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Comparative Evaluation of Dentinal Tubule Occlusion Using Herbal Desensitizing Agents With Diode Laser Assisted and Non-laser Assisted Conditions: Confocal Laser Scanning Microscopic Study

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Research Article

Keywords: Dentinal hypersensitivity, Dentinal tubule occlusion, CPP-ACP, Herbal dentifrices, Propolis, Miswak, Diode laser, Confocal laser scanning microscopy

Posted Date: May 29th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-9548249/v1

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Additional Declarations: No competing interests reported.

Abstract

Introduction:

Dentinal hypersensitivity is a common clinical condition characterized by pain due to exposed dentin in response to external stimuli. Contemporary management focuses on reducing dentinal permeability by occluding these tubules. Recently, herbal desensitizing agents and adjunctive laser therapy have gained attention due to their biocompatibility and potential effectiveness.

Aim

To evaluate and compare the efficacy of two herbal dentifrices (Edinora Sensipro and Bentodent) and a CPP-ACP–based agent (GC Tooth Mousse), with and without diode laser application, in occluding dentinal tubules.

Method

Fifty-four dentin disc samples prepared from extracted human teeth were randomly allocated into three groups (n = 18) based on the desensitizing agent used. Each group was further subdivided into laser and non-laser subgroups (n = 9). Samples were brushed twice daily with toothpastes for 14 days, followed by diode laser application (940 nm) in designated groups on day1 and 14. Dentinal tubule occlusion was assessed using confocal laser scanning microscopy (CLSM) after rhodamine dye application. Data were analyzed using two-way ANOVA and post-hoc tests.

Results

All groups demonstrated increased dentinal tubule occlusion at Day 14. GC Tooth Mousse showed the highest occlusion, followed by Bentodent and Edinora. Laser application significantly enhanced occlusion in selected groups.

Conclusion

Diode laser application may enhance desensitizing effects. CPP-ACP exhibited superior tubule occlusion, while herbal dentifrices showed moderate efficacy.

Introduction

Dentin hypersensitivity (DH) presents as a short, sharp pain arising from exposed dentin in response to stimuli such as thermal, chemical, tactile, evaporative, or osmotic triggers acting on exposed dentinal

surfaces.[1] The most widely accepted explanation is Brännström's hydrodynamic theory, which proposes that external stimuli cause movement of fluid within dentinal tubules, stimulating pulpal mechanoreceptors and resulting in pain.[2] Temperature changes influence the direction of fluid movement, with heat producing inward flow and cold causing outward movement.[3]

Management of DH mainly focuses on either occluding dentinal tubules to limit fluid movement or reducing pulpal nerve excitability. However, the wide range of available desensitizing agents can make treatment selection challenging for clinicians.[3,4]

The growing interest in herbal products has drawn attention to natural agents such as miswak (*Salvadora persica*), incorporated in Edinora Sensipro toothpaste. Miswak contains calcium, phosphate, fluoride, silica, tannins, sterols, and flavonoids, which may contribute to dentinal tubule sealing.[5] Propolis, present in Bentodent toothpaste and derived from *Apis mellifera*, promotes protein coagulation and mineral deposition within dentinal tubules while also providing antimicrobial and anti-inflammatory properties.[6]

In addition, synthetic remineralizing agents such as CPP-ACP in GC Tooth Mousse release calcium and phosphate ions that form apatite crystals, leading to effective tubule occlusion.[7] The use of a 940-nm diode laser as an adjunctive therapy may further enhance tubule sealing through dentin surface melting and coagulation of tubular fluid.[8] Confocal Laser Scanning Microscopy (CLSM) allows detailed three-dimensional evaluation of dentinal tubule occlusion.[9,10]

Accordingly, this study evaluates miswak-, propolis-, and CPP-ACP-based agents with and without diode laser application using CLSM analysis.

Materials and Methods

Method of Data Collection

A convenience sampling technique was used for sample selection. A total of 30 extracted human caries free teeth with intact crowns were collected and sectioned to obtain 54 dentin samples for the study. The teeth included were caries-free with intact crowns and showed no evidence of attrition, abrasion, or erosion. The 54 samples were randomly divided into three main groups (n = 18 per group).

Group I: Edinora Sensipro toothpaste

Group Ia: Diode laser application on Day 1 and Day 14 (n = 9)

Group Ib: No laser application (n = 9)

Group II: Bentodent Desensitizer toothpaste

Group IIa: Diode laser application on Day 1 and Day 14 (n = 9)

Group IIb: No laser application (n = 9)

Group III (Control): GC Tooth Mousse

Group IIIa: Diode laser application on Day 1 and Day 14 (n = 9)

Group IIIb: No laser application (n = 9)

Procedure

Dentine discs preparation

Thirty extracted human teeth meeting the inclusion criteria were collected and autoclaved before preparation. Sections were performed using a water-cooled diamond disc to prevent overheating. Two horizontal cuts were made at the cementoenamel junction (CEJ) and the middle third of the crown. A third section separated the mesial and distal halves, producing 54 standardized crown specimens. Specimens were mounted in modeling wax blocks with the occlusal surface facing upward to ensure proper alignment during the experiment.

The buccal enamel surface was removed using a high-speed, water-cooled diamond bur to expose a uniform dentin surface while minimizing excessive tissue removal.

To remove the smear layer, 17% ethylenediaminetetraacetic acid (EDTA) was applied for 1 minute, followed by thorough rinsing with distilled water. Specimens were then allocated into six experimental subgroups (n = 9). After treatment, samples were immersed in artificial saliva and stored at room temperature to simulate oral conditions.

Artificial Saliva Preparation

Specimens were stored in artificial saliva prepared according to the formulation described by Fusayama et al. The initial pH was 5.2 and was adjusted to 7.2 by gradual addition of 1 ml sodium hydroxide (NaOH). The artificial saliva was replaced every 24 hours throughout the experimental period.[11]

Brushing Technique

All specimens were brushed with the assigned toothpaste twice daily for 2 minutes with a 12-hour interval using an electric toothbrush. A pea-sized amount of toothpaste was applied, and brushing was performed at a 90° angle to the dentin surface for 14 days. After brushing, specimens were rinsed and stored again in artificial saliva.

Laser Application

Following toothpaste application, diode laser application was performed on Day 1 and Day 14. Irradiation was carried out in non-contact mode for 60 seconds after brushing.

The Biolase eEpic™ X diode laser was used at a wavelength of 940 nm (± 10 nm) with an output power of 1–1.25 W in continuous wave mode. A 300-µm optical fiber was used without direct contact with the specimen surface with distance of 5mm.

Dye Application

After the final toothpaste and laser application, rhodamine dye was applied for 24 hours to facilitate evaluation of dye penetration.

Assessment of Dentinal Tubule Occlusion

Dentinal tubule occlusion was evaluated using confocal laser scanning microscopy (CLSM). Specimens were labelled with 0.1% (w/w) rhodamine B isothiocyanate dye to assess penetration depth. Imaging was performed in fluorescent mode using a helium–neon laser as the excitation source.

Statistical Analysis:

Data was recorded specimen-wise as a single post-treatment (Day 14) measurement of occluded dentinal tubule area (µm²). Samples were categorized by Material (Edinora, Bentodent, GC Mousse) and Laser irradiation (Yes/No), forming six subgroups (n = 9 each; total n = 54). A two-way ANOVA was performed to assess the effects of Material, Laser, and their interaction. Post-hoc comparisons were conducted using Tukey's HSD or Holm adjustment. Results were expressed as mean ± SD, with significance set at $\alpha = 0.05$.

Results

Two-way ANOVA demonstrated a very large main effect of material on occlusion ($F(2,48) = 657.23$, $p < 0.0001$, partial $\eta^2=0.9648$) and a significant main effect of laser irradiation ($F(1,48) = 39.23$, $p < 0.0001$, partial $\eta^2=0.4498$) from *Table 1*. Importantly, there was a significant Material × Laser interaction ($F(2,48) = 4.80$, $p = 0.0125$, partial $\eta^2=0.1668$), indicating that the magnitude of laser-related improvement differed by material. Consistent with this interaction, material-wise simple effects (Yes − No) showed that laser did not significantly change Bentodent (MD = + 13,820.74 µm²; 95% CI − 11,747.99 to 39,389.49; Holm-adjusted $p = 0.2687$), but significantly increased occlusion for Edinora (MD = + 63,043.47 µm²; 95% CI 31,748.94 to 94,337.997; Holm-adjusted $p = 0.0012$) and GC Mousse (MD = + 47,988.97 µm²; 95% CI 35,643.79 to 60,334.15; Holm-adjusted $p < 0.0001$).(Fig. 1)

Discussion

Dentinal hypersensitivity is best explained by the hydrodynamic theory, where pain results from fluid movement within exposed dentinal tubules, which is commonly seen in the cervical region of young and middle-aged individuals and is managed by reducing dentinal permeability to limit pulpal mechanoreceptor activation.[12–15] Consequently, current management of DH is directed toward either physically occluding dentinal tubules or modulating pulpal nerve responsiveness.[16,17]

The present study was designed to evaluate the tubule-occluding efficacy of herbal toothpastes as a mechanism to reduce dentin permeability. The findings demonstrated statistically significant improvement in dentinal tubule occlusion from baseline to 14 days across all evaluated parameters. These results are consistent with previous reports by Almas and Miglani et al., who emphasised the importance of tubule-occluding agents in reducing dentin permeability and clinical hypersensitivity. [6,18]

In addition to chemical desensitising agents, adjunctive physical modalities, such as diode lasers, have recently gained attention for managing DH. Diode lasers primarily act through photothermal interaction with dentin, inducing melting and subsequent recrystallisation of intra- and peritubular dentin.[19] This process results in narrowing or sealing of dentinal tubules and reduction in dentinal fluid movement. Patil et al. reported significantly fewer open dentinal tubules after diode laser application than after iontophoresis or in untreated controls, indicating a superior occluding potential.[20] Khare et al. also observed improved outcomes when diode laser irradiation was used along with propolis-based desensitising dentifrices.[21] These findings highlight the potential of diode lasers as an effective adjunct capable of enhancing the therapeutic effects.

Assessment of dentinal tubule occlusion has traditionally relied on scanning electron microscopy (SEM); however, advanced imaging modalities such as confocal laser scanning microscopy (CLSM) provide additional insight into dentin permeability. CLSM enables three-dimensional visualization of dentinal tubules and facilitates evaluation of fluid penetration using fluorescent tracers.[9,10] Therefore, CLSM was selected for the present study, as it allows a more comprehensive assessment of tubule occlusion and fluid penetration as seen in *Fig. 2*.

GC Tooth Mousse containing casein phosphopeptide–amorphous calcium phosphate (CPP-ACP) used as the control in the present study. CPP-ACP functions by stabilizing calcium and phosphate ions in a bioavailable form, thereby promoting remineralization.[7] Upon interaction with saliva, amorphous calcium phosphate releases these ions, which subsequently convert into apatite crystals that deposit on dentin surfaces and within dentinal tubules. The resulting intratubular mineral plugs reduce dentin permeability and act as a reservoir of calcium and phosphate ions. The findings of this study align with those of Ghafournia and Devadiga et al., who reported effective dentinal tubule occlusion and reduction in hypersensitivity following the use of CPP-ACP.[7,16] Despite its established efficacy, CPP-ACP requires repeated application and may show variable resistance to acidic dissolution.[9] These limitations have encouraged exploration of alternative natural formulations that may provide comparable benefits with improved patient acceptance.

Bentodent desensitizing toothpaste, formulated with bentonite clay, propolis, and guava leaf extract, represents one such herbal formulation. Due to the limited availability of published literature on this product, it was included in the present investigation. Bentodent demonstrated a significant reduction in open dentinal tubules over the 14-day evaluation period. These findings correspond with those of Kar et al., who reported considerable tubule occlusion with Bentodent, although slightly less than NovaMin-containing formulations.[22]

The desensitizing efficacy of Bentodent can be attributed to the combined action of its constituents. Bentonite clay possesses a fine particulate structure and strong adsorptive capacity, allowing it to mechanically penetrate dentinal tubules and form a smear-like barrier over exposed dentin.[18] Propolis, a resinous bee-derived substance rich in flavonoids and phenolic compounds, further enhances tubule sealing through protein coagulation and mineral deposition. Naghsh et al. reported that propolis-based toothpaste significantly reduced the proportion of open dentinal tubules, demonstrating efficacy comparable to 5% sodium fluoride varnish.[13] The adhesive nature of propolis facilitates enhanced retention within tubules, while its antimicrobial and anti-inflammatory properties contribute to stabilization of pulpal tissues.[20,23]

Edinora toothpaste containing miswak (Salvadora persica) was another herbal dentifrice evaluated in this study. Miswak has long been recognized for its oral health benefits and contains calcium, phosphate, fluoride, silica, tannins, and other bioactive compounds. In the present study, Edinora demonstrated a significant improvement in dentinal tubule occlusion over the 14-day period. Khunkar and Linjawi previously reported complete blockage of dentinal tubules following the application of miswak extract on root dentin.[24] Mineral components such as calcium and phosphate contribute to intratubular precipitation, while silica may facilitate smear layer formation.[4,24]

However, comparatively lower tubule occlusion was observed with Edinora in the present study. This finding may be attributed to formulation-related factors. Miswak primarily exerts its effect through organic bioactive compounds that promote protein precipitation rather than extensive mineral deposition.[23] The concentration and bioavailability of calcium and phosphate in miswak-based formulations may be lower than those present in remineralizing systems such as CPP-ACP. Additionally, silica-mediated smear layer formation may produce superficial occlusion rather than deep intratubular sealing.[19] Unlike propolis-containing formulations, miswak lacks strongly bioadhesive resinous components that enhance deposit retention within dentinal tubules. These factors may collectively explain the relatively lower occlusion observed despite its documented antimicrobial and desensitizing properties.

Overall, the findings of the present study support previous evidence indicating that both conventional and herbal dentifrices reduce dentinal hypersensitivity primarily through dentinal tubule occlusion.[1,9] Herbal formulations offer additional advantages such as improved biocompatibility, reduced adverse effects, and the presence of multiple synergistic bioactive constituents. Parimala et al. highlighted that

herbal toothpastes possess analgesic, anti-inflammatory, antimicrobial, and tubule-occluding properties, making them suitable for long-term use.[23]

The superior dentinal tubule occlusion observed with CPP-ACP in this study can be attributed to its ability to stabilize and release bioavailable calcium and phosphate ions, promoting intratubular apatite formation and deeper mineral deposition. This mechanism provides more stable and acid-resistant occlusion compared with surface-level blockage. In contrast, Bentodent demonstrated moderate efficacy, primarily due to mechanical occlusion by bentonite clay and protein coagulation induced by propolis, resulting mainly in superficial sealing. Edinora, containing miswak, showed comparatively lower occlusion, likely due to limited mineral bioavailability and reliance on smear-layer formation and organic precipitation rather than sustained intratubular remineralization.

Certain limitations should be acknowledged. The 14-day evaluation period restricts assessment of long-term durability under clinical conditions. Acidic and abrasive challenges simulating dietary exposure and tooth-brushing were not incorporated, preventing evaluation of deposit stability.

Within these limitations, the present study demonstrated that two commercially available herbal dentifrices were capable of promoting dentinal tubule occlusion. Furthermore, existing evidence suggests that diode laser application may enhance tubule sealing compared with non-laser approaches. Collectively, these findings support the role of both conventional and herbal agents in the management of dentinal hypersensitivity through dentinal tubule occlusion.

Conclusion

Overall, dentinal tubule occlusion at Day 14 is highest with GC Mousse and lowest with Edinora. Laser irradiation enhances occlusion for Edinora and GC Mousse but not for Bentodent, yielding a significant Material × Laser interaction.

Declarations

Conflict of Interest

We declare that there are no competing interests related to this study.

Funding Declaration: The authors received no financial support for the research, authorship, and/or publication of this article.

Author Contribution

M.V. – Conception and design of the work; methodology development; data curation; formal analysis; investigation; drafting of the manuscript.A.T.– Conception and design of the work; methodology development; supervision; critical review and editing of the manuscript.V.K.– Formal analysis; investigation; interpretation of data; visualization; critical review and editing of the manuscript.T.M. –

Visualization; critical review and editing of the manuscript.M.B.-Critical review and editing of the manuscript.

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Table

Table 1: **Mean occluded area (μm²) with and without laser for each material at Day-14**

Material	Without Laser (Mean ± SD), µm²	With Laser (Mean ± SD), µm²	Laser Gain (Yes−No), µm² (95% CI)	Holm-adj p	Interpretation
Bentodent	525,622.92 ± 22,770.35	539,443.67 ± 28,120.78	+13,820.74 (−11,747.99 to 39,389.49)	0.2687	**No significant laser effect**
Edinora	351,298.26 ± 29,301.79	414,341.73 ± 33,207.17	+63,043.47 (31,748.94 to 94,337.997)	0.0012	**Laser significantly improves occlusion**
GC Mousse	653,841.66 ± 16,825.78	701,830.63 ± 4,701.82	+47,988.97 (35,643.79 to 60,334.15)	<0.0001	**Laser significantly improves occlusion**

Figures

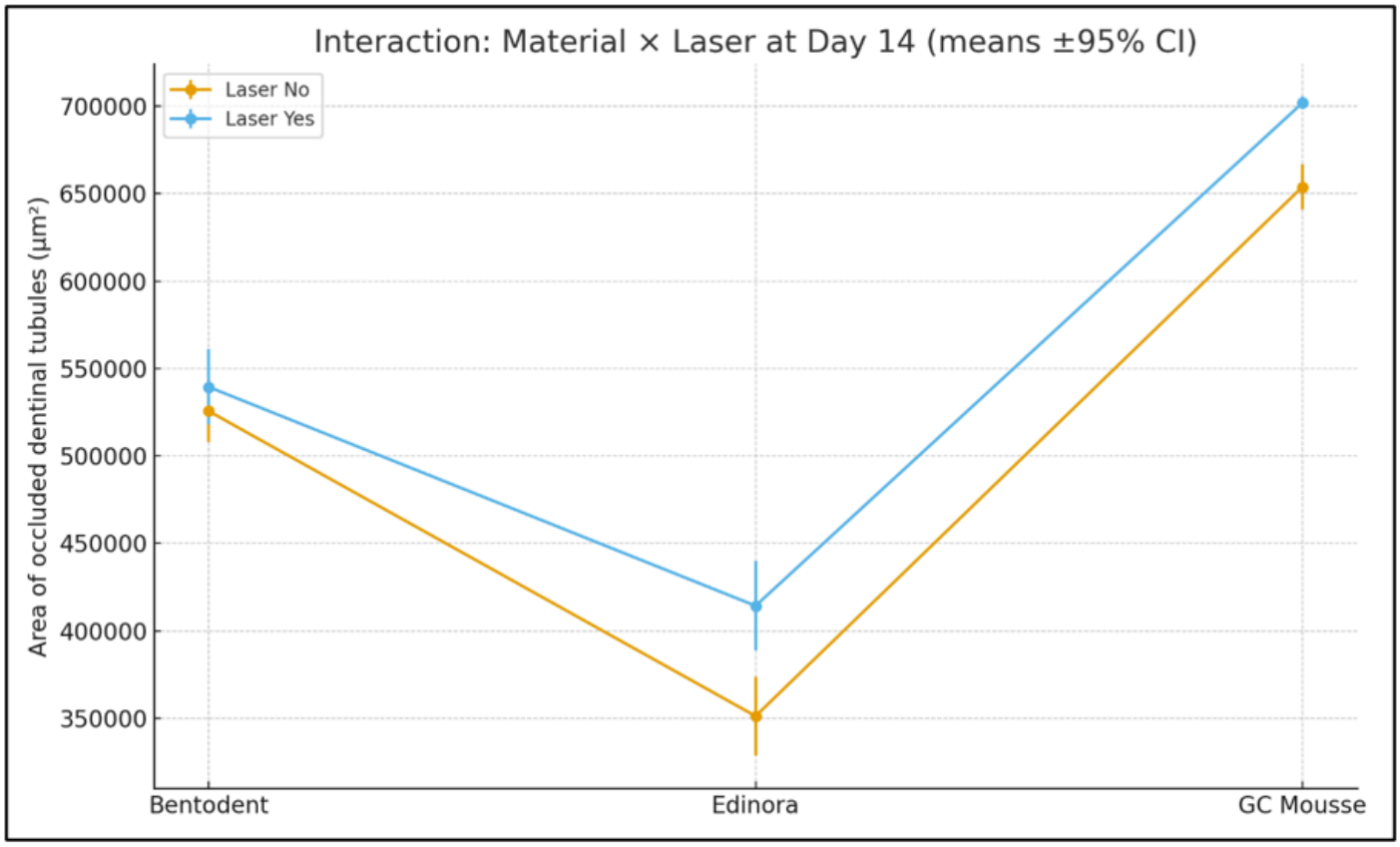


Figure 1

Interaction plot (means ±95% CI) for Material × Laser at Day 14. The divergence at Edinora and GC Mousse illustrates the significant interaction.

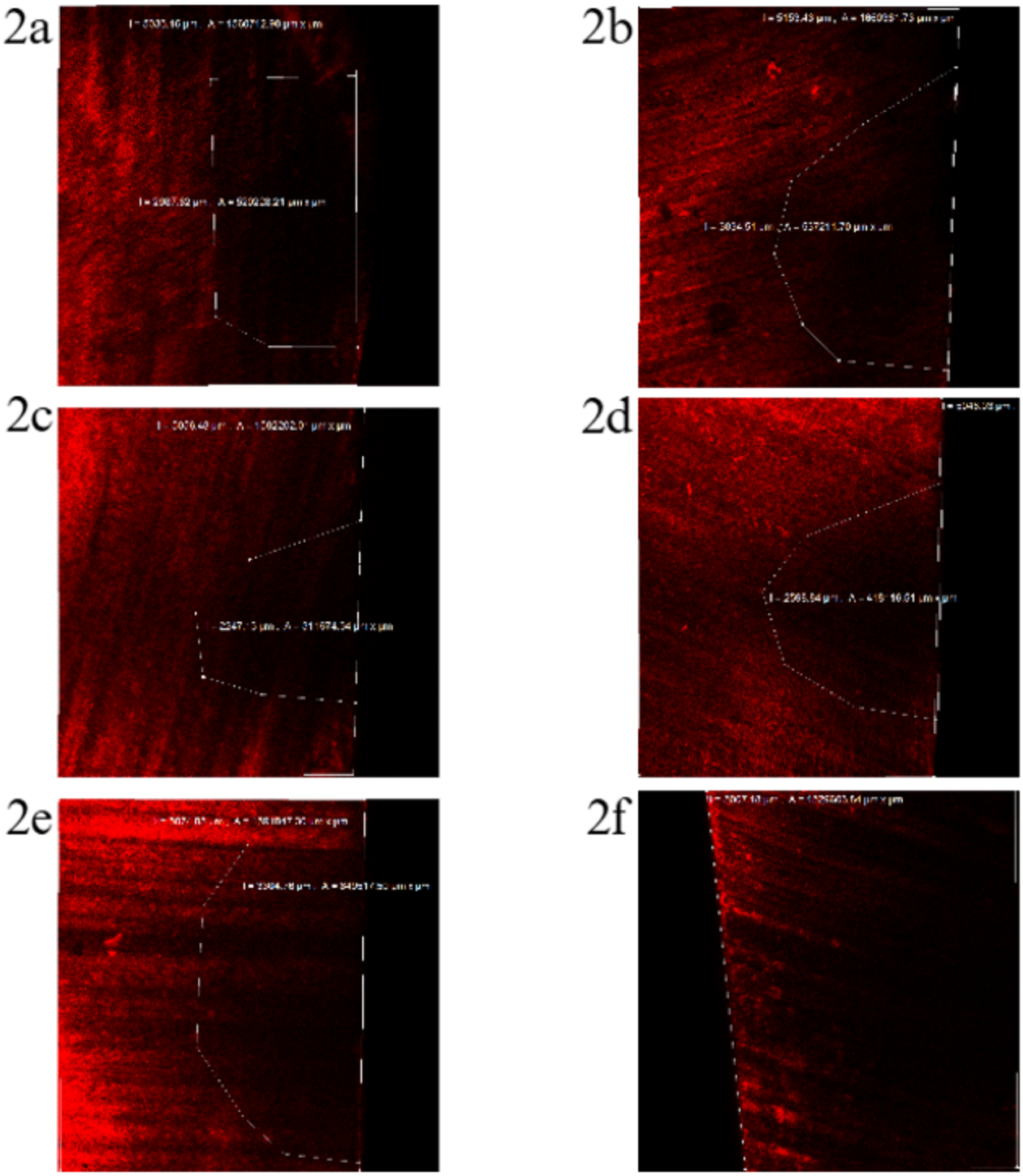


Figure 2

Representative CLSM images of dentinal tubule penetration. 2a: Bentodent toothpaste without laser; 2b: Bentodent toothpaste with laser; 2c: Edinora toothpaste without laser; 2d: Edinora toothpaste with laser; 2e: GC tooth mousse without laser; 2f: GC tooth mousse with laser.