنية الكيمياء المناعية النسيجية للكشف عن تعبير Runx2 وALP وOCN، كما تم تقييم درجات التفاعل المناعي (IRS) بطريقة شبه كمية، وُحُللت البيانات إحصائيًا باستخدام اختبار Kruskal-Wallis test عند مستوى معنوية ($P < 0.05$). أظهرت مجموعة الهيدروكسي أباتيت البقري-الجيلاتين-فيتامين د ارتفاعًا معنويًا في درجات IRS الخاصة بـ Runx2 وALP وOCN مقارنة بمجموعة السيطرة ($P < 0.05$)، في حين لم تُسجل فروق ذات دلالة إحصائية بينها وبين مكملات الكالسيوم التجارية، مما يشير إلى تقارب الفعالية في تحفيز التكوين العظمي. ويُستنتج أن الجمع بين الهيدروكسي أباتيت البقري والجيلاتين وفيتامين د يسهم بفاعلية في تسريع تجدد العظام من خلال تعزيز التمايز العظمي وتكوين المصفوفة خارج الخلوية ونضج الخلايا البانية للعظم، مما يجعله خيارًا واعدًا لتعزيز التئام العظام بفعالية مماثلة لمكملات الكالسيوم المتوافرة تجاريًا.