ORIGINAL ARTICLE

# Demineralized dentin matrix (DDM) from human teeth increases osteoblasts and type i collagen density after tooth extraction: an experimental study

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## ABSTRACT

**Introduction:** Post-extraction cavities must be promptly treated to minimize alveolar ridge resorption. Various bone graft materials can be used to encourage bone regeneration in perialveolar defects. Demineralized Dentin Matrix (DDM) is a bone graft material found in human tooth dentin containing type I collagen. The content of DDM is expected to show an increase in mediators that form bone, such as osteoblasts, thereby accelerating the bone healing process. The study aims to analyze the effect of DDM on osteoblast count and type I collagen density during post-extraction bone healing. **Methods:** This true experimental study used guinea pigs with extracted left mandibular incisors. Sockets were filled with (1) polyethylene glycol gel (control group, n=9) or (2) DDM gel (treatment group, n=9). The gel was inserted into the socket until it was full and then sutured with non-absorbable silk. The guinea pigs were euthanized on days 7, 14, and 21 for osteoblast counting and type I collagen density measurement. One-way ANOVA was used to assess osteoblast numbers, while the Kruskal-Wallis test was applied to analyze type I collagen density. **Results:** The treatment group exhibited a higher osteoblast count on day 7 (48.73), day 14 (79.00), and day 21 (89.66) compared to the control group (day 7: 33.00, day 14: 59.6, day 21: 78.27). A statistically significant difference was observed between the treatment and control groups in osteoblast count ($p = 0.000$) and type I collagen density ($p = 0.009$). **Conclusion:** DDM increases osteoblast numbers and type I collagen density on days 7, 14, and 21 post extraction, potentially enhancing bone remodeling.

## KEYWORDS

Demineralized dentin matrix (DDM), human teeth, osteoblast, type I collagen.

## INTRODUCTION

Tooth extraction is a surgical procedure to remove teeth from their sockets, typically performed as a last resort when teeth cannot be preserved through other treatments. Common indications include persistent primary teeth, supernumerary teeth, crowding, severe periodontal disease, fractured teeth, periapical abscesses, deep caries, teeth on fracture lines, impacted teeth, orthodontic needs, prosthetic requirements, pre-radiotherapy preparation, prophylactic extraction, and retained root fragments. Studies show significant alveolar bone resorption post-extraction, with losses of 31.6% at 3 months, 42.4% at 6 months, and 50.73% at 12 months.[1,2]

Dental sockets formed after tooth extraction should be treated immediately to prevent alveolar bone resorption. One common post-extraction complication is a dry socket, which occurs when a blood clot fails to form, exposing the underlying bone and soft tissue to the oral environment, leading to chemical stimulation and pain. Post-tooth extraction cavities should be promptly treated to minimize alveolar ridge resorption. The reduction in bone volume following extraction typically measures 1.5-2 mm vertically and 40-50% horizontally within 6 months. Bone repair in cases of reduced bone volume can be facilitated using bone grafting techniques. Socket preservation is a procedure in which graft material is placed into the extraction socket to minimize bone and soft tissue loss, thereby preserving bone volume and structure for optimal aesthetic and functional outcomes. Various bone graft materials can enhance bone regeneration in alveolar defects surrounding the tooth socket.[3,4,5]

Efforts to promote bone regeneration in perialveolar bone defects can involve various types of bone graft materials, including autografts, allografts, and xenografts. However, each of these materials has limitations, such as high cost, risk of disease transmission, and limited osteoinductive capacity. Autografting, while considered the gold standard for bone formation, presents drawbacks including donor site morbidity, limited availability, and irregular absorption rates. Consequently, various allogeneic replacement materials have been developed as alternatives to stimulate osteoblast proliferation and migration. Xenografts, bone graft materials derived from animals, have also been extensively investigated. While bovine grafts are used as bone substitutes, they can sometimes elicit rejection reactions. Therefore, a wide array of allogenic substitution materials has been developed as alternative candidates to stimulate the migration and proliferation of osteoblasts.[2,6,7]

Bone grafting is considered the gold standard in bone therapy, as it provides structural support, fills bone defects, accelerates healing, and enhances the bone regeneration response through its osteogenic, osteoinductive, and osteoconductive properties. Tooth grafts are classified as allografts, as they originate from the same species but different individuals. Demineralized dentin matrix (DDM) is a bone graft material derived from human tooth dentin. The advantages of using DDM include its osteoinductive and osteoconductive properties, reduced need for anesthesia, shorter surgical time, minimized blood loss, and avoidance of secondary surgical sites. Tooth grafts are indeed classified as allografts because they are sourced from other individuals within the same species.[2,7,8]

Dentin from human teeth can be used as a graft material or as a source of growth factors in bone tissue engineering. Various forms of dentin have been investigated for their potential as bone substitutes. Dentin is also a vital composite material, hydrated and possessing diverse structural components and properties. As a group of tooth structures, dentin has a composition nearly analogous to bone, comprising approximately 70% hydroxyapatite, 20% collagen, and 10% body fluid by volume and weight.[7] Specifically, human tooth dentin consists of 70% inorganic material and 20% organic material, with type I collagen constituting 90% of the organic content, alongside Bone Morphogenetic Protein

(BMP) and 10% water. The microstructure of dentin consists of collagen fiber tissue and contains various growth factors, such as BMP (Bone Morphogenetic Protein), IGF-2 (Insulin Growth Factor-2), PDGF (Platelet-Derived Growth Factor), TGF-ß (Tumor Growth Factor-ß), and FGF (Fibroblast Growth Factor). The insertion of Demineralized Dentin Matrix (DDM) into the alveolus after tooth extraction aims to preserve bone quality, optimize horizontal and vertical bone dimensions, and accelerate soft tissue recovery.

This is due to the presence of type I collagen within the dentin structure, which can help enhance collagen synthesis during the bone healing process. Dentin contains several growth factors also found in bone, such as insulin-like growth factor-1 (IGF-1), insulin-like growth factor-2 (IGF-2), transforming growth factor-beta (TGF-β), Fibroblast Growth Factor (FGF), Platelet-Derived Growth Factor (PDGF), Parathyroid Bone Morphogenetic Proteins (BMPS), and certain members of the Growth Differentiation Factor (GDF). Consequently, recent research has demonstrated favorable outcomes following the use of dentin as a bone graft, establishing its clinical safety and significant bone formation capacity.[9,10,11]

Demineralized Dentin Matrix (DDM) placed in a post-extraction tooth socket is intended to preserve bone quality, improve vertical and horizontal bone dimensions, and facilitate soft tissue healing. These effects are attributed to the presence of type I collagen within the dentin structure, which can help increase collagen synthesis during the bone healing process. The ability of DDM to form bone is expected to manifest as an increase in bone formation mediators such as NFkB, RANKL, osteoprotegerin, osteoblasts, fibroblasts, and collagen, all of which play crucial roles in the bone healing process.[10,11]

The bone healing process consists of three stages: inflammation, proliferation, and remodeling. Osteoblasts gradually adhere to the surface of the bone graft matrix, spreading based on the graft's surface properties and secreting adhesive components of the extracellular matrix (ECM), a process mediated by focal adhesion formation. Osteoblasts are bone-forming cells essential for bone metabolism, including alveolar bone, which supports teeth. Osteoblast proliferation typically begins around day 7, peaking between days 21 and 28. In bone defect healing studies, type I collagen first appears during the proliferation (days 3-5), peaks in expression (days 7-14), and continues rapid accumulation until day 21.

Therefore, type I collagen density can be effectively evaluated at 7, 14, and 21 days post-extraction.[12,13] Osteoblasts synthesize and store type I collagen as the main constituent of bone matrix in the formation of bone tissue. The increasingly dense collagen fibers show that the injured tissue has healed.[14,15] Given the osteoconductive and osteoinductive properties of DDM derived from human teeth, which enable new bone growth, previous research has primarily focused on osteoblast counts in extraction sockets.

However, this study aims to investigate both osteoblast numbers and the decrease in osteoclast numbers during bone healing following extraction. Therefore, this research seeks to determine the effect of DDM on osteoblast count and type I collagen density in the bone healing process after guinea pig (*Cavia cobaya*) tooth extraction.

## METHODS

This study uses a true experimental method with a post-test design only with a control group design. The sampling techniques based on the calculation formula for the sample size above, the sample of each group amounted to 3 samples guinea pigs (*Cavia cobaya*). In this study, there were 6 treatment groups so the total sample was 18 samples of guinea pigs (*Cavia cobaya*). The Demineralized Dentin Matrix (DDM) was prepared at the Dr. Soetomo Hospital. Subsequently, the DDM gel formulation was carried out at the Biochemistry Laboratory of the

Faculty of Medicine, Airlangga University. Animal experimentation was conducted at the Experimental Animal Biochemistry Laboratory of the Faculty of Medicine, Airlangga University, while the preparation of specimens and histopathological examination were performed at the Anatomy Pathology Laboratory of the Faculty of Medicine, Airlangga University.

For the preparation of Demineralized Dentin Matrix (DDM) gel, all equipment was sterilized in an oven at 105°C for 3 hours. PEG material and the active ingredients were weighed on an analytical balance according to the required calculations. PEG 400 and PEG 4000 were then mixed in a porcelain dish, heated in a water bath at 0°C, and stirred until a homogeneous mixture was obtained. This mixture was removed from the water bath and stirred until it cooled. The weighed active ingredients were gradually added, and the mixture was stirred until homogeneous. The resulting PEG gel was transferred into plastic pots, ready for application. PEG was used as a base for its ability to facilitate cellular fusion through cell membranes and to dissolve water-insoluble substances.[16]

Eighteen male guinea pigs (*Cavia cobaya*), aged 3-4 months and weighing 250-300 grams, were selected based on the inclusion criteria of being healthy. The animals were acclimatized for 7 days in cages maintained at a temperature of 18-26°C with adequate ventilation. They were fed fresh vegetables three times daily as a source of vitamin C and provided with standard distilled water to ensure a uniform sample before the experiment. Prior to tooth extraction, the guinea pigs were injected with anesthesia, consisting of ketamine 50 mg/kg body weight combined with xylazine 5 mg/kg body weight (with a ratio of 10:1) intraperitoneally. Following correct needle placement, the anesthetic was administered. Once the guinea pigs were unconscious, the lower left incisors were extracted using a needle holder, and the sockets were irrigated with sterile distilled water.

The guinea pigs (*Cavia cobaya*) that underwent left lower incisor extraction were randomly divided into two groups (n=9 per group): the treatment group (T group) received Demineralized Dentin Matrix (DDM) gel, and the control group (C group) received PEG gel. The respective gels were inserted into the extraction sockets until full using a sterile syringe, and the wounds were subsequently sutured with non-absorbable suture. Observations were conducted on days 7, 14, and 21 post-extraction to assess type I collagen density and osteoblast counts. On each of these days, the guinea pigs in the respective treatment and control subgroups were euthanized via intraperitoneal injection of ketamine anesthesia at a dose of 0.4 mL per 400 g of body weight (four times the initial anesthetic dose).

Following euthanasia, tissue samples were collected by making a vertical cut in the jaw in the region of the extraction socket, either mesially or distally. The prepared specimens were then stained using Masson trichrome (MT) to visualize type I collagen density and Hematoxylin and eosin (H&E) to count osteoblasts. The staining results were observed under a light microscope at 400x magnification in five randomly selected fields of view by two observers.

The histopathological structure of osteoblasts in alveolar bone is characterized by single-nucleated, flattened cells located in the endosteum, with red-bluish cytoplasm. Type I collagen appears as fiber with stains blue, sinuous, and inelastic fibers composed of collagen proteins (white fibers).

## RESULTS

The mean and standard deviation (SD) of osteoblast cell counts and collagen type I density in the control group (C) and the treatment group (T) on days 7, 14, and 21 are presented in Table 1 and Table 3.

**Table 1. Mean ± SD number of osteoblast cells on days 7, 14, and 21**

| Group | Day 7 | Day 14 | Day 21 |
|---|---|---|---|
| C | 33.00±8.90 | 59.6±8.32 | 78.27±1.13 |
| T | 48.73±8.23 | 79.00±2.55 | 89.66±1.13 |

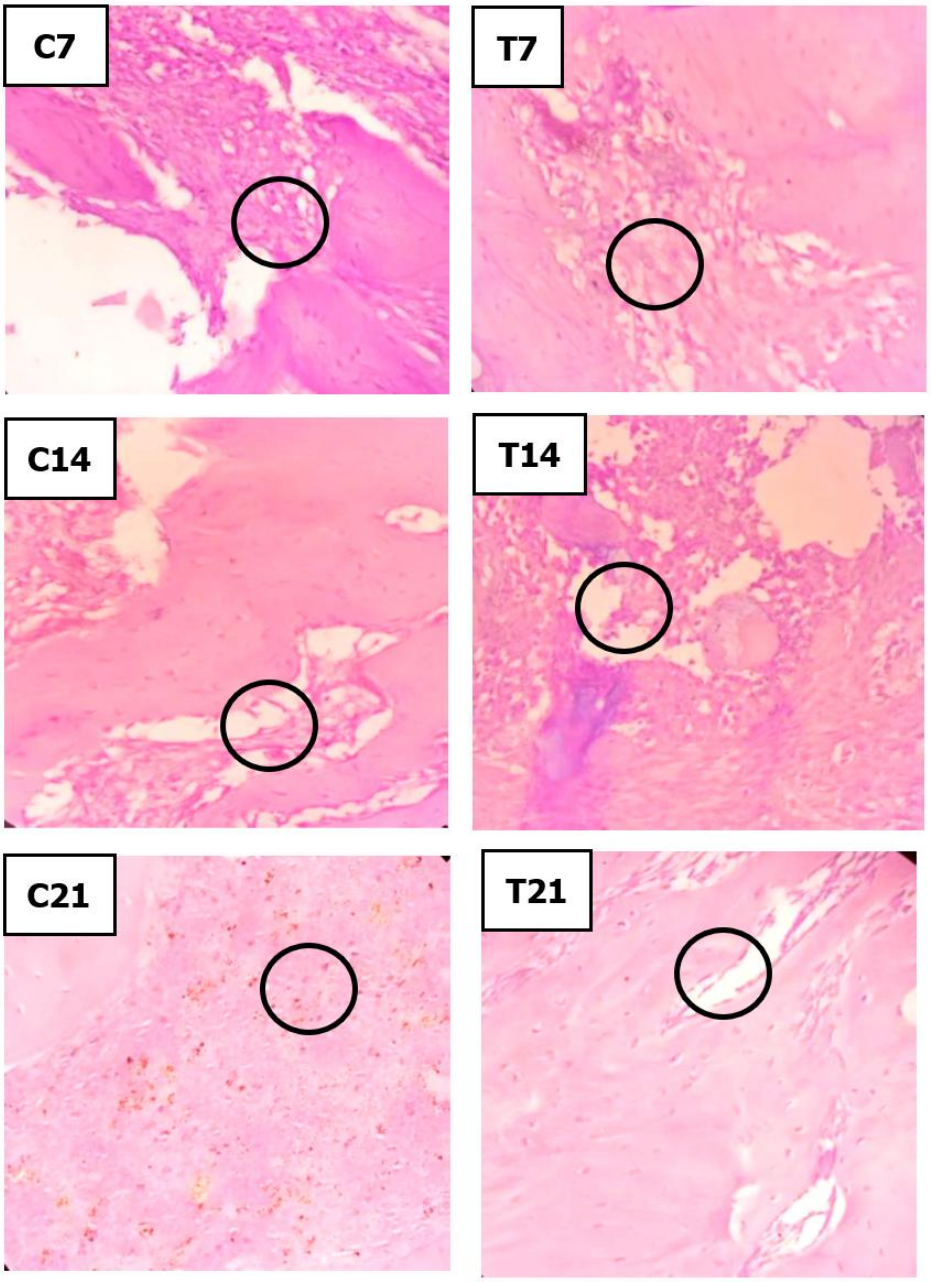


**Figure 1.Histopathological images showing osteoblasts (black circle) within the extraction sockets of guinea pig (*Cavia cobaya*) teeth post-extraction in the treatment (T) and control (C) groups under a light microscope at 400x magnification on days 7, 14, and 21. The treatment group shows more osteoblast cells than the control group on days 7, 14, and 21.**

The One-way ANOVA test revealed a significant difference in the number of osteoblast cells on days 7, 14, and 21 ($p = 0.000$, ($p < 0.05$). This indicates that the application of DDM had a significant effect on increasing the number of osteoblasts during bone healing after tooth extraction.

**Table 2. One-Way ANOVA Test Results**

| ANOVA Test | |
|---|---|
| **Sig. p** | **0,000** |

**Table 3. Mean ± SD Collagen Density Type I on days 7, 14, and 21**

| Group | Day 7 | Day 14 | Day 21 |
|---|---|---|---|
| C | 0,00 ± 0,00 | 1,00 ± 0,00 | 1,00 ± 0,00 |
| T | 1,00 ± 0,00 | 1,67 ± 0,57 | 2,00 ± 0,00 |

The histopathological examination on days 7, 14, and 21 can be observed in Figure 2, showing type I collagen fibers stained blue.

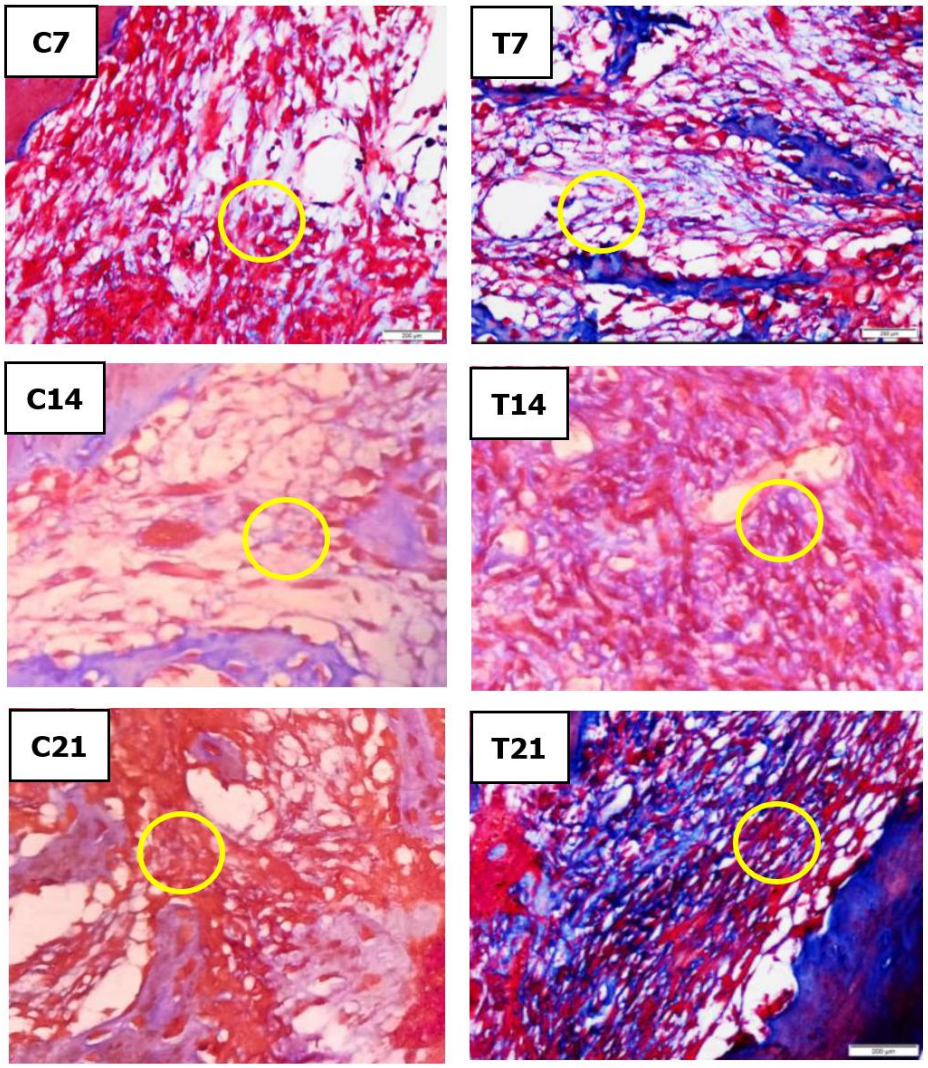


**Figure 2.** Histopathological images showing type I collagen (yellow circle) within the extraction sockets of guinea pig (*Cavia cobaya*) teeth post-extraction in the treatment (T) and control (C) groups under a light microscope at 400x magnification on days 7, 14, and 21. The treatment group (right side of the image) exhibits a higher density of type I collagen compared to the control group on days 7, 14, and 21.

The Kruskal-Wallis test results (Table 4) indicate a statistically significant difference in the density of type I collagen between the control and treatment groups (p = 0.009).

**Table 4. Kruskal-Wallis Test Results**

| | Significance Value |
|---|---|
| Mean Type I Collagen | 0.009 |

## DISCUSSION

The research findings on the effect of Demineralized Dentin Matrix (DDM) on type I collagen and osteoblasts during post-extraction bone healing in guinea pig (*Cavia*

*cobaya*) teeth demonstrated a statistically significant difference ($p<0.05$) between the DDM gel and the PEG gel control regarding the density of type I collagen (table 2) and the number of osteoblasts on days 7, 14, and 21 (table 1). These results indicated that in the treatment group receiving DDM gel, osteoblast cell counts increased progressively on days 7, 14, and 21 following dental extraction. These findings are consistent with Kardikadewi's research, which suggests that Demineralized Dentin Sponge Material (DDMS) can increase osteoblast activity by promoting Runx2 expression during osteoblasticogenesis in the bone healing process.

Runx2, a key transcription factor in skeletal framework development belonging to the Runx protein family (Runx1, Runx2, and Runx3), is involved in the proliferation of osteoblast progenitors derived from mesenchymal cells. Runx2 expression is regulated at the preosteoblastic stage, reaches its peak during the immature osteoblast stage, and is finally regulated during maturation for osteoblastic matrix formation, subsequently influencing the Bone Morphogenetic Protein (BMP) gene.

BMP is a protein that plays an important role in bone formation and regeneration. Demineralized Dentin Matrix (DDM) is a synthetic bone replacement membrane that comes from dentin and has a composition similar to human bone. This collagen type I (Col-i) complex can release growth factors such as BMP and has important biological effects.[17,18]

DDM possesses osteoconductive and osteoinductive properties and can be obtained from human dentin. Its osteoinductive capacity facilitates differentiation into mature osteoblast cells, while its osteoconductive properties aid in cell adhesion, proliferation, and extracellular matrix formation from the material. On day 7, osteoblast proliferation and woven bone formation from socket edges towards the bone trabeculae were still ongoing.[18,19] The results of this study also prove that on day 7 there was an increase in the number of osteoblasts in the control group that did not apply DDM (table 1).

Osteoblast cells contributed to the mineralization of soft callus by secreting type 1 collagen continuously, leading to woven bone formation. On day 14, osteoblasts actively transformed woven bone into lamellar bone. Woven bone is an immature bone with randomly oriented collagen fibers, whereas lamellar bone consists of organized cortical bone with properly aligned collagen fibers. Firdaus indicates that day 14 marks the end of the inflammatory phase and the beginning of the repair phase, characterized by cellular interactions stimulating growth factors, cytokines, and receptors that induce mesenchymal cell differentiation into osteoblasts, fibroblasts, and chondroblasts.

Growth factors such as BMP-2 and TGF-β within hydroxyapatite stimulate new bone formation, resulting in an increased number of osteoblasts. This is consistent with this research's findings of increased osteoblasts in the DDM group, as DDM contains growth factors, including BMP-2 and TGF-β. BMP-2.[19,20] The results of this study also prove that on day 14 there was an increase in the number of osteoblasts in the control group that did not apply DDM (table 1).

By day 21, osteoblast cells began to become embedded within bone trabeculae, forming osteoids. The presence of spicules compacting into bone trabeculae was evident. Once osteoid was formed, osteoblast cells became trapped and differentiated into osteocytes. On day 21, the growth of osteoblasts started to stabilize in the treatment group where osteoblast cells had begun to be trapped in the matrix as osteocytes. Osteocytes originate from osteoblast cells in lacunae and are located in the bone matrix lamella.

Osteocytes function in calcium and phosphate metabolism and respond to mechanical stimuli in bone healing. Vidahayati demonstrated that hydroxyapatite use leads to the formation of bone trabeculae and the transformation of bone into an immature state by day 21. This is in line with the present research, showing increased osteoblast activity and new bone formation.[20,21] The results of this study

also prove that on day 21 there was an increase in the number of osteoblasts in the control group that did not apply DDM (table 1).

The research results depicted in Figure 2 show a mean type I collagen density score of 1.00 in the treatment group on day 7, followed by a significant increase to 2.00 by day 21. In contrast, the control group had a mean type I collagen density of 0.00 on day 7, showing a significant increase to 1.00 on days 14 and 21. Adventa's research regarding the use of hydroxyapatite in the defect area, type 1 collagen first appears on days 3 to 5 and then experiences peak expression on days 7 to 14.

Type 1 collagen continues to increase rapidly until day 21 and is followed by a slower accumulation until day 90. The Minamizato study concluded that DDM administration can increase bone density after clinical application of DDM prepared immediately after tooth extraction for bone augmentation, taking advantage of the relatively short preparation time due to partial demineralization. DDM, as introduced in this study, is an efficient, safe, and sensible bone replacement.

As a result, this material has the potential to be one of the options as a bone replacement in implant dentistry, where the content of DDM contains growth factor and hydroxyapatite so that it can accelerate the process of bone healing and bone remodeling.[8,28] These results indicate that the treatment group receiving DDM gel was more effective in increasing type I collagen density compared to the control group receiving PEG gel.

This is likely because DDM gel contains dentin, which possesses inductive properties similar to bone, is biocompatible and has biochemical characteristics akin to the bone, consisting of approximately 80% hydroxyapatite crystals and 20% type I collagen. Dentin also contains various proteins, including bone sialoprotein, osteopontin, osterix, dentin sialoprotein, and osteocalcin, considered effective bone graft materials.

Furthermore, these materials are osteoinductive and osteoconductive due to the presence of growth factors and contain living bone-forming cells such as pre-osteoclasts and pre-osteoblasts, which can rapidly activate bone growth. These findings are consistent with Bao's study, which showed that DDM can release type 1 collagen and BMP, inducing greater cell migration, including osteoblasts.

DDM of a specific size can accelerate the differentiation of mesenchymal cells into osteoblasts, thereby aiding the process of bone formation. DDM also releases BMP-2 at relatively high concentrations from day 1 to day 13, and recombinant BMP-2 is commonly used in culturing osteoprogenitor cells to induce osteoblast differentiation via the SMAD pathway. Numerous studies have demonstrated similarities between dentin and bone. Recently, a barrier membrane derived from dentin, acting as a collagen osteo-inductive membrane, has shown successful results in bone regeneration. [22,23]

Demineralized Dentin Matrix (DDM) stimulates bone formation both in vitro and in vivo by increasing the expression of the Bone Morphogenetic Protein-2 (BMP-2) gene in bone cells, which subsequently enhances the expression of type I collagen and vascular endothelial growth factor (VEGF), an important factor in inducing osteoblast differentiation and stimulating bone formation. The results of this study also prove that on the 7$^{th}$, 14$^{th}$, and 21$^{st}$ days, there was an increase in the number of osteoblasts and increased collagen density in the control group that did not apply DDM (table 1 & table 3). Kabir et al. Reported that the use of DDM autograft in powder form placed in post-dental extraction sockets resulted in gradual resorption and replacement by new bone (46-74%) through osteoinduction and osteoconduction. A six-year follow-up using cone-beam computed tomography (CBCT) in these patients revealed the formation of bone similar to iliac crest corticocancellous bone.[24,25]

The duration of administration can affect the results of the study. Type I collagen density is one of the important parameters and indicators in the wound healing process. In the wound healing process, type I collagen formation begins

around day 3 and increases significantly by day 7 post-injury. Fibroblasts differentiate to form the extracellular matrix and start producing tropocollagen on day 3, which is then processed into collagen. Day 7 marks the peak of the proliferation phase, where type I collagen accumulates, providing strength to the injured area. By day 7, epithelial regrowth begins, accompanied by a decrease in inflammatory cells and an increase in connective tissue.

The new connective tissue primarily consists of type III collagen initially, but type I collagen accumulation rapidly increases, peaking between days 7 and 14 and continuing to increase until day 21, constituting about 75% of the total collagen in granulation tissue. This study also proved that there was an increase in collagen density on day 7 and followed on day 14 and day 21 in the socket after tooth extraction of the treatment group applied with DDM than in the control group that was not given DDM (table 3). A limitation of this study is that bone remodeling occurs between weeks 8 and 12. Therefore, future research should extend the observation period to include day 28, as osteoblast formation and type I collagen density may become more significant at this stage.[2,26,27,28]

In the study to see the significant differences between treatments regarding type I collagen, it showed that the research data was not normally distributed or homogeneous ($p<0.05$) so the data analysis with nonparametric analysis used the *Kruskal-Wallis* test, while the study on osteoblasts showed normal and homogeneous distributed research data ($p>0.05$) so that it was followed by parametric analysis with One Way ANOVA.

The limitation of this research is that the remodeling phase or maturation phase is the last phase of the wound healing process that can last for months and is declared ending if all signs of inflammation have been lost after the injury, so further research needs to be done on demineralized dentin matrix (DDM) with a time evaluation of more than 21 days.

## CONCLUSION

The study demonstrates that Demineralized Dentin Matrix (DDM) significantly increases osteoblast numbers and type I collagen density at 7, 14, and 21 days post-extraction. These findings suggest DDM's potential as an effective bone graft material to enhance and accelerate alveolar bone remodeling following tooth extraction.

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**Author Contributions**
Research articles with several authors: RKD, SO, ES, and AG conceived and designed the study. RKD, NS, and SA conducted data collection. NS, SA, and NH analyzed and interpreted data. RG, RKD, NS, and SA wrote the final draft. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript. RKD sent the paper for publication.
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**Informed Consent Statement:** Not applicable" for studies not involving humans
**Data Availability Statement:** The results reported support data can be found, including links to a data collection that is publicly archived, analyzed, or produced during the study.
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