Odovtos-International Journal of Dental Sciences (Odovtos-Int. J. Dent. Sc.), Online First, 2026. ISSN: 2215-3411
https://doi.org/10.15517/nwaze102

ODOVTOS
International Journal of Dental Sciences

FOd Facultad de Odontología

https://revistas.ucr.ac.cr/index.php/Odontos

BASIC RESEARCH:

Effects of *Aloe vera* Gel on Osteoclast and Osteoblast Activity and Receptor Activator of Nuclear Factor Kappa-B Ligand Expression During Bone Remodeling Following Orthodontic Temporary Anchorage Device Placement: An *In Vivo* Study

Efectos del gel de *Aloe vera* sobre la actividad de osteoclastos y osteoblastos y la expresión del ligando del receptor activador del factor nuclear kappa-B durante la remodelación ósea posterior a la colocación de dispositivos de anclaje temporal en ortodoncia: Estudio *in vivo*

Pepi Riyani Siregar[1] https://orcid.org/0009-0000-3594-7884
Baharuddin M. Ranggang[1] https://orcid.org/0009-0002-6573-8922
Nasyrah Hidayati[1] https://orcid.org/0009-0001-3488-7952
Eddy Heriyanto Habar[1] https://orcid.org/0000-0002-4228-0624
Eka Erwansyah[1] https://orcid.org/0000-0002-1760-2409
Ardiansyah S. Pawinru[1] https://orcid.org/0000-0002-4763-5439
Zilal Islamy Paramma[1] https://orcid.org/0009-0003-2089-5235

[1]Department of Orthodontic. Faculty of Dentistry, Hasanuddin University, Makassar, Indonesia.

Correspondence to: Baharuddin M. Ranggang - baharuddinfkg@unhas.ac.id

Received: 7-VIII-2025 Accepted: 14-XI-2025

ABSTRACT: The biomedical use of *Aloe vera* continues to expand, including in orthodontics where its role in bone remodeling is of particular interest. This study aimed to evaluate the effect of *Aloe vera* gel on osteoclast, osteoblast, and RANKL expression following Temporary Anchorage Device (TAD) placement. Thirty male New Zealand White rabbits were randomly assigned to control and treatment groups. TADs were placed in the maxillary buccal region, and the treatment group received 50% Aloe vera gel topically. Each rabbit in the treatment group received 0.5 g of 50% *Aloe vera* gel topically around the TAD site twice daily. Histological analysis and ELISA were conducted on days 3, 7, and 14. The results showed a significant increase in osteoblast counts on days 7 and 14 ($p<0.05$). Osteoclast counts remained elevated on day 3 but were significantly lower than the control group ($p < 0.05$), followed by a further decrease on day 14. RANKL expression increased moderately over time ($R^2=0.517$). Regression analysis demonstrated a time-dependent effect of *Aloe vera* on osteoblasts ($R^2=0.834$) and osteoclasts ($R^2=0.928$). These findings suggest that *Aloe vera* gel supports bone remodeling by relatively suppressing osteoclast activity, enhancing osteoblast response, and modulating RANKL expression proportionally, making it a promising adjunct for improving TAD stability in clinical orthodontics.

Copyright (c) 2026 Pepi Riyani Siregar, Baharuddin M. Ranggang, Nasyrah Hidayati, Eddy Heriyanto Habar, Eka Erwansyah, Ardiansyah S. Pawinru, Zilal Islamy Paramma.

Odovtos -Int J Dent Sc endorses to CC-BY-NC-SA 4.0.

KEYWORDS: *Aloe vera*; Bone remodeling; Osteoblasts; Osteoclasts; RANKL; Temporary Anchorage Device (TAD).

RESUMEN: El uso biomédico del *Aloe vera* continúa expandiéndose, incluido su empleo en ortodoncia, donde su papel en la remodelación ósea resulta de especial interés. Este estudio tuvo como objetivo evaluar el efecto del gel de *Aloe vera* sobre la actividad de osteoclastos, osteoblastos y la expresión de RANKL tras la colocación de dispositivos de anclaje temporal (TAD, por sus siglas en inglés). Treinta conejos machos New Zealand fueron asignados aleatoriamente a grupos control y tratamiento. Los TAD se colocaron en la región bucal maxilar, y el grupo de tratamiento recibió gel de *Aloe vera* al 50% de forma tópica. Cada conejo del grupo tratado recibió 0.5 g de gel de *Aloe vera* al 50% aplicado tópicamente alrededor del sitio del TAD dos veces al día. Se realizaron análisis histológicos y ELISA en los días 3, 7 y 14. Los resultados mostraron un aumento significativo en el recuento de osteoblastos en los días 7 y 14 ($p<0.05$). El recuento de osteoclastos se mantuvo elevado en el día 3, pero fue significativamente menor que en el grupo control ($p<0.05$), seguido de una disminución adicional en el día 14. La expresión de RANKL aumentó moderadamente con el tiempo ($R^2=0.517$). El análisis de regresión mostró un efecto dependiente del tiempo del *Aloe vera* sobre los osteoblastos ($R^2=0.834$) y los osteoclastos ($R^2=0.928$). Estos hallazgos sugieren que el gel de *Aloe vera* favorece la remodelación ósea al suprimir relativamente la actividad osteoclástica, potenciar la respuesta osteoblástica y modular proporcionalmente la expresión de RANKL, lo que lo convierte en un coadyuvante prometedor para mejorar la estabilidad de los TAD en la práctica clínica ortodóntica.

PALABRAS CLAVE: *Aloe vera*; Remodelación ósea; Osteoblastos; Osteoclastos; RANKL; Dispositivo de anclaje temporal (TAD).

INTRODUCTION

Orthodontic mini-implants, also known as miniscrews or temporary anchorage devices (TADs), create absolute anchorage (1). Errors in orthodontic treatment, including the use of devices such as TADs, can lead to complications such as root resorption, pain, temporomandibular joint (TMJ) disorders, or even implant failure if the procedures are not performed correctly. Therefore, it is crucial for clinicians to understand bone biomechanics, proper TAD placement site selection, and the biological risks associated with each procedure (2). The direct placement of TADs into the alveolar bone can trigger a local inflammatory response that stimulates bone remodeling through osteoclast and `osteoblast activity (3). One of the main biological processes occurring during the use of TADs is bone remodeling, in which osteoclast and osteoblast cells play crucial roles in continuous bone resorption and formation. Osteoclasts are responsible for bone resorption, while osteoblasts function in forming new bone (4).

Bone remodeling is an adaptive response of bone to mechanical pressure or microenvironmental changes around the TAD. The insertion of a TAD can cause microtrauma that triggers inflammatory activity (5). One important biological pathway in bone remodeling is the RANK/RANKL/OPG pathway. Receptor Activator of Nuclear Factor-κB Ligand (RANKL) is a cytokine from the

Tumor Necrosis Factor (TNF) family that stimulates osteoclast differentiation and activation by binding to its receptor, RANK. Osteoprotegerin (OPG) acts as a decoy receptor that inhibits the RANKL-RANK interaction, thereby regulating the rate of osteoclastogenesis. This pathway is an important molecular indicator in bone remodeling (6). Osteoclast activation causes temporary bone resorption, followed by osteoblast activity to form new bone as an adaptive response to the mechanical stress from the TAD. The balance between these two cellular activities is essential to maintain long-term TAD stability and integrity (5).

Aloe vera is known for its various therapeutic properties, including anti-inflammatory effects, wound healing, and tissue balance regulation (7). *Aloe vera* gel contains active compounds such as polysaccharides, flavonoids, and amino acids, which have the potential to modulate biological responses in bone tissue (8). Several studies support the potential of *Aloe vera* to accelerate healing and bone remodeling. Tahmasebi *et al*. (9) demonstrated that *Aloe vera* could increase the number of osteoblasts in injured bone areas. Tabatabaeian M. & Esfahanian V (10) also found that *Aloe vera* gel significantly reduced inflammation and accelerated bone regeneration in experimental animals. A study by Chithra *et al*. (11) demonstrated that topical application of *Aloe vera* enhances collagen synthesis, particularly type I collagen, and improves extracellular matrix organization in healing wounds of rats, supporting its potential in bone and tissue regeneration. Additionally, Iosageanu *et al*. (12) showed that polysaccharide extracts derived from *Aloe vera* gel significantly enhanced fibroblast proliferation and migration, which are both essential processes in wound healing and extracellular matrix regeneration, potentially involving increased hyaluronic acid activity. Similar findings were reported by Yao *et al*. and Jiang *et al*. (13-14), who found that *Aloe vera* could increase bone mineral density and the expression of osteogenic proteins such as ALP, COL1, and RUNX2. Furthermore, a histological study by Taalab *et al*. (15) on animal models showed that Aloe vera used as an adjuvant with β-TCP significantly enhanced interradicular bone formation.

However, the findings regarding the effects of *Aloe vera* on bone regeneration are not entirely consistent. Soares *et al*. (16) reported that topical application of *Aloe vera* did not result in a significant difference in bone healing speed compared to the control group. Similarly, Paul *et al*. (17) concluded that the effectiveness of *Aloe vera* in musculoskeletal regeneration strongly depends on dose and formulation, as demonstrated in a Wistar albino rat model of inflammatory arthritic injury. In addition, Jiang *et al*. (18) found that low-dose Aloe vera polysaccharide treatment did not significantly affect osteoblast count compared to the control group, suggesting a limited osteogenic response under certain dosage conditions. Likewise, Korani *et al*. (19) reported that *Aloe vera* exhibited the lowest osteogenic potential among several natural substances studied, with honey and propolis showing superior bone regeneration outcomes.

Based on this background and the inconsistency of findings from previous studies, further investigation is necessary to evaluate the effect of *Aloe vera* gel application on bone remodeling in the context of Temporary Anchorage Device (TAD) placement. To date, no studies have specifically quantified the number of osteoclasts and osteoblasts following topical *Aloe vera* gel application after TAD insertion. In addition to cellular markers, the RANK/RANKL/OPG signaling pathway plays a pivotal role in bone remodeling regulation, where RANKL (Receptor Activator of Nuclear Factor κB Ligand) is a key molecule involved in osteoclast differentiation and activation. However, the potential modulatory effect of *Aloe vera* gel on RANKL expression during orthodontic-induced bone remodeling has not yet been clearly elucidated. Therefore, this *in vivo* study was conducted to determine the effect of *Aloe vera* gel application

on osteoclast count, osteoblast count, and RANKL expression during the bone remodeling process after TAD placement, aiming to fill this scientific gap and provide a foundation for the potential use of natural biomaterials as adjuvant therapy in orthodontic practice.

MATERIALS AND METHODS

STUDY DESIGN AND ETHICS

This experimental laboratory study used a post-test only control group design. Ethical approval was obtained (No. 089/KEPK FKG-RSGM UH/EA/III/2025).

SAMPLE SIZE

A total of 30 healthy male New Zealand White rabbits weighing 1.5-2 kg were used as experimental units. The animals were randomly allocated into two groups: a control group (n=15) and a treatment group (n=15) that received 50% Aloe vera gel. Each group was further subdivided into three subgroups (n=5 per time point) for histological evaluation on days 3, 7, and 14, resulting in 30 animals in total for the entire experiment.

The sample size was determined based on previous *in vivo* studies evaluating bone remodeling and osteoblast/osteoclast responses in rabbit models, which commonly used five animals per subgroup to achieve reliable histomorphometric data. Although no formal a priori power calculation was performed, the chosen sample size followed established methodological standards to ensure valid statistical comparisons using independent t-tests and regression analyses, while adhering to the 3Rs principle for animal research.

INCLUSION AND EXCLUSION CRITERIA

The inclusion criteria were healthy male New Zealand White rabbits aged 4-6 months and weighing 1.5-2 kg, clinically free from oral or systemic diseases, and acclimatized for 7 days under controlled laboratory conditions.

The exclusion criteria were (1) rabbits showing signs of illness or anatomical abnormalities, and (2) rabbits with TADs that dislodged before the scheduled observation. These criteria were established a priori to ensure animal welfare and maintain the reliability of histological outcomes.

PREPARATION OF 50% ALOE VERA EXTRACT

Fresh *Aloe vera* leaves were taxonomically verified by the Chemical Testing Laboratory, Department of Agricultural Technology, Politeknik Pertanian Negeri Pangkajene Kepulauan, South Sulawesi, Indonesia confirming the species as Aloe barbadensis Miller. The gel was separated from the washed leaves, dried at 50 °C for 72 h, powdered, and extracted with distilled water at 70 °C using the maceration method. 20 Filtrates from two sequential macerations were combined and concentrated using a water bath at 70 °C. A 50% extract solution was prepared by diluting 50 g of the concentrate in 100 mL distilled water. Sterilization was performed using sequential membrane filtration (0.45 μm followed by 0.22 μm) until particle free and sterile.

TAD PLACEMENT AND GEL APPLICATION

Temporary Anchorage Devices (TADs; Mico One®, 8 mm length, 1.5 mm diameter) made of stainless steel SUS316L were inserted into the

maxillary buccal alveolar bone approximately 1 cm posterior to the incisors, as illustrated in Figure 1. SUS316L was chosen for its excellent biocompatibility and corrosion resistance, minimizing the risk of additional inflammatory reactions. Each rabbit in the treatment group received 0.5 g of 50% Aloe vera gel topically around the TAD site twice daily using sterile cotton swabs.

Figure 1. A. Experimental animals were fitted with Temporary Anchorage Device (TAD) on the right maxilla (red circle), B. Screw Driver.

HISTOLOGICAL EXAMINATION (HE)

Histological sample processing was carried out at the Anatomical Pathology Laboratory, Hasanuddin University Teaching Hospital. On experimental days 3, 7, and 14, animals from both control and treatment groups were euthanized following subcutaneous injection of atropine sulfate, succeeded by intramuscular administration of a castran-ketamine combination for deep anesthesia. Euthanasia was performed via cardiac air embolism. Maxillary bone segments (approximately 1×2 cm), centered around the TAD insertion sites, were excised and fixed in 10% neutral buffered formalin (pH 7.0) for 24-48 hours. Specimens underwent decalcification and dehydration through graded ethanol series (70% to absolute), followed by xylene clearing and paraffin embedding at 56-58°C. Paraffin blocks were sectioned using a rotary microtome at a thickness of 10 μm in a vertical plane relative to the TAD placement. Sections were floated in a water bath (38-40°C), mounted on gelatin-coated slides, and dried overnight at 38-40 °C. Routine hematoxylin-eosin (H&E) staining was performed following sequential deparaffinization (xylene I-III), rehydration (absolute to 70% ethanol), hematoxylin staining, eosin counterstaining, and final clearing in xylene. Coverslips were applied using a mounting medium. Osteoclasts appeared as large multinucleated cells attached to resorbing bone surfaces, while osteoblasts were small cuboidal cells aligned along forming bone surfaces.

Each histological slide was labeled with a unique identification code indicating the animal number, experimental group (control or treatment), and observation day (3, 7, or 14). Coding and randomization were performed by a blinded technician to prevent observer bias during microscopic evaluation. Histological observations were performed using a light microscope (Leica ICC50W, Leica Camera, Germany) at 400× magnification to quantify cellular expression. Quantification was carried out using the Immuno Ratio Scale (IRS) software program. The expression level was determined based on the intensity and distribution of brown-colored staining within the tissue sections, expressed as an integrated value per specimen.

Tissue specimens were obtained from the sites where the Temporary Anchorage Devices

(TADs) had been removed. Observations were performed in three representative fields of view corresponding to the cervical, middle, and apical thirds of the peri-TAD bone area. For each section, five random microscopic fields were evaluated, and the mean cell count was calculated. All evaluations were conducted independently by two blinded observers, and inter-observer consistency was verified before statistical analysis.

MOLECULAR ANALYSIS (RANKL EXPRESSION)

To assess RANKL expression, enzyme-linked immunosorbent assay (ELISA) was performed using Rabbit RANKL ELISA Kit 96T (Reed Biotech, USA). The specific ELISA plate used was the pre-coated 12×8 well strips, Cat. No.: MBGAM0002, Lot: M170425E6, Exp: 20260516. Blood serum samples were obtained from the central ear artery. All reagents and standards were prepared according to the manufacturer's protocol. Serum samples and standards were pipetted into the microplate wells pre-coated with specific antibodies. After incubation and washing, HRP-conjugated detection antibodies were added, followed by the substrate solution. The reaction was terminated with stop solution, and absorbance was read at 450 nm using a microplate reader. The concentration of RANKL in each sample was calculated based on a standard curve generated from the provided standards. All procedures were carried out at the Hasanuddin University Teaching Hospital.

STATISTICAL ANALYSIS

Osteoclast and osteoblast counts were analyzed using SPSS software version 25.0 (IBM Corp., Armonk, NY, USA). The normality of the data was assessed using the Shapiro-Wilk test, and Levene's test was used to evaluate the homogeneity of variances. Data that were normally distributed and homogeneous were analyzed using the independent samples t-test to compare the control and treatment groups. In addition, linear regression analysis was performed to evaluate the time-dependent effect of *Aloe vera* application on cell counts. RANKL expression was measured using the enzyme-linked immunosorbent assay (ELISA) method, and the results were statistically analyzed using the same SPSS software. A p-value of <0.05 was considered statistically significant.

RESULTS

Figure 2 histological evaluation was conducted to examine the morphological characteristics of bone tissue following Temporary Anchorage Device (TAD) placement in both control and Aloe vera-treated groups. Figure 2 presents representative hematoxylin-eosin (H&E)-stained sections consolidated into three panels: the Control group, the *Aloe vera*-treated Experimental group, and a comparative view summarizing overall tissue morphology. The micrographs illustrate sequential cellular and structural patterns across the observation period of 3, 7, and 14 days.

In the early observation period, bone tissue exhibited irregular surfaces with multinucleated cells distributed along resorption lacunae. These cells were frequently positioned adjacent to shallow depressions on the bone surface. Small cuboidal cells were also present, aligned near areas of early matrix deposition. The surrounding connective tissue displayed moderate cellularity and visible vascular channels.

As the observation period progressed, surface-lining cells became more uniformly distributed along the bone margins. The cuboidal cells appeared denser and occupied broader regions, while the bone matrix adjacent to them demonstrated more homogeneous eosinophilic staining. Multinucleated cells persisted but were less frequent and limited to discrete resorption sites.

In the final observation period, bone tissue showed a compact, uniformly stained matrix with a continuous layer of lining cells along the bone surface. The intertrabecular spaces contained fewer stromal cells compared with earlier stages. While small gaps between lining cells remained visible in the control group, the Aloe vera-treated group exhibited a more cohesive and organized cellular layer.

All micrographs were captured at 400× magnification. Figure 2 provides a visual representation of the progressive histological changes and cellular distribution observed throughout the experimental timeline.

Table 1 presents the mean osteoclast and osteoblast counts in the control and Aloe vera-treated groups across days 3, 7, and 14. Osteoclast numbers were consistently and significantly lower in the treatment group compared to the control group at all time points (Day 3: 12.80 ± 3.11 *vs*. 8.00 ± 1.00; Day 7: 6.60 ± 1.34 *vs*. 4.40 ± 0.55; Day 14: 4.40 ± 0.89 *vs*. 0.80 ± 1.10; $p<0.05$).

Conversely, osteoblast counts were significantly higher in the treatment group than in the control group at the same time points (Day 3: 41.20 ± 21.29 *vs* 130.40 ± 50.82; Day 7: 171.80 ± 18.17 *vs*. 222.40 ± 22.51; Day 14: 215.60 ± 22.11 *vs*. 319.20 ± 36.46; $p<0.05$).

Table 2 summarizes the mean RANKL levels in the control and treatment groups. RANKL expression was significantly higher in the Aloe vera group compared to control on days 3, 7, and 14 (0.043 ± 0.005 *vs*. 0.022 ± 0.009; 0.036 ± 0.004 *vs*. 0.020 ± 0.006; 0.029 ± 0.005 *vs*. 0.016 ± 0.004, respectively), with $p<0.05$ for all comparisons.

Table 3 presents the comparison of osteoclast and osteoblast counts in the treatment group over the observation period. Osteoblast numbers increased significantly from 130.40±50.82 on day 3, to 222.40±22.51 on day 7, and 319.20±36.46 on day 14. Conversely, osteoclast numbers decreased significantly from 8.00±1.00 on day 3, to 4.40±0.55 on day 7, and 0.80±1.10 on day 14. RANKL expression showed a slight increase over time, from 0.020±0.008 on day 3 to 0.023±0.013 on day 14.

Regression analysis demonstrated a significant time-dependent effect on osteoblast counts (R^2=83.4%, $p<0.05$) and osteoclast counts (R^2=92.8%, $p<0.05$). RANKL expression exhibited a moderate time-dependent correlation (R^2=51.7%), indicating a less pronounced but consistent regulatory response over time.

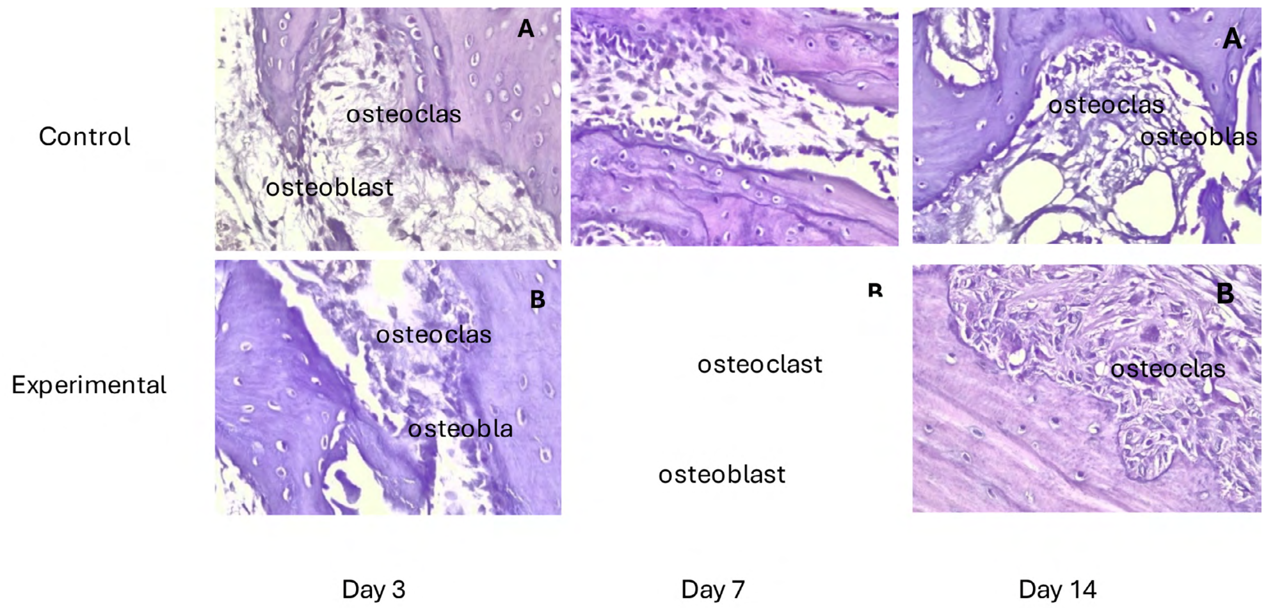

Figure 2. Histological evaluation of osteoblast and osteoclast activity at different observation periods. Representative H&E-stained sections of bone tissue from the Control Group (upper row) and the Experimental Group treated with *Aloe vera* (lower row) at 3, 7, and 14 days post-treatment. Osteoblasts (yellow boxes) line the bone matrix, while osteoclasts (red boxes) are located in resorption lacunae. Progressive changes in bone remodeling and cellular organization are observed over time. Original magnification: 400×.

Table 1. Mean Osteoclast and Counts by Group and Observation Time (mean ± SD).

	Osteoclasts		
Times	**Control**	**Experimental (*Aloe vera*)**	**P value**
Day-3	12.80 ± 3.11	8.00 ± 1.00	0.001
Day-7	6.60 ±1.34	4.40 ± 0.55	0,009
Day-14	4.40 ±0.89	0.80±1.10	0,000
	Osteoclasts		
Times	**Control**	**Experimental (*Aloe vera*)**	**P value**
Day-3	41.20 ± 21.29	130.40 ± 50.82	0.007
Day-7	171.80 ±18.17	222.40 ± 22.51	0,004
Day-14	215.60 ±22.11	319.20±36.46	0,001

*Independent sample t-test, significant ($p<0.05$).

Table 2. Mean RANKL Counts by Group and Observation Time (mean ± SD).

	RANKL		
Groups	**Control**	**Experimental (*Aloe vera*)**	**P value**
Day-3	0.022 ± 0.009	0.043 ± 0.005	0.002
Day-7	0.020 ± 0.006	0.036 ± 0.004	0,001
Day-14	0.016 ± 0.004	0.029± 0.005	0,002

*Independent sample t-test, significant ($p<0.05$).

Table 3. Effect of osteoclast, osteoblast and RANKL counts in each treatment group based on observation time.

Groups	Observation Period			R Square value
	Day-3	Day-7	Day14	
Osteoclasts	8.00 ± 1.00	4.40 ± 0,55	0.80 ± 1.10	0,928
Osteoblast	130.40 ± 50.82	222.40 ± 22.51	319.20 ± 36.46	0.834
RANKL	0.020 ± 0.008	0.021 ± 0.011	0.023 ± 0.013	0,517

* Linear regression.

DISCUSSION

The placement of Temporary Anchorage Devices (TADs) induces localized mechanical stress and an inflammatory response that triggers bone remodeling through osteoclast and osteoblast activity. In this study, the topical application of 50% *Aloe vera* gel significantly decreased osteoclast counts while increasing osteoblast numbers over time, accompanied by a moderate rise in RANKL expression. These findings indicate that *Aloe vera* regulates the balance between bone resorption and formation, promoting osteoblastic activity during the healing phase and supporting a stable remodeling process around TADs.

The biological mechanism responsible for these effects is associated with *Aloe vera*'s bioactive compounds-particularly acemannan and aloin-which stimulate osteoblast differentiation through the upregulation of Runx2, BMP-2, and Osterix. At the same time, these compounds inhibit pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6. This dual mechanism suppresses osteoclastogenesis by modulating the RANK/RANKL/OPG pathway and enhances bone matrix deposition. The moderate increase in RANKL observed in this study likely represents a physiological adaptation to maintain bone turnover homeostasis rather than excessive resorption, reflecting a controlled remodeling process within the peri-TAD bone microenvironment.

These results are consistent with previous studies reporting that *Aloe vera* polysaccharides enhance osteogenesis through the BMP-2/Smads and Wnt/β-catenin signaling pathways (Yao *et al*., 2022; Wu *et al*., 2020). In contrast, Soares *et al*. (2019) and Paul *et al*. (2021) observed variable outcomes, possibly due to differences in *Aloe vera* concentration, formulation, or exposure duration. The 50% formulation used in the present study appears to provide an optimal balance, stimulating bone regeneration while minimizing inflammatory responses.

This study has several limitations. It was conducted on an animal model, which limits the direct translation of findings to human clinical situations. The analysis was restricted to histological cell counts and ELISA-based RANKL quantification without molecular-level confirmation of gene or protein expression. Furthermore, the 14-day observation period only represents the early phase of bone remodeling; thus, long-term effects of Aloe vera on bone maturation and TAD stability remain unverified. Future research with extended observation periods and molecular pathway analyses is required to further elucidate the precise osteogenic mechanisms of *Aloe vera*.

In summary, topical *Aloe vera* gel enhances bone remodeling following TAD placement by suppressing osteoclast activity, promoting osteoblast differentiation, and modulating RANKL expres-

sion in a controlled manner. These biological effects highlight *Aloe vera*'s potential as a natural bioactive adjunct to accelerate peri-implant bone healing and improve TAD stability in orthodontic treatment.

CONCLUSION

There were significant differences in osteoclast, osteoblast, and RANKL expression following *Aloe vera* gel application during bone remodeling with TAD placement on days 3, 7, and 14. The duration of *Aloe vera* administration significantly reduced osteoclasts, increased osteoblasts, and moderately elevated RANKL expression. This proportional rise in RANKL suggests Aloe vera may regulate bone remodeling through the RANK/RANKL/OPG pathway and contribute to improved TAD stability.

CONFLICT OF INTEREST: There is no conflict of interest.

AUTHOR CONTRIBUTION STATEMENT: Conceptualization: P.R.S., B.M.R.; Investigation, Resources: P.R.S., E.H.H., A.S.P. and Z.I.P.; Methodology: P.R.S., E.H.H., E.E., A.S.P. and Z.I.P.; Validation: P.R.S. and E.E.; Visualization: P.R.S. and A.S.P.; Writing-original draft and review & editin: P.R.S., B.M.R., N.H., E.H.H., E.E., A.S.P. and Z.I.P.; Formal analysis: B.M.R., N.H. and E.E.; Funding acquisition: P.R.S. and B.M.R.; Software: A.S.P.; Project administration: P.R.S.; Supervision: P.R.S. B.M.R. and N.H.

ACKNOWLEDGMENTS: We thank the Orthodontic Department at Hasanuddin University for their laboratory support.

REFERENCES

1. Erwansyah, E., Ranggang, B. & Bakri, R., 2023. Orthodontic mini-implants: principles and applications. Journal of Orthodontic Research, 12 (2), pp.45-53.
2. Pawinru, H., Habar, D. & Wahyuni, S., 2023. Complications of temporary anchorage devices in orthodontics. Dental Sciences Review, 9 (1), pp.15-22.
3. Jeon H.H., Park J.G., Ahn S.J., et al. Mechanistic insight into orthodontic tooth movement: a review of alveolar bone remodeling involving osteoclastogenesis and osteogenesis. Int J Mol Sci. 2021; 22 (1): 508-526. PMID: 33923725.
4. Omi M, Mishina Y. Roles of osteoclasts in alveolar bone remodeling: focus on inflammation, mechanotransduction and RANKL/OPG regulation. Genesis. 2022; 60 (8-9): e23490. PMID: 35757898.
5. Zhao, L., Chen, Y. & Li, X., 2021. Inflammatory microenvironment and bone remodeling around orthodontic TADs. Orthodontics & Craniofacial Research, 24 (3), pp.315-323.
6. Rehatta R., Gunawan T., Malik A. The role of RANK/RANKL/OPG signaling in bone remodeling induced by mechanical stress. J Bone Metab. 2025; 30 (2): 89-98.
7. Matei C.E., Visan A.I., Cristescu R. Aloe Vera polysaccharides as therapeutic agents: benefits vs side effects. Polysaccharides. 2025; 6 (2): 36.
8. Patel, S., Yadav, R. & Gupta, A., 2024. Aloe vera and its role in bone regeneration: A review. Journal of Bone Research, 39 (7), pp.1023-1034.

9. Tahmasebi A., Shapouri Moghadam A., Enderami S.E., Islami M., Kaabi M., Saburi E., Mansouri V. Aloe vera-derived gel-blended poly(3-hydroxybutyrate-co-3-hydroxyvalerate) nanofibrous scaffold for bone tissue engineering. ASAIO J. 2020; 66 (8): 966-973. doi: 10.1097/MAT.0000000000001094
10. Tabatabaeian M., Esfahanian V. Effect of acemannan/aloe vera on bone regeneration specially in the oral and maxillofacial region: a literature review. J Res Dent Maxillofac Sci. 2023; 8 (3): 226-35. doi: 10.61186/jrdms.8.3.226
11. Chithra P., Sajithlal G.B., Chandrakasan G. Influence of Aloe vera on collagen characteristics in healing dermal wounds in rats. J Ethnopharmacol. 1998; 59 (3): 179-186. doi:10.1016/S0378-8741(97)00122-8
12. Iosageanu A., Mihai E., Seciu-Grama A.M., Utoiu E., Gaspar-Pintiliescu A, Gatea F, et al. In vitro wound-healing potential of phenolic and polysaccharide extracts of Aloe vera gel. J Funct Biomater. 2024; 15 (9): 266. doi: 10.3390/jfb15090266
13. Yao X., Zhang Y., Ge C., Zhao Y., Wang D., Sun Y. et al. Aloe polysaccharide promotes osteogenesis potential of adipose-derived stromal cells via BMP-2/Smads and prevents ovariectomy-induced osteoporosis. Bioengineering (Basel). 2022; 9 (12): 758.
14. Jiang L., Yang M., Xu Y., Zhang H., Chen Y. et al. Polysaccharides from Aloe vera improve bone regeneration by upregulating Runx2 and osteocalcin expression in rats with pulpitis. Acta Cir Bras. 2022; 37 (9): e370906.
15. Taalab MR, Ragab RA, Ghallab NA, Saafan AM, Elgendy EA. Histomorphometric and immunohistochemical evaluation of Aloe vera gel combined with β-tricalcium phosphate in treatment of grade II furcation defects. Sci Rep. 2023; 13 (1): 4032.
16. Soares I.M.V., Abuna G., Sari D.M., et al. Treatment of bone defects with MSCs, Hemospon®, and Aloe vera gel in rat calvarial defects: bone formation was not significantly different between groups. J Appl Oral Sci. 2019; 27: e20180305
17. Paul S., Paul S., Khan M.T.A., Islam A. Chanda J., Kabir S.M.H., et al. Dose-dependent modulation of inflammation and gene expression by Aloe vera gel homogenate in Freund's complete adjuvant-induced arthritic rats. Future J Pharm Sci. 2021; 7: 60. doi: 10.1186/s43094-020-00163-6
18. Jiang L., Yang M., Xu Y., Zhang H., Chen Y., et al. Polysaccharides from Aloe vera target the Wnt/β-catenin pathway to impact tooth density of pulpitis rats. Acta Cir Bras. 2022; 37 (12): e371202.
19. Korani S., et al. Applications of honeybee-derived products in bone tissue regeneration: honey and propolis outperform Aloe vera. Bone Rep. 2024;13:100523.
20. Ritchie C. Temporary anchorage devices in orthodontics: A review of their use and effects. Orthod Update. 2023; 16 (3): 125-30. PMID: 37201230.
21. Matsunaga S., Kido M.A., Saito M. Effects of mechanical stress on osteoclasts and osteoblasts. J Bone Miner Metab. 2017; 35 (3): 251-60. PMID: 28180988.
22. Roberts-Harry D., Sandy J. Orthodontics. Part 10: anchorage control and distal movement. Br Dent J. 2004; 196 (5): 255-63. PMID: 15039727.
23. Surjushe A., Vasani R., Saple D.G. Aloe vera: a short review. Indian J Dermatol. 2008; 53 (4): 163-6. PMID: 19882025.
24. Hamman J.H. Composition and applications of Aloe vera leaf gel. Molecules. 2008; 13 (8): 1599-616. PMID: 18794790.
25. Choi S., Lee S.K., Kim Y.W., et al. Aloe vera and its components modulate osteogenic activity through BMP-2, Runx2, and osterix. J Clin Biochem Nutr. 2011; 49 (3): 122-8. PMID: 22174599.
26. Boonyagul S., Srisatjaluk R.L., Amornchat C., et al. Acemannan stimulates prolifera-

tion and osteogenic differentiation of human mesenchymal stem cells. J Med Assoc Thai. 2014; 97 Suppl 2: S26-34. PMID: 25365971.

27. Elkasas R.M., El-Deeb M.A., Fathalla M. Effect of Aloe vera gel on socket healing following tooth extraction: A histological and histomorphometric study. Arch Oral Biol. 2020; 110: 104598. PMID: 31982593.
28. Jeon G.C., Cha J.Y., Lim W.H., et al. The effect of mechanical force on periodontal tissue remodeling during orthodontic tooth movement. J Periodontal Implant Sci. 2021; 51 (1): 3-12. PMID: 33725256.
29. Shields E.D. A morphogenetic hypothesis for bone remodeling. Anat Rec. 2001; 263 (2): 107-17. PMID: 11559902.
30. Boyle W.J., Simonet W.S., Lacey D.L. Osteoclast differentiation and activation. Nature. 2003; 423 (6937): 337-42. PMID: 12748652.
31. Nazir SF, Khan MM, Khan SN, et al. Aloe vera modulates bone remodeling markers via OPG/RANKL/RANK pathway. Biomed Res Int. 2020;2020:1–9. PMID: 32934845.
32. Wu Y., Quan Q., Zeng B., et al. Aloe vera polysaccharides enhance bone regeneration via BMP-2/Smads and Wnt/β-catenin pathways. Int J Biol Macromol. 2020; 149: 1009-17. PMID: 31918088.
33. Zhang X., Chen Y., Zhu X., et al. Osteogenic potential of Aloe vera-modified scaffolds in bone tissue engineering. Biomed Pharmacother. 2022; 145: 112461. PMID: 34736078.
34. Reddy R.L., Reddy M., Reddy M. Comparative evaluation of bone regeneration using natural substances-Aloe vera, honey, and propolis in extraction sockets. J Clin Diagn Res. 2016; 10 (5): ZC55-60. PMID: 27437337.