ORIGINAL ARTICLE

Effect of apatite cement on osteoblast cell numbers in alveolar sockets after tooth extraction in rabbits: an experimental study

Andi Supriatna[1]
Rina Putri Noer Fadilah[2]
Zwista Yulia Dewi[3]
Nindya Yuniar[4]
Dinda Kurnia Fitri[5]

[1]Department of Periodonsia, Faculty of Dentistry, Universitas Jenderal Achmad Yani, Indonesia
[2]Departement Dental Public Health, Faculty of Dentistry, Universitas Jenderal Achmad Yani, Indonesia
[3]Department of Dental Material, Faculty of Dentistry, Universitas Jenderal Achmad Yani, Indonesia
[4]Undergraduate student, Faculty of Dentistry, Universitas Jenderal Achmad Yani, Indonesia
[5]Department of Oral & Craniofacial Sciences, Faculty of Dentistry, University Malaya

*Correspondence:
andi.supriatna@lecture.unjani.ac.id

Received: 11 May 2026
Revised: 20 June 2026
Accepted: 20 July 2025
Published: 31 July 2026
DOI:10.24198/pjd.vol38no2.70901.

p-ISSN 1979-0201
e-ISSN 2549-6212

Citation:
Supriatna, A, Fadilah, RPN, Dewi, ZY, Yuniar, N, Fitri, DK. Effect of apatite cement on osteoblast cell numbers in alveolar sockets after tooth extraction in rabbits: an experimental study. Padjajaran J. Dent. July. 2026; 38(2): 255-267.

ABSTRACT

Introduction: Tooth extraction damages soft and hard tissues, followed by alveolar bone resorption, which can interfere with the healing process and prosthetic rehabilitation. One effort to minimize alveolar bone resorption is the use of graft materials such as apatite cement, which is biocompatible and osteoconductive. This study aimed to analyze the effect of apatite cement on osteoblast cell numbers during alveolar socket healing following tooth extraction in rabbits. **Methods:** This study used a true experimental design with a post-test-only control group design. The study objects were biological materials placed in the alveolar sockets of New Zealand White rabbits, which were divided into a control group, a tooth extraction group without apatite cement administration, and a tooth extraction group with apatite cement administration. Histological observations were conducted on days 3, 7, and 14, representing the inflammatory, proliferative, and early remodeling phases of rabbit socket healing, respectively. The data were analyzed statistically using normality and homogeneity tests, followed by the one-way ANOVA statistical test. **Results:** The mean osteoblast cell counts in the apatite cement group were significantly higher than those in the control group on days 3, 7, and 14. The highest osteoblast count was observed on day 14, with mean values of 28.93 ± 3.57 cells/field in the apatite cement group compared with 0.80 ± 0.35 cells/field in the control group ($p < 0.05$). One-way ANOVA demonstrated significant differences among the experimental groups. **Conclusion:** Apatite cement administration increased osteoblast cell numbers in the alveolar socket after tooth extraction in rabbits, suggesting its potential to promote early osteogenic activity during the initial stages of socket healing.

KEYWORDS

Alveolar socket, apatite cement, osteoblasts, rabbits, tooth extraction.

INTRODUCTION

Tooth extraction is the procedure of removing one or more teeth from their sockets.[1] According to the 2023 Indonesian Health Survey (SKI), 31.9% of the Indonesian population has undergone tooth extraction.[2] Indications for tooth extraction include teeth that cannot be restored, cavities, periodontal disease, persistent deciduous teeth, dental abscesses, impacted teeth, teeth with increased mobility, orthodontic treatment, patients requiring prosthetic rehabilitation, dental trauma, and tumors.[1]

According to research conducted by Dewi et al., periodontal disease was the most common indication for tooth extraction, accounting for 1,465 teeth (67.67%).[3] The tooth extraction process causes damage to both soft and hard tissues.[4] Bone resorption is a natural physiological process that occurs after tooth extraction and cannot be avoided. Horizontal and vertical alveolar ridge bone loss of 30% occurs within the first 6 months, and continues until reaching approximately 50% loss within 12 months post-extraction.[5] This alveolar bone loss

affects aesthetics, function, quality of life related to oral health, and may hinder prosthetic tooth replacement therapy due to alveolar bone resorption following tooth extraction.[6]

Socket preservation is a procedure performed on the tooth socket by placing graft material after tooth extraction to reduce bone and soft tissue loss.[7] Bone graft material serves as an additive that promotes the formation of natural bone and new bone growth, thereby aiding bone regeneration.[8] Generally, there are four types of bone grafts: alloplast, allograft, autograft, and xenograft.[9] Alloplast is a material commonly used for bone grafting.[10]

Alloplast materials are synthetic substances designed to contain organic and inorganic components in proportions nearly identical to human bone.[11] This material has standardized quality, is free from the risk of infection, and does not require additional surgical intervention compared to allografts, xenografts, and autografts.[11-12]

One type of alloplastic material is apatite cement, a self-setting paste-like material consisting of a mixture of calcium phosphate and liquid, which, after hardening, forms hydroxyapatite (HAp:$Ca_{10}(PO_4)_6(OH)_2$). This material has excellent flow properties, allowing it to fill bone defects in narrow and irregular spaces. Apatite cement is a biocompatible material, meaning that it can be absorbed by the body without causing harmful reactions and can degrade over time, being replaced by natural bone tissue. This material is also osteoconductive, aiding in the growth of new bone by stimulating osteoblast cells.[13-14]

The success of bone grafting is marked by its ability to stimulate osteoblast activity. A bone graft is expected to serve as a suitable substrate for the attachment of pre-osteoblasts, which will differentiate into osteoblasts.[15] Osteoblasts originate from osteoblastic proliferation and develop from bone marrow mesenchymal cells. Osteoblasts produce and regulate the bone matrix and play a role in mineralization during bone remodeling.[16]

Previous studies have demonstrated that apatite cement possesses the ability to promote cell proliferation and adhesion, thereby enhancing osteoblast activity.[17] The osteoconductive properties of apatite cement provide a framework or scaffold compatible with the composition of new bone surrounding the bone graft, thereby supporting the differentiation of mesenchymal cells to grow along the surface of the graft material.[13]

Previous studies have shown that the application of apatite cement increases the trabecular thickness of rabbit alveolar bone after extraction compared to that in rabbits without apatite cement application. The osteoconductive properties of apatite cement provide a favorable scaffold for cell attachment and bone ingrowth, thereby supporting the healing process without directly inducing osteogenic differentiation.[15-17]

Previous studies have demonstrated that apatite cement is a biocompatible and osteoconductive graft material that supports alveolar bone healing following tooth extraction. However, evidence regarding its effect on osteoblast cell numbers during the different stages of alveolar socket healing remains limited.

The novelty of this study lies in the temporal histological evaluation of osteoblast cell numbers on days 3, 7, and 14 after tooth extraction, representing the inflammatory, proliferative, and early remodeling phases of socket healing in New Zealand White rabbits. This approach provides a better understanding of the biological response to apatite cement throughout the healing process. Therefore, this study aimed to analyze the effect of apatite cement administration on osteoblast cell numbers in the rabbit alveolar sockets following tooth extraction.

METHODS

This study was a true experimental study conducted at the Laboratory of the Faculty of Health Sciences, Universitas Jenderal Achmad Yani, Indonesia, using stored biological specimens obtained from a previous true experimental study.

Histological observations of osteoblast cell numbers in the alveolar sockets were performed on specimens collected on days 3, 7, and 14 following tooth extraction to evaluate the effect of apatite cement on the early stages of alveolar socket healing.

The specimens consisted of alveolar sockets from adult New Zealand White rabbits that had been fixed in 10% neutral buffered formalin. The inclusion criteria were stored biological specimens with complete documentation (including the date of specimen collection), documented consent from the previous study, and specimens adequately preserved in 10% neutral buffered formalin. Specimens contaminated with microorganisms or foreign materials, as well as damaged or incomplete specimens, were excluded from the study.

The sample size for each treatment group was calculated in a previous study using Mead's formula. In this study, three groups were used, consisting of biological materials from rabbit alveolar sockets without treatment, biological materials from rabbit alveolar sockets following tooth extraction but without administration of apatite cement, and biological materials from rabbit alveolar sockets following tooth extraction and administration of apatite cement.

The sample size for this study was determined using Mead's formula obtained from previous studies as follows: $E = N - T$, where E represents the degrees of freedom for error in the analysis of variance (ANOVA), N represents the total number of experimental animals, and T represents the number of treatment groups. The value of E is recommended to be within the range of 10 and 20 ($10 < E < 20$); if E is less than 10, the number of test animals is considered insufficient, which may reduce the statistical power of the study, whereas if E is greater than 20, the number of animals will not provide a significant increase in the statistical significance of the results, thus contradicting the principles of scientific ethics.[17]

In this study, three treatment groups were included: (1) an untreated control group representing normal alveolar bone, (2) an extraction-only group without apatite cement administration, and (3) an extraction group receiving apatite cement. The untreated control group was included to provide baseline histological characteristics of normal alveolar bone and to allow a comprehensive comparison of osteoblast cell counts among normal conditions, socket healing following tooth extraction, and socket healing with apatite cement administration. The sample size was determined using Mead's formula ($E = N - T$), where E represents the error degree of freedom for error, N represents the total number of experimental animals, and T represents the number of treatment groups. Three treatment groups were included in this study ($T = 3$), and the value of E was set to 15, which fell within the recommended range of 10–20. Based on this calculation, the minimum sample size required was 18 animals.

Based on calculations using Mead's formula, the sample size for this study was determined to be a minimum of 18 animals. The sampling technique used in previous animal studies was simple random sampling, in which the selection process ensured that all subjects had an equal chance of receiving treatment. The independent variable in this study was the administration of apatite cement, and the dependent variable was the number of osteoblasts present in the alveolar socket.

Apatite cement consists of tetracalcium phosphate anhydride and dicalcium phosphate anhydride (TTCP and DCPA, respectively) mixed with a $Na_{18}H_{12}PO_4$ solution. An analytical balance was used to measure the weight (in grams) and weight ratios. Osteoblasts are mononuclear cells derived from mesenchyme. These cells are found on the surface of bone tissue undergoing growth because osteoblasts play a role in bone matrix formation, so these cells are found on the surface of bone tissue. Osteoblasts are arranged in a single layer with a cuboidal or short cylindrical shape, featuring a round nucleus and basophilic cytoplasm at the apical end of the cell, and the Golgi complex is located in the basal region.[18]

Osteoblast measurements were performed using a light microscope, yielding cell counts and cell count ratios. The equipment used in this study included an

autoclave, tissue labels, a closed decalcification container, anatomical forceps, tissue cassettes, a base mold, an incubator or paraffin oven, beakers, a microtome, microscope slides, and an Olympus CX23 light microscope. The materials used in this study included masks, gloves, stored biological materials, 10% neutral formalin, 10% EDTA, litmus paper or a pH meter, absolute alcohol (100% ethanol), 96%, 95%, 80%, 70% ethanol, xylene, paraffin, ethanol, and Hematoxylin and Eosin (HE).

The biological material consisted of alveolar sockets from New Zealand White rabbits that had been immersed in 10% neutral formalin. In the previous study, the following procedures were performed: The instruments used were cleaned and sterilized using an autoclave, which is a sterilization device that uses steam under pressure at 121°C for 30 min. The cleaned instruments were then placed in sterilization pouches meeting American Dental Association (ADA) standards and packaged to prevent contamination.[19]

The preparation of apatite cement involved mixing equal molar (equimolar) amounts of (TTCP ($Ca_4(PO_4)_2O$) and (DCPA ($CaHPO_4$). Equimolarity is derived from the formula Molarity = g/Mr, where Mr is the relative molecular mass of TTCP and DCPA. DCPA was produced by heating DCPD at 300°C for 40 min. The heating process aimed to remove the water of crystallization present in DCPD, according to reaction $CaHPO4{\cdot}2H2O$ (DCPD) → CaHPO4 (DCPA). TTCP was produced by heating a mixture of DCPA and calcium carbonate ($CaCO_3$) in a 1:1.27 weight ratio at 1500°C for 6 h in an oven, followed by cooling at room temperature in a desiccator to obtain the synthesized product: $2CaHPO_4 + 2CaCO_3 \rightarrow Ca_4(PO_4)_2O + H_2O+2CO_2$.

TTCP was ground to a particle size of 17 µm, and DCPA was ground to a particle size of 1 µm using ethanol. Subsequently, the samples were dried in a dehydrator at 80°C. The final yields of the two products were 1.36 g of DCPA and 3.66 g TTCP, which were mixed and sterilized with gamma rays, and the apatite cement powder was stored in a desiccator.[14-15] Sample preparation involved preparing a 0.2 M $Na_{18}H_{12}PO_4$ solution by mixing 0.2 mol/L Na_2HPO_4. Next, 30 µL of liquid with 90 mg of powder on a mixing glass an L/P ratio of 0.3, and stirred with a spatula. When mixed with water, the material forms a moldable paste that begins to set after 15–17 min. Mixing of the apatite cement and SCPC was performed by stirring until homogeneous for 30 seconds.[13]

The test animals were 5–7 month-old New Zealand White rabbits that had been acclimated to the laboratory environment for one week. New Zealand White rabbits were selected because they are widely used in bone regeneration and biomaterial studies owing to their relatively large size, ease of handling, rapid bone turnover, and well-characterized healing patterns. Their alveolar bone anatomy and remodeling characteristics make them a suitable model for evaluating early socket healing following tooth extraction in humans. The rabbits were fed a standard diet of commercial feed and Reverse Osmosis (RO) drinking water ad libitum daily.[23]

The rabbit tooth extraction process began with tissue disinfection using 70% alcohol, followed by general anesthesia via intramuscular injection of 10% ketamine at a dose of 20–40 mg/kg body weight and xylazine at a dose of 3–5 mg/kg body weight to provide analgesic and sedative effects.[20-21] Tooth extraction was performed on mandibular incisors using deciduous extraction forceps and a periodontal elevator to separate the connective tissue attached to the tooth, followed by luxation using careful unidirectional motion to avoid root fracture. The extraction socket was irrigated with saline solution, and bleeding was controlled with sterile gauze.[21-22]

Apatite cement bone graft material was applied according to the test group to the extraction socket site. The application is performed once bleeding has ceased and the socket is clean. The apatite cement was applied to the alveolar socket using an Ash 49 or a dental irrigation syringe by first loading the paste-form apatite cement into the syringe, then inserting the syringe tip into the socket,

and gently pushing with the plunger until set. The amount of material applied was approximately 3 mg until the alveolar socket was filled. The test animals were administered CO_2 gas and then anesthetized intramuscularly with a lethal dose of ketamine. Rabbit death was confirmed by the absence of respiration. Decapitation was performed by cutting the lower jaw of the rabbit at the site of the socket extraction using a small saw.[23-25]

The preparation of histological specimens began with initial fixation, which involved preserving the cellular structure of the tissue using a 10% neutral formalin solution. The tissue was decalcified with 10% EDTA at a pH of 7.0–7.4 in distilled water or aquades in a sealed container, which was then incubated at room temperature (±25°C) for 5–7 d, depending on the size of the tissue and the concentration, until the tissue softened. Decalcification was performed to soften the tissue; the tissue was ensured to be soft by pressing it with a needle or forceps or by using a chemical method involving the addition of 5% ammonium oxalate; if a white precipitate (Ca-oxalate) appeared, decalcification was not yet complete.

After decalcification was complete, the tissue was rinsed under running water for 6–12 h to remove residual EDTA and prevent tissue damage caused by residual acid. The tissue was vertically cut at the center of the socket using a scalpel. Dehydration was performed by immersion in 70% alcohol for 45 min, 80% alcohol for 45 min, and 96% alcohol for 15 min, carried out in stages twice, and 100% (absolute alcohol) for 30 min in stages using absolute alcohol I, II, and III.

Next, clearing was performed with xylene to remove the residual alcohol solution, which was performed three times in three separate tubes for 45 min each. Before embedding, infiltration was performed to remove the clearing solution and prevent tissue structure damage during sectioning using paraffin I and paraffin II. The tissue was placed into a base mold, liquid paraffin was poured into the mold with the cassette placed on the surface, and it was allowed to harden, forming a paraffin block. Tissue sections were prepared using a microtome in the vertical direction with a thickness of 4–6 mm. The sections were smoothed onto a glass slide by soaking in warm water in a beaker at a temperature of approximately 40–45°C.[23,26]

Before staining, deparaffinization was performed by soaking in xylene to remove residual paraffin so that the stain could be properly absorbed. Soaking in xylene was performed in stages using Xylene I and Xylene II for 5 min, followed by rehydration using alcohol, absolute alcohol, 95%, and 70%. Next, the tissue was soaked in distilled water for 30 s to achieve maximum rehydration. The tissue specimens were stained with hematoxylin for 10 min to stain the cell nuclei, making them appear blue-violet, followed by bluing under running water to ensure the subsequent staining remained stable.

The specimen was re-stained with eosin to stain the cytoplasm pink to red for 1 min. After staining was complete, dehydration was performed with 70%, 95%, and absolute alcohol to remove water from the tissue so that it appeared transparent. The specimens were mounted in Entellan to protect the tissue and ensure that the specimens remained stable and durable, and were dried in a paraffin oven.[27]

Histological sections were stained with hematoxylin and eosin (H&E) and examined under a light microscope at magnification of 40x, 100x, and 400x. The number of osteoblasts was evaluated at 400x magnification across five fields of view during bone remodeling.[23,26] Osteoblasts were identified based on their characteristic morphology, appearing as mononuclear cuboidal cells with basophilic cytoplasm and eccentrically located nuclei lining the surface of the newly formed bone trabeculae. Cell identification and counting were performed according to the established histological criteria.

The data obtained by the researchers were analyzed using statistical tests with the assistance of IBM SPSS Statistics 25 software. In this study, because the number of samples tested was <50, the Shapiro-Wilk test was used for normality,

and Levene's test was used for homogeneity. The analysis used was a One-Way ANOVA test, yielding $p<0.05$, indicating an effect of the treatment.

This study used stored biological material in the form of alveolar bone from New Zealand White rabbits. The stored biological material (rabbit alveolar bone) was used to investigate the effect of apatite cement on the number of osteoblasts in the alveolar socket following rabbit tooth extraction. Observations were conducted on days 3, 7, and 14, followed by histological examination to determine the osteoblast count.

RESULTS

A total of 18 rabbit alveolar bone specimens were evaluated in this study. Histological observations revealed differences in osteoblast cell numbers between the untreated and apatite cement-treated groups during the observation period. Representative histological sections are presented in Figure 1, and the quantitative analysis of osteoblast cell counts is shown in the subsequent figures and tables.

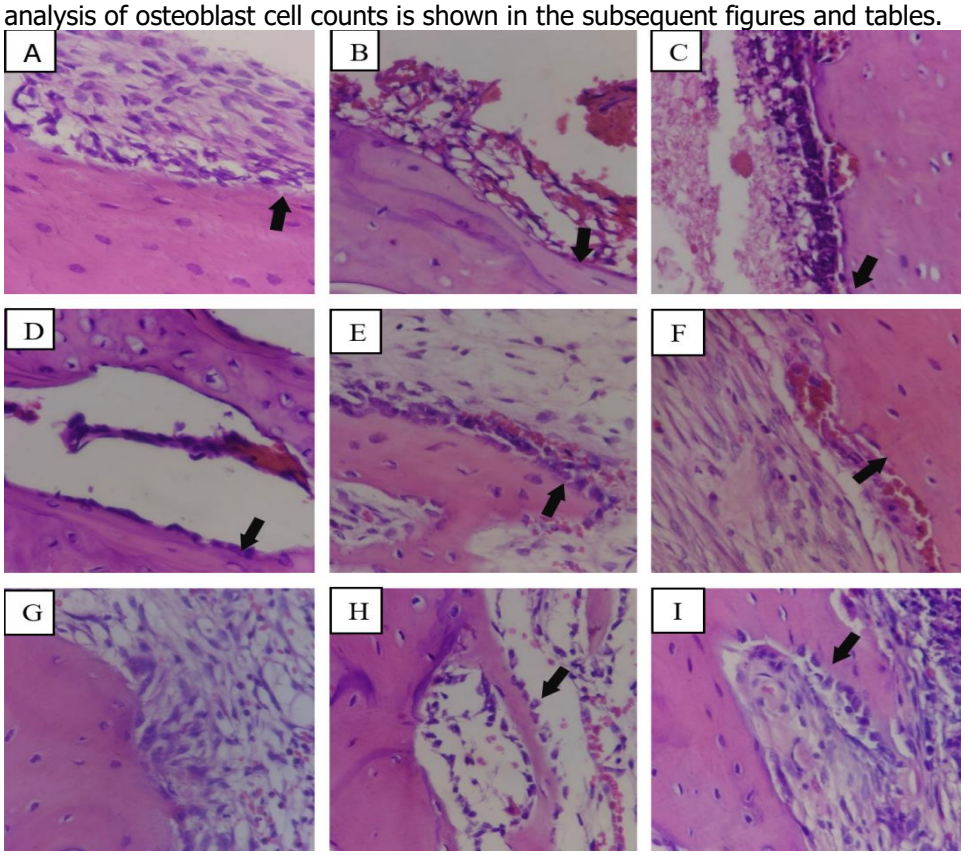

Figure 1. Results of Examination for Each Histological Group. Microscopic findings of an alveolar socket specimen stained with hematoxylin and eosin at 400x magnification. The black arrow indicates the osteoblasts in the area surrounding the socket. A. Group 0, day 3; B. Group 1, day 3; C. Group 2, day 3; D. Group 0, day 7; E. Group 1, day 7; F. Group 2, day 7; G. Group 0, day 14; H. Group 1, day 14; I. Group 2, day 14.

The results of the examination are shown as microscopic images of the alveolar socket with hematoxylin and eosin (H&E) staining at 400x magnification, organized by group and observation time. The arrangement of the images from left to right shows Groups 0 (without tooth extraction), 1 (tooth extraction without apatite cement), and 2 (tooth extraction with apatite cement), whereas the arrangement from top to bottom represents the observation times on days 3, 7, and 14. These images demonstrate the presence of osteoblasts (black arrows) around the surface of the alveolar bone socket. In the first row, showing observations on day 3, Group 0 (without tooth extraction) exhibited a relatively intact alveolar bone surface with a layer of osteoblasts arranged parallel to the bone contours. Osteoblasts appeared oval to cuboidal in shape, with basophilic

nuclei that adhered directly to the eosinophilic bone matrix. In Group 1 (tooth extraction without apatite cement), osteoblasts were still recognizable on the alveolar bone surface, although their arrangement appeared less homogeneous than that in the untreated group.

Osteoblasts appeared to follow the bone contours, which had begun to undergo morphological changes with a more uneven distribution. In Group 2 (apatite cement treatment), osteoblasts were clearly visible along the alveolar bone surface and the area around the socket, with a relatively dense cellular arrangement that followed the bone margins. In the second row, showing observations on day 7, Group 0 (without tooth extraction) demonstrated the presence of osteoblasts on the alveolar bone surface with a stable and continuous arrangement. Osteoblasts appear to maintain an orientation parallel to the bone surface. In Group 1 (tooth extraction without apatite cement), osteoblasts were more clearly visible in several areas of the alveolar bone surface, forming a layer of cells that followed the uneven contours of the bone.

The osteoblasts in this group appeared scattered and did not always form a layer. In Group 2 (tooth extraction with apatite cement), osteoblasts appeared to form a more organized layer around the bone surface and alveolar socket, with cells appearing close to each other and following the contours of the bone matrix. In the third row, showing observations on day 14, Group 0 (without tooth extraction) showed osteoblasts on the alveolar bone surface, although their appearance was more integrated with the existing bone tissue structure.

Osteoblasts followed the bone surface, which appeared smoother and more compact. In Group 1 (tooth extraction without apatite cement), osteoblasts were still identified around the alveolar bone surface and socket area, with a cellular arrangement following the bone contours that appeared more regular than at previous observation time points. In Group 2 (tooth extraction with apatite cement), osteoblasts were clearly visible around the alveolar bone surface with a relatively even distribution and a cellular arrangement that followed smoother bone contours.

The results of microscopic observations included quantitative counting of osteoblasts. The quantitative analysis was performed by counting osteoblasts in five randomly selected fields of view. The results are presented in Table 1.

Table 1. Overview of the average number of osteoblasts in each group

Variable		*Mean±SD*
Group 0	Day 3	0.83±0.46
	Day 7	0.80±0.50
	Day 14	0.80±0.35
Group 1	Day 3	13.33±1.09
	Day 7	18.13±0.56
	Day 14	20.63±0.92
Group 2	Day 3	16.13±0.76
	Day 7	22.90±1.17
	Day 14	28.93±3.57

Table 1 shows that Group 0 (untreated) exhibited a very low mean number of osteoblasts that remained relatively stable at each observation time point, with an mean osteoblast count of 0.83 on day 3, which then decreased to 0.80 on day 7 and remained at 0.80 on day 14. In Group 1 (tooth extraction without apatite cement), there was an increase in the mean number of osteoblasts at each observation time point. The mean osteoblast count was 13.33 on day 3, increasing to 18.13 on day 7, and to 20.63 on day 14. In Group 2 (tooth extraction with apatite cement), there was alson an increase in the mean number of osteoblasts at each observation time point. The mean osteoblast count was 16.13 on day 3, then increased to 22.90 on day 7, and further increased to 28.93 on day 14. The

differences in the mean number of osteoblasts on days 3, 7, and 14 for each group are shown in Figure 2.

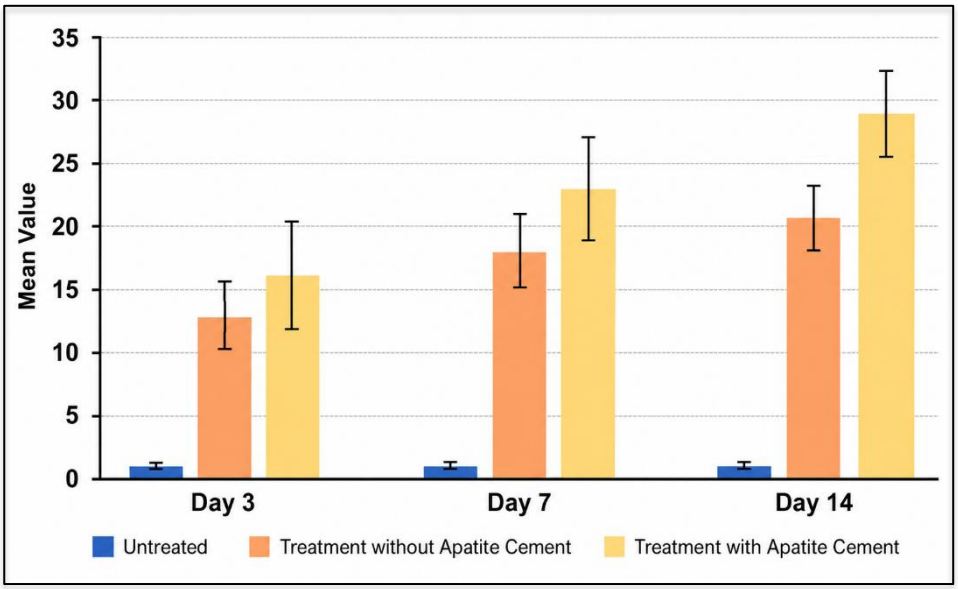

Figure 2. Average number of osteoblasts.

The graph in Figure 2 shows that the highest mean osteoblast count was found in the tooth extraction group administered apatite cement (Group 2) on day 14, with a count of 28.93 ± 3.57. The data were then subjected to a normality test using the Shapiro-Wilk test because the dataset contained fewer than 50 observations, and to a homogeneity test using the Levene's Test, as shown in Table 2.

Tabel 2. Results of the normality and homogeneity test

Variable		*P Value* (Normality Test)	*P Value* (Homogeneity Test)
Osteoblasts day 3	Group 0	0.801	
	Group 1	0.610	0.170
	Group 2	0.169	
Osteoblasts day 7	Group 0	0.110	
	Group 1	0.643	0.060
	Group 2	0.499	
Osteoblasts day 14	Group 0	0.607	
	Group 1	0.939	0.080
	Group 2	0.654	

Normality and homogeneity tests were conducted to determine whether the data were normally distributed and homogeneous (Table 2). The sample size in this study was 18 samples (<50); therefore, the Shapiro-Wilk test was used to assess normality. Based on the Shapiro-Wilk test, $p > 0.05$, indicating that the data were normally distributed. Based on the homogeneity test using Levene's test, $p>0.05$, indicating that the data were homogeneous. Subsequently, a One-Way ANOVA was conducted to compare the groups (Table 3).

Table 3. Effect of apatite cement on osteoblast numbers

Variable		F (ANOVA)	*P Value*
Osteoblasts day 3	Group 0		
	Group 1	598.467	0.001
	Group 2		
Osteoblasts day 7	Group 0		
	Group 1	1243.053	0.001
	Group 2		
Osteoblasts day 14	Group 0		
	Group 1	273.954	0.001
	Group 2		

Table 3 shows that the largest F-value was observed on day 7, at 1243.05, indicating the greatest variation in the number of osteoblasts among the groups, with a p-value of 0.001 ($p < 0.05$), indicating a statistically significant difference. These findings indicate that apatite cement had a significant effect on the number of osteoblasts in the alveolar socket following rabbit tooth extraction. Subsequently, a Tukey post-hoc test with least significant difference (LSD) was performed, as shown in Table 4.

Table 4. The Effect of apatite cement accros groups

	Variabel	*P Value*
Osteoblasts day 3	Group 1 dan 2	0.00
	Group 0 dan 2	0.00
	Group 0 dan 1	0.00
Osteoblasts day 7	Group 1 dan 2	0.00
	Group 0 dan 2	0.00
	Group 0 dan 1	0.00
Osteoblasts day 14	Group 1 dan 2	0.00
	Group 0 dan 2	0.00
	Group 0 dan 1	0.00

Table 4 shows a statistically significant difference ($p<0.05$) between Group 0 (untreated), Group 1 (tooth extraction without apatite cement), and Group 2 (tooth extraction with apatite cement).

DISCUSSION

These findings suggest that the biomaterial accelerates physiological bone regeneration by facilitating osteogenic cell activity within the extraction socket. In contrast, the untreated control group exhibited only basal osteoblast activity, which likely reflects normal physiological bone turnover required to maintain bone matrix synthesis and mineralization rather than active regenerative processes.[13,15] The biomaterial significantly improved the healing process compared to leaving the socket untreated. These results are consistent with previous studies demonstrating that calcium phosphate-based biomaterials, including apatite cement, enhance osteoblast proliferation, differentiation, and mineralization during the early stages of alveolar bone healing.[37]

Alveolar socket healing following tooth extraction occurs through a series of overlapping biological phases, including hemostasis, inflammation, proliferation, and bone remodeling. During the first 1–3 days, the extraction socket is characterized by the formation of blood clots and infiltration of inflammatory cells. Between days 3 and 7, granulation tissue gradually replaces the blood clot, accompanied by angiogenesis and the recruitment of mesenchymal stem cells. These cells subsequently differentiate into osteoblasts, initiating new bone formation along the socket wall. By day 14, osteoblast activity markedly increases, resulting in the active deposition of osteoid and progressive mineralization of the newly formed bone. Therefore, observations on days 3, 7, and 14 represent key stages of early alveolar bone healing and osteogenesis.[28,29]

Consistent with this biological healing sequence, the extraction-only group (Group 1) demonstrated a gradual increase in osteoblast numbers from day 3 to day 14, reflecting the normal physiological progression of socket healing. These findings are consistent with a previous study showing that osteoblast recruitment and new bone formation increase progressively during the early stages of alveolar socket healing following tooth extraction. Osteoblast activity gradually increases during the proliferative phase of socket healing as woven bone begins to replace the initial blood clot and granulation tissue. The progressive increase in osteoblast numbers observed in the present study therefore reflects the normal pattern of physiological bone repair in untreated extraction sockets.[38,39] Tooth extraction initiates an inflammatory response that stimulates the release of cytokines and growth factors, including BMPs, TGF-β, PDGF, and VEGF, which promote

mesenchymal stem cell recruitment and osteoblast differentiation during the proliferative phase of bone healing.[28,29]

The tooth extraction group with apatite cement (Group 2) exhibited a greater increase in osteoblast cell numbers than that without apatite cement throughout the observation period. This finding is consistent with previous studies demonstrating that calcium phosphate- and apatite-based biomaterials enhance early osteoblast recruitment and bone regeneration due to their excellent osteoconductive properties. For example, Bohner et al. reported that calcium phosphate cements provide a favorable microenvironment for osteoblast attachment and differentiation, thereby facilitating early bone healing. Similarly, Liu et al. demonstrated that hydroxyapatite-based biomaterials significantly increased osteoblast proliferation and mineralized tissue formation during the early phases of bone repair. The present findings further support these observations, suggesting that apatite cement accelerates osteogenesis by providing an osteoconductive scaffold that enhances osteoblast adhesion, proliferation, and extracellular matrix deposition, thereby promoting earlier and more extensive new bone formation.[33,34]

Osteoblasts are the primary cells involved in the bone formation process, synthesizing and secreting a bone matrix rich in type I collagen, and playing a role in regulating the mineralization of new bone formation.[30] Based on the results shown in Table 3, there was a significant difference between the untreated group, the group of tooth extraction without apatite cement, and the group of tooth extraction with apatite cement, indicating that apatite cement had a significant effect on the number of osteoblasts in the alveolar socket following tooth extraction in rabbits. This finding is consistent with previous studies reporting that calcium phosphate-based biomaterials promote early osteoblast recruitment and differentiation by providing an osteoconductive surface that supports new bone formation.[30,31] Furthermore, the greater osteoblast number observed in the group of tooth extraction with apatite cement are consistent with earlier reports demonstrating that apatite-based biomaterials enhance the early phase of bone healing compared with the group of tooth extraction untreated extraction sockets.[31,32]

The differences in the mean osteoblast cell number on days 3, 7, and 14 in the tooth extraction group without apatite cement (Table 1) indicate that healing time influences the increase in osteoblast activity. On day 3, the osteoblast cell number was at its lowest due to the ongoing healing phase, which was dominated by the inflammatory response and granulation tissue formation. Consequently, the number of maturing osteoblasts remained low, and proliferation had not yet been activated. This finding is consistent with previous reports showing that pro-inflammatory cytokine-induced MMP-13 expression increases during the early stages of healing, indicating the presence of the inflammatory phase.[31]

By day 7, the osteoblast cell number increased due to the resolution of the inflammatory phase and the onset of the reparative phase, which was marked by the initiation of the mineralization process, thereby promoting the migration and differentiation of mesenchymal cells into osteoblasts to enhance bone formation activity. This finding is consistent with previous studies demonstrating increased BMP expression by day 7, which promotes osteoblast differentiation and activation.[32] On day 14, the mean osteoblast cell number was at its highest due to the proliferation and differentiation of osteoblasts during bone healing, accompanied by increased expression of growth factors that stimulate osteoblast activity.

During the proliferative phase, osteoblasts are arranged on the surface of the newly formed bone and play a role in collagen matrix synthesis and osteoid mineralization, resulting in a higher osteoblast volume density. In this phase, there is an increase in the differentiation of mesenchymal cells into pre-osteoblasts and mature osteoblasts, marked by the activation of RUNX2, which acts as the primary regulator of osteoblastogenesis. Additionally, there is an increase in growth factor

activity, particularly TGF-β, which plays a role in stimulating osteoblast proliferation and differentiation. Previous studies have reported that BMP levels decrease by day 14 as osteoblasts enter the maturation phase and become actively involved in bone matrix formation.[26,32]

Based on the data analysis, there was a significant difference in the mean osteoblast cell number among the treatment groups at each observation time point (days 3, 7, and 14). The significant difference in osteoblast cell number between the groups in the one-way ANOVA indicates different biological responses during the bone regeneration process. Therefore, the alternative hypothesis (H1) was accepted, indicating that apatite cement had an effect on osteoblast cell number in the alveolar socket following tooth extraction in rabbits.

The present findings are consistent with previous studies demonstrating that calcium phosphate-based biomaterials, including apatite and hydroxyapatite, enhance alveolar bone healing by providing an osteoconductive scaffold that supports osteoblast recruitment and new bone formation. Experimental animal studies have reported significantly greater osteoblast activity and accelerated bone regeneration in extraction sockets treated with hydroxyapatite-based graft materials compared with untreated sockets. Likewise, recent reviews have concluded that synthetic calcium phosphate biomaterials promote favorable biological responses during alveolar socket healing by facilitating bone regeneration and preserving alveolar architecture. These findings support the present study, in which the tooth extraction with apatite cement showed significantly higher osteoblast cell numbers during the inflammatory, proliferative, and early remodeling phases of socket healing compared with the control group.[33,34]

The increase in osteoblast cell numbers observed in the tooth extraction with apatite cement may be attributed to the osteoconductive properties of the biomaterial, which provide a favorable surface for cell attachment, proliferation, and extracellular matrix deposition. This biological response facilitates early bone formation and supports the transition from the inflammatory to the proliferative and early remodeling phases of socket healing.

This study had several limitations. First, it only evaluated osteoblast cell number as an indicator of bone regeneration, without assessing other parameters such as bone density, immunohistochemical analysis, and the activity of other cells, such as osteoclasts. Second, the observation period was limited to days 3, 7, and 14; therefore, the findings do not fully describe the long-term and comprehensive bone remodeling process, during which alveolar bone maturation and hard tissue stabilization occur over several weeks to months following tooth extraction. Finally, the use of stored biological specimens may have potential for changes in tissue morphology due to the fixation process and long-term storage, which may have affected histological examination.

However, because the observation period was limited to 14 days, these findings should not be interpreted as evidence of complete bone regeneration. Further studies with longer follow-up periods and additional histological, radiographic, and biomechanical assessments are required to evaluate the long-term regenerative potential and clinical effectiveness of apatite cement before its routine clinical application. The application of apatite cement significantly increased osteoblast cell numbers during the early stages of alveolar socket healing following tooth extraction in rabbits. The increased osteoblast cell number indicate enhanced early osteogenic activity, suggesting that apatite cement provides an osteoconductive environment that supports initial bone formation.

CONCLUSION

The application of apatite cement significantly increased osteoblast cell numbers during the early stages of alveolar socket healing following tooth extraction in rabbits. The increased osteoblast cell numbers indicate enhanced

early osteogenic activity, suggesting that apatite cement provides an osteoconductive environment that supports initial bone formation. However, because the observation period was limited to 14 days, these findings should not be interpreted as evidence of complete bone regeneration in the study. Further studies with longer follow-up periods and additional histological and radiographic assessments are required to evaluate the long-term regenerative potential of apatite cement.

The implication of this study is that apatite cement may serve as a promising biomaterial for alveolar socket preservation by promoting early bone healing after tooth extraction. These findings provide preclinical evidence supporting the potential application of apatite cement as an alternative bone graft substitute in regenerative dentistry and contribute to the development of calcium phosphate–based biomaterials for clinical bone regeneration.

Acknowledgement: The authors declare no acknowledgements.
Author Contributions: Conceptualization, A.S.; methodology, A.S., R.P.N.F., and Z.Y.D.; investigation, R.P.N.F., Z.Y.D., N.Y., and D.K.F.; formal analysis, R.P.N.F.; writing original draft preparation, R.P.N.F.; writing review and editing, A.S., R.P.N.F., Z.Y.D., N.Y., and D.K.F.; supervision, A.S.; project administration, A.S. All authors have read and agreed to the published version of the manuscript.
Funding: This research received no external funding.
Institutional Review Board Statement: This study received ethical approval from the Health Research Ethics Committee of the Faculty of Medicine at Jenderal Achmad Yani University, under reference number 003/UH4.01/2026
Informed Consent Statement: "Not applicable"
Data Availability Statement: All data supporting the findings of this study are presented within the article. Additional relevant data are available from the corresponding author upon reasonable request and in accordance with ethical guidelines and participant privacy protection
Conflicts of Interest: The authors declare no conflicts of interest.

REFERENCES

1. Ahmed N, Lal A, Shakeel M, Cyrus D, Zehra FT, Ayub A. Prevalence of types, frequency and risk factors for complications after exodontia. Pak J Med Dent. 2021;10(1). https://doi.org/10.36283/pjmd10-1/008
2. Ministry of Health of the Republic of Indonesia. Indonesia health survey 2023. Jakarta: Ministry of Health of the Republic of Indonesia; 2023.
3. Dewi CD, Syamsudin E, Hadikrishna I. Characteristics of patients and indications of tooth extraction at the Exodontia Clinic, Padjadjaran University Dental Hospital. J Kedokt Gigi Univ Padjadjaran. 2022;34(2):152. https://doi.org/10.24198/jkg.v34i2.37719
4. Sa'adah N, Ridwan RD, Diyatri I, Rohmaniar PD, Adriansyah AA. Potential of stem cells from human exfoliated deciduous teeth (SHED)-derived secretome gel in wound healing process post tooth extraction. J Int Dent Med Res. 2024;17(1):71-6.
5. Lin HK, Pan YH, Salamanca E, Lin YT, Chang WJ. Prevention of bone resorption by HA/β-TCP + collagen composite after tooth extraction: A case series. Int J Environ Res Public Health. 2019;16(23):4616. https://doi.org/10.3390/ijerph16234616
6. Avila-Ortiz G, Couso-Queiruga E, Stuhr S, Chambrone L. Long-term outcomes of post-extraction alveolar ridge preservation and alveolar ridge reconstruction followed by delayed implant placement: A systematic review. Periodontol 2000. 2025;90(1):1-12. https://doi.org/10.1111/prd.12642
7. Fee L. Socket preservation. Br Dent J. 2017;222(8):579-82. https://doi.org/10.1038/sj.bdj.2017.355
8. Ashfaq R, Kovács A, Berkó S, Budai-Szűcs M. Developments in alloplastic bone grafts and barrier membrane biomaterials for periodontal guided tissue and bone regeneration therapy. Int J Mol Sci. 2024;25(14):7746. https://doi.org/10.3390/ijms25147746
9. Kijartorn P, Wongpairojpanich J, Thammarakcharoen F, Suwanprateeb J, Buranawat B. Clinical evaluation of 3D printed nano-porous hydroxyapatite bone graft for alveolar ridge preservation: A randomized controlled trial. J Dent Sci. 2022;17(1):194-203. https://doi.org/10.1016/j.jds.2021.05.003
10. Timothius CJC, Kilic HN, Gandhi KK, Kakar A, John V. Particulate bone graft materials for periodontal and implant surgery: A narrative review and case series. Dent Rev. 2023;3(2):100068. https://doi.org/10.1016/j.dentre.2023.100068
11. Fukuba S, Okada M, Nohara K, Iwata T. Alloplastic bone substitutes for periodontal and bone regeneration in dentistry: Current status and prospects. Materials (Basel). 2021;14(5):1096. https://doi.org/10.3390/ma14051096
12. Dali SSM, Wong SK, Chin KY, Ahmad F. Osteogenic properties of calcium phosphate cement doped with synthetic materials: A structured narrative review of preclinical evidence. Int J Mol Sci. 2023;24(8):7161. https://doi.org/10.3390/ijms24087161
13. van Oirschot B, Mikos AG, Liu Q, van den Beucken JJP, Jansen JA. Fast-degradable calcium phosphate cement for maxillofacial bone regeneration. Tissue Eng Part A. 2023;29(5-6):161-71. https://doi.org/10.1089/ten.TEA.2022.0177
14. Pagani S, Salerno M, Filardo G, Locs J, van Osch GJVM, Vecstaudza J, et al. Human osteoblasts' response to biomaterials for subchondral bone regeneration in standard and aggressive environments. Int J Mol Sci. 2023;24(19):14764. https://doi.org/10.3390/ijms241914764

15. Sudimantini LM, Wirata IW, Gunawan IWNF, Sumadi IWJ, Marmanto TS. Dog femoral osteoblast number post transplantation of demineralized porcine cortical bone xenograft. Bul Vet Udayana. 2022;14(2):97-102. https://doi.org/10.24843/bulvet.2022.v14.i02.p05
16. Dewi P, Sabri M, Rahmi E, Jalaluddin M, Asmilia N, Azhar A. Density of lumbar vertebral bone in ovariectomized rats (Rattus norvegicus) given Cissus quadrangularis Salisb extract. J Med Vet. 2017;11(1):39-44. https://doi.org/10.21157/j.med.vet..v11i1.4117
17. Hardono T, Supriyadi K. Modifikasi autoclave berbasis ATmega328 (suhu). Med Tek J Tek Elektromedik Indones. 2020;1(2):60-5. https://doi.org/10.18196/mt.010210
18. Adhikara AG, Maharani AP, Puspitasari A, Nuswantoro NF, Juliadmi D, Maras MAJ, et al. Bovine hydroxyapatite for bone tissue engineering: Preparation, characterization, challenges, and future perspectives. Eur Polym J. 2024;214:113171. https://doi.org/10.1016/j.eurpolymj.2024.113171
19. Feranisa A, Indraswary R, Anggraini S. Effects of chitosan nano mouth spray on epithelial thickness in the socket wound healing: An in vivo study. Medali J. 2022;4(Suppl 1):104-12. https://doi.org/10.30659/medali.4.3.104-112
20. Mavi E, Hocaoglu PT. Effects of a single intravenous dose of zoledronic acid on bone healing following tooth extraction in ovariectomized rabbits. Saudi Dent J. 2021;33(7):724-30. https://doi.org/10.1016/j.sdentj.2020.03.016
21. MacBeth ND, Donos N, Mardas N. Alveolar ridge preservation with guided bone regeneration or socket seal technique: A randomized single-blind controlled clinical trial. Clin Oral Implants Res. 2022;33(7):681-99. https://doi.org/10.1111/clr.13933
22. Niemiec BA, Gawor J, Nemec A, Clarke DE, McHugh N, Tutt C, et al. World small animal veterinary association global dental guidelines. J Small Anim Pract. 2020;61(7):E36-E161. https://doi.org/10.1111/jsap.13132.
23. Nguyen Thi Thuy Duong, Ngo Thi Quynh Trang, Nguyen Mai Anh, Tran Tan Tai, Nguyen Thanh Tung. Research on the model of mandibular alveolar bone defect in rabbits. J Med Pharm. 2024;14(2):66. https://doi.org/10.34071/jmp.2024.2.9
24. Fischer KR, Solderer A, Arlt K, Heumann C, Liu CC, et al. Bone envelope for implant placement after alveolar ridge preservation: A systematic review and meta-analysis. Int J Implant Dent. 2022;8:56. https://doi.org/10.1186/s40729-022-00453-z
25. Ma ZY, Zhang XF, Hu YZ, Zhu MD, Jin J, Qian P. Comparison of staining quality between rapid and routine hematoxylin and eosin staining of frozen breast tissue sections: An observational study. J Int Med Res. 2024;52(6):3000605241259682. https://doi.org/10.1177/03000605241259682
26. Kim JM, Lin C, Stavre Z, Greenblatt MB, Shim JH. Osteoblast-osteoclast communication and bone homeostasis. Cells. 2020;9(9):2073. https://doi.org/10.3390/cells9092073
27. Jafer MA, Salem RM, Hakami FB, Ageeli RE, Alhazmi TA, Bhandi S, et al. Techniques for extraction socket regeneration for alveolar ridge preservation. J Contemp Dent Pract. 2022;23(2):245-50. https://doi.org/10.5005/jp-journals-10024-3247
28. Kenkre JS, Bassett JHD. Bone remodelling cycle. Ann Clin Biochem. 2018;55(3):308-27. https://doi.org/10.1177/0004563218759371
29. Damayanti MM, Rachmawati M. Immunohistochemical evaluation of matrix metalloproteinase-13 on rabbit (Oryctolagus cuniculus) socket healing after application of platelet-rich fibrin with and without hydroxyapatite. F1000Res. 2022;11:29. https://doi.org/10.12688/f1000research.74094.1
30. Keranmu D, Nuermuhanmode N, Ainiwaer A, et al. Clinical application of concentrated growth factors combined with bone substitute in alveolar ridge preservation of anterior teeth. BMC Oral Health. 2022;22:54. https://doi.org/10.1186/s12903-022-02091-8
31. Maula N, Waty MU, Dewi RK, Oktawati S, Gani A, Suhartono E, et al. The impact of chitosan derived from black soldier fly (Hermetia illucens) pupae on bone remodeling post-tooth extraction: An in vivo study. Padjadjaran J Dent. 2025;37(1):59-68. https://doi.org/10.24198/pjd.vol37no1.59308
32. Davda S, Shado R, Novo Pereira I, Madruga D, Hassan H. Vertical and horizontal bone loss following alveolar socket preservation using bone grafts and autologous platelet concentrates versus bone grafts alone: A systematic review and meta-analysis. BDJ Open. 2025;11(1):11. https://doi.org/10.1038/s41405-025-00306-y
33. Bohner M, Santoni BLG, Dobelin N. β-Tricalcium phosphate for bone substitution: Synthesis and properties. Acta Biomater. 2020;113:23-41. https://doi.org/10.1016/j.actbio.2020.06.022
34. Liu Y, Lim J, Teoh SH. Development of clinically relevant scaffolds for vascularised bone tissue engineering. Biotechnol Adv. 2013;31(5):688-705. https://doi.org/10.1016/j.biotechadv.2012.10.003
35. Mohd N, Razali M, Ghazali MJ, Kasim NHA. 3D-printed hydroxyapatite and tricalcium phosphate-based scaffolds for alveolar bone regeneration in animal models: A scoping review. Materials (Basel). 2022;15(7):2621. https://doi.org/10.3390/ma15072621
36. Zubaidah N, Pratiwi DD, Masa MMSN, Setiawatie EM, Kunarti S. The osteogenesis mechanisms of dental alveolar bone socket post induction with hydroxyapatite bovine tooth graft: An animal experimental study in Rattus norvegicus strain Wistar. Eur J Dent. 2023;17(3):871-80. https://doi.org/10.1055/s-0042-1756691
37. Hou X, Zhang L, Zhou Z, Luo X, Wang T, Zhao X, et al. Calcium phosphate-based biomaterials for bone repair. J Funct Biomater. 2022;13(4):187. https://doi.org/10.3390/jfb13040187
38. Kim S, Song M, Williams D, Du W, Cho I, Bae E, et al. Early cellular events of osteomucosal healing in the tooth extraction socket. PLoS One. 2025;20(8). https://doi.org/10.1371/journal.pone.0328459
39. Gomes PS, Daugela P, Poskevicius L, Mariano L, Fernandes MH. Molecular and cellular aspects of socket healing in the absence and presence of graft materials and autologous platelet concentrates: A focused review. J Oral Maxillofac Res. 2019;10(3). https://doi.org/10.5037/jomr.2019.10302