IJPST
Indonesian Journal of Pharmaceutical Science and Technology

Research Article
Vol. 13 No. 2 (2026):173-179
https://doi.org/10.24198/ijpst.v13i2.70155

Effects of Platelet Rich Plasma and Hydroxyapatite Implants Derived from Cuttlefish Bone (*Sepia Sp*) on Bone Generation and Osteogenic Activity

Azhar Firdaus[1], Erwin Erwin[2], Sofia Sofia[3,4*], and Zinatul Hayati[5,6]
[1]Master's Program in Biomedical Science, Faculty of Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia.
[2]Laboratory of Clinic and Surgery, Faculty of Veterinary Medicine, Syiah Kuala University.
[3]Department of Public Health, Division of Environmental Health, Faculty of Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia.
[4]Doctor of Medical Science Program, Faculty of Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia.
[5]Infectious Disease Laboratory, Universitas Syiah Kuala, Banda Aceh, Indonesia.
[6]Department of Microbiology, School of Medicine, Universitas Syiah Kuala, Banda Aceh, 23111, Indonesia

Abstract
Bone defects require biocompatible biomaterials that support osteoconduction and tissue regeneration. Hydroxyapatite (HA) derived from cuttlebone (*Sepia sp.*) offers high calcium content and a porous structure with potential as a bone implant, while Platelet Rich Plasma (PRP) contains growth factors that enhance regeneration. This study aimed to evaluate the effects of PRP and cuttlebone HA on bone defect healing in rats using histopathological features. Nine male Wistar rats were divided into three groups: K1 (negative control), K2 (HA + PRP), and K3 (HA). Alveolar bone defects (5 mm in diameter) were surgically created, and treatments were applied accordingly. Samples were evaluated on day 30 using hematoxylin-eosin staining and histomorphometric analysis. Parameters assessed included osteoblast, osteocyte, and osteoclast counts and neovascularization in the implant bone. One-way ANOVA revealed significant differences in osteoblast (p-value =0.000), osteoclast (p-value =0.033), and osteocyte (p-value =0.001) counts across groups. Kruskal-Wallis test showed significant difference in neovascularization (p-value =0.047). Histopathological examination demonstrated no necrosis and increased bone marrow proliferation in the K2 group. This study indicates that cuttlebone-derived hydroxyapatite holds potential as a regenerative implant, particularly when combined with PRP.

Keywords: bone defects, cuttlefish bone, histopathology, hydroxyapatite, platelet rich plasma,

Article History:
Submitted 17 March 2026
Revised 18 April 2026
Accepted 2 May 2026
Published 30 June 2026

*Corresponding author:
sofia@usk.ac.id

Citation:
Firdaus A, Erwin E, Sofia S, Hayati Z. Effects of Platelet Rich Plasma and Hydroxyapatite Implants Derived from Cuttlefish Bone (*Sepia Sp*) on Bone Generation and Osteogenic Activity. Indonesian Journal of Pharmaceutical Science and Technology. 2025 : 13 (2), 173-179.

Efek Pemberian Platelet Rich Plasma dan Implan Hidroksiapatit dari Tulang Sotong (*Sepia sp*)

Abstrak
Defek tulang memerlukan biomaterial yang biokompatibel dan mampu mendukung osteokonduksi serta regenerasi jaringan. Hidroksiapatit (HA) yang berasal dari tulang sotong (*Sepia sp.*) memiliki kandungan kalsium tinggi dan struktur berpori yang berpotensi menjadi implan tulang, sedangkan Plasma Kaya Trombosit (PKT) mengandung faktor pertumbuhan yang dapat meningkatkan regenerasi. Penelitian ini bertujuan menilai pengaruh PKT dan HA tulang sotong terhadap penyembuhan defek tulang pada tikus putih berdasarkan gambaran histopatologi. Sebanyak 9 ekor tikus Wistar jantan dibagi menjadi tiga kelompok: K1 (kontrol negatif), K2 (HA + PKT), dan K3 (HA). Defek tulang alveolar dibuat dengan diameter 5 mm secara operatif dan perlakuan diaplikasikan sesuai kelompok. Sampel dievaluasi pada hari ke-30 menggunakan pewarnaan hematoksilin eosin dan pemeriksaan histomorfometri. Parameter yang diamati meliputi jumlah osteoblas, osteosit, osteoklas dan neovaskularisasi pada tulang implan. Hasil uji One-way ANOVA menunjukkan terdapat perbedaan jumlah sel osteoblas (p-value =0,000), osteoklas (p-value =0,033), dan osteosit (p-value =0,001) antara ketiga kelompok. Uji Kruskal-Wallis menunjukkan perbedaan signifikan neovaskularisasi antar kelompok (p-value =0,047). Pengamatan histopatologis tulang implan menunjukkan tidak terdapat gambaran nekrosis dan peningkatan proliferasi bone marrow pada kelompok K2. Penelitian ini menunjukkan biomaterial hidroksiapatit dari tulang sotong berpotensi dikembangkan sebagai implan regeneratif terutama bila dikombinasikan dengan PKT.

Kata Kunci: defek tulang, hidroksiapatit, histopatologi, tulang sotong, plasma kaya trombosit

1. Introduction

Alveolar bone defects frequently occur due to periodontal disease, trauma, infection, or surgical procedures, leading to loss of structural support and disruption of periodontal tissue function. Periodontitis is the primary cause of alveolar bone destruction driven by chronic inflammation triggered by bacterial infection and dysregulated immune responses. This condition enhances osteoclast activity, impairs osteoblast function, and results in bone defects that are difficult to heal without regenerative intervention.[1–4]

Bone regeneration is a complex mechanism involving interactions among osteoblasts, osteoclasts, osteocytes, immune cells, and various cytokines and growth factors. The bone matrix, composed of collagen and hydroxyapatite crystals, serves as both a structural framework and a substrate for cell adhesion during new tissue formation.[5,6] In extensive bone defects, natural healing capacity is often insufficient, necessitating biomaterials that support osteoconduction and accelerate bone formation.[7]

Hydroxyapatite (HA) has long been used as an implant biomaterial in orthopedics and dentistry due to its biocompatibility, osteoconductivity, and structural similarity to bone mineral. Cuttlebone (*Sepia sp*.) is an emerging natural HA source rich in calcium carbonate, featuring a porous structure that supports cell proliferation and differentiation. Its use offers a cost-effective, and environmentally friendly alternative.[8,9]

Beyond inorganic biomaterials, platelet-rich plasma (PRP) is widely used as a regenerative adjuvant, containing growth factors such as PDGF, TGF-β, VEGF, EGF, IGF-1, FGF, and cytokines that modulate inflammation. These compounds enhance cell proliferation, osteoblast differentiation, angiogenesis, and extracellular matrix formation, thereby accelerating bone healing.[10,11]

Previous studies in white rats demonstrated that combining PRP with carbonate hydroxyapatite accelerates bone defect closure by reducing inflammation and promoting new tissue formation. Similar findings were reported in clinical studies following third molar extraction, where PRP-HA combinations resulted in faster regeneration of hard and soft tissues compared to controls. These results indicate that PRP-HA combinations are superior to PRP or HA alone.[12]

However, research evaluating cuttlebone-derived HA combined with PRP remains limited, particularly in histopathological assessments. Addressing this knowledge gap is essential given the need for effective, accessible, and affordable local biomaterials. Accordingly, this study aims to assess the effects of platelet-rich plasma combined with cuttlebone-derived hydroxyapatite on bone defect healing in white rats through histopathological analysis.

2. Materials and Method

2.1. Tools

This study employed a microtome (Leica 2235®, Germany), light microscope (Olympus®, Japan), slide warmer (Jisico®, Korea), and paraffin section mounting water bath (Electrothermal®, UK). Tissue embedding utilized a paraffin embedding system (Slee Paraffin Embedding Set, PT Rayty Brothers®). Supplementary laboratory tools included tissue cassettes, standard glassware, scalpels, forceps, tweezers, and conventional histopathological equipment.

2.2. Materials

Research materials included: Hydroxyapatite (HA) derived from cuttlebone (*Sepia sp*.), processed through calcination and CaO-H_3PO_4 reaction to form solid implants. Platelet-rich plasma (PRP) was obtained from rat blood via differential centrifugation to achieve a high platelet concentration.

2.3. Methods

The study subjects consisted of 9 male Wistar white rats aged 2–3 months, weighing 150–200 g, obtained from the Animal Experiment Unit, Faculty of Veterinary Medicine, Universitas Syiah Kuala. This research has been approved by the Laboratory Animal Research Ethics Committee, Faculty of Veterinary Medicine, Universitas Syiah Kuala (No. 454/KEPH/XII/2025). All animal housing and surgical procedures were conducted in accordance with the principles of the Guide for the Care and Use of Laboratory Animals.

2.3.1. Hydroxyapatite Implant Preparation

High-quality, clean, and intact cuttlebone obtained from fish markets was selected, weighed (500 g), washed with distilled water to remove debris and organic matter, and dried naturally under sunlight until completely dry (moisture <5%). The dried cuttlebone was ground in a blender or mortar into a fine powder (<100 µm particle size), sieved for homogeneity, and pre-heated in an oven at 200°C for 2 hours to eliminate residual water and organic compounds.

The cuttlebone powder underwent calcination at 1000°C for 3 hours to form calcium oxide (CaO), then reacted with phosphoric acid (H_3PO_4). CaO and H_3PO_4

were weighed and added to a 250 mL chemical glass with 50 mL 98% ethanol, stirred, and heated. H_3PO_4 solution was dripped at 1 mL/min into the CaO-ethanol mixture, stirred and heated at 60°C for 1 hour, then left for 24 hours. The mixture was stirred and heated for 30 minutes, producing a precipitate dried at 100°C. The HA powder was furnace-heated at 400°C, 600°C, and 900°C for 2 hours each to optimize crystallinity and phase. The final product was crushed into 0.5–1 mm implant granules, sterilized by autoclave (121°C, 15 minutes) before surgical application.

2.3.2. PRP preparation

Approximately 10 mL of rat blood was collected using sterile equipment into collection tubes and centrifuged at 100 rpm for 10 minutes to separate the platelet-rich plasma The upper platelet-poor plasma was removed, and PRP underwent secondary centrifugation at 2000 rpm for 10 minutes to pellet platelets. Supernatant plasma was discarded, leaving high-concentration PRP.

2.3.3. Surgical Procedure and Implantation

Rats received premedication, then intramuscular anesthesia (ketamine 50 mg/kg BW, xylazine 5 mg/kg BW). The right mandible was shaved (3×3 cm), disinfected with 70% alcohol, incised (2 cm), and retracted to expose the bone. A 5 mm diameter bone defect was drilled 2 cm from the ear (measured with calipers). Implant materials application.

Group 1 (K1): Defect cleaned with 0.9% NaCl (negative control).
Group 2 (K2): Defect filled with 0.2 mL PRP + 0.1 g cuttlebone HA granules (PRP injected around HA powder placed with forceps).
Group 3 (K3): 0.1 g HA granules only.

This study design specifically excludes a PRP-only group to focus on evaluating the osteoconductive effects of cuttlebone-derived hydroxyapatite (HA) as the primary biomaterial. The three-group comparison (control vs. HA alone vs. HA+PRP) effectively demonstrates HA's standalone regenerative capacity (K3) while assessing the synergistic enhancement provided by PRP growth factors when combined with HA scaffold (K2). This approach isolates HA's scaffold properties (porosity, calcium release, structural support) as the baseline therapeutic intervention, confirming its efficacy independent of bioactive factors before evaluating combination superiority.

Wounds were closed layer with 4-0 resorbable sutures. Post-operative care included 5 days of antibiotics, anti-inflammatory drugs, and vitamin C.

2.3.4. Histopathological Analysis

After 30 days, rats were sacrificed, alveolar bone harvested, fixed, and decalcified. Histological preparations involved fixation, dehydration (graded ethanol 70%-absolute, 1 hour each), clearing (xylene, 3×45 minutes), embedding (paraffin at 60°C for 30 minutes, blocked), sectioning (3 µm rotary microtome), and Hematoxilyn & Eosin staining. Slides were examined under a Meiji Techno microscope (400×), digitally photographed. Parameters assessed were osteoblast, osteoclast, osteocyte.

2.3.5. Data Analysis

Data obtained from histopathological examinations including osteoblast, osteocyte, and osteoclast counts and neovascularization, were analyzed using IBM SPSS Statistics Version 28.0 (IBM Corp., Armonk, NY, USA).Preliminary analysis included the Shapiro-Wilk test to evaluate normality and Levene's test to assess variance homogeneity across groups. For normally distributed data, one-way ANOVA was conducted with a significance threshold of $p < 0.05$, followed by post-hoc Duncan testing when significant. Non-normally distributed data were analyzed using the Kruskal-Wallis test at $p < 0.05$, with the Mann-Whitney U test used as post-hoc analysis.[13]

3. Result

Statistical analysis revealed that normal distributions for osteoblast, osteoclast, and osteocyte data. Data are presented as mean ± standard deviation (mean ± SD). Meanwhile, neovascularization data showed non-normal distribution and are displayed as median (minimum-maximum).

Histopathological analysis using one-way ANOVA demonstrated significant differences in osteoblast, osteoclast, and osteocyte parameters, while the Kruskal-Wallis test also revealed significant differences in neovascularization parameters.

Table 1 shows day-30 histopathological examination results, with the lowest average osteoblast count in K2 (PRP+HA), followed by K3 (HA) and K1 (negative control) in the first row. Meanwhile, the highest mean values for average osteoclasts, osteocytes, and neovascularization were observed in K3 (HA), followed by K2 (PRP+HA) and K1 (negative control) in the second, third, and fourth rows, respectively.

Post-hoc Duncan test results for osteoblasts showed significant differences between K2 (PRP+HA) and K3 (HA) groups compared with the K1 (negative control). Duncan post-hoc analysis for osteoclasts revealed

differences between K3 (HA) and both K1 (negative control) and K2 (PRP+HA). The Duncan test for osteocytes showed significant differences between K3 (HA) and K2 (PRP+HA) compared with K1 (negative control). Mann-Whitney test for neovascularization parameters showed significant differences between K3 (HA) and K1 (negative control).

Figure 1 represents the histopathological findings of implant bone tissue across treatment groups. Observations assessed cellular activity in bone healing, including the presence and distribution of osteoblasts, osteoclasts and osteocytes, as well as neovascularization. Histological differences between groups reflect the influence of PRP and HA combination on the bone regeneration process.

4. Discussion

Alveolar bone healing is a dynamic process involving inflammation, proliferation, differentiation, angiogenesis, and remodeling, culminating in mature bone formation. In this study, cuttlebone hydroxyapatite (HA) served as an osteoconductive scaffold, while platelet-rich plasma (PRP) provided growth factors to enhance regeneration. Thus, histopathological findings should be interpreted not only based on cell quantity but also according to the healing phase at the time of observation.

Evaluation of osteoblasts, osteoclasts, osteocytes, and neovascularization represents key aspects of bone formation, resorption, maturation, and vascularization, thereby demonstrating the effectiveness of PRP combined with cuttlebone HA in alveolar bone regeneration.

4.1. Osteoblasts

Osteoblasts physiologically appear during weeks 1-2 of bone healing. The low osteoblast count in K2 (PRP+HA) indicates that PRP combined with HA accelerates bone regeneration. This aligns with PRP's mechanism, obtaining PDGF, TGF-β, IGF, and other mediators that promote early proliferation while accelerating differentiation and mineralization, rapidly advancing the formation phase toward maturation.[14,15] Karakayali et al. (2022) found that PRP administration in rabbits at week 2 significantly increased bone density ($p<0.001$), confirming that PRP enhances osteogenesis and bone mineral density during healing.[16]

HA inhibits progression on bone defect through osteoconduction, osteoinduction, and controlled biodegradability, the thereby supporting natural bone regeneration. Growth factors like FGF-2 further enhance HA's role in fibroblast proliferation during bone and surrounding tissue regeneration.[17]

Cuttlebone HA regeneration begins in the early phase (0-7 days) with osteoblast adhesion and proliferation on scaffold surfaces, marked by increased type I collagen expression from CHA pore mechanical stimulation. The intermediate phase (7-21 days) features osteoblast differentiation with elevated alkaline phosphatase (ALP) and osteocalcin activity, supported by new vascularization for osteogenesis nutrition. During remodeling (21-56 days), HA degrades slowly (<1%

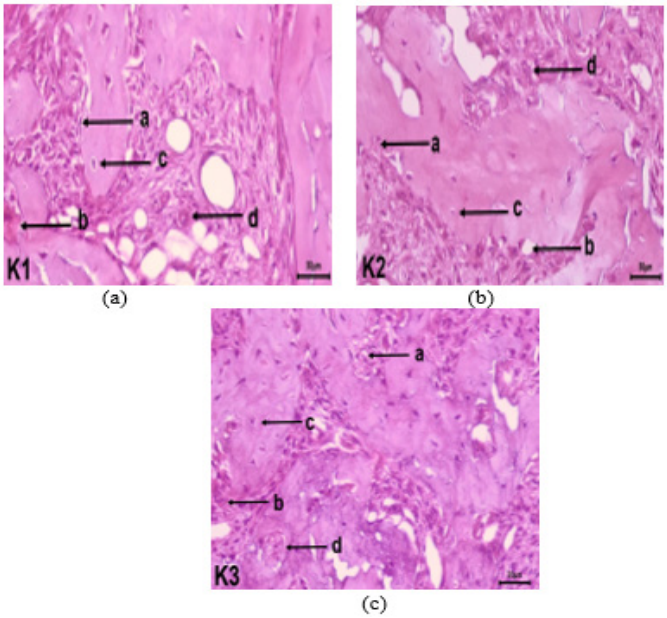

Figure 1. Histopathological Features of Implant Bone. Groups K1 (Negative Control), K2 (Platelet Rich Plasma + Hydroxyapatite), K3 (Hydroxyapatite). (a) Osteoblasts, (b) Osteoclasts, (c) Osteocytes, (d) Neovascularization. Hematoxylin & Eosin (H&E) staining at 400× magnification.

per week) and is gradually replaced by woven bone maturing into lamellar bone via endosteal osteoblast activity, forming complete functional Haversian systems.[18]

Previous studies show nano-HA (nHA) effectively manages osteoporosis through osteoinductive mechanisms, stimulating osteoblast proliferation, differentiation, and bone mineralization. nHA enhances mineral deposition in resorption areas, inhibits excessive osteoclast activity, and accelerates osteoporotic fracture healing via porous scaffolds supporting bone healing. Combining nHA with chitosan or mesenchymal stem cells significantly increases osteocyte density and trabecular bone volume in rat osteoporosis models.[19]

HA heated at 900°C optimizes bone regeneration with pure crystallinity, high porosity (100-600 µm pores), and superior bioactivity supporting osteoblast proliferation and osseointegration. Cuttlebone calcined at 900°C produces pure HA phase (XRD-confirmed) with 4.36 MPa compressive strength suitable for alveolar spongiosa bone, outperforming <900°C (cytotoxic) or >1000°C (low porosity).[20]

The low osteoblast count in K2 (PRP+HA) at day 30 suggests most osteoblasts have differentiated into mature osteocytes, resulting in fewer visible active osteoblasts histologically. This aligns with PRP accelerating transition from early callus to lamellar bone maturation, resulting in faster osteoblast dynamics than HA alone or control. Additionally, 900°C-calcined cuttlebone HA provides a stable, alveolar bone-compatible mineral framework, enabling PRP growth factors to function effectively within a microenvironment that support cell adhesion, proliferation, and differentiation. Thus, PRP+cuttlebone HA combination accelerates alveolar bone defect healing while improving tissue maturity, positioning it as a promising implant candidate for future oral surgery and dental implant procedures.

4.2. Osteoclast

High osteoclast counts in K3 indicate HA's standalone effectiveness in supporting osteogenic differentiation, though studies show PRP+growth factor combinations produce superior final bone quality compared to HA alone.[18,21]

Previous research supports the idea that PRP+cuttlebone HA reduces osteoclast numbers by creating a more progressive healing environment that promotes bone maturation and stabilization, reducing remodeling needs. PRP stimulates growth factors, accelerating callus maturation, bone marrow formation, and vascularization, so by day 56 sampling, intensive remodeling has passed, and osteoclast activity relatively declines.[22]

High K3 (HA) osteoclast counts indicate active bone resorption at day 30. Porous cuttlebone HA scaffolds (100-600 µm) require osteoclasts for resorption and integration with native bone, hence higher activity than controls. Conversely, K2 (PRP+HA) shows fewer osteoclasts, as PRP growth factors (PDGF, TGF-β) accelerate callus maturation and matrix strengthening, reducing early resorption some osteoclasts already apoptotic or inactive. A day-14 study found increased osteoblasts and decreased osteoclasts in HA implants used as sockets post-mandibular incisor extraction.[23] This matches natural patterns where HA temporarily triggers osteoclast formation via RANKL/OPG balance, while PRP creates a bone formation-supportive environment preventing excessive resorption.[24] These differences prove PRP+HA superiority for alveolar bone clinical applications by achieving optimal osteoblast-osteoclast balance, producing faster-maturing, mechanically stronger regenerated bone without excessive resorption risk.

4.3. Osteocytes

Cuttlebone hydroxyapatite (HA) supports osteoblast formation and differentiation into osteocytes—mature bone cells essential for tissue maintenance. As a calcium phosphate mineral mimicking bone's inorganic component, HA is osteoconductive, promoting osteoblast and osteocyte differentiation, making it ideal for mandibular bone defect implants.[25]

Studies show cuttlebone HA enhances osteoblast proliferation and bone matrix mineralization, accelerating osteoblast-to-osteocyte transformation during regeneration. Its natural pore structure facilitates optimal osteocyte growth and the formation of new bone. Thus, cuttlebone HA effectively supports osteocytes and comprehensive bone regeneration.[22]

PRP+HA combination accelerates organized bone formation and defect stability, reducing stimuli for reactive bone spicule (osteocyte) formation. Consequently, osteocyte counts/scores may be lower than HA-alone groups still in reactive callus and coarser remodeling phases.[18]

High K3 (HA) osteocyte averages reflect cuttlebone HA's dominant osteoconductive role, providing stable scaffolds for osteoblast-to-mature osteocyte differentiation, particularly during day-30 callus and remodeling phases. Meanwhile, K2 (PRP+HA) more rapidly enters organized bone maturation, reducing reactive bone spicule formation stimuli with relatively

lower reactive osteocytes counts than standalone HA, though histological bone tissue quality is more mature and near-normal.[23,26] This confirms cuttlebone HA as the primary osteoconductive framework, with PRP as a biological modulator that accelerates the transition from reactive callus formation to stable, functional lamellar bone.

4.4. Neovascularization

Studies show sea-derived hydroxyapatite (including cuttlebone) is osteoconductive and stimulates angiogenesis by increasing VEGF and osteonectin expression—critical for new blood vessel formation, including neovascularization during bone healing.[27]

Previous research demonstrates that cuttlebone HA more effectively stimulates new bone growth and implant bone apposition than raw cuttlebone due to its porous structure (100-600 µm), facilitating new blood vessel growth.[22]

Early HA-alone groups trigger extensive neovascularization within the callus, meeting the immature tissue nutritional needs, as evidenced by increased blood vessels in histology sections.[18] PRP addition accelerates inflammation and new vessel formation with faster callus maturation. At day-30 evaluation, tissue resembles normal bone with fewer visible new vessels as some undergo remodeling into organized haversian systems with smaller lumen.[22] Clinical PRP heterogeneity also underscores documenting platelet concentration and centrifugation methods for cross-study comparisons.[28]

High K3 (HA) neovascularization averages reflect an active, relatively immature callus phase with high blood supply needs, a triggering extensive, unorganized capillary tissue formation. Conversely, K2 (PRP+HA) shows histologically fewer new vessels, likely reflecting faster maturation, with some neovascularization transformed into organized Haversian systems with smaller lumens not fully counted as “new vessels” in semiquantitative scoring. Thus, quantitative neovascularization differences between K3 and K3 and K2 do not simply indicate standalone HA is “better,” but reflect dominant day-30 bone healing phases—K3 in active vascular callus phase, K2 shifted toward remodeling/maturation resembling normal bone vascular patterns. This supports PRP+HA’s role in accelerating the transition from reactive neovascularization to ward mature, functional bone tissue beyond merely increasing new vessel numbers.

5. Conclusion

This study demonstrates that the combination of platelet-rich plasma (PRP) and cuttlebone-derived hydroxyapatite (HA) provides the most effective outcome in accelerating bone defect healing in white rats. This combination enhances osteoblast, osteoclast, and osteocyte differentiation, neovascularization, reduces necrosis, and increases bone marrow proliferation in the implant bone. The PRP and cuttlebone HA combination shows great potential as a regenerative biomaterial for bone defect healing. For future research, we recommend biomechanical evaluation of regenerated bone strength, long-term studies (>90 days) to assess lamellar bone stability, phase I clinical trials in humans for periodontal applications, and optimization of PRP concentration with standardized platelet counting to enhance inter-study reproducibility.

Conflict of Interest

The authors declare no conflicts of interest, either financial or non-financial, that could have affected the execution or interpretation of this research.

Reference

1. Lang NP, Bartold PM. Periodontal health. 2017;(May):9-16.
2. Wulandari P. The aging process and its relation to periodontal conditions. Explor Immunol. 2023 Jun;3:207–16.
3. Usui M, Onizuka S, Sato T, Kokabu S, Ariyoshi W, Nakashima K. Mechanism of alveolar bone destruction in periodontitis — Periodontal bacteria and inflammation. Jpn Dent Sci Rev. 2021 Nov;57:201–8.
4. Helmi MF, Huang H, Goodson JM, Hasturk H, Tavares M, Natto ZS. Prevalence of periodontitis and alveolar bone loss in a patient population at Harvard School of Dental Medicine. BMC Oral Health. 2019 Dec;19(1):254.
5. Soliman AM, Barreda DR. Acute Inflammation in Tissue Healing. Int J Mol Sci. 2023;24(1):641
6. Yang N, Liu Y. The Role of the Immune Microenvironment in Bone Regeneration. Int J Med Sci. 2021;18(16):3697–707.
7. Khotib J. Acceleration of Bone Fracture Healing through the Use of Natural Bovine Hydroxyapatite Implant on Bone Defect Animal Model. 2019;55(3).
8. González JA, Vallejo JR. The Use of Shells of Marine Molluscs in Spanish Ethnomedicine: A Historical Approach and Present and Future Perspectives. Pharmaceuticals (Basel). 2023;16(10):1503.
9. Battistella E, Mele S, Foltran I, Lesci IG, Roveri N, Sabatino P, et al. Cuttlefish Bone Scaffold for Tissue Engineering: A Novel Hydrothermal Transformation, Chemical-Physical, and Biological Characterization. J Appl Biomater Funct Mater. 2012 Jan;10(2):99–106.
10. Bacevich B, Smith R, Reihl A, Mazzocca A, Hutchinson I. Advances with Platelet-Rich Plasma for Bone Healing. Biol Targets Ther. 2024 Jan;Volume 18:29–59.
11. Kaushik A, Kumaran MS. Platelet-Rich Plasma: The Journey so Far! Indian Dermatol Online J. 2020;11(5):685–92.

12. Jennes D, M. Philip L, K. Venugopal S, Anoop S, S. Devi S, et al. Radiographic evaluation of healing of critical size calvarial defect treated with silica-collagen-hydroxyapatite biocomposite graft and homologous platelet-rich plasma in rat model. J Vet Anim Sci. 2025;56(4):593–9.
13. Hamada C. Statistical analysis for toxicity studies. J Toxicol Pathol. 2018;31(1):15–22.
14. Chen J, Liu B, He J, Wei Y, He Y, Zhou H, et al. Multimodal mechanisms of platelet-rich plasma in bone defect repair: Angiogenesis, inflammation modulation, and metabolic regulation.. Regen Ther. 2025;30(October):920–32.
15. Rodriguez IA, Kalaf EAG, Bowlin GL, Sell SA. Platelet-Rich Plasma in Bone Regeneration : Engineering the Delivery for Improved Clinical Efficacy. 2014;2014:392398.
16. Karakayal M. Effect of platelet-rich plasma on bone regenerate consolidation in distraction osteogenesis : An experimental study in rabbits. 2022;56(1):8–13.
17. Widiyanti P. Sintesis dan Karakterisasi Komposit Hidroksiapatit dari Tulang Sotong (Sepia sp.)-Kitosan untuk Kandidat Aplikasi Bone Filler. Jurnal Fisika dan Terapannya. 2013;1(2):82–96.
18. Al-rawe RA, Al-rammahi HM, Cahyanto A, Ma A, Liew YM. Cuttlefish-Bone-Derived Biomaterials in Regenerative Medicine, Dentistry, and Tissue Engineering: A Systematic Review. 2024;1–40.
19. Febriani R, Reindah JJ, Aprilia NA. Efektivitas Nano-Hydroxyapatite Sebagai Upaya Kuratif Fracture Osteoporosis Melalui Mekanisme Osteokonduktif Bone Healing (Scaffold): Tinjauan Sistematis. JIMKI. 2025;11(2):31–44.
20. Supardi A, Nisa ZI, Hikmawati D. Synthesis of Nanohydroxyapatite from Cuttlefish Bone (Sepia sp.) Using Milling Method. 2019;2019.
21. Aminatun A, Handayani DEF, Widiyanti P, Winarni D, Siswanto S. In vivo approach on femur bone regeneration of white rat (Rattus norvegicus) with the use of hydroxyapatite from cuttlefish bone (Sepia spp.) as bone filler. Vet World. 2019 Jun;12(6):809–16.
22. Mansouri K, Fattahian H, Mansouri N. The Role of Cuttlebone and Cuttlebone Derived Hydroxyapatite with Platelet Rich Plasma on Tibial Bone Defect Healing in Rabbit : An Experimental Study Tavşanlarda Mürekkepbalığı Kabuğu ve Kan Pulcuğundan Zengin Plazma İle Birlikte Mürekkep Balığı Kaynaklı. 2018;24(1):107–15.
23. Widarena R, Marlie H, Rubianto M, Setiawatie EM. Number of Osteoblasts and Osteoclasts in Combination Carbonate Hydroxyapatite , Platelet Rich Fibrin (PRF) and Antioxidant in Socket Rats Wistar After Tooth Extraction. 2021;4(1):15–8.
24. Setyoko B, Lufhf A, Verisandri A, Damayanti AT, Fitriana FA. Effect of Carbonated Hydroxyapatite Synthesis From Cuttlefish Shells on Orthodontic Relapse Prevention: In Silico Study. 2023;10(1):19–27.
25. Kennedy MM, Mikos AG. Biomaterials-based Approaches to Mandibular Tissue Engineering: Where We Were, Where We Are, Where We Are Going. Regen Biomater. Apr 2025;12:rbaf024.
26. Pang B, Xian J, Chen J, Ng L, Li M, Zhao G, et al. Cuttlefish Bone-Derived Calcium Phosphate Bioceramics Have Enhanced Osteogenic Properties. J Funct Biomater. 2024;15(8):212.
27. Ashrin MN, Fitriana A, Nuriyanto AF, Ariani MD, Kridanto MJ. The Potential Effect Of Applying Scaffold Hydroxyapatite Derived From Portunus Pelagicus Crab Shells On Osteonectin And VEGF-B Expression.. 2025;15(06):234–9.
28. Bacevich BM, David R, Smith J, Reihl AM, Mazzocca AD, Hutchinson ID. Biologics : Targets and Therapy Advances with Platelet-Rich Plasma for Bone Healing. 2024.