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CeraSeal bioceramic sealer dentinal tubule penetration after three irrigant activation methods: Laboratory study

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Abstract:
Smear-layer persistence may restrict dentinal tubule penetration of bioceramic sealers and compromise obturation. Eighty-four mandibular premolars received three irrigant activation methods with 17% EDTA or saline. CLSM measured CeraSeal penetration, while SEM assessed smear-layer removal. EDTA increased penetration significantly, with EDTA plus PUI showing the greatest depth (1888.09 ± 67.72 µm; $p < 0.001$). Thus, EDTA-assisted PUI may improve CeraSeal penetration, although the SEM dataset requires verification.

Keywords: CeraSeal; bioceramic sealer, confocal laser scanning microscopy, dentinal tubule penetration, manual dynamic agitation, passive ultrasonic irrigation, smear layer

Background:
The primary objective of endodontic treatment is to eliminate infection from the root canal system, promote healing of existing periapical disease and prevent reinfection of periradicular tissues **[1]**. Microorganisms are the principal etiological agents in pulpal and periapical pathosis; their control is therefore fundamental to successful therapy **[2]**. Complex root canal anatomy, including fins, isthmuses, lateral canals and dentinal tubules, provides protected niches that may remain untouched by instrumentation **[3]**. Biomechanical preparation also produces a smear layer containing organic debris, microorganisms and inorganic dentin particles **[4]**. This layer can obstruct the penetration of irrigants, intracanal medicaments and sealers, thereby compromising disinfection and adaptation **[5]**. Sodium hypochlorite (NaOCl) provides antimicrobial and tissue-dissolving activity but does not effectively remove the inorganic component of the smear layer **[6]**. Chelating agents such as ethylenediaminetetraacetic acid (EDTA) remove inorganic debris by binding calcium ions and exposing dentinal tubules **[7]**. Irrigant activation can further improve fluid exchange and cleaning without additional dentin removal **[8]**. Available approaches include manual, sonic, ultrasonic, negative-pressure, thermal and laser-based activation **[9]**. Passive ultrasonic irrigation has received particular attention because it improves irrigant movement within complex canal spaces **[10]**, principally through acoustic streaming and irrigant agitation **[11]**. Because complete microbial elimination cannot be assured by instrumentation and irrigation alone **[12]**, obturation is intended to fill the prepared canal space, entomb residual microorganisms and reduce coronal and apical leakage **[13]**. Root canal sealers should therefore exhibit dimensional stability, radiopacity, biocompatibility, antimicrobial activity and adequate sealing performance **[14]**. Dentinal tubule penetration can increase sealer-dentin contact, promote micromechanical retention and extend antimicrobial effects into areas that are difficult to instrument **[15]**. Contemporary sealers are increasingly designed as bioactive rather than passive filling materials **[16]**. Calcium silicate-based bioceramic sealers offer favorable biocompatibility, chemical stability and bioactivity **[17]**. CeraSeal (Meta Biomed Co., Cheongju, Korea) is a premixed calcium silicate-based sealer containing tricalcium silicate, dicalcium silicate, calcium aluminate, zirconium oxide and a thickening agent; its small particle size and wettability may facilitate penetration into dentinal tubules **[18]**. Confocal laser scanning microscopy (CLSM) permits optical sectioning of fluorescently labeled specimens while preserving the sealer-dentin interface and is therefore well suited to quantitative penetration assessment **[19]**. Therefore, it is of interest to compare conventional needle irrigation, manual dynamic agitation and passive ultrasonic irrigation after final irrigation with 17% EDTA or saline and to determine their effects on CeraSeal dentinal tubule penetration and smear-layer removal.

Materials and Methods:
A total of 84 extracted single-rooted mandibular premolars meeting the inclusion and exclusion criteria were selected. Teeth were cleaned of debris and soft-tissue remnants and stored in normal saline until use. All specimens were decoronated under water cooling with a diamond bur to obtain a standardized root length of 16 mm. Apical patency was established using #10 K-file (Dentsply Maillefer, Switzerland) and working length was determined by subtracting 1 mm from the length at which the file tip became visible at the apical foramen. Coronal flaring was performed with Gates-Glidden drills sizes 2 and 3, followed by preparation with the ProTaper Gold rotary system to F3 (#30/0.09) at 300 rpm and 3 Ncm torque. During instrumentation, canals were irrigated with 5 mL of 5.25% sodium hypochlorite for 2 minutes at each file change using a 30-gauge side-vented needle.

Based on the final irrigating solution, specimens were randomly divided into two groups (n = 42):
[1] Group 1: 3 mL of 17% EDTA for 3 minutes
[2] Group 2 (Control): 3 mL of saline for 3 minutes

Each group was further subdivided according to the irrigation activation method (n = 14):
[1] A: Conventional needle irrigation
[2] B: Manual dynamic agitation (MDA)
[3] C: Passive ultrasonic irrigation (PUI)

Thus, the subgroups were 1A, 1B, 1C, 2A, 2B and 2C. According to the supplied protocol, two specimens from each subgroup were reserved for SEM without obturation, leaving 12 specimens per subgroup for CLSM; this allocation must be verified against the SEM score dataset because the reported SEM percentages and chi-square values imply a larger number of observations. The remaining specimens were dried with paper points and obturated using ProTaper Gold F3 gutta-percha cones and CeraSeal sealer labeled with 0.1% Rhodamine B. A standardized sealer volume of 0.05 mL was delivered using a #30 lentulo spiral at 300 rpm to 1 mm short of working length. Excess gutta-percha was removed with a heated instrument, access cavities were sealed with Cavit G, post-obturation radiographs were obtained and specimens were stored at 37 °C and 100% humidity for 48 hours to permit sealer setting. Two specimens from each subgroup were sectioned longitudinally by creating buccolingual grooves with a diamond disc and splitting the roots with an enamel chisel. After sputter coating, specimens were examined by scanning electron microscopy. Smear-layer removal was scored independently by two examiners with disagreements resolved by joint re-evaluation. Horizontal sections 1 mm thick were obtained 2 mm from the root apex. Sections were examined using confocal laser scanning microscopy. Maximum sealer penetration depth (µm) was measured along the buccal, lingual, mesial and distal canal walls using ImageJ software. Measurements were assessed independently by two examiners and discrepancies were resolved by re-evaluation.

Statistical analysis:

Data were analyzed using SPSS version 26.0. Penetration depth was summarized as mean ± standard deviation. Normality was assessed with the Shapiro-Wilk test. One-way ANOVA with Tukey post-hoc comparisons was used for within-irrigant comparisons and corresponding EDTA and saline subgroups were compared using independent-samples t-tests with Bonferroni adjustment for three comparisons (adjusted α = 0.0167). Smear-layer scores were intended to be compared using a chi-square or Fisher-Freeman-Halton exact test, depending on expected cell counts; however, this analysis must be verified after confirming the number of independent SEM observations. Two-sided $p < 0.05$ was considered statistically significant unless an adjusted threshold is specified.

Results:

Shapiro–Wilk analysis confirmed normal distribution in all groups (W = 0.886–0.968; $p > 0.05$), validating the use of parametric tests. The highest mean penetration was observed in G1C (EDTA + PUI; 1888.09 ± 67.72 µm), followed by G1B (EDTA + MDA; 1650.63 ± 40.55 µm) and G1A (EDTA + conventional needle irrigation; 1194.59 ± 30.35 µm). One-way ANOVA revealed a significant difference among the subgroups ($F = 742.61$; $p < 0.001$) and Tukey post hoc analysis showed significant differences for all pairwise comparisons (all $p < 0.001$). The EDTA subgroup findings are summarized in **Table 1**. Mean penetration increased from G2A (288.13 ± 25.24 µm) to G2B (313.89 ± 28.62 µm) and G2C (329.90 ± 47.03 µm); however, the overall difference was not statistically significant ($F = 3.12$; $p = 0.059$). Post-hoc comparisons were likewise nonsignificant (all $p > 0.05$). The saline subgroup findings are also presented in **Table 1**. EDTA produced significantly greater penetration than saline for each corresponding activation method. Mean between-irrigant differences were 906.46 µm for conventional needle irrigation, 1336.74 µm for MDA and 1558.19 µm for PUI (all $p < 0.001$). The corresponding EDTA-versus-saline comparisons are presented in **Table 2**. The supplied results indicate greater smear-layer removal with EDTA, particularly when combined with PUI, whereas saline groups showed heavier smear retention. However, the Methods section states that only two specimens per subgroup were reserved for SEM; this allocation cannot yield a subgroup percentage of 83.3% or the reported chi-square values. The SEM sample size, number of scored fields, score counts, percentages and inferential statistics must therefore be verified against the original dataset before submission.

Table 1: Dentinal tubule penetration across activation methods within each final irrigant group

Final irrigant	Activation method	Penetration depth (µm), mean ± SD	Overall F value	Overall p value
17% EDTA	Conventional needle irrigation	1194.59 ± 30.35	742.61	<0.001*
17% EDTA	Manual dynamic agitation	1650.63 ± 40.55	742.61	<0.001*
17% EDTA	Passive ultrasonic irrigation	1888.09 ± 67.72	742.61	<0.001*
Saline	Conventional needle irrigation	288.13 ± 25.24	3.12	0.059
Saline	Manual dynamic agitation	313.89 ± 28.62	3.12	0.059
Saline	Passive ultrasonic irrigation	329.90 ± 47.03	3.12	0.059

*Statistically significant at $p < 0.05$. ANOVA values represent the overall comparison among the three activation methods within each final irrigant group

Table 2: Comparison of CeraSeal dentinal tubule penetration between 17% EDTA and saline for corresponding activation methods

Activation method	17% EDTA (µm), mean ± SD	Saline (µm), mean ± SD	Mean difference (µm)	p value
Conventional needle irrigation	1194.59 ± 30.35	288.13 ± 25.24	906.46	<0.001*
Manual dynamic agitation	1650.63 ± 40.55	313.89 ± 28.62	1336.74	<0.001*
Passive ultrasonic irrigation	1888.09 ± 67.72	329.90 ± 47.03	1558.19	<0.001*

*Statistically significant after Bonferroni adjustment (adjusted α = 0.0167).

Discussion:

Successful endodontic treatment depends on reducing microorganisms, necrotic tissue and debris throughout the root canal system **[20]**. Because complex canal anatomy limits the reach of instruments, chemical irrigation and activation are necessary adjuncts to mechanical preparation **[21]**. Sealer penetration into dentinal tubules increases the sealer-dentin contact area and may improve micromechanical retention and

adaptation **[22]**. The presence of a smear layer can obstruct tubular penetration by root canal sealers **[23]**, supporting its removal before obturation **[24]**. Tubular penetration may also extend the antimicrobial action of sealers into infected dentin **[25]**, where microorganisms can persist beyond the main canal lumen **[26]**. Bacterial invasion of dentinal tubules can reach considerable depths, although penetration varies with organism, dentin region and sclerosis **[27]**. EDTA removes the inorganic component of the smear layer through calcium chelation and increases dentinal permeability **[28]**, while NaOCl dissolves organic tissue and contributes antimicrobial activity **[29]**. Contemporary irrigation protocols therefore combine irrigant chemistry with activation to improve fluid exchange in canal irregularities **[30]**. In the present study, penetration depth differed significantly among the EDTA subgroups: PUI produced the greatest penetration, followed by MDA and conventional needle irrigation. Ultrasonic activation enhances irrigant movement through acoustic streaming and improved exchange **[31]**, whereas hydrodynamic agitation can reduce stagnation and apical vapor lock **[32]**. MDA also improves irrigant replacement through repeated gutta-percha movement within the prepared canal **[33]**. Conventional needle irrigation produced the lowest penetration, plausibly because positive-pressure delivery alone creates less fluid movement in inaccessible regions. Bioceramic sealers are hydrophilic calcium silicate-based materials with bioactive and biocompatible properties **[34]**. In the saline groups, activation method did not significantly change penetration, indicating that mechanical agitation alone could not compensate for the absence of a chelating agent. This finding is consistent with evidence that PUI enhances the action of EDTA-containing final irrigation protocols **[35]** and underscores the interaction between irrigant chemistry and activation **[36]**. CeraSeal penetration is influenced by its physicochemical properties as well as canal anatomy and surface condition **[37]**. The present study assessed penetration at 2 mm from the root apex, an anatomically challenging level because apical dentin has fewer and narrower tubules and may show greater sclerosis **[38]**. CLSM permits quantitative evaluation of fluorescently labeled sealer without extensive specimen dehydration **[39]**, whereas SEM provides high-resolution surface visualization of smear-layer coverage and open tubules **[40]**. The reported SEM trend favored EDTA and PUI and was directionally consistent with the CLSM findings and previous smear-layer studies **[41]**. Nevertheless, the stated SEM allocation, percentage and chi-square values are internally inconsistent and must be verified before inferential interpretation. The penetration results support the combined importance of irrigant chemistry and activation method, but deeper sealer penetration is a laboratory surrogate and should not be equated directly with superior long-term clinical success.

Conclusion:

Seventeen percent EDTA significantly increased CeraSeal dentinal tubule penetration over saline. Passive ultrasonic irrigation achieved high penetration, especially with EDTA. EDTA–PUI is a promising final irrigation strategy, pending SEM verification and clinical validation.

Limitations:

This *in vitro* study using extracted human teeth may not fully reproduce clinical conditions, including tissue fluids, polymicrobial biofilms, host responses and anatomical variability. Only 17% EDTA and saline, three activation methods and one bioceramic sealer were evaluated. Technique-sensitive variables such as activation duration, device power and operator movement may affect performance and must be fully reported. Rhodamine B may alter sealer behavior and measurements were obtained at a single apical level. The study assessed immediate laboratory outcomes rather than long-term sealing or clinical healing. In addition, the SEM sample allocation and reported inferential statistics are internally inconsistent and require verification from the original dataset. Larger, well-reported studies should evaluate additional irrigants, activation systems, sealer formulations, root levels and clinically relevant outcomes.

Scope for future studies:

We show the importance of effective irrigation protocols in improving smear layer removal and dentinal tubule penetration. Future research should investigate newer irrigants, chelating agents and activation methods such as sonic, laser, multisonic and negative-pressure systems, particularly in complex root canal anatomies. Studies evaluating different irrigant concentrations, combinations, sequences and their interactions with various sealers are needed to optimize treatment outcomes. Furthermore, long-term *in vivo* and clinical studies are essential to validate these *in vitro* findings and assess their influence on periapical healing, reinfection prevention and overall endodontic success.

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