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RESEARCH ARTICLE

Energy Dispersive Spectroscopy of Open Dentinal Tubules on Application of Nanocrystalline Hydroxyapatite: An In Vitro Study

[version 1; peer review: 2 approved]

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v1 **First published:** 12 May 2026, **15**:720
https://doi.org/10.12688/f1000research.173988.1
Latest published: 12 May 2026, **15**:720
https://doi.org/10.12688/f1000research.173988.1

Abstract

Background

Dentinal hypersensitivity is an enigmatic problem that has long bewildered the dental profession and is one domain where man has not yet achieved the elusive “gold standards” of management. Recently, lasers have been used owning to their various modes of action to reduce hypersensitivity. Thus, the aim of the present in vitro study is to evaluate and compare the effect diode laser along with and without Nanocrystalline Hydroxyapatite powder on occlusion of dentinal tubules under scanning electron microscope (SEM).

Methodology

Twenty specimens were obtained from dentin discs of 2 mm thickness prepared from the cemento-enamel junction (CEJ) portion of 20 extracted human third molar teeth. The specimens were divided in to four groups of 5 specimens each. The specimens were etched in 37% phosphoric acid for 60 seconds to simulate hypersensitivity condition. Specimens in Group 1 (control) received no further treatment. Group 2

Open Peer Review

Approval Status ✓ ✓

	1	2
version 1 12 May 2026	✓ view	✓ view

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(DL) specimens received irradiation with 810 nm diode laser at an output power of 1 W for 60 seconds in continuous wave, non-contact mode. Specimens in Group 3 received treatment with a single application of nanocrystalline hydroxyapatite powder alone, whereas, Group 4 received a combination treatment of Diode laser irradiation followed by application of nanocrystalline hydroxyapatite powder.

Results

The average tubular diameter of dentinal tubules observed in the specimens belonging to Group 1 (control) was 3.14 microns, and in the Group 2 (diode laser) the average dentinal tubular diameter to be 2.28 microns. Mean diameter of dentinal tubules in Group 3 (nanoHA alone) was 2.41 microns. The specimens belonging to Group 4 (DL + nanoHA) showed average dentinal tubular diameter were 1.74 microns.

Conclusion

The single application of nanocrystalline hydroxyapatite powder and diode laser irradiation, showed significantly greater tubular occlusion and increased reduction in dentinal tubular diameters showing greater potential in occluding open dentinal tubules when compared to diode laser alone.

Keywords
Dentin Hypersensitivity; Diode laser; Scanning electron microscope; nanocrystalline hydroxyapatite

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Author roles: Rawat T: Conceptualization, Data Curation, Methodology, Project Administration, Writing – Original Draft Preparation; **Srinath R**: Conceptualization, Data Curation, Methodology, Supervision; **Praveen NC**: Conceptualization, Data Curation, Methodology, Supervision; **Amisha BS**: Investigation, Validation, Visualization; **Murugendrappa Gowdar I**: Formal Analysis, Validation, Writing – Original Draft Preparation, Writing – Review & Editing; **Almalki SA**: Formal Analysis, Visualization; **Gufran K**: Writing – Original Draft Preparation, Writing – Review & Editing; **G V U**: Writing – Review & Editing; **Nadar B**: Writing – Review & Editing

Competing interests: No competing interests were disclosed.

Grant information: The authors extend their appreciation to Prince Sattam bin Abdulaziz University for funding this research work through the project number (PSAU/2025/01/5130). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.
The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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How to cite this article: Rawat T, Srinath R, Praveen NC *et al.* **Energy Dispersive Spectroscopy of Open Dentinal Tubules on Application of Nanocrystalline Hydroxyapatite: An In Vitro Study [version 1; peer review: 2 approved]** F1000Research 2026, **15**:720 https://doi.org/10.12688/f1000research.173988.1

First published: 12 May 2026, **15**:720 https://doi.org/10.12688/f1000research.173988.1

Introduction

Dentin hypersensitivity is indeed a prevalent issue in dental practice, defined as pain resulting from exposed dentin reacting to various stimuli—chemical, thermal, tactile, or osmotic—without any underlying dental pathology.[1] The exposure of dentinal tubules to the oral environment can occur due to several factors, including parafunctional habits (such as bruxism), improper tooth brushing techniques, excessive consumption of acidic beverages, and frequent use of certain mouthwashes.[2] This exposure can lead to both physiological and psychological discomfort, significantly impacting an individual's oral health-related quality of life. The wide range in prevalence reported in a recent study between 1.3% and 91% highlights the variability in individual experiences.[3]

Dentin hypersensitivity indeed develops in two phases, with the first phase being lesion localization. During this phase, various factors—such as attrition, abrasion, erosion, and gingival recession—result in the loss of enamel and cementum. This loss exposes the underlying dentin, which can lead to a thinning of the calcified smear layer that normally provides some protection to the dentinal tubules. As the protective layers are compromised, the dentin becomes more susceptible to external stimuli, leading to increased fluid movement within the dentinal tubules. This fluid movement activates the sensory nerves, resulting in the characteristic pain associated with dentin hypersensitivity.[4] The second phase of dentin hypersensitivity, known as lesion initiation, occurs when the protective barriers such as tubular plugs and the smear layer are removed. As these protective layers are compromised, the dentinal tubules become fully exposed to the external environment. This exposure allows external stimuli to directly affect the dentin and pulp. The heightened sensitivity in this phase is due to the direct stimulation of the nerve endings within the dentin and the pulp, leading to significant discomfort or pain.[5]

The findings from scanning electron microscopy and dye penetration studies indicate that hypersensitive dentin has a significantly higher number of tubules and larger diameters compared to non-sensitive dentin. Specifically, hypersensitive dentin features eight times more tubules and tubules that are twice the diameter of those in non-sensitive dentin. The increased tubule diameter is particularly noteworthy, as fluid flow through these tubules is proportional to the fourth power of the radius. This means that even a small increase in diameter can lead to a substantial rise in fluid flow—doubling the diameter results in a 16-fold increase in fluid movement.[6]

The management of dentinal hypersensitivity focuses on alleviating pain or discomfort through two primary strategies such as occluding agents and direct ionic diffusion.[7] Various laser types have been tested for DH treatment, including Neodymium- or Erbium-doped yttrium-aluminum garnet (Nd:YAG and Er:YAG), CO_2, He-Ne, and diode (ie, GaAlAs) lasers, with various energy settings and with wavelengths ranging from 632.8 nm (He-Ne) to 10,600 nm (Er:YAG, CO_2). The mechanism of laser effects on dentin hypersensitivity is thought to be the laser- induced occlusion or narrowing of dentinal tubules as well as direct nerve analgesia.[8] They cause occluding of tubules through coagulation of proteins which diminishes the fluid movement, causing partial sub-melting of the denuded dentin and discharging of the internal tubular nerve.

Diode lasers have emerged as a promising option for treating dentinal hypersensitivity due to their specific parameters, which result in fewer damaging changes to the root surface and minimal temperature increases in the irradiated area.[9] This makes them a safer and more efficient choice compared to other laser types. Given that microstructural changes in dentinal tubules are not clearly observable in in-vivo studies, in vitro research becomes essential. Scanning electron microscopy (SEM) is used in these studies to closely examine the occlusion of dentinal tubules under high magnification, providing detailed insights into the effectiveness of different treatment modalities.[10,11] The aim of the present study is to evaluate the efficacy of diode laser treatment, both with and without the application of nanocrystalline hydroxyapatite crystals, in occluding dentinal tubules. By comparing these two approaches, the study seeks to determine whether the addition of hydroxyapatite enhances the occlusive effects of the diode laser, thus improving outcomes for patients with dentinal hypersensitivity. The findings from this research could provide valuable guidance for clinical practices aimed at managing this condition effectively.

Materials and methods

The present invitro study, adhered to the Declaration of Helsinki and was approved by the Institutional Ethical Committee, College of Dental Sciences, Davangere (Approval No: CODS/263/2020–2021). Written informed consent was obtained from all participants for use of extracted teeth and for publication of data. Twenty extracted human third molars were included in the study. Teeth presenting carious lesions, intrinsic stains, restorations, or abnormal morphology were excluded. From the coronal portion above the cemento-enamel junction, dentin discs of 2 mm thickness were prepared using diamond cylindrical burs with a high-speed handpiece.[12]

The discs were sequentially polished with silicon carbide papers of 400, 600, 800, and 1000 grit to produce a standardized smear layer. Each disc was sectioned into four quadrants using a diamond disc bur, and the quadrants were randomly allocated to four groups, ensuring that specimens for both control and test groups originated from the same tooth.[13] The groups were defined as follows: Group 1 – Control (no treatment), Group 2 – Diode laser, Group 3 – Nanocrystalline Hydroxyapatite (Sigma Aldrich, USA), and Group 4 – Diode laser combined with Nanocrystalline Hydroxyapatite. Following allocation, specimens were mounted on glass slides with cyanoacrylate adhesive and ultrasonicated in distilled water for two minutes to eliminate residual smear layer.[12]

The specimens were etched with 37% phosphoric acid for 60 s to simulate hypersensitivity conditions[14] and stored in artificial saliva throughout the experimental period.[15] Specimens in Groups 2 and 4 were irradiated twice for 5 s using a diode laser (810 nm, 1 W) [Picasso, AMD lasers, USA] delivered through a 300 µm optic fiber attached to a straight handpiece. The laser tip was positioned perpendicular to the dentin surface at a 1 mm distance to avoid contamination. Following treatment, discs were dehydrated at 70 °C for 24 h, mounted on stubs with double-faced carbon tape, sputter-coated with a 30 µm gold-platinum layer in a vacuum apparatus, and examined under SEM.

SEM analysis

Specimens were fixed in 2.5% glutaraldehyde (0.1 M phosphate buffer, pH 7.4) for 24 h, washed, and dehydrated through graded alcohols before mounting on SEM stubs. After air-drying (48 h), they were sputter-coated with 30–40 nm gold (BAL-TEC SCD-500) and examined under SEM (JEOL JSM-IT300, 15 kV). Tubular diameter and occlusion were assessed by scanning central areas to obtain circular tubule cross-sections,[12] and representative photomicrographs were captured at 1500× (Figures 1 and 2).

Calculation of number of tubules

Tubules were manually counted in photomicrographs, and the total number per sq. mm was calculated using the formula[16]:

Total number of tubules per sq.mm = 1000000 × N/area of the photomicrograph in sq. microns. ('N' being the number of tubules observed in the specimen).

Measurement of diameter of tubules

Tubule diameters were measured using IMAGE J software. The largest diameter across each tubule was recorded to minimize error from oblique sections, with calibration based on the photomicrograph scale bar.[17] Measurements were converted to microns accordingly (Figures 3 and 4).

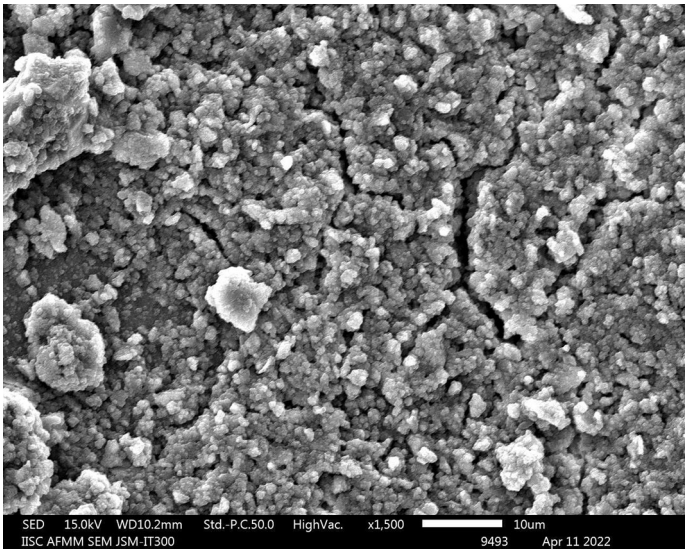


Figure 1. SEM micrograph showing diameter of dentinal tubules.

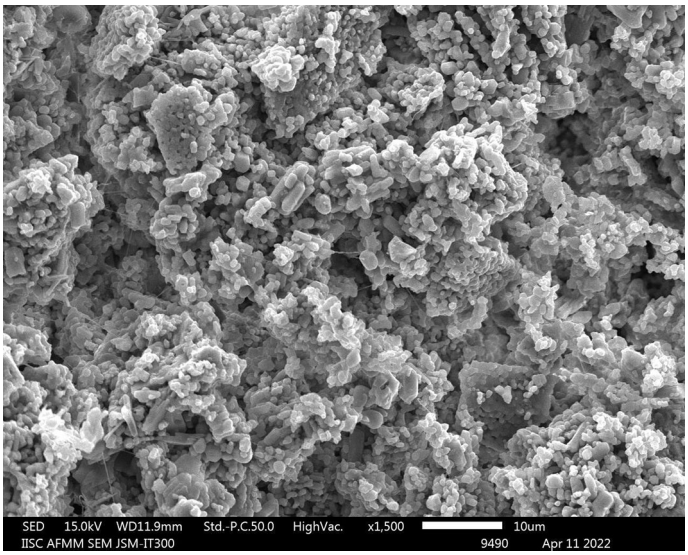
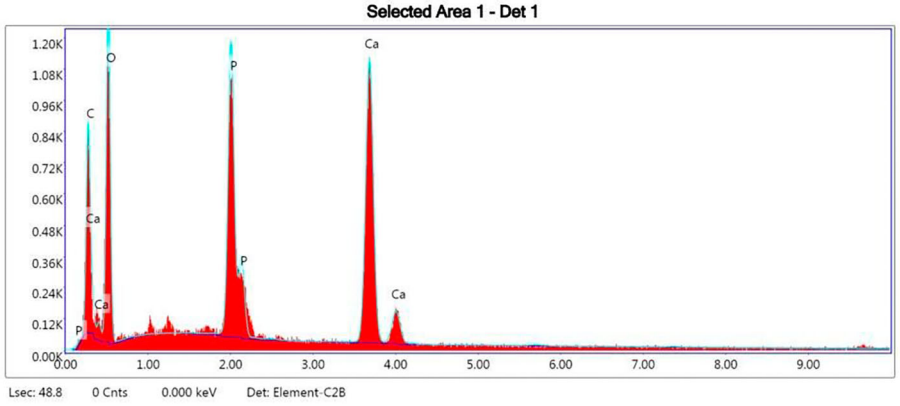


Figure 2. **Surface topography of dentin demonstrating tubule occlusion.**



eZAF Smart Quant Results

Element	Weight %	Atomic %	Net Int.	Error %	Kratio	Z	A	F
C K	16.52	27.65	76.06	11.27	0.0510	1.1106	0.2776	1.0000
O K	37.56	47.21	164.43	10.81	0.0724	1.0589	0.1818	1.0000
P K	14.26	9.26	216.11	3.36	0.1206	0.9167	0.9143	1.0080
CaK	31.65	15.88	262.66	2.95	0.2816	0.8964	0.9881	1.0041

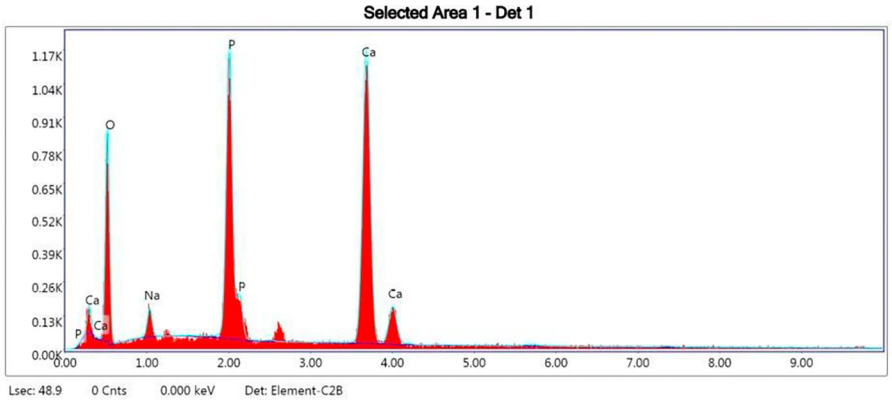
Figure 3. **EDS spectrum of dentin showing major elements in tubular structures.**

Grading tubule patency

Tubule patency was graded as follows: Grade A: Smear layer with some tubules just apparent, Grade B: Less than or equal to 10 tubules visible with majority occluded, Grade C: Greater than 10 tubules visible with majority occluded, Grade D: Less than or equal to 10 tubules visible with majority patent, Grade E: Greater than 10 tubules visible with majority patent.[18]

Statistical analysis

The statistical analysis was performed using SPSS v 20 (IBM Corporation, New York, USA). Kruskal Wallis H test was used to find out the difference in occlusive effects of diode laser with or without the application of nanocrystalline hydroxyapatite crystals. The significance level was fixed at $p < 0.05$.



eZAF Smart Quant Results

Element	Weight %	Atomic %	Net Int.	Error %	Kratio	Z	A	F
O K	34.47	54.23	109.68	11.15	0.0647	1.1008	0.1705	1.0000
NaK	2.32	2.54	16.02	13.77	0.0105	0.9966	0.4549	1.0015
P K	19.13	15.55	218.49	3.60	0.1653	0.9548	0.8983	1.0077
CaK	44.09	27.69	276.76	2.96	0.4032	0.9345	0.9762	1.0026

Figure 4. EDS quantification of mineral and trace elements within dentinal tubules.

Results

The combination of the diode laser and nanocrystalline hydroxyapatite resulted in a significantly lower number of dentinal tubules (56.1 ± 22.4) compared to the diode laser alone (101.8 ± 49.9) and nanocrystalline hydroxyapatite alone (80.2 ± 27.6). The observed differences were statistically significant, with a p-value of 0.01, indicating that the combined treatment was more effective in reducing dentinal tubule density than either treatment used independently (Table 1).

The combination of the diode laser and nanocrystalline hydroxyapatite resulted in a significantly lower diameter of dentinal tubules (1.74 ± 0.23) compared to the diode laser alone (2.28 ± 0.33) and nanocrystalline hydroxyapatite alone (2.41 ± 0.35). The differences in tubule diameter among the groups were statistically significant, with a p-value of 0.00, highlighting the effectiveness of the combined treatment in reducing tubule size (Table 2).

Table 1. Comparison of mean number of dentinal tubules between the groups.

Groups	Mean ± SD	p-value
Control	105.2 ± 19.3	0.01
Diode laser	101.8 ± 49.9	
Nanocrystalline Hydroxyapatite alone	80.2 ± 27.6	
Both Diode laser and Nanocrystalline Hydroxyapatite	56.1 ± 22.4	

Table 2. Comparison of mean diameter of dentinal tubules between the groups.

Groups	Mean ± SD (microns)	p-value
Control	3.14 ± 0.60	0.01
Diode laser	2.28 ± 0.33	
Nanocrystalline Hydroxyapatite alone	2.41 ± 0.35	
Both Diode laser and Nanocrystalline Hydroxyapatite	1.74 ± 0.23	

Discussion

The present study highlights the effectiveness of using a combination of diode laser and nanocrystalline hydroxyapatite for reducing dentinal tubular diameter. The use of a diode laser at 810 nm with an output power of 1 W for 60 seconds in continuous, non-contact mode seems to provide optimal conditions for sealing dentin tubules, as supported by previous findings from Umana et al.[19] The laser's wavelength, which falls between 800 and 980 nm, demonstrates poor absorption in water and hydroxyapatite, allowing for deeper penetration and minimal thermal damage to surrounding dental tissues. The resultant increase in temperature effectively melts and reduces the diameter of the dentinal tubules, providing a potential solution for dentinal hypersensitivity while maintaining pulp vitality.

The combination therapy group demonstrated more substantial dentinal tubule occlusion than other groups. The findings are in line with the study conducted by Shamel et al.[20] The robustness of Diode Laser over hydroxyapatite nanocrystals might be related to photothermal processes, which heat and dissolve the hard tissue on the surface.[21] The capacity of nano-HAP to create hydroxyapatite plugs that obstruct dentinal tubules and a coating of mineral hydroxyapatite is what accounts for its efficacy in lowering DHS.

The formation of a hydroxyapatite (HAP) layer on teeth is supported by various studies indicating that nano-hydroxyapatite (nano-HAP) serves as a reservoir for calcium and phosphate ions. This helps maintain a state of supersaturation in saliva, which encourages mineral deposition on tooth surfaces.[22,23] Research shows that using nano-HAP toothpaste can elevate calcium levels in saliva, enhancing this effect.[23] Additionally, nano-HAP can fill micropores on teeth, acting as a template for apatite deposition by attracting calcium and phosphate ions from saliva and other sources. This process promotes the integrity and growth of crystal structures within the tooth tissue.[24]

It is widely recognized that Dentin Hypersensitivity (DHS) occurs when external stimuli lead to rapid fluid movement within exposed dentinal tubules, activating sensory nerve receptors in the pulp and causing sharp pain. Plugging these tubules with mineral hydroxyapatite (HAP) can significantly reduce their permeability, thereby minimizing fluid disturbance and alleviating DHS.[25] The findings of the current study align with previous research demonstrating that nano-HAP, whether in toothpaste or topical cream form, effectively reduces DHS.[26–32]

A key strength of our study was the use of Scanning Electron Microscopy (SEM), which offers numerous advantages such as a non-destructive approach, high resolution, three-dimensional imaging, and detailed topographical information.[33,34] Additionally, various in vitro studies were conducted to analyze the mechanisms of action and desensitizing properties of the agents used. These included hydrostatic fluid filtration systems, attenuated total reflection Fourier transform infrared spectroscopy, energy dispersive X-ray analysis, confocal laser scanning microscopy, and electron spectroscopy analysis.[35–37] This comprehensive methodology enhances the reliability and depth of our findings. The primary limitation of our study is the small sample size and its in vitro design. Future research should concentrate on the clinical efficacy of diode lasers with varying wavelengths, as well as the potential adverse effects of laser application on dentinal tubules. This will provide a more comprehensive understanding of the practical implications and safety of these treatments.

Conclusion

The findings indicate that the combination of nanocrystalline hydroxyapatite powder and diode laser irradiation results in significantly better dentinal tubule occlusion compared to the diode laser alone. This suggests a synergistic effect between the two treatments, enhancing their ability to reduce dentinal tubular diameters and potentially improving the overall effectiveness in managing conditions like dentin hypersensitivity. The occlusion of open dentinal tubules is crucial for protecting the pulp from external stimuli and reducing sensitivity. The use of nanocrystalline hydroxyapatite alongside laser treatment may not only improve the structural integrity of dentin but also contribute to long-term outcomes in dental health.

Ethical approval and consent to participate

The study was conducted in accordance with the Declaration of Helsinki and this study was approved by ethical committee of College of Dental Sciences, Davangere, with the approval number CODS/263/2020–2021. Written Informed consent was obtained from the participants for the use of their extracted teeth.

Consent for publication

All the participants gave consent for the publication of the data.

Data availability

Underlying data

Figshare – Energy Dispersive Spectroscopy of Open Dentinal Tubules on Application of Nanocrystalline Hydroxyapatite: An in vitro Study. https://doi.org/10.6084/m9.figshare.30762686.v1[38]

This project contains following underlying data:

- study-data.xlsx.

Data are available under the terms of the Creative Commons Zero "No rights reserved" data waiver (CC BY 4.0 Public domain dedication).

Extended data

Figshare – Energy Dispersive Spectroscopy of Open Dentinal Tubules on Application of Nanocrystalline Hydroxyapatite: An in vitro Study (Informed consent form). https://doi.org/10.6084/m9.figshare.32064924.v2[39]

This project contains following extended data

- Informed consent form.docx

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Current Peer Review Status: ✔ ✔

Version 1

Reviewer Report 22 June 2026

https://doi.org/10.5256/f1000research.191855.r484410

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✔ **Sangamesh Ningappa Chinnannavar**
Kalinga Institute of Dental Sciences, Odisha, India

• **Title and Abstract:**
The title and abstract effectively summarize the key aspects of the study and provide a clear overview of its scope and objectives. However, there appears to be a discrepancy between the laser parameters reported in the Abstract and those described in the Methods section. Kindly review and correct this inconsistency.
• **Introduction:**
The introduction is well-structured and logically organized, supported by relevant and appropriate citations. It effectively establishes the background and rationale for the study while highlighting the importance of further research in this area.
• **Need for the Study:**
The rationale for the study is well justified with a sound scientific basis that emphasizes its clinical and academic significance. The study addresses an important gap in the existing literature and contributes valuable insights to the field.
• **Methodology:**
The methodology is clearly described and reproducible. The study design, sampling methods, and research procedures are adequately detailed. The statistical analysis performed is appropriate and robust, supporting the validity and reliability of the findings.
• **Tables and Figures:**
The tables and figures are relevant, well presented, and aligned with the study objectives. They effectively support the interpretation of the results. Since the Kruskal–Wallis test demonstrated statistical significance, kindly mention the post hoc test performed for multiple comparisons.
• **Discussion:**
The discussion is comprehensive and provides a meaningful interpretation of the study findings. The comparison with previous studies is appropriate and helps place the results within the context of existing scientific literature.
• **Conclusion:**
The conclusion is consistent with the study findings and appropriately summarizes the key outcomes. The inferences drawn are logical and supported by the results presented in the study.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Oral Medicine and Radiology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 15 June 2026

https://doi.org/10.5256/f1000research.191855.r484413

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Janardhana Amaranath B.J.
Rama University Kanpur, Uttar Pradesh, India

The manuscript is well-structured and maintains clarity and transparency throughout the reporting. The title and abstract are informative and provide a concise overview of the study objectives, methodology, and principal findings.

The introduction effectively establishes the need for the study by clearly outlining the research aim and objectives. The background information is relevant and adequately justifies the significance of the study within the current scientific context.

The authors have described the validation process of the data collection instrument in detail, which significantly enhances the reliability and credibility of the study. The methodology is clearly

presented and reflects careful planning and execution. The inclusion of additional details regarding the random allocation procedure, allocation concealment, and examiner blinding during SEM image acquisition and measurements would further enrich the methodological description and provide greater clarity for readers. Overall, the study is well designed, scientifically sound, and contributes valuable findings to the field, making it suitable for indexing.

The statistical methods used are clearly specified and appear appropriate for the type of data analyzed. The results are systematically presented in well-organized tables with comprehensive and relevant information.

The discussion provides a concise interpretation of the key findings and appropriately relates them to previous literature. Relevant studies with similar findings have been adequately cited, resulting in a balanced and informative discussion.

The manuscript also offers useful recommendations for future research based on the study findings and acknowledged limitations. The references are appropriately formatted and adhere to the journal guidelines.

Is the work clearly and accurately presented and does it cite the current literature?
Yes

Is the study design appropriate and is the work technically sound?
Yes

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Yes

If applicable, is the statistical analysis and its interpretation appropriate?
Yes

Are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions drawn adequately supported by the results?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: in the specialty of periodontics

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

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