The effect of locally applied ferulic acid on osteoblast proliferation and trabecular bone thickness in a rabbit model

Mohanad Imad Nsaif[1]
Saif Saad Ali Al-Jewari[2]
Abdulsattar Salim Mahmood[3]

[1] Ministry of Health – Anbar Health Directorate, Anbar, Iraq
[2] Department of Oral and Maxillofacial Surgery, College of Dentistry, University of Mosul, Mosul, Nineveh, Iraq
[3] Department of Oral and Maxillofacial Surgery, College of Dentistry, University of Mosul, Mosul, Nineveh, Iraq

Corresponding Author: Mohanad Imad Nsaif
e-mail: mohanad.22dep10@student.uomosul.edu.iq

Abstract

***Background*: The preservation of the alveolar ridge after tooth extraction is essential for maintaining bone volume, which is vital for subsequent implant placement. Ferulic acid (FA), a polyphenol distinguished by its antioxidant and osteogenic properties, has the potential to augment bone regeneration. Nevertheless, its specific role in alveolar bone healing warrants further investigation.**
***Purpose*: This study assesses the effects of locally administered FA in conjunction with demineralized freeze-dried bone allograft (DFDBA) on osteoblast proliferation (Ki-67 expression) and trabecular bone thickness within a rabbit model.**
***Materials and Methods*: This in vivo experimental animal study was conducted using twenty male New Zealand white rabbits (1.5–2 kg), which were allocated into four groups (n=5): Group A received 2% FA + 98% DFDBA, Group B received 4% FA + 96% DFDBA, Group C was treated with DFDBA alone (positive control), and Group D underwent natural healing (negative control). The alveolar sockets were treated accordingly and sutured following extraction of the upper central incisor. Histological analysis involved hematoxylin and eosin (H&E) staining and immunohistochemical assessment of Ki67 expression to evaluate osteoblast proliferation. Trabecular bone thickness was measured through digital histomorphometry. Statistical analyses were performed using appropriate parametric or non-parametric tests.**
***Results*: Groups receiving FA (2% and 4%) demonstrated significantly higher Ki67 expression and trabecular bone thickness compared to the DFDBA-only and natural healing groups ($p < 0.05$). The 4% FA group exhibited the most pronounced osteogenic response.**
***Conclusion*: Local application of FA, particularly at 4%, enhances osteoblast proliferation and trabecular bone thickness in alveolar ridge preservation. This suggests its potential as an adjunct in post-extraction bone regeneration strategies.**

Key words: Ferulic acid, Alveolar Ridge Augmentation, Allografts, Ki-67 Antigen, Bone Regeneration.

Introduction

Ferulic acid (4-hydroxy-3-methoxy cinnamic acid) was initially isolated from Ferula foetida (1). It is a derivative of caffeic acid commonly found in plants, fruits, and various beverages, including coffee and beer (2, 3). The biological effects of ferulic acid began to be recognized in the 1970s, when researchers in Japan first identified its antioxidant properties in rice oil (4). Furthermore, ferulic acid may inhibit platelet aggregation

How to Cite

Mohanad Imad Nsaif, Saif Saad Ali Al-Jewari, Abdulsattar Salim Mahmood. **The effect of locally applied ferulic acid on osteoblast proliferation and trabecular bone thickness in a rabbit model.**
Oral and Implantology
Vol. 17 No. 3 (2025), 282-293.
DOI 10.11138/oi.v17i3.148

and the synthesis of thromboxane-like molecules, thereby potentially reducing the risk of thrombosis (5). Additionally, ferulic acid has been shown to negatively regulate osteoclast activity (6).

Additionally, phenolic acids stimulate bone formation via specific signaling pathways (7). As a demineralized freeze-dried bone allograft (DFDBA), demyelinated bone matrix (DBM) constitutes an allograft. DFDBA offers an osteoconductive surface and is a significant source of osteoinductive elements (8). Consequently, it promotes mesenchymal cell migration, adhesion, and osteogenesis following implantation in well-vascularized bone (9). It further facilitates endochondral bone regeneration in regions that otherwise fail to produce bone. DFDBA was treated with an acidic solution to remove mineral ingredients while retaining most proteinaceous components of bone, together with low amounts of calcium-based solids, inorganic phosphates, and trace cellular debris (10). DFDBA possesses a propensity for accelerated degradation, thereby facilitating the creation of new bone (11). Consequently, commercially manufactured allograft-retained proteins can influence cellular activity in vivo (12, 13). The primary advantage of allografts lies in their mechanical qualities, which are comparable to those of autogenous bone, and their potential to include the collagenous matrix and proteins present in normal bone, despite lacking vital cells. Their handling qualities are similar to those of autologous bone (14). Bones are a particular type of connective tissue in the human body, characterized by calcium reinforcement and the presence of specialized bone cells. Bone tissue undergoes continuous remodeling, including formation and resorption, which is essential for maintaining the integrity and architecture of the skeleton (15). Optimal bone health depends on a balanced diet rich in nutrients, regular weight-bearing activity, and sufficient hormonal equilibrium (16-19). Bone remodeling is an unpredictable process wherein osteoclasts remove old or damaged bone, which is subsequently replaced by new bone tissue produced by osteoblasts (20). The Ki-67 protein functions as a crucial proliferation marker in disease. Ki-67 was first recognized as an antigen for a monoclonal antibody found in the nucleus of growing cells (21). Although antibodies targeting Ki-67 are essential tools in cancer detection, the role of this protein remained unclear for an extended period. The sole relevant characteristic of Ki-67 was its absence in quiescent cells and its presence during cellular proliferation (21). The protein is expressed in isoforms of 320 and 359 kDa, derived from differentially spliced mRNA variants encoded by the human MKI67 gene (22). Both isoforms of Ki-67 function similarly as surfactants, maintaining the separation of mitotic chromosomes following nuclear envelope breakdown. Ki-67 forms the perichromosomal protein compartment by binding to protein phosphatase 1 (23).

Materials and Methods

This study received approval from the Ethical and Scientific Research Committee of the Department of Oral and Maxillofacial Surgery at the College of Dentistry, University of Mosul, Iraq (Approval No. UoM 24/1028 dated 02/09/2024). All procedures adhered to the ARRIVE guidelines for animal research, ensuring humane treatment, minimization of pain, and compliance with institutional animal welfare protocols. The materials used included ferulic acid (FA), obtained from Taslan Chemicals® (USA) with a purity of 99% ($C_{10}H_{10}O_4$), and demineralized freeze-dried bone allograft (DFDBA), supplied by CenoBone™ Freeze-Dried Bioimplants (UK).

Procedure

This **in vivo experimental animal study** used twenty male New Zealand white rabbits (1.5–2 kg). They were housed under standard conditions with free access to water and a controlled diet and classified as follows:

The rabbits were randomly divided into four groups (n = 5 per group). Group A received 2% ferulic acid graft mixed with demineralized freeze-dried bone allograft (DFDBA). Group B received 4% ferulic acid mixed with DFDBA. Group C was treated with DFDBA alone, and Group D served as the natural healing (negative control) group. Following **anesthesia and analgesia** administration with **Ketamine**: 10–50 mg/kg intramuscularly (Turkey), **Xylazine**: 3–5 mg/kg intramuscularly (Ireland), and **Lidocaine 2%:** local infiltration (UAE). Once sedation was achieved in the rabbits, the extraction of the upper central incisor was performed with caution to prevent any fracture of the bony plates. The alveolar sockets were debrided to remove residual soft tissue, including the periodontal ligament and lamina dura, using a bone curette and spoon excavator, and subsequently filled according to the respective treatment assigned to each group. The sockets were then sutured using 4/0 silk sutures, and the animals were monitored post-operatively (see Figure 1).

Following a period of 21 days, the animals were humanely euthanized, and bone specimens were subsequently collected and fixed in 10% buffered formalin. The samples underwent decalcification in 10% formic acid and were embedded in paraffin. Histological sections of 5 μm thickness were prepared and stained with hematoxylin and eosin for the purpose of measuring trabecular bone thickness. Additionally, immunohistochemical staining was performed to assess Ki67

Trabecular Bone Thickness Measurement:

Quadrant Partitioning

Each histological slide is divided into four quadrants and further subdivided into four sub-quadrants (Al Hijazi & Salim, 2010) (37). To ensure unbiased measurement of trabecular thickness (μm), measurements were taken in three randomly selected fields within each sub-quadrant, utilizing ProView™ digital morphometry software (Optica Microscopes, Italy). The mean trabecular thickness per slide was then calculated from a total of 48 measurements (4 quadrants × 4 sub-quadrants × 3 fields).

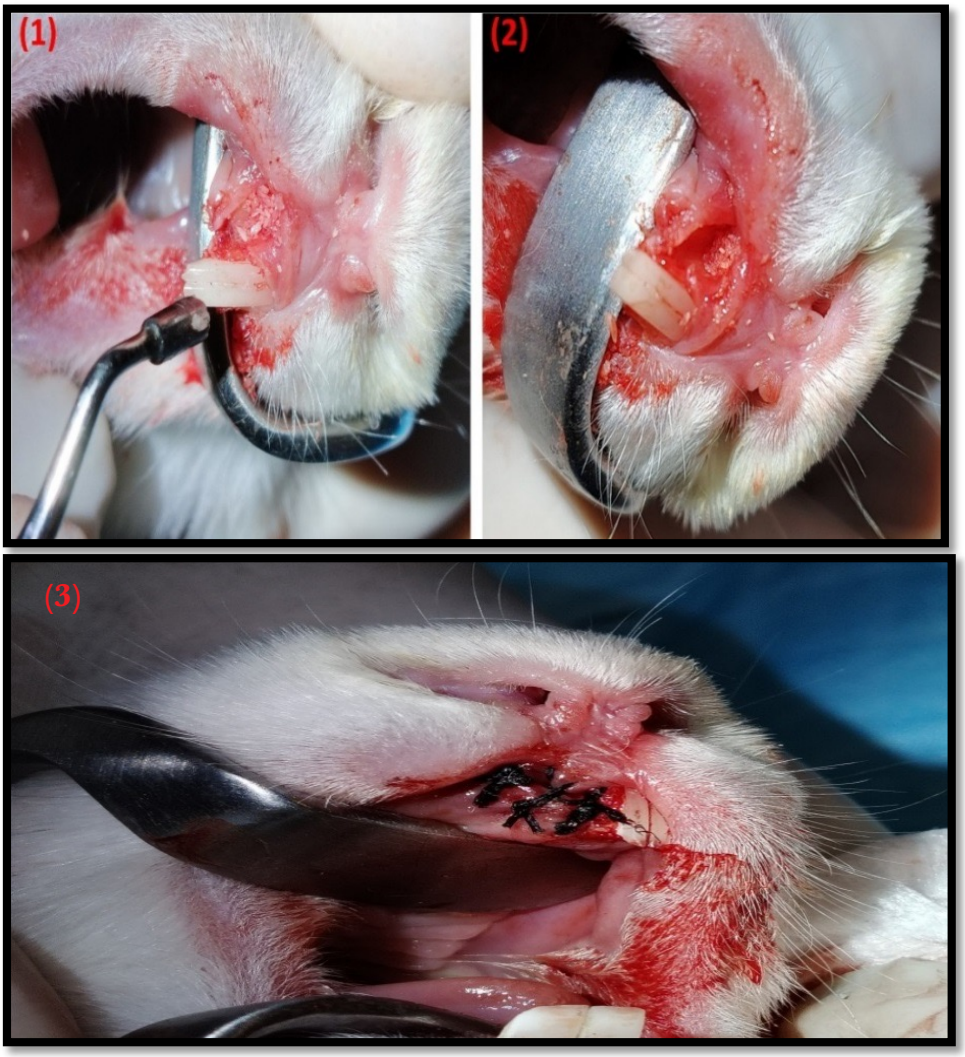

Figure 1. Augmentation and Suturing Procedures. **1:** Packing the Augmented Mixture**:** Demonstrates the placement of the augmentation material into the dental socket until it is fully packed. **2:** Final Lock Before Suturing: Shows the stabilized augmented mixture within the socket, prepared for suturing. **3:** Simple Continuous Suturing Technique: Illustrates the application of a simple continuous suture using silk thread to ensure a watertight seal within the treated socket.

Ki-67 Immunohistochemistry:

Scoring: The scoring criteria for Immunohistochemistry (IHC) for **Ki-67** in this study were adopted in accordance with **Li YX et al.** (2015) (24), as delineated in Table 1.

Statistical Analysis

Statistical analyses were conducted utilizing **IBM SPSS Statistics 28** for the evaluation of histological data, including trabecular bone thickness and Ki-

Table 1. Scoring criteria of Immunohistochemistry (IHC) for Ki-67 in this study

Staining positive cells		Staining intensity		Final score product	
Percent (%)	**Score 1**	**Intensity**	**Score 2**	**Score 1 × Score 2**	**Score 3**
<5%	0	Absent	0	0–1	0 (-)
6–25%	1	Weak	1	2–4	1+ (+)
26–50%	2	Moderate	2	5–8	2+ (++)
51–75%	3	Strong	3	9–12	3+ (+++)
76–100%	4				

Scoring Methodology: Final score (Score 3) = (Percentage of positive cells Score 1) × (Staining intensity Score 2). Example 1: <5% cells (Score 1 = 0) + Weak intensity (Score 2 = 1) → 0 × 1 = 0 (Score 3 = 0 (-)). Example 2: 80% cells (Score 1 = 4) + Strong intensity (Score 2 = 3) → 4 × 3 = 12 (Score 3 = 3+ (+++)) (24).

67. The normality of data distribution was examined through the **Shapiro-Wilk test**, while the homogeneity of variances was assessed using **Levene's test**.
A non-parametric **Kruskal-Wallis H test** was conducted for inter-group comparisons, subsequently followed by **Mann-Whitney U tests** with **Bonferroni correction** for pairwise analyses. Statistical significance was established at $p \leq 0.05$ (significant) and $p \leq 0.01$ (highly significant).

Results

All rabbits tolerated the surgical procedures well; no intraoperative or postoperative complications were observed.
Quantitative analysis demonstrated significant differences among the groups in both markers: trabecular bone thickness **(**see Table 2**)** and Ki67 expression (see Table 3).
The results were expressed as (Mean **± SD),** where:

a. Group B (4% FA + DFDBA) exhibited the highest trabecular bone thickness (**719.2 ± 509.5 µm**) and Ki67 expression (**2.8 ± 0.44**), see **(Figure 2).**
b. Group A (2% FA + DFDBA) with **234.4 ± 91.2 µm** bone thickness and **2.1 ± 0.54** Ki67 score, see **(Figure 3)**.
c. Group C (DFDBA alone) showed moderate values (**258.1 ± 56.7 µm** bone thickness, **1.6 ± 0.54** Ki67), see **(Figure 4)**.
d. Group D (natural healing) demonstrated the lowest outcomes (**101.4 ± 18.5 µm** bone thickness, **0.4 ± 0.54** Ki67), see **(Figure 5)**.

A dose-dependent response was observed: Group B demonstrated superior performance compared to Group A in trabecular thickness ($p = 0.008$) and Ki67 expression ($p = 0.003$). Relative to Group C, Group B exhibited a **2.8-fold increase in bone thickness** and a **1.7-fold enhancement in Ki67 scores**, thereby underscoring the synergistic effect of FA in conjunction with DFDBA. Statistical validation confirmed substantial effect sizes (trabecular thickness: Cohen's $d = 2.1$; Ki67: $r = 0.72$) and high reproducibility (ICC = 0.92). Furthermore, **Figure 6** presents the Comparative Histograms for both markers.

Discussion

Previous research has confirmed the essential role of cellular proliferation markers and bone microarchitecture in evaluating bone regeneration. In this study, Ki67 expression was employed to assess osteogenic cell proliferation, while trabecular bone thickness was used as a measure of the quality of new bone tissue. The results demonstrate that bioactive interventions—specifically, the supplementation of ferulic acid (FA) alongside demineralized freeze-dried bone allograft (DFDBA)—lead to a dose-dependent enhancement of both parameters.
In the negative control group (Group D), the trabecular bone thickness was significantly reduced (101.4±18.5µm), with negligible Ki67 expression (0.4±0.54). The findings align with the clinical observations of Marian et al. (2024) (25) and the spontaneous healing pattern seen by Martins et al.

Table 2. Trabecular Bone Thickness Across Experimental Groups

Group	Mean ± SD (µm)	Median (IQR)	Significance*
Group D (Control)	101.4 ± 18.5	93.0 (80–120)	a
Group C (DFDBA)	258.1 ± 56.7	258 (170–339)	b
Group A (2% FA)	234.4 ± 91.2	246 (145–285)	b
Group B (4% FA)	719.2 ± 509.5	598 (327–1224)	c

*Different superscript letters denote significant differences (Mann-Whitney U test with Bonferroni correction, $p < 0.01$).

Table 3: Ki67 Expression Scores Across Experimental Groups

Group	Mean ± SD	Median (IQR)	Significance*
Group D (Control)	0.4 ± 0.54	0.4 (0.2–0.6)	a
Group C (DFDBA)	1.6 ± 0.54	1.6 (1.2–2.0)	b
Group A (2% FA)	2.1 ± 0.54	2.1 (1.8–2.4)	c
Group B (4% FA)	2.8 ± 0.44	2.8 (2.6–3.0)	d

*Different superscript letters denote significant differences (Mann-Whitney U test with Bonferroni correction, $p < 0.01$).

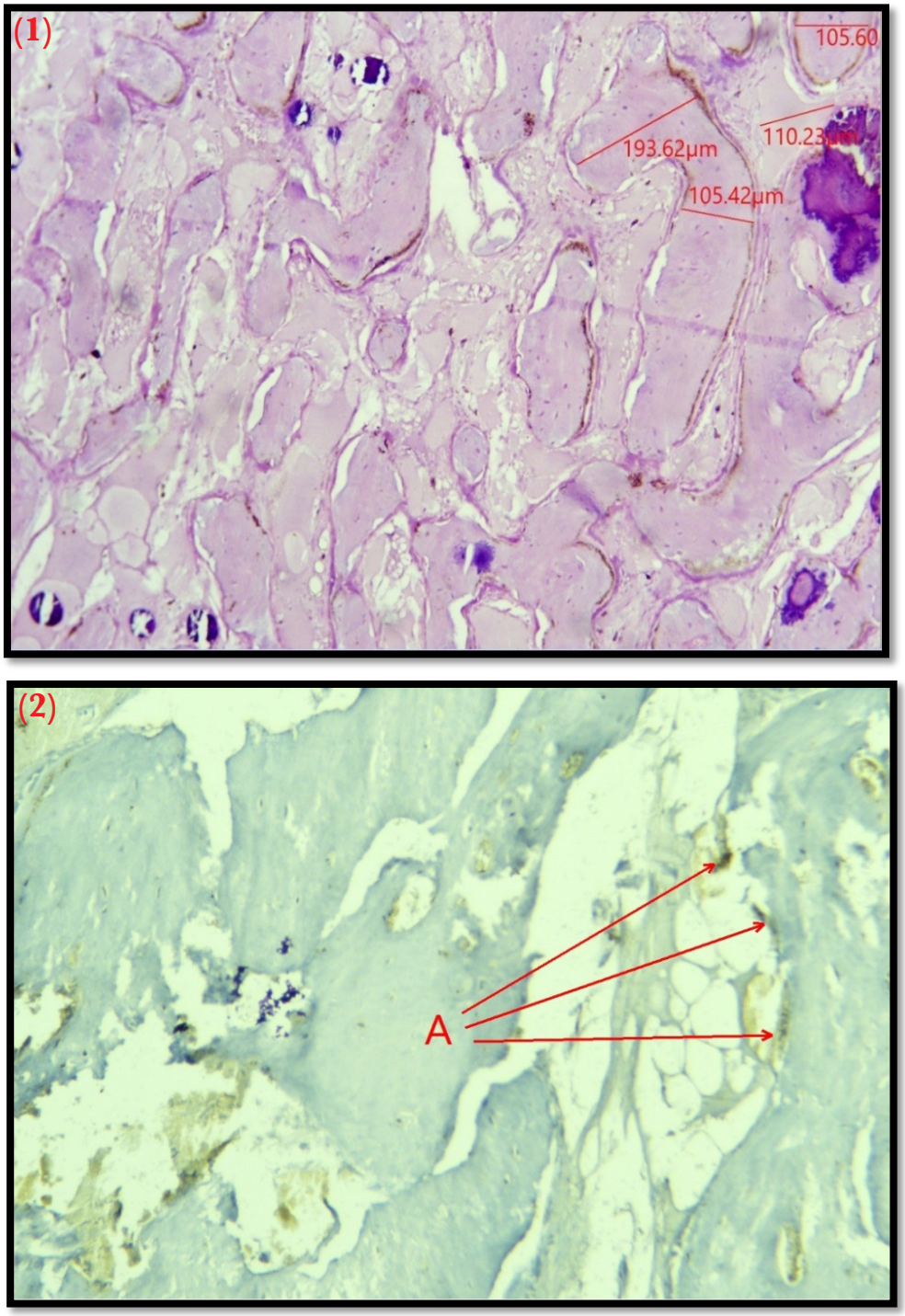

Figure 2. Histological and Immunohistochemical Analysis of Group B, **1:** Trabecular Bone Formation**:** Digital histomicrograph at 10x magnification displaying new trabecular bone formation in Group B. Measurements in micrometers (µm) were performed using the Pro-View application from Optica®, **2:** Ki67 Expression: Immunohistochemical staining for Ki67 in Group B at 10x magnification, highlighting Ki67 expression in newly formed osteoblasts.

(2022) (26), indicating that natural healing results in insufficient osteoblast recruitment and considerable alveolar ridge erosion. The diminished Ki67 expression in this cohort highlights the restricted inherent regeneration potential of untreated extraction sockets.

In Group C (Positive Control: 100% DFDBA), a modest increase in Ki67 expression (1.6±0.54) was observed, reflecting the passive osteoconductive effect of DFDBA that recruits osteoprogenitor cells but lacks potent mitogenic stimuli. This finding is consistent with Parashis et al. (2004) (27), who reported that DFDBA achieves only partial defect fill in human intrabony lesions without the presence of growth factors. Additionally, in Group C (100% DFDBA), trabecular bone thickness increased to an intermediate level (258.1±56.7μm), indicating that while DFDBA provides an osteoconductive scaffold,

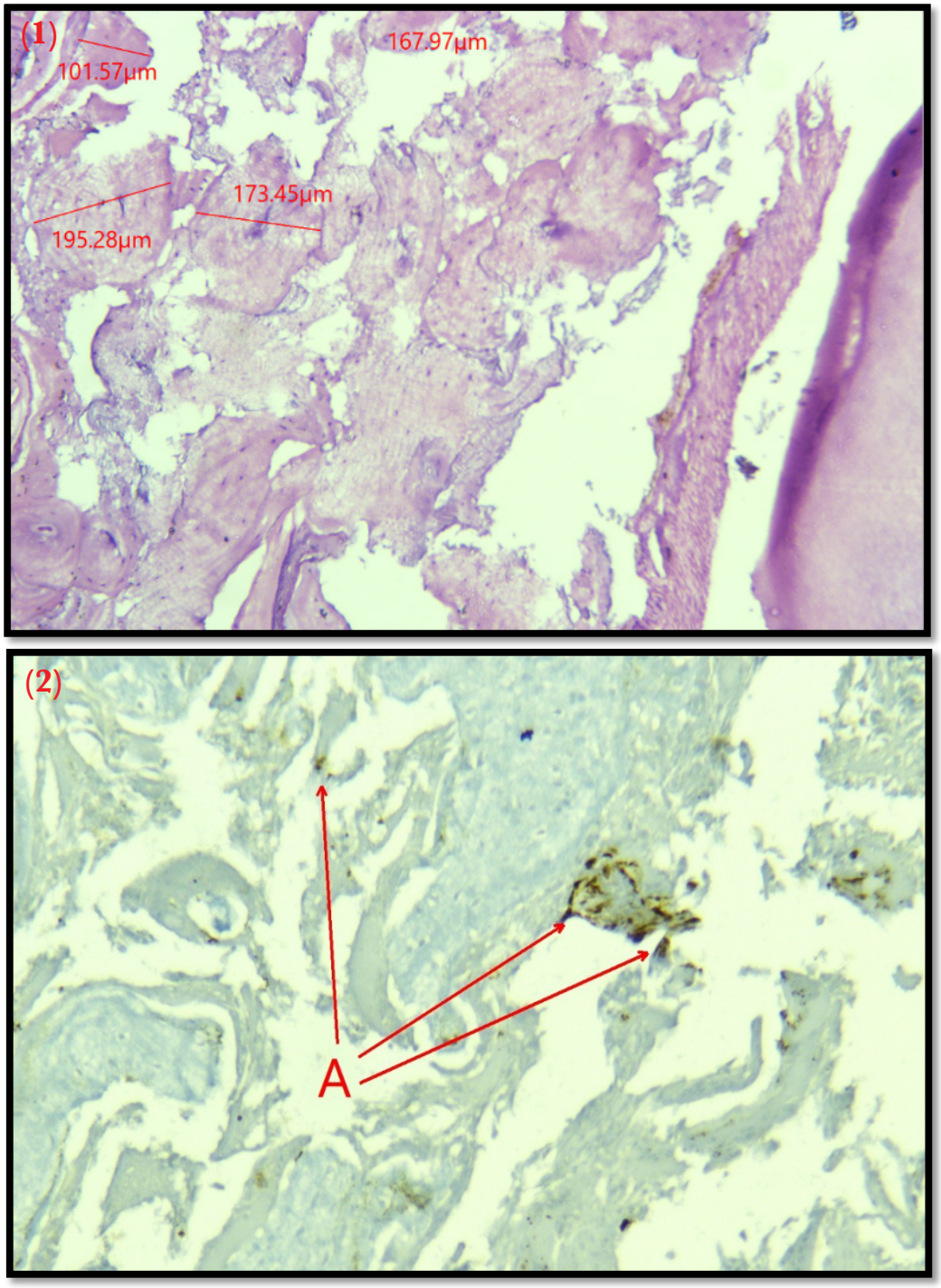

Figure 3. Histological and Immunohistochemical Analysis of Group A. **1:** Trabecular Bone Formation: A digital histomicrograph at 10x magnification illustrating new trabecular bone formation in Group A. Measurements in micrometers (µm) were conducted using the Pro-View application from Optica®. **2:** Ki67 Expression: Immunohistochemical staining for Ki67 in Group A at 10x magnification, emphasizing Ki67 expression in newly formed osteoblasts.

its efficacy remains limited without supplementary bioactive stimulation. These observations align with the findings of Keshavarzi et al. (2023) (28). Paradoxically, the trabecular thickness in Group C exceeded that of Group A (234.4±91.2μm), likely owing to residual DFDBA particles resembling native bone structure, whereas FA facilitated accelerated remodeling of the scaffold into biologically active tissue. In Group A (2% FA + 98% DFDBA), trabecular thickness reached 234.4±91.2μm—a moderate enhancement which may be attributable to the protective effects of FA on collagen and its role in suppressing osteoclast-mediated bone resorption, as described by Doss et al. (29). Group B (4% FA + 96% DFDBA) demonstrated a substantial increase in trabecular bone thickness (719.2±509.5μm). This pronounced enhancement

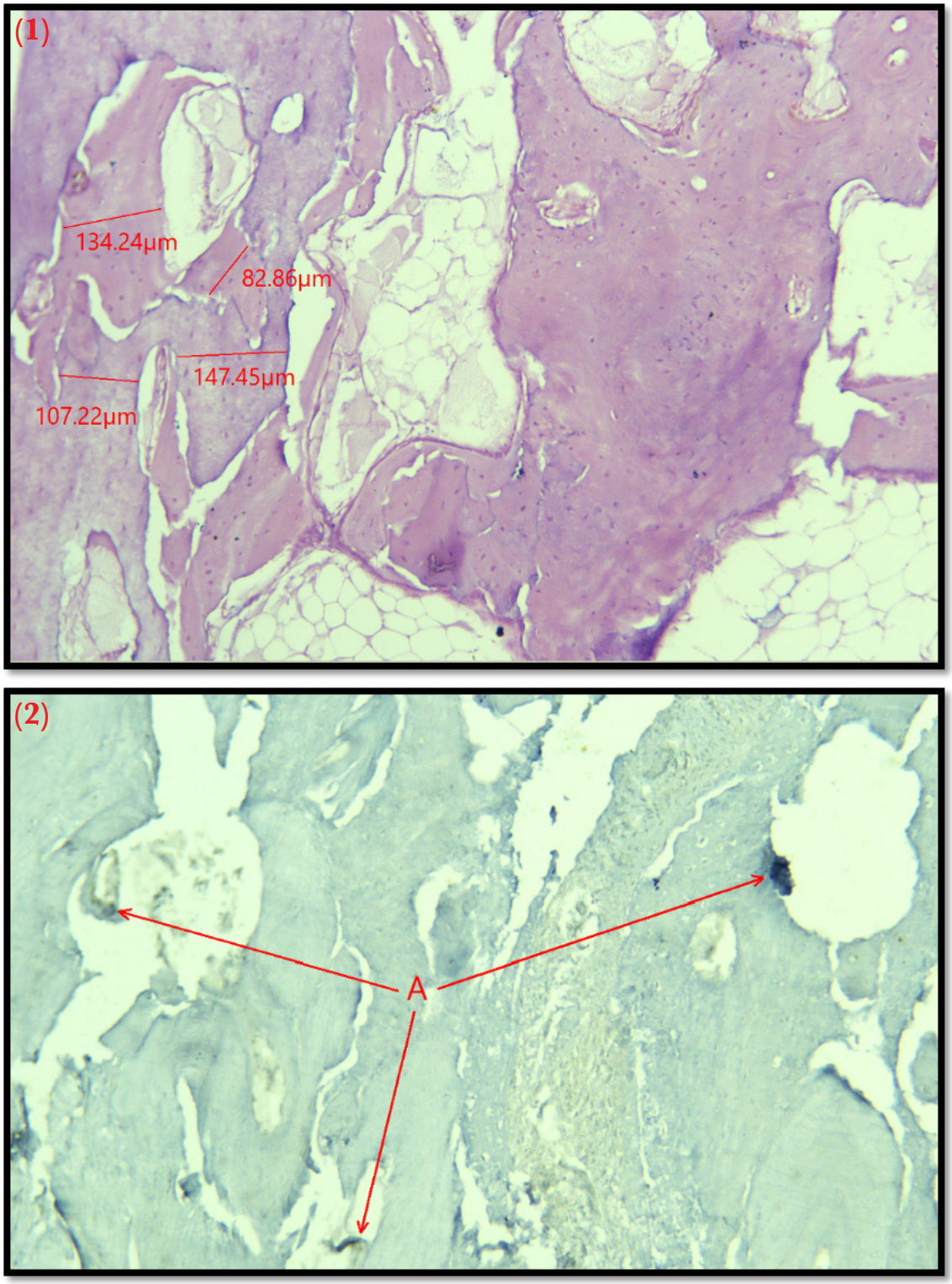

Figure 4. Histological and Immunohistochemical Analysis of Group C, **1:** Trabecular Bone Formation: Digital histomicrograph at 10x magnification displaying new trabecular bone formation in Group C. Measurements in micrometers (µm) were performed using the Pro-View application from Optica®. **2:** Ki67 Expression: Immunohistochemical staining for Ki67 in Group C at 10x magnification, highlighting Ki67 expression in newly formed osteoblasts.

corroborates the dose-dependent impact of FA and is supported by the studies of Wagle et al. (2021) (30) and Sassa et al. (2003) (31), which reported that higher concentrations of FA notably stimulate the proliferation of bone marrow-derived mesenchymal stem cells and enhance bone mineral density. Furthermore, FA possesses the capacity to protect collagen from degradation (32) and to promote the organization of a collagen-rich extracellular matrix (33), likely contributing to the superior bone quality observed in

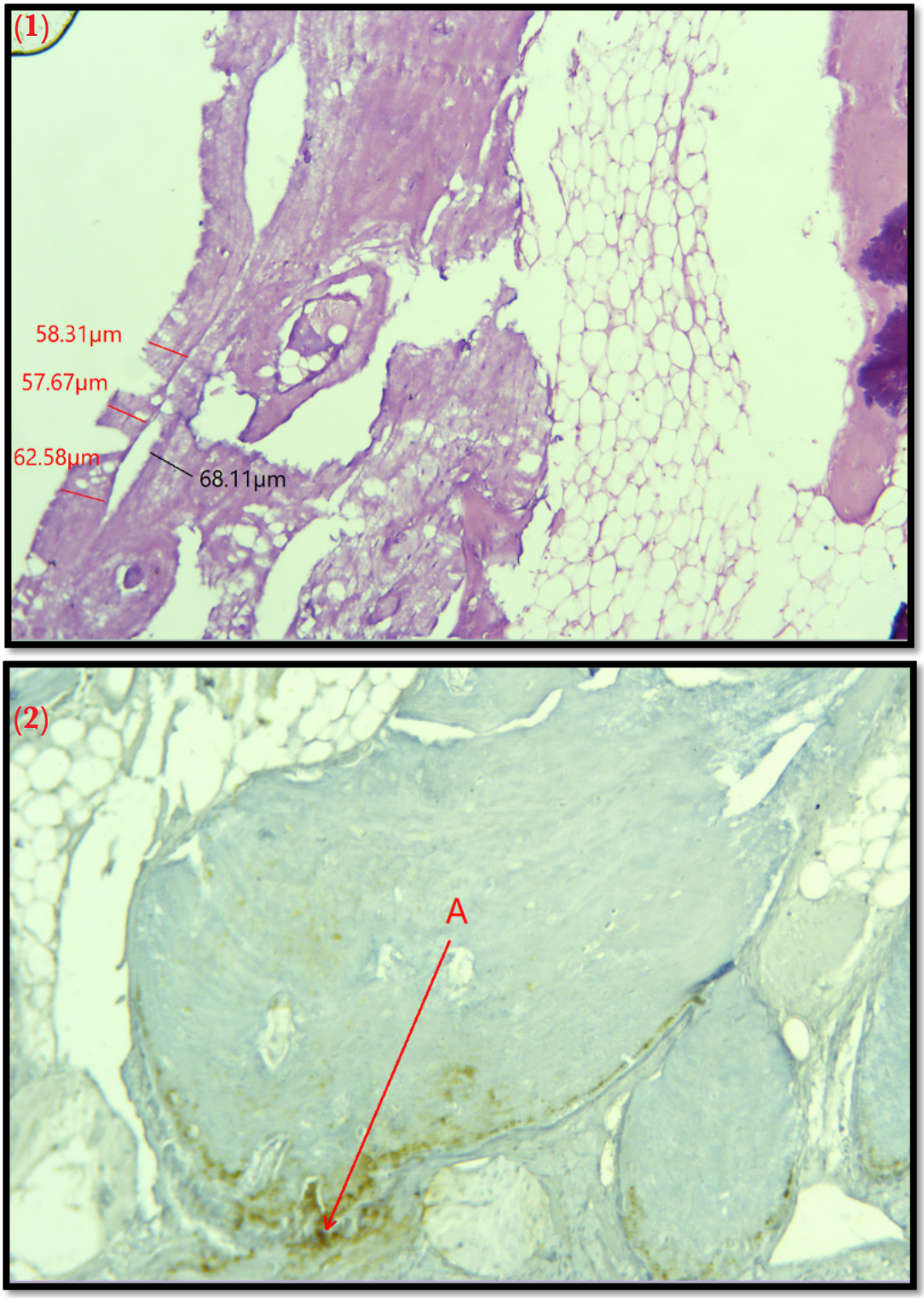

Figure 5. Histological and immunohistochemical analysis of Group D. **1:** Trabecular bone formation: Digital histomicrograph at 10x magnification illustrating thin strips of trabecular bone in the group. Measurements in micrometers (µm) were conducted using the Pro-View application from Optica®. **2:** Ki67 expression: Immunohistochemical staining for Ki67 in Group D at 10x magnification, demonstrating the lowest Ki67 expression among the groups in newly formed osteoblasts.

this group. The addition of ferulic acid to the grafting material markedly increased cellular proliferation. In Group A (2% FA + 98% DFDBA), Ki67 expression rose to 2.1±0.54, suggesting that even a low concentration of FA can augment osteoblast proliferation. This observation aligns with the findings of Liu et al. (2018) (34), who reported that elevated Ki67 levels are closely associated with increased osteoblast proliferation and expedited bone formation. This outcome underscores the osteoinductive potential of ferulic acid. In Group

Figure 6. Comparative Histograms. **1.** Trabecular Bone Thickness Across Groups**:** A histogram comparing trabecular bone thickness measurements among Groups A, B, C, and D. **2.** Ki67 Expression Across Groups**:** A histogram illustrating the comparative Ki67 expression levels among Groups A, B, C, and D**.**

B, the dose-dependent upregulation of Ki67 (2.8 ± 0.44) correlates with FA's ability to activate the ERK signaling pathway, as demonstrated in the study of Zhou et al. (2021) (35), thereby promoting osteoblast proliferation. Concurrently, FA's antioxidant properties mitigate oxidative stress, which is known to impair osteoblast function, as evidenced by Ghaisas et al. (2014), who reported that FA reduces oxidative stress and significantly promotes wound healing in diabetic rats. This mechanism may further support osteoblast survival by alleviating oxidative stress within bone microenvironments (36).

Collectively, the concurrent increase in Ki67 expression and trabecular bone thickness across the experimental groups underscores the potential of ferulic acid to accelerate and enhance bone regeneration. The comparatively low values in the control group (Group D) illustrate the limitations of natural healing. In contrast, the significant improvements seen with higher concentrations of FA (particularly in Group B) highlight its promise as a bioactive adjunct in regenerative bone therapies. Future studies should aim to elucidate the molecular mechanisms through which FA modulates cell proliferation and matrix deposition, ultimately optimizing its clinical application in alveolar socket preservation and other bone repair settings.

Mechanistic Insights

The beneficial effects of FA on bone regeneration extend beyond its capacity to promote cellular proliferation. The robust antioxidant properties of FA enable it to neutralize free radicals and reduce reactive oxygen species (ROS), thereby alleviating oxidative stress. FA effectively inhibits collagen degradation, presumably by decreasing the activity of matrix metalloproteinases (MMPs) (32), which are vital for maintaining the extracellular matrix. Collagen provides the structural framework necessary for osteoblast adhesion and new bone formation. By preserving collagen integrity, FA fosters a conducive environment for osteoblast proliferation, as evidenced by increased Ki67 expression, and supports the development of durable trabecular bone. The synergistic advantages of FA, including its antioxidant, anti-inflammatory, and collagen-preserving effects, markedly enhance bone regeneration when administered alongside DFDBA.

Critical Evaluation of Methods and Limitations

While our findings are robust, considerations regarding methodology merit discussion. The employment of a rabbit model, although a standard approach for alveolar bone research, restricts direct applicability to human clinical outcomes. Furthermore, the 21-day observation window captures the initial phases of healing but does not delineate long-term remodeling processes. The significant variability observed in trabecular thickness within Group B (SD: 509.5 μm) may be indicative of technical challenges associated with fluorescence analysis (FA) distribution within the graft or measurement inconsistencies inherent to histomorphometry. To mitigate these issues, future investigations should incorporate micro-computed tomography (micro-CT) for three-dimensional bone assessment and establish standardized protocols for FA-graft homogenization.

Inter-observer agreement reinforced the reliability of Ki67 scoring (ICC: 0.92); however, the subjective interpretation of immunohistochemical staining persists as a potential source of bias. The implementation of automated image analysis tools could serve to further augment reproducibility.

Clinical Implications

The notable improvements in trabecular bone thickness and Ki67 expression observed in the FA-treated groups hold considerable therapeutic importance. Increased osteoblast proliferation and enhanced bone formation may contribute to improved alveolar ridge retention, thereby increasing the success rates of subsequent dental implant procedures. These findings suggest that FA may serve as a potent bioactive supplement in regenerative dentistry, especially for patients with impaired healing abilities (e.g., diabetics, smokers).

Conclusion

In summary, the localized administration of FA with DFDBA significantly enhances alveolar ridge preservation through the stimulation of osteoblast proliferation, as evidenced by increased Ki67 expression, and by improving trabecular bone thickness. The dose-dependent improvements underscore FA's potential as a bioactive supplement in bone regeneration techniques. This research offers novel insights into FA's dual role as an osteoproliferative and antioxidant agent, presenting a cost-effective approach to mitigating post-extraction alveolar resorption—a persistent issue in clinical dentistry. Furthermore, this study advances regenerative protocols by bridging the divide between scaffold-based and bioactive therapies, thereby paving the way for enhanced implant outcomes and patient care.

Author contributions

Lecturer Dr. Saif Saad Ali Al-Jewari (BDS, F.I.B.M.S) and Dr. Abdulsattar Salim Mahmood (B.D.S, M.Sc, Ph.D) are contributed for Methodology, Review and Supervision.

Ethics approval

All procedures performed were per the ethical standards of our institutional research committee at the University of Mosul, College of Dentistry, Department of Oral and Maxillofacial Surgery.

The local ethical committee No: UoM 24/1028 approved the study on 02/09/2024.

Competing interest

The authors declare that they have no competing interests.

Availability of data

The datasets collected and/or analyzed during the current study are available from the corresponding author on request. The corresponding author had full access to all the data in the study and took responsibility for the integrity of the data and the accuracy of the data analysis.

Funding

This research received no specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

1. Kumar, N.; Pruthi, V. Potential applications of ferulic acid from natural sources. Biotechnol. Rep. 2014, 4, 86-93. https://doi.org/10.1016/j.btre.2014.09.002 https://doi.org/10.1016/j.btre.2014.09.002
2. Rechner, A. R.; Kuhnle, G.; Hu, H.; Roedig-Penman, A.; van den Braak, M. H.; Moore, K. P.; et al. The metabolism of dietary polyphenols and the relevance to circulating levels of conjugated metabolites. Free Radic. Res. 2002, 36(11), 1229-1241. DOI not available https://doi.org/10.1080/246-1071576021000016472
3. Mancuso, C.; Santangelo, R. Ferulic acid: pharmacological and toxicological aspects. Food Chem. Toxicol. 2014, 65, 185-195. https://doi.org/10.1016/j.fct.2013.12.024 https://doi.org/10.1016/j.fct.2013.12.024
4. Zhao, Z.; Moghadasian, M. H. Chemistry, natural sources,

dietary intake and pharmacokinetic properties of ferulic acid: A review. Food Chem. 2008, 109(4), 691-702. https://doi.org/10.1016/j.foodchem.2008.01.057 https://doi.org/10.1016/j.foodchem.2008.01.057
5. Li, D.; Rui, Y. X.; Guo, S. D.; Luan, F.; Liu, R.; Zeng, N. Ferulic acid: A review of its pharmacology, pharmacokinetics and derivatives. Life Sci. 2021, 284, 119921. https://doi.org/10.1016/j.lfs.2021.119921 https://doi.org/10.1016/j.lfs.2021.119921
6. Vimalraj, S.; Saravanan, S.; Hariprabu, G.; Yuvashree, R.; Kanna, S. K. A.; Sujoy, K.; et al. Kaempferol-zinc(II) complex synthesis and evaluation of bone formation using zebrafish model. Life Sci. 2020, 256, 117993. https://doi.org/10.1016/j.lfs.2020.117993 https://doi.org/10.1016/j.lfs.2020.117993
7. Kalinowska, M.; Piekut, J.; Bruss, A.; Follet, C.; Sienkiewicz-Gromiuk, J.; Świsłocka, R.; et al. Spectroscopic (FT-IR, FT-Raman, 1H, 13C NMR, UV/VIS), thermogravimetric and antimicrobial studies of Ca(II), Mn(II), Cu(II), Zn(II) and Cd(II) complexes of ferulic acid. Spectrochim. Acta A Mol. Biomol. Spectrosc. 2014, 122, 631-638. https://doi.org/10.1016/j.saa.2013.11.110 https://doi.org/10.1016/j.saa.2013.11.110
8. Jaiswal, Y.; Kumar, S.; Mishra, V.; Bansal, P.; Anand, K. R.; Singh, S. Efficacy of decalcified freeze-dried bone allograft in the regeneration of small osseous defect: A comparative study. Natl. J. Maxillofac. Surg. 2017, 8(2), 143-148. https://doi.org/10.4103/njms.NJMS_45_17 https://doi.org/10.4103/0975-5950.221714
9. Naidu, P. Allografts in Periodontal Regeneration. Madr. J. Case Rep. Stud. 2019, 3, 121-125. DOI not available https://doi.org/10.18689/mjcrs-1000130
10. Zhang, H.; Yang, L.; Yang, X. G.; Wang, F.; Feng, J. T.; Hua, K. C.; et al. Demineralized bone matrix carriers and their clinical applications: an overview. Orthop. Surg. 2019, 11(5), 725-737. https://doi.org/10.1111/os.12529 https://doi.org/10.1111/os.12529
11. Johnson, T. M.; Berridge, J. P.; Baron, D. Protocol for maintaining alveolar ridge volume in molar immediate implant sites. Clin. Adv. Periodontics 2017, 7(4), 207-214. https://doi.org/10.1902/cap.2017.160062 https://doi.org/10.1902/cap.2017.170026
12. Shigeyama, Y.; D'Errico, J. A.; Stone, R.; Somerman, M. Commercially-prepared allograft material has biological activity in vitro. J. Periodontol. 1995, 66(6), 478-487. https://doi.org/10.1902/jop.1995.66.6.478 https://doi.org/10.1902/jop.1995.66.6.478
13. Vaid, T.; Kumar, S.; Mehta, R.; Shah, S.; Joshi, S.; Bhakkand, S.; et al. Clinical and radiographic evaluation of demineralized freeze-dried bone allograft with concentrated growth factor versus concentrated growth factor alone in the treatment of intrabony defects. Med. Pharm. Rep. 2021, 94(2), 220. https://doi.org/10.15386/mpr-1794 https://doi.org/10.15386/mpr-1718
14. Sanz, M.; Dahlin, C.; Apatzidou, D.; Artzi, Z.; Bozic, D.; Calciolari, E.; et al. Biomaterials and regenerative technologies used in bone regeneration in the craniomaxillofacial region. J. Clin. Periodontol. 2019, 46(Suppl. 21), 82-91. https://doi.org/10.1111/jcpe.13049 https://doi.org/10.1111/jcpe.13049
15. Raggatt, L. J.; Partridge, N. C. Cellular and molecular mechanisms of bone remodeling. J. Biol. Chem. 2010, 285(33), 25103-25108. https://doi.org/10.1074/jbc.R109.041087 https://doi.org/10.1074/jbc.R109.041087
16. Hart, N. H.; Newton, R. U.; Tan, J.; Rantalainen, T.; Chivers, P.; Siafarikas, A.; et al. Biological basis of bone strength: anatomy, physiology and measurement. J. Musculoskelet. Neuronal Interact. 2020, 20(3), 347-371. DOI not available
17. Nordin, M. Basic Biomechanics of the Musculoskeletal System; 2nd ed.; Lippincott Williams & Wilkins: Philadelphia, 2001; pp 267-275.
18. Crockett, J. C.; Rogers, M. J.; Coxon, F. P.; Hocking, L. J.; Helfrich, M. H. Bone remodelling at a glance. J. Cell Sci. 2011, 124(Pt 7), 991-998. https://doi.org/10.1242/jcs.063032 https://doi.org/10.1242/jcs.063032
19. Eriksen, E. F. Cellular mechanisms of bone remodeling. Rev. Endocr. Metab. Disord. 2010, 11, 219-227. https://doi.org/10.1007/s11154-010-9153-1 https://doi.org/10.1007/s11154-010-9153-1
20. Zhu, L.; Tang, Y.; Li, X. Y.; Keller, E. T.; Yang, J.; Cho, J. S.; et al. Osteoclast-mediated bone resorption is controlled by a compensatory network of secreted and membrane-tethered metalloproteinases. Sci. Transl. Med. 2020, 12(529), eaaw6143. https://doi.org/10.1126/scitranslmed.aaw6143 https://doi.org/10.1126/scitranslmed.aaw6143
21. Uxa, S.; Castillo-Binder, P.; Kohler, R.; Stangner, K.; Müller, G. A.; Engeland, K. Ki-67 gene expression. Cell Death Differ. 2021, 28(12), 3357-3370. https://doi.org/10.1038/s41418-021-00823-x https://doi.org/10.1038/s41418-021-00823-x
22. Scholzen, T.; Gerdes, J. The Ki-67 protein: from the known and the unknown. J. Cell. Physiol. 2000, 182(3), 311-322. https://doi.org/10.1002/(SICI)1097-4652(200003)182:3<311::AID-JCP1>3.0.CO;2-9 https://doi.org/10.1002/(SICI)1097-4652(200003)182:3<311::AID-JCP1>3.0.CO;2-9
23. Booth, D. G.; Takagi, M.; Sanchez-Pulido, L.; Petfalski, E.; Vargiu, G.; Samejima, K.; et al. Ki-67 is a PP1-interacting protein that organises the mitotic chromosome periphery. Elife 2014, 3, e01641. https://doi.org/10.7554/eLife.01641 https://doi.org/10.7554/eLife.01641
24. Li, Y. X.; Zhang, L.; Simayi, D.; Zhang, N.; Tao, L.; Yang, L.; et al. Human papillomavirus infection correlates with inflammatory Stat3 signaling activity and IL-17 level in patients with colorectal cancer. PLoS One 2015, 10(2), e0118391. https://doi.org/10.1371/journal.pone.0118391 https://doi.org/10.1371/journal.pone.0118391
25. Marian, D.; Toro, G.; D'Amico, G.; Trotta, M. C.; D'Amico, M.; Petre, A.; et al. Challenges and innovations in alveolar bone regeneration: A narrative review. Medicina 2024, 61(1), 20. https://doi.org/10.3390/medicina61010020 https://doi.org/10.3390/medicina61010020
26. Martins, J. R.; Wagner, T. P.; Vallim, A. C.; Konflanz, W.; Schwendicke, F.; Celeste, R. K.; et al. Comparison of the efficacy of different techniques to seal the alveolus. J. Clin. Periodontol. 2022, 49(7), 694-705. https://doi.org/10.1111/jcpe.13643 https://doi.org/10.1111/jcpe.13628
27. Parashis, A.; Andronikaki-Faldami, A.; Tsiklakis, K. Clinical and radiographic comparison of three regenerative procedures. Int. J. Periodontics Restorative Dent. 2004, 24(1), 81-90. DOI not available
28. Keshavarzi, F.; Ahrari, F.; Seyedmajidi, M.; Ziaei, M.; Bijani, A.; Jenabian, N. CenoBone® with and without PRGF in ridge preservation. Dent. Res. J. (Isfahan) 2023, 20, 26. https://doi.org/10.4103/1735-3327.372649 https://doi.org/10.4103/1735-3327.372649
29. Doss, H. M.; Samarpita, S.; Ganesan, R.; Rasool, M. Ferulic acid suppresses osteoclast differentiation via NF-κB inhibition. Life Sci. 2018, 207, 284-295. https://doi.org/10.1016/j.lfs.2018.06.013 https://doi.org/10.1016/j.lfs.2018.06.013
30. Wagle, S.; Sim, H. J.; Bhattarai, G.; Choi, K. C.; Kook, S. H.; Lee, J. C.; et al. Ferulic acid protects against irradiation-induced bone damage. Antioxidants (Basel) 2021, 10(8), 1166. https://doi.org/10.3390/antiox10081166 https://doi.org/10.3390/antiox10081166
31. Sassa, S.; Kikuchi, T.; Shinoda, H.; Suzuki, S.; Kudo, H.; Sakamoto, S. Preventive effect of ferulic acid on bone loss. In Vivo 2003, 17(3), 277-280. DOI not available
32. Jayamani, J.; Naisini, A.; Madhan, B.; Shanmugam, G. Ferulic acid as a collagen fibril formation inhibitor. Int. J. Biol. Macromol. 2018, 113, 277-284. https://doi.org/10.1016/j.ijbiomac.2018.02.134 https://doi.org/10.1016/j.ijbiomac.2018.02.134
33. Trombelli, L.; Farina, R.; Marzola, A.; Bozzi, L.; Liljenberg,

B.; Lindhe, J. Remodeling of human extraction sockets. J. Clin. Periodontol. 2008, 35(7), 630-639. https://doi.org/10.1111/j.1600-051X.2008.01246.x https://doi.org/10.1111/j.1600-051X.2008.01246.x

34. Liu, Z.; Chang, H.; Hou, Y.; Wang, Y.; Zhou, Z.; Wang, M.; et al. miR-26a overexpression in BMSCs facilitates bone regeneration. Mol. Med. Rep. 2018, 18(6), 5317-5326. https://doi.org/10.3892/mmr.2018.9582 https://doi.org/10.3892/mmr.2018.9582
35. Zhou, W.; Chen, B.; Shang, J.; Li, R. Ferulic acid attenuates osteoporosis via GSK-3β/Lrp-5/ERK pathways. Physiol. Int. 2021, 108(3), 317-341. https://doi.org/10.1556/2060.2021.00174
36. Ghaisas, M. M.; Kshirsagar, S. B.; Sahane, R. S. Wound healing activity of ferulic acid in diabetic rats. Int. Wound J. 2014, 11(5), 523-532. https://doi.org/10.1111/iwj.12009 https://doi.org/10.1111/iwj.12009
37. Salim, A. S.; Al Hijazi, A. Effect of calcium phosphate ceramic on tooth socket healing. J. Baghdad Coll. Dent. 2010, 22, 57-61. DOI not available