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Effect of Adjunctive Antimicrobial Photodynamic Therapy on Early Trabecular Radiographic Healing of Mandibular Third-Molar Extraction Sockets Using Quantitative Cone-Beam Computed Tomography: A Quasi-Experimental Study

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ABSTRACT

Objective:
To evaluate the effect of adjunctive antimicrobial therapy(aPDT) on trabecular bone healing of mandibular third-molar extraction sockets using standardized grey-value (Gv) analysis on cone-beam computed tomography (CBCT).

Design:
Prospective Quasi-experimental study.

Place and Duration:
Dow university of health sciences and Liaquat college of medicine and dentistry, Karachi, Pakistan (January-June 2026)

Methods:
Of 110 patients requiring surgical extraction of a mesio-angular impacted mandibular third molar, 98 completed follow-up (48 control,50 intervention). The intervention group received photosensitizer (Tolonium chloride blue) was applied into extraction socket for 30 seconds and later rinsed with saline. The socket was then activated by a 635 nm diode laser using device Lasotronix (SMART MDR) at 20mW for 60 secondsbefore suturing; controls received standard care without laser application. Trabecular grey valuewas measured on same CBCTunit pre-operatively and at 1-month with identical exposure parameters. Independent- and paired-samples t-tests, chi-square tests and Cohen's d were used (α=0.05).

Results:
Groups were comparable at baseline for age, gender, side and pre-operative Gv (all $p>0.05$). At 1 month, mean trabecular Gv was significantly higher in the intervention group than in controls (112.7 ± 22.0 vs 41.2 ± 12.2; $p<0.001$; Cohen's d=3.99).

Conclusion:
Single-session adjunctive aPDT applied at the time of mandibular third-molar surgery was associated with significantly higher early quantitative trabecular bone density on CBCT at 1 month, suggesting an accelerative effect on the initial phase of alveolar socket healing.

Keywords:antimicrobial photodynamic therapy, third molar, alveolar bone healing, cone-beam computed tomography, grey value.

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INTRODUCTION

Surgical removal of the mandibular third molar is among the most frequently performed procedures in oral and maxillofacial surgery.(1) The third molar is the tooth most commonly affected by impaction, with reported prevalence of 26–64% and a female predilection, and the mandibular arch is affected roughly twice as often as the maxillary arch.(2)Mesio-angular impaction is the pattern most frequently reported in Pakistan, occurring in 41.7–51.1% of cases.(2, 3) Surgical extraction, which often requires flap elevation, osteotomy and tooth sectioning, produces variable local tissue trauma and is associated with pain, swelling, trismus,

delayed soft-tissue healing, alveolar osteitis and localized infection.(3-5)

Socket healing is a sequential biological process spanning clot formation, inflammatory-cell migration, granulation-tissue formation, angiogenesis, osteoblastic activity, osteoid deposition, mineralization and remodeling.(6) Local microbial burden and inflammation may influence the pace and quality of this sequence, so interventions that reduce local bacterial load could theoretically improve the biological outcome of healing.(6, 7) CBCT permits standardized, quantitative, three-dimensional assessment of the extraction region through grey values (Gv), an analogue of the Hounsfield unit that is scanner- and protocol-dependent and therefore comparable only when acquisition parameters are held constant.(8, 9) On this scale, bone is classified as D1 (>1250 Gv), D2 (850–1250 Gv), D3 (350–850 Gv) or D4 (150–350 Gv).(10, 11)

Antimicrobial photodynamic therapy is a minimally invasive technique in which a non-toxic photosensitizer is excited by light of an appropriate wavelength, generating reactive oxygen species that disrupt bacterial cell membranes.(12)Beyond its antibacterial action, aPDT may accelerate alveolar healing through photobiomodulation: reduction of pro-inflammatory cytokines, stimulation of mesenchymal stem cells, increased mitochondrial ATP production, enhanced mineralization and vascular endothelial growth factor–driven neovascularization.(13) Because it acts locally rather than systemically, aPDT avoids the gastrointestinal effects and resistance risk associated with systemic antibiotics, making it attractive for accessible sites such as the third-molar socket.(13)

Existing clinical evidence on aPDT after third-molar surgery has focused predominantly on postoperative pain, swelling, trismus, periodontal parameters and quality of life.(14, 15)A recent randomized trial by Pardo et al. reported favorable periodontal and clinical outcomes for the adjacent second molar but did not identify a significant short-term radiographic bone-healing benefit.(16) Quantitative, standardized radiographic data on the initial trabecular healing response to aPDT remain scarce, and none exist from a Pakistani population. This study used a single standardized CBCT protocol to quantify trabecular Gv change one month after mandibular third-molar extraction, comparing sockets managed with and without adjunctive aPDT.

MATERIAL AND METHOD:

A prospective quasi-experimental non-randomized, alternate-allocation study was conducted in the Department of Oral and Maxillofacial Surgery, Ishrat-ul-Ebad Khan Institute of Oral Health Sciences, Liaquat College of Medicine and Dentistry, and Dow Dental College, Karachi, Pakistan, from January to June 2026, after approval by the Institutional Review Board with the reference number(DSH/IRB/2026/0061) of Liaquat College of Medicine and Dentistry. Written informed consent was obtained from all participants.

Sample size was estimated using OpenEpi version 3.01.(17) By assuming a prevalence of 50% and 95% confidence level, the initial sample size was calculated as 384 patients. Since the study population was finite, the Finite Population Correction (FPC) was applied resulting in a final adjusted sample size of 98 patient.Of 110 patients enrolled, 12 (10.9%) were lost to follow-up, leaving 98 for analysis (48 control, 50 aPDT).

Eligible participants were aged 17–60 years, ASA I or II, with a unilateral mesio-angular impacted mandibular third molar (Winter classification),(18)at Pell and Gregory level B,(3) visible in the oral cavity, requiring surgical extraction, willing to provide radiographic and surgical consent, and with controlled diabetes or hypertension where present.Patients with uncontrolled systemic disease, HIV infection, organ transplantation, psychiatric disorders, dental abscess or fistula, limited mouth opening, drug allergy, pregnancy/lactation, or temporomandibular joint disorder were excluded.

Every alternate participant was assigned to the intervention group and received antimicrobial photodynamic therapy. At the start of the procedure, each patient was anesthetized with inferior alveolar nerve block and long buccal nerve anesthesia. A three-sided buccal flap was raised and buccal bone guttering was performed with the slow speed handpiece. Bone obstructing the path of removal was removed to the level of cemento-enamel junction. The third molar was surgically extracted. Each participant is divided into two groups i.e., control group and an intervention group.

The control group received standard treatment after extraction of mandibular third molar tooth. The standard protocol was to curette the extraction socket for debris removal that accelerate the healing process. The socket was rinsed with normal saline.

The sutures were placed to provide primary closure of soft tissues. The standard post-operative instructions were given to each patient.

In the intervention group, 0.5 ml of a Lasotronix-specified photosensitizer (Tolonium chloride) was applied into the extraction socket for 30 seconds. The photosensitizer was then thoroughly rinsed away using normal saline. A 635 nm red diode laser was calibrated for photo-activated disinfection. The laser probe was used in non-contact mode at a distance of approximately 5 mm from the bone surface. The socket was directly irradiated using the Lasotronix (SMART MDR) device at an output of 20 mW for 60 seconds (2 × 30 seconds, total energy 1.2 J) with constant beam irradiation over the surgical area before suturing. The socket was closed with sutures and standard post-operative instructions were provided.

Highly focused small-volume CBCT was used to assess initial bone healing. Trabecular bone Gv was recorded from a defined region of interest (ROI) within the extraction socket using pre-operative CBCT (acquired for treatment planning) and a follow-up CBCT at one month. All scans for all participants were acquired on the same CBCT unit (Defence Diagnostic Radiology Laboratory) with identical exposure parameters to preserve inter-subject comparability of Gv, since grey values are not standardized across scanners. (8, 9) Radiation exposure followed the ALARA principle.

The ROI measured 4 × 4 cm, encapsulating all necessary anatomical boundaries within 40 mm horizontally and 40 mm vertically when properly centered over the impacted mandibular third molar. Its boundaries were: superiorly, above the occlusal plane of the mandibular molars; inferiorly, past the inferior alveolar nerve canal and the dense cortical bone of the inferior border of the mandible; anteriorly, capturing the entire mandibular second molar and the distal root of the first molar; and posteriorly, extending into the ascending ramus to fully visualize the deep bone guttering distal to the third molar. Scans were acquired on a Carestream (CS 9300) unit under identical conditions (tube voltage 90 kV, tube current 15 mA, slice thickness 1 mm, slice interval 1 mm). Coronal, axial and cross-sectional images were obtained, and bone density was recorded in grey values. Grey value was read by positioning the cursor at the center of the extraction socket on each scan.

The surgeon and the patient were not blinded because of the nature of the procedure. However, the CBCT assessor was blinded to group allocation, and data were analyzed using coded treatment groups by a statistician independent of treatment allocation.

Data were analyzed using IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY). Continuous variables are presented as mean ± standard deviation and compared between groups by the independent-samples t-test, with Welch's correction indicated unequal variances; pre–post comparisons used the paired-samples t-test. Categorical variables were compared by the Pearson chi-square test. Percentage change in Gv from baseline was computed for each patient as ((Gv at one month − Gv pre-operative) / Gv pre-operative) × 100. Effect sizes are reported as Cohen's d between groups and d(z) within groups, and mean differences with 95% confidence intervals. A p-value < 0.05 was considered significant.

RESULTS

Of 110 patients enrolled (50 control, 60 aPDT), 12 (10.9%) did not attend the one-month CBCT, two controls (4.0%) and ten aPDT patients (16.7%) — leaving 98 participants for analysis (48 control, 50 aPDT). The groups were comparable at baseline in age, sex, side and pre-operative trabecular Gv (all p > 0.05; Table I).

Pre-operative trabecular Gv was 613.06 ± 126.95 in controls and 606.98 ± 151.07 in the aPDT group (mean difference 6.08 Gv, 95% CI; t= 0.22, p = 0.830), both means lying within the D3 (bone type) range.

At one month, mean trabecular Gv was significantly higher after aPDT (67.06 ± 13.28) than in controls (42.13 ± 13.28): mean difference 24.94 Gv 95% CI, t= 9.29, p < 0.001, Cohen's d = 1.88, equivalent to approximately 59% more residual radiographic density. The absolute change did not differ significantly between groups, a metric whose variance is inflated by the wide spread of baseline values (279–950 Gv). Levene's test indicated unequal variances for this variable (variance ratio 3.03), so Welch's correction was applied: t= 7.45, p < 0.001, d = 1.49 (Table II)

Baseline values were statistically indistinguishable (p = 0.830), whereas at one month the aPDT group retained significantly higher grey values (mean difference 24.94 Gv; p < 0.001; Cohen's d = 1.88). (Figure 1A) The relative radiographic deficit was significantly smaller after aPDT (−88.29 ± 3.84%

vs −92.98 ± 2.20%; Welch t= 7.45; p < 0.001; Cohen's d = 1.49). (Figure 1B)

Table I. Baseline demographic and radiographic characteristics of the groups.

Variable	Control group (n = 48)	aPDT group (n = 50)	Test statistic	p-value
Age (years), mean ± SD	39.40 ± 8.14	38.44 ± 9.44	t = 0.54	0.593
Age range (years)	26–59	18–58	—	—
Male, n (%)	28 (58.3)	24 (48.0)	$\chi^2(1) = 1.05$	0.305
Female, n (%)	20 (41.7)	26 (52.0)		
Right side, n (%)	25 (52.1)	25 (50.0)	$\chi^2(1) = 0.04$	0.837
Left side, n (%)	23 (47.9)	25 (50.0)		
Pre-operative trabecular Gv, mean ± SD	613.06 ± 126.95	606.98 ± 151.07	t= 0.22	0.830
Pre-operative trabecular Gv, range	375–850	279–950	—	—

aPDT = antimicrobial photodynamic therapy; Gv = grey value; SD = standard deviation. Continuous variables compared by independent-samples t-test; categorical variables by Pearson chi-square test. p < 0.05 considered significant.

Table II. Trabecular grey-value outcomes within and between groups

Outcome	Control group (n = 48)	aPDT group (n = 50)	Mean difference (95% CI)	Test statistic	p-value	Cohen's d
Pre-operative Gv, mean ± SD	613.06 ± 126.95	606.98 ± 151.07	6.08 (−49.99 to 62.15)	t(96) = 0.22	0.830	0.04
1-month post-operative Gv, mean ± SD	42.13 ± 13.28	67.06 ± 13.28	24.94 (19.61 to 30.26)	t(96) = 9.29	**< 0.001**	1.88
Absolute change in Gv, mean ± SD	−570.94 ± 123.44	−539.92 ± 147.96	31.02 (−23.74 to 85.78)	t(96) = 1.12	0.264	0.23
Percentage change in Gv (%), mean ± SD	−92.98 ± 2.20	−88.29 ± 3.84	4.69 (3.43 to 5.94)	Welch t(78.8) = 7.45	**< 0.001**	1.49
Within-group change, mean (95% CI)	−570.94 (−606.78 to −535.10)	−539.92 (−581.97 to −497.87)	—	t(47) = 32.05; t(49) = 25.80	**< 0.001; < 0.001**	d(z) 4.63; 3.65

aPDT = antimicrobial photodynamic therapy; CI = confidence interval; Gv = grey value; SD = standard deviation. Mean difference is expressed as aPDT minus control. Between-group comparisons used the independent-samples t-test; Welch's correction was applied to percentage change because Levene's test indicated unequal variances (variance ratio 3.03). Within-group comparisons used the paired-samples t-test. Bold indicates statistical significance at p < 0.05.

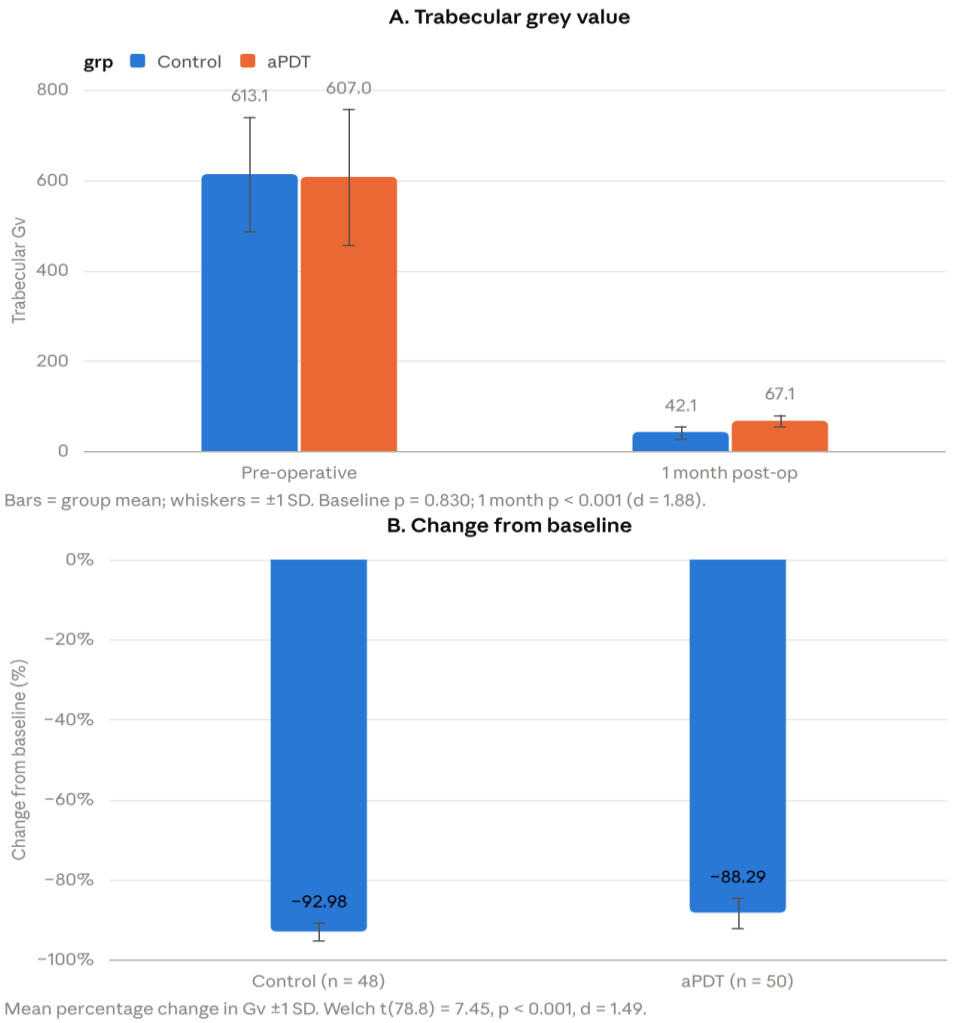


Figure 1. Trabecular grey-value outcomes in the control and aPDT groups. **(A)** Mean trabecular grey value (Gv) within the extraction-socket region of interest pre-operatively and at one month after surgery, by group; bars represent group means and whiskers represent ± 1 standard deviation. **(B)** Mean percentage change in trabecular Gv from baseline to one month, by group; whiskers represent ± 1 standard deviation.

DISCUSSION

In this quasi-experimental comparison of 98 patients undergoing mandibular third-molar surgery, adjunctive single-session aPDT was associated with a significantly higher trabecular Gv at 1 month than conventional management, with a very large effect size (d= 1.88). Because pre-operative Gv was statistically comparable between groups, this difference is unlikely to reflect baseline imbalance and is more consistent with a biological effect of the intervention on the trajectory of early socket mineralization.

This finding extends the existing literature. Pardo et al., in a randomized trial using periodontal indices on the adjacent second molar, found favorable clinical effects of aPDT but no significant short-term radiographic bone-healing benefit.(16) The apparent discrepancy may relate to outcome selection: periodontal probing parameters capture soft-tissue and pocket-level change around an adjacent tooth, whereas the present study measured trabecular mineral density directly within the socket using a quantitative, scanner-standardized Gv protocol, which may be more sensitive to early biological differences in bone turnover. Earlier work on aPDT emphasized clinical parameters rather than quantitative bone density.(12, 19)

A plausible biological explanation is photobiomodulation. In vitro and animal evidence indicates that red and near-infrared light of the wavelength range used in aPDT stimulates mitochondrial ATP synthesis, modulates reactive-oxygen-species signaling, and promotes osteogenic differentiation of mesenchymal and adipose-derived stem cells, with measurable increases in alkaline phosphatase activity and mineralized-nodule formation.(20, 21) Concurrently, the antimicrobial photodynamic effect reduces the local bacterial burden that would otherwise sustain inflammation

and delay the transition from the inflammatory to the proliferative and mineralisation phases of healing.(22, 23) The combination of reduced local infection and direct photobiostimulation of osteoprogenitor activity offers a coherent mechanism for the higher early Gv observed in the aPDT group.

The very steep percentage reduction in Gv seen in both groups at 1 month (−93% control, −81% aPDT) is expected and reflects the normal biology of a fresh socket: pre-operative Gv represents intact, mineralized alveolar bone, whereas the 1-month scan captures an early, still hypo-mineralized repair matrix that has not yet approached the density of native bone. This pattern is consistent with radiographic socket-healing literature showing that grey-value or Hounsfield-unit density recovers gradually over weeks to months rather than immediately.(19) The significant between-group difference in the magnitude of this early reduction, rather than in absolute density itself, is therefore the more clinically meaningful signal of an accelerated healing trajectory in the aPDT group.

Methodologically, several considerations affect interpretation. First, allocation was alternate rather than randomized, which introduces potential for selection bias despite the demonstrated baseline comparability of groups. Second, all scans were acquired on a single CBCT unit with fixed parameters, which was a deliberate design choice to preserve Gv comparability(24),but limits generalizability to other machines or protocols; multi-center validation using standardized or relative Gv scales(25),would strengthen external validity. Third, the 12% attrition rate, while modest, could have introduced attrition bias if loss to follow-up was not random. Fourth, follow-up was limited to a single 1-month time point; longer-term data would clarify whether the early radiographic advantage in the aPDT group persists, narrows, or predicts clinically important endpoints such as reduced alveolar osteitis or improved eventual bone quality for future implant placement.(19) Fifth, microbiological confirmation of bacterial load reduction, histomorphometry validation, and inflammatory biomarker data were not obtained in this study and would help substantiate the proposed mechanism.(26)

Despite these limitations, the large effect size and biological plausibility support aPDT as a promising, low-risk adjunct at the time of third-molar surgery, particularly in settings where minimizing systemic antibiotic use is desirable.(27) Future randomized, blinded, multi-center trials with longer follow-up, standardized or relative Gv reporting, and paired microbiological and clinical outcomes are warranted to confirm these findings and to define optimal photosensitizer and laser-dosimetry parameters for socket healing.

CONCLUSION

In this quasi-experimental study of mandibular third-molar extraction sockets, adjunctive single-session antimicrobial photodynamic therapy was associated with significantly higher quantitative trabecular bone density on standardized CBCT at 1 month compared with conventional management, despite comparable baseline characteristics. These findings suggest that aPDT may accelerate the early mineralization phase of alveolar socket healing and support its consideration as a simple, minimally invasive surgical adjunct, pending confirmation in randomized, blinded trials with longer radiographic follow-up.

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Author's Contribution:
Farwa Waqar: Conceptualization and data collection,
Usman Ghani Anwar: Literature review, drafting and revisions.

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