ORIGINAL ARTICLE

Effect of demineralized dentin matrix and chitosan of black soldier fly on osteoblast and osteoclast activity in post-extraction socket preservation: an experimental study

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ABSTRACT

Introduction: Alveolar bone remodeling is crucial because it represents a key component of oral rehabilitation. Dentin Demineralization Matrix (DDM) and chitosan Black Soldier Fly (BSF) pupae have osteoinductive and osteoconductive properties, influencing osteoblast and osteoclast activity. The objective of this study was to analyze the effectiveness of a combined gel of DDM and chitosan BSF pupae on osteoblast and osteoclast activity. **Methods:** Eighteen guinea pigs were divided into control (C) and treatment (T) groups. The mandibular left incisor was extracted, and in the C group, the socket was filled with polyethylene glycol (PEG) gel as a placebo, then sutured using non-absorbable silk. In the T group, the socket was applied with chitosan BSF pupae gel and DDM, then sutured with non-absorbable silk. The samples were euthanized on days 7, 14, and 21, followed by histological evaluation with Hematoxylin & Eosin (H&E). Data were analyzed using the Kruskal-Wallis test due to non-normal distribution. **Results:** There were significant increases in the number of osteoblasts and a decrease in the number of osteoclasts over time between days 7, 14, and 21. Kruskal-Wallis analysis showed a statistically significant difference ($p=0.011$, $p<0.05$). **Conclusion:** Application of a combined DDM and BSF-chitosan pupae enhanced osteoblastic activity while suppressing osteoclastic activity after tooth extraction. These findings indicate its potential as a biomaterial candidate for alveolar bone regeneration and future regenerative applications.

KEYWORDS

Demineralized, dentin matrix, chitosan black, soldier fly, osteoblasts, osteoclasts, bone remodeling

INTRODUCTION

Alveolar bone provides foundational stability for teeth, allowing for effective mastication and phonetic function. This specialized bone forms the sockets and plays an important role in overall oral function.[1,2] Various factors can compromise alveolar bone health, leading to defects that trigger a continuous bone remodeling response. This process aids in preserving bone density and strength.[1] Such defects should be managed promptly to prevent further bone resorption. The most common method to address this condition is via bone grafting.[1] A bone graft serves to act as a scaffold and to provide support for the formation of new bone.[3]

The gold standard for bone grafting remains the autograft. However, autografts are limited in availability, and bone harvesting is associated with pain and donor site morbidity.[4] Therefore, alternative biomaterials have been explored to overcome these limitations. Other types of bone grafts are developed from a combination of two biological sources, including human and animal tissues. Natural polymers are widely used in bone remodeling because their structural and chemical similarity to the extracellular matrix (ECM) enhances osteoinductive potential.[5]

Among various natural polymer sources, the black soldier fly (BSF) has gained attention as a sustainable source of chitosan for biomedical applications. The BSF is an insect that, upon reaching the imago stage, leaves its pupa. The pupa consists of 35% chitin, which can be converted into chitosan through a deacetylation process. Chitosan has been recognized as a natural polysaccharide that forms a copolymer composed of 2-acetamido-D-glucose and 2-amino-D (usually more than 80%) linked together by β(1→4) glycosidic bonds. [6-8] Chitosan has numerous favorable properties, such as biodegradable, biocompatible, antibacterial, antimicrobial, antifungal, analgesic, antitumor, high bioavailability, good water permeability selectivity, and high chemical resistance. In addition, chitosan is suitable for bone regeneration due to its strong osteointegration and osteoconductive properties, which promote osteoblast growth and decrease osteoclast activity.[6-11]

In addition to BSF chitosan, another biomaterial that exhibits osteoinductive and osteoconductive properties is demineralized dentin matrix (DDM). DDM is derived from human tooth dentin after extraction, which is subsequently demineralized by immersion in acid to remove its mineral content.[12] The demineralization process enables dentin to release growth factors, which induce osteoinductive and osteoconductive properties.[13] Based on its chemical composition, dentin is similar to bone but differs in its structure, containing 70% inorganic components (such as hydroxyapatite) and 20% organic components (90% collagen I, a small amount of collagen III and V, as well as non-collagenous proteins, and 10% water).[14,15] DDM acts as a scaffold, as dentin promotes bone tissue formation (osteoconduction), but dentin also releases growth factors that encourage bone formation (osteoinduction).[13]

Both DDM and Chitosan have individual limitations that can complement each other when combined. Since bacterial activity can inhibit bone remodeling, a scaffold with antibacterial properties is required. This deficiency in DDM can be compensated by combining it with Chitosan, which exhibits antibacterial activity. Chitosan also has a limitation of low mechanical resistance due to its rigid and brittle membrane. This limits its ability to function effectively as a standalone osteoconductive scaffold.[16]

Chitosan requires the addition of other biomaterials, such as DDM, to improve mechanical properties and prevent easy degradation. The combination of these two materials aims to achieve optimal biomaterial characteristics for bone remodeling. However, limited studies have explored the synergistic effect of combining DDM with BSF chitosan on alveolar bone healing post-tooth extraction. Given the increasing need for biocompatible and sustainable graft alternatives, exploring natural sources such as BSF chitosan combined with DDM represents a promising direction for bone regeneration research.

One of this study's novelties is the use of a combined material of DDM and chitosan from BSF pupae to accelerate bone remodeling. While previous studies have investigated the use of chitosan from BSF pupae alone, none has explored its combination with DDM. For example, Maula (2025) demonstrated that BSF pupae chitosan increases osteoblast numbers and decrease osteoclast activity after tooth extraction, thereby accelerating bone regeneration. Similarly, Gao (2019) concluded that DDM enhances bone regeneration, and Dewi (2025) reported that DDM increases collagen type I density and osteoblast numbers post-extraction. [17-19]

The hypothesis of this study is that the combined use of DDM and BSF-derived chitosan is more effective than either material alone. The research also examines the biomolecular pathways involved, including osteoblast and osteoclast activity. The findings are expected to contribute to the development of regenerative biomaterial-based tissue engineering materials more effective and accessible for clinical applications in dentistry, particularly in socket preservation and the acceleration of alveolar bone healing. This study aims to analyze the effects of combining DDM and chitosan from BSF pupae on accelerating alveolar bone remodeling post-extraction

METHODS

This experimental laboratory study was conducted with a posttest control group design. Eighteen male guinea pigs obtained from the Airlangga University Veterinary Medicine Department met the inclusion criteria: healthy, weighing 200-300 grams, and aged 3-4 months. The exclusion criteria were guinea pigs that died before 7 days of the adaptation period. Guinea pigs were used because of their adequate size, low cost, ease of handling, and physiological characteristics that resemble those of humans, making them suitable for testing.[17,18] The guinea pigs were divided into a control group (C) (n=9) and a treatment group (T) (n=9).

A combination gel containing DDM and chitosan from BSF pupae was prepared by mixing the two ingredients with PEG 400 and PEG 4000 until a homogeneous consistency was achieved. Guinea pigs were anesthetized with ketamine (50 mg/kg body weight) and xylazine (5 mg/kg body weight) administered intramuscularly. The left mandibular incisor was extracted, and the socket was irrigated with sterile distilled water. In group C, PEG gel was applied to the socket to completely fill the and then sutured with nonabsorbable silk sutures. In group T, a combination of DDM gel and BSF pupae chitosan was applied to the socket until filled and then sutured with nonabsorbable silk suture. The guinea pigs were fed a standard diet and vitamins throughout the study until euthanasia on days 7, 14, and 21.

The bone samples underwent standard histological processing, including tissue fixation, decalcification, washing, embedding, labeling, sectioning, slide mounting, and staining. Hematoxylin-eosin (HE) staining was performed on tissue sections in the apical third of the socket to analyze the number of osteoclasts and osteoblasts. Osteoclasts are giant multinucleated motile cells that play an essential role in matrix resorption during bone growth and remodeling. Osteoblasts microscopically have a cuboidal to cylindrical shape with a size of 30-80 µm and a purplish color, located on the surface of the bone matrix in closely arranged rows.[20] Osteoclasts and osteoblasts were observed in 5 fields of view using a light microscope (OLYMPUS XC10 series, equipped with OlyVIA software) with 400x magnification at the Research Center Laboratory, Faculty of Dentistry, Airlangga University, by researchers, analysts, and dentists specializing in anatomical pathology. [18,21,22,24]

RESULTS

Osteoblast calculation results showing the mean and standard deviation in the C group and T group on days 7, 14, and 21.

Table 1. Mean and standard deviation for osteoblast differences

Group	Mean and Standard Deviation		
	7	14	21
Control (C)	1.66 ± 0.577	2.00 ± 1.00	2.66 ± 0.577
Treatment (T)	9.66 ± 1.154	12.00 ± 1.00	13.00 ± 2.00

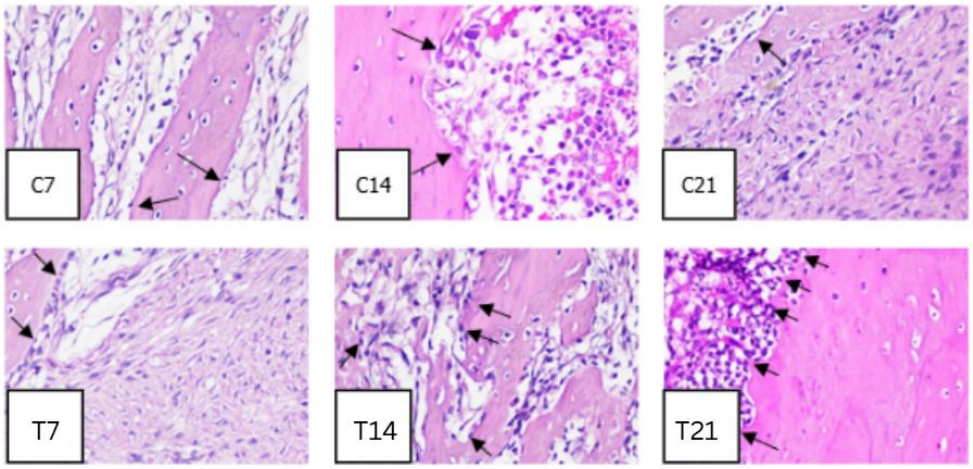

Figure 1. Representative microscopic images of osteoblasts with HE staining and 400x magnification.(C7): The control group day 7. (C14): The control group day 14. (C21): The control group on day 21. (T7): the treatment group day 7. (T14): the treatment group day 14 and (T21): the treatment group day 21

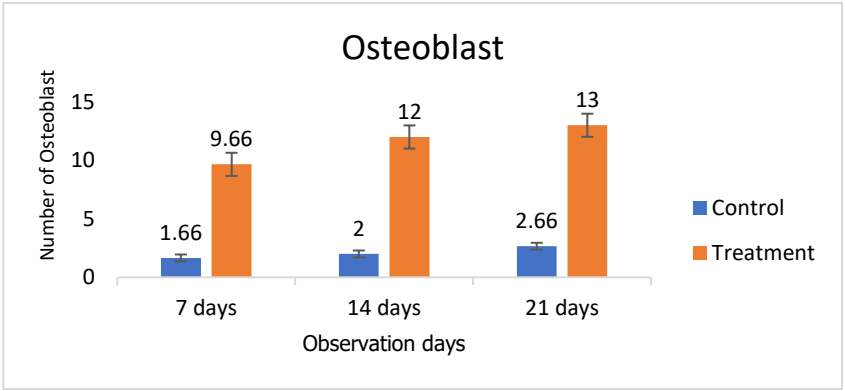

Figure 2. Representative microscopic images of osteoblasts with HE staining and 400x magnification. (C7): The control group day 7. (C14): The control group day 14. (C21): The control group on day 21. (T7): the treatment group day 7. (T14): the treatment group day 14 and (T21): the treatment group day 21

Based on Table 1, Figure 1, Figure 2, the number of osteoblasts differed in each group. The figures also show that the lowest number of osteoblasts was in the C7 group, with an average of 1.66 ± 0.57, and the highest number was in the T21 group, with an average of 13.00 ± 200. Osteoblasts in the control group were fewer than those in the treatment group on days 7, 14, and 21. These data indicate an increase in osteoblasts from days 7, 14, and 21 in both the C group and the T group.

Osteoblast data analysis was performed using normality and homogeneity tests, and nonparametric methods such as Kruskal-Wallis and Mann-Whitney to ensure valid and homogeneous results. Table 2 shows pairs of groups consisting of C14-T7, C2-T7, C2-T14, and C21-T21, which showed no significant difference with p-values of 0.064, 0.297, 0.122, and 0.069, respectively. Conversely, C7-T7, C7-T14, C7-T21, C14-T14, and C14-T21 showed differences. The difference between C7 and T7 had a p-value of 0.037, between C7 and T14 with a p-value of 0.010, and between C7 and T21 with a p-value of 0.004. There was also a significant difference between C14 and T14, with a p-value of 0.018, and between C14 and

T21, with a p-value of 0.009. Osteoclast calculation results showing the mean and standard deviation in the C group and T group at days 7, 14, and 21.

Table 2. Mann-Whitney test results for differences in osteoblast counts

Group	C7	C14	C21	T7	T14	T21
C7		0.817	0.417	0.037*	0.010*	0.004*
C14	0.817		0.297	0.064	0.018*	0.009*
C21	0.417	0.297		0.297	0.122	0.069
T7	0.037*	0.064	0.297		0.615	0.439
T14	0.010*	0.018*	0.122	0.615		0.787
T21	0.004*	0.009*	0.069	0.439	0.787	

*: Groups in pairs have significant differences

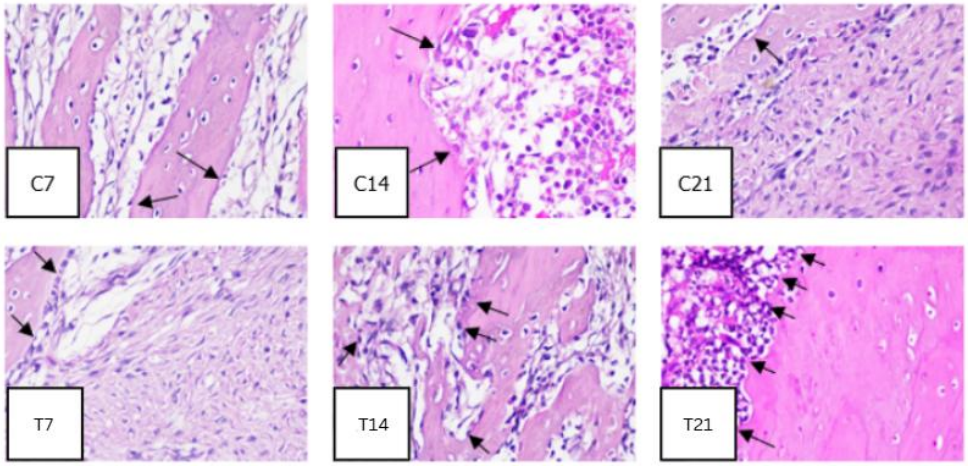

Figure 3. Representative microscopic images of osteoclasts with HE staining and 400x magnification. (C7): The control group day 7. (C14): The control group day 14. (C21): The control group on day 21. (T7): the treatment group day 7. (T14): the treatment group day 14 and (T21): the treatment group day 21

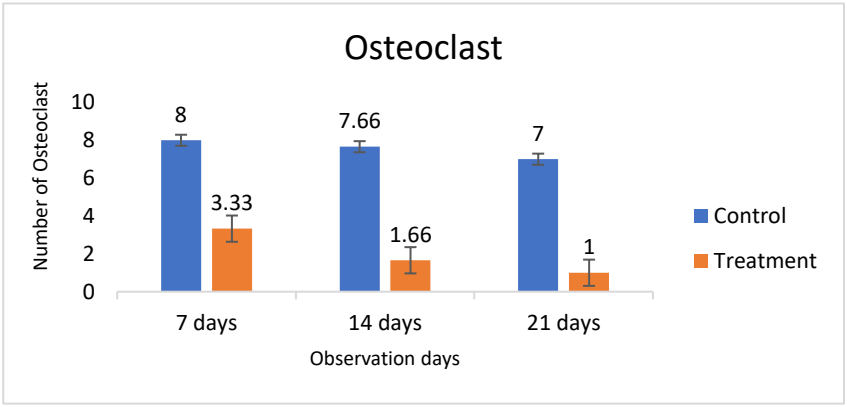

Figure 4. Representative microscopic images of osteoclasts with HE staining and 400x magnification. (C7): The control group day 7. (C14): The control group day 14. (C21): The control group on day 21. (T7): the treatment group day 7. (T14): the treatment group day 14 and (T21): the treatment group day 21

Based on Figures 3 and 4 and Table 3, there were differences in the number of osteoclasts in each group. The figure also shows that the lowest average osteoclast count was found in group T21 (1.00), and the highest average was found in group C7 (8.00). On the same day of observation, osteoclast counts in group C showed a lower count than group T. Osteoclast counts decreased in groups C and T on days 7, 14, and 21.

Osteoclast data analysis was performed using normality and homogeneity tests, followed by nonparametric methods such as Kruskal-Wallis and Mann-Whitney to ensure valid results.

Table 3. Mean and standard deviation for osteoclast differences

Group	Mean and Standard Deviation		
	7	14	21
Control (C)	8.00 ± 1.00	7.66 ± 0.57	7.00 ± 1.00
Treatment (T)	3.33 ± 0.57	1.66 ± 0.57	1.00 ± 0.00

Table 4. Results of Mann Whitney test for osteoclast differences

Group	C7	C14	C21	T7	T14	T21
C7		0.846	0.670	0.096	0.013*	0.003*
C14	0.846		0.535	0.141	0.022*	0.006*
C21	0.670	0.535		0.295	0.063	0.020*
T7	0.096	0.141	0.295		0.416	0.201
T14	0.013*	0.022*	0.063	0.416		0.642
T21	0.003*	0.006*	0.020*	0.201	0.642	

*: Groups in pairs have significance differences

Based on Figure 3, Figure 4 and Table 3, there were differences in the number of osteoclasts in each group. The figure also shows that the lowest average osteoclast count was found in group T21 (1.00), and the highest average was found in group C7 (8.00). On the same day of observation, group C showed a lower count than group T. Osteoclast counts decreased in groups C and T on days 7, 14, and 21. Osteoclast data analysis was performed using normality and homogeneity tests followed by Kruskal-Wallis and Mann-Whitney tests to confirm statistically reliable results.

DISCUSSION

As shown in Table 1, the number of osteoclasts observed on day 7 in the control group (C7 = 8.00 ± 1.00) was higher than in the treatment group (T7 = 3.33 ± 0.57). Following tooth extraction, the socket enters the early healing phase dominated by inflammation lasting up to approximately seven days. This result aligns to Novy et al. (2025), chitosan administration reduces the production of pro-inflammatory cytokines such as TNF-α and IL-6. These cytokines promote osteoclast differentiation and activation via the RANKL pathway. Therefore, the suppression of cytokine secretion inhibits osteoclast formation and activity, as reflected in the reduced number of osteoclasts. In addition, the decreased pro-inflammatory cytokine levels are inversely correlated with osteoprotegerin (OPG) expression, which further suppresses osteoclastic resorption. These findings indicate that the DDM BSF chitosan gel effectively limits osteoclastic activity during the initial inflammatory phase of socket healing.[25,26]

Subsequently, the number of osteoblasts increased as bone formation progressed. The average number of osteoblasts presented in Table 2 on day 7 in the control group (C7 = 1.66 ± 0.57) was lower than in the treatment group (T7 = 9.66 ± 1.15). This finding aligns with Kim et al. (2020), who developed a chitosan-based hydrogel modified with heparin to stabilize BMP-2 activity in demineralized bone matrix (DBM). Their study demonstrated that the hydrogel enhanced osteoblast differentiation and mineral deposition in mesenchymal stem cells by inhibiting noggin (a BMP antagonist). Thus, the DDM–BSF chitosan gel used in this study likely supports early osteoblastic activity while simultaneously attenuating osteoclastic resorption, accelerating the overall bone remodeling process.[28]

A histological review by Kim et al. (2019) further supports this mechanism, showing that DDM can act as a carrier for recombinant human BMP-2 (rhBMP-2) and stimulate new bone formation through controlled osteoclastic resorption. This resorption enlarged dentinal tubules, releasing endogenous BMPs, and promoting subsequent osteoblastic activity during the bone remodeling phase. [13]

Table 2 demonstrates that on day 14, the number of osteoblasts in T14 (12.00±1,00) was more than in C14 (2,00±1,00). This indicates that T14 has greater osteoblast activity supporting new bone remodeling than C14. In contrast to the continuously increasing osteoblast counts, as can be seen in Table 1, the number of osteoclasts decreased from day 7 to day 14 in both the control and treatment groups. On day 14, the mean osteoclast count in the control group (C14 = 7.66 ± 0.57) was higher than in the treatment group (T14 = 1.66 ± 0.57). This pattern indicates that the combination gel not only reduced osteoclast activity in the early healing phase (day 7) but also maintained this effect through day 14, thereby supporting bone formation. Statistical analysis on Table 3 and 4 showed a significant difference between the control and treatment groups on day 14 for both the increase in osteoblasts (p = 0.018) and the decrease in osteoclasts (p = 0.022).

The results presented in Table 1 indicate that number of osteoclasts continued to decline on day 21, with the lowest count observed in the treatment group (T21 = 1.00 ± 0.00), a value significantly different from the control group (C21 = 2.66 ± 0.57; p = 0.020). This decrease reflects the transition from the resorption phase to the remodeling phase of bone healing. Dewi *et al*. (2025) reported that osteoclast activity peaks around day 7 and gradually declines as inflammatory cytokine levels decrease. By day 21, osteoclastic resorption had subsided, allowing the bone formation process to predominate.[29]

In contrast, the number of osteoblasts reported in Table 2 and figure 2 on day 21 reached its highest mean value in the treatment group (T21 = 13.00 ± 2.00), surpassing the control group (C21 = 2.66 ± 0.57). These findings are consistent with the study by Putranto *et al*. (2022), which demonstrated that chitosan derived from marine shells enhances osteoblast proliferation and differentiation during bone remodeling. The overall results shown in Tables 3 and 4 indicate that BSF chitosan gel application increased osteoblast numbers on days 7, 14, and 21, while decreasing osteoclast counts on days 7 and 14, with statistically significant differences (p < 0.05), suggesting that BSF chitosan supports bone regeneration.[30]

However, the Mann–Whitney analysis revealed no significant difference between C21 and T21 in osteoblast numbers. According to Sa'diyah *et al*. (2020), this outcome may be attributed to the bone remodeling stage, in which osteoblasts begin to mature into osteoid and subsequently differentiate into osteocytes. Thus, by day 21, both groups may have entered the remodeling phase, where bone formation stabilizes and cellular activity reaches homeostasis.[31]

This early increase in osteoclasts is consistent with Fayyad et al. (2024), who conducted a systematic review evaluating the effectiveness of chitosan in promoting tooth extraction socket healing. The study concluded that chitosan alone significantly accelerates hemostasis and enhances wound healing while reducing postoperative pain and inflammation compared to controls. By comparison, the combination of DDM and BSF chitosan in the present study demonstrated not only accelerated healing but also a significant increase in osteoblast activity and suppression of osteoclasts. This suggests that adding DDM enhances the regenerative potential of chitosan through its osteoinductive and osteoconductive properties, resulting in more effective bone remodeling than chitosan alone.[32]

The present study was limited by the short observation period and small sample size, which may affect the generalizability of the results. Further studies with larger samples and longer follow-up periods are needed to confirm these findings.

CONCLUSION

The application of a gel combining DDM and chitosan BSF pupae increased osteoblastic activity and reduced osteoclast activity following tooth extraction. These findings imply its potential as a biomaterial candidate for alveolar bone regeneration and future clinical applications.

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Data Availability Statement: The results reported support data can be found, including links to a data collection that is publicly archived that is analyzed or produced during the study
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