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ANTIOXIDANT FINAL IRRIGATION AND ULTRASONIC ACTIVATION MODIFY DENTIN AND ENHANCE BOND[1]

A IRRIGAÇÃO FINAL ANTIOXIDANTE E A ATIVAÇÃO ULTRASSÔNICA MODIFICAM A DENTINA E MELHORAM A ADESÃO

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ABSTRACT

Objective: This study evaluated the effect of antioxidant-based final irrigation protocols, with or without ultrasonic activation, on the bond strength of an epoxy resin–based sealer. **Methods**: Ninety human single-rooted premolars were prepared with sodium hypochlorite and EDTA and randomly assigned to 10 groups (n = 9) according to the final irrigant (EDTA, sodium ascorbate, apple cider vinegar, sodium thiosulfate, or sodium metabisulfite), with or without passive ultrasonic activation. Canals were obturated with gutta-percha and AH Plus. Push-out bond strength was measured in the cervical, middle, and apical thirds. Failure modes were classified, and 2 representative apical specimens per group were qualitatively examined by scanning electron microscopy (SEM). Data were analyzed using one-way and repeated-measures ANOVA ($\alpha = 0.05$). **Results**: Final irrigation protocols significantly influenced push-out bond strength ($P < .001$). Bond strength increased from the cervical to the apical third. Antioxidant regimens, particularly sodium ascorbate and sodium metabisulfite, produced higher bond strength values, whereas sodium thiosulfate and apple cider vinegar showed lower bond strength. Cohesive failures predominated. SEM revealed persistent smear layer remnants and debris in all groups. **Conclusions**: Antioxidant and chelating irrigants improved epoxy resin sealer adhesion, and ultrasonic activation promoted more homogeneous bonding. Sealer retention was more strongly associated with chemical dentin conditioning than with smear layer removal.

Keywords: Root canal irrigation. Push-out bond strength. Epoxy resin sealer.

RESUMO

Objetivo: Este estudo avaliou o efeito de protocolos de irrigação final à base de antioxidantes, com ou sem ativação ultrassônica, na resistência de união de um cimento obturador à base de resina epóxi. **Métodos:** Noventa pré-molares humanos unirradiculares foram preparados com hipoclorito de sódio e EDTA e distribuídos aleatoriamente em 10 grupos (n = 9) de acordo com o irrigante final (EDTA, ascorbato de sódio, vinagre de maçã, tiossulfato de sódio ou metabissulfito de sódio), com ou sem ativação ultrassônica passiva. Os canais foram obturados com guta-percha e AH Plus. A resistência de união por push-out foi mensurada nos terços cervical, médio e apical. Os modos de falha foram classificados e 2 espécimes apicais representativos por grupo foram examinados qualitativamente por microscopia eletrônica de varredura (MEV). Os dados foram analisados utilizando ANOVA de uma via e de medidas repetidas (α = 0,05). **Resultados:** Os protocolos de irrigação final influenciaram significativamente a resistência de união por push-out ($P < 0,001$). A resistência de união aumentou do terço cervical para o terço apical. Os regimes antioxidantes, particularmente o ascorbato de sódio e o metabissulfito de sódio, produziram valores de resistência de união mais elevados, enquanto o tiossulfato de sódio e o vinagre de maçã apresentaram menor resistência de união. Predominaram as falhas coesivas. A microscopia eletrônica de varredura (MEV) revelou remanescentes persistentes da camada de smear e detritos em todos os grupos. **Conclusões:** Os irrigantes antioxidantes e quelantes melhoraram a adesão do cimento epóxi, e a ativação ultrassônica promoveu uma adesão mais homogênea. A retenção do cimento esteve mais fortemente associada ao condicionamento químico da dentina do que à remoção da camada de smear.

Palavras-chave: Irrigação do canal radicular. Resistência de união por push-out. Cimento epóxi.

INTRODUCTION

Effective chemomechanical debridement is fundamental for predictable endodontic success. Although contemporary nickel–titanium instrumentation improves canal shaping, mechanical preparation alone cannot fully address the anatomical complexity of the root canal system. Isthmuses, fins, and recesses frequently remain untouched, allowing the persistence of biofilm, debris, and smear layer that compromise disinfection and sealing[1-3]. Therefore, chemical irrigation is essential to supplement instrumentation and improve canal cleanliness.

Sodium hypochlorite (NaOCl) remains the irrigant of choice because of its broad antimicrobial activity and ability to dissolve organic tissues[4]. However, it does not remove the inorganic component of the smear layer and induces oxidative alterations in dentin collagen. Residual oxygen and chloramine by-products interfere with resin polymerization and reduce the adhesion of epoxy resin–based sealers[5,6]. Ethylenediaminetetraacetic acid (EDTA) is routinely used to remove the smear layer; nevertheless, prolonged chelation may promote dentin erosion and mineral loss, resulting in irregular substrate conditions and inconsistent bonding performance[7-9].

Passive ultrasonic irrigation (PUI) enhances irrigant penetration, acoustic streaming, and debris removal, improving dentin surface cleanliness compared with syringe irrigation[10-12]. Despite these improvements, chemical alterations induced by NaOCl may still compromise dentin reactivity and sealer adhesion.

Antioxidant or neutralizing solutions have therefore been proposed to reverse NaOCl-induced oxidation and restore dentin reactivity. Sodium ascorbate has shown partial recovery of bonding; however, its effects remain inconsistent and protocol-dependent[13,14], highlighting the need for more effective and stable reducing agents. Sodium metabisulfite ($Na_2S_2O_5$) is a strong oxygen-scavenging and reducing agent that neutralizes residual oxidizing species and restores resin polymerization at the dentin interface. Its post-bleaching application has been shown to recover resin–dentin bond strength by eliminating residual oxygen and reversing adhesive inhibition[15].

Because dentin surface chemistry directly determines the sealer–dentin interface, push-out bond strength testing provides a quantitative measure of interfacial retention, whereas scanning electron microscopy (SEM) enables qualitative evaluation of smear layer removal and tubule exposure[16-17]. Whether antioxidant-based final irrigation protocols enhance sealer bonding under clinically relevant conditions remains unclear.

Accordingly, this study comparatively investigated multiple reducing agents, including sodium metabisulfite, under standardized endodontic irrigation conditions with or without passive ultrasonic activation. Bond strength and dentin surface morphology were evaluated using push-out testing and SEM. The null hypothesis was that neither the final irrigant nor ultrasonic activation would influence bond strength or dentin surface characteristics.

MATERIALS AND METHODS

Study Design and Specimen Preparation

This in vitro experimental study included 90 extracted human single-rooted mandibular premolars stored in 0.1% thymol solution at room temperature until use. Teeth exhibiting a single straight canal, similar root lengths, and no cracks, resorptions, calcifications, or previous endodontic treatment were selected according to established criteria for push-out bond strength testing[16,18]. Crowns were removed under water cooling using a diamond disc, and root length was standardized to 17 mm.

Working length was established with a size 10 K-file positioned 1 mm short of the apical foramen. Root canals were prepared using the ProTaper Gold system

(Dentsply Sirona, Ballaigues, Switzerland) following the manufacturer's instructions. During instrumentation, canals were irrigated with freshly prepared 2.5% sodium hypochlorite (NaOCl) (total volume: 5 mL) delivered 2 mm short of the working length using a side-vented needle. The smear layer was subsequently removed with 17% EDTA for 1 minute, followed by saline irrigation[4,7].

Final Irrigation Protocols and Obturation

Specimens were randomly allocated to 10 experimental groups (n = 9 teeth per group) using a computer-generated randomization sequence according to the final irrigant and the use of PUI. Final irrigation volume and time were standardized to 5 mL for 1 minute for all groups.

The groups were defined as follows: G1, 17% EDTA; G2, EDTA + PUI; G3, 10% sodium ascorbate; G4, sodium ascorbate + PUI; G5, apple cider vinegar; G6, apple cider vinegar + PUI; G7, 10% sodium thiosulfate; G8, sodium thiosulfate + PUI; G9, 6% sodium metabisulfite; G10, sodium metabisulfite + PUI.

PUI was performed using an ultrasonic endodontic tip positioned 2 mm short of the working length without contacting the canal walls, as previously described[10,11].

After irrigation, canals were flushed with saline, dried with sterile paper points, and obturated using a single-cone technique with gutta-percha and an epoxy resin–based sealer (AH Plus Jet; Dentsply Sirona). Specimens were stored at 37 °C and 100% relative humidity for 7 days to ensure complete sealer setting.

Push-out Bond Strength Testing

Roots were sectioned perpendicular to their long axis using a low-speed saw under water cooling to obtain 1.0-mm-thick slices from the cervical, middle, and apical thirds. The most coronal and apical slices were discarded. Slice thickness was verified with a digital caliper (±0.01 mm accuracy). Two slices per third were tested.

To prevent pseudo-replication, the tooth was defined as the experimental and statistical unit. Bond strength values from slices of the same tooth were averaged, and a single mean value per tooth was used for analysis.

Push-out testing was performed using a universal testing machine (Instron, Norwood, MA, USA) at a crosshead speed of 1 mm/min. Bond strength (MPa) was calculated by dividing the maximum load at failure (N) by the bonded interface area. The bonded area was determined using the formula for the lateral surface of a truncated cone.

Failure modes were classified under a stereomicroscope at 25× magnification by an examiner blinded to group allocation.

SEM

Two apical specimens per group were randomly selected, longitudinally sectioned, dehydrated, sputter-coated with gold, and examined by scanning electron microscopy. SEM analysis was qualitative and descriptive and was not intended for inferential comparisons.

Statistical Analysis and Sample Size Justification

Bond strength data were analyzed using the tooth as the statistical unit. Normality and homogeneity were assessed using Shapiro–Wilk and Levene tests. Intergroup comparisons were performed using one-way ANOVA, and intragroup comparisons among root thirds were conducted using repeated-measures ANOVA followed by Tukey post hoc tests ($\alpha = 0.05$). Statistical analyses were performed using SPSS version 26.0 (IBM Corp., Armonk, NY, USA).

Sample size was based on variability reported in previous studies. Post-hoc power analysis confirmed adequate statistical power (>80%) to detect medium effect sizes (Cohen's $f \geq 0.40$).

RESULTS

Table 1 and Figure 1 present the mean ± SD bond strength values. Significant differences were detected among groups in all root thirds ($P < .001$).

In the cervical third, values ranged from 0.8 ± 0.3 MPa (G8) to 6.5 ± 1.3 MPa (G10). In the middle third, values ranged from 0.9 ± 0.3 MPa (G7) to 14.5 ± 1.2 MPa (G9). In the apical third, values ranged from 1.6 ± 0.5 MPa (G7) to 18.0 ± 1.2 MPa (G3). Additional values were 17.0 ± 1.2 MPa (G1) and 16.0 ± 1.3 MPa (G10).

Repeated-measures ANOVA showed differences among thirds within all groups ($P < .001$). Most groups showed higher bond strength in the apical third. Higher overall mean values were recorded for G1, G2, G3, G9, and G10. The lowest overall mean was observed in G7.

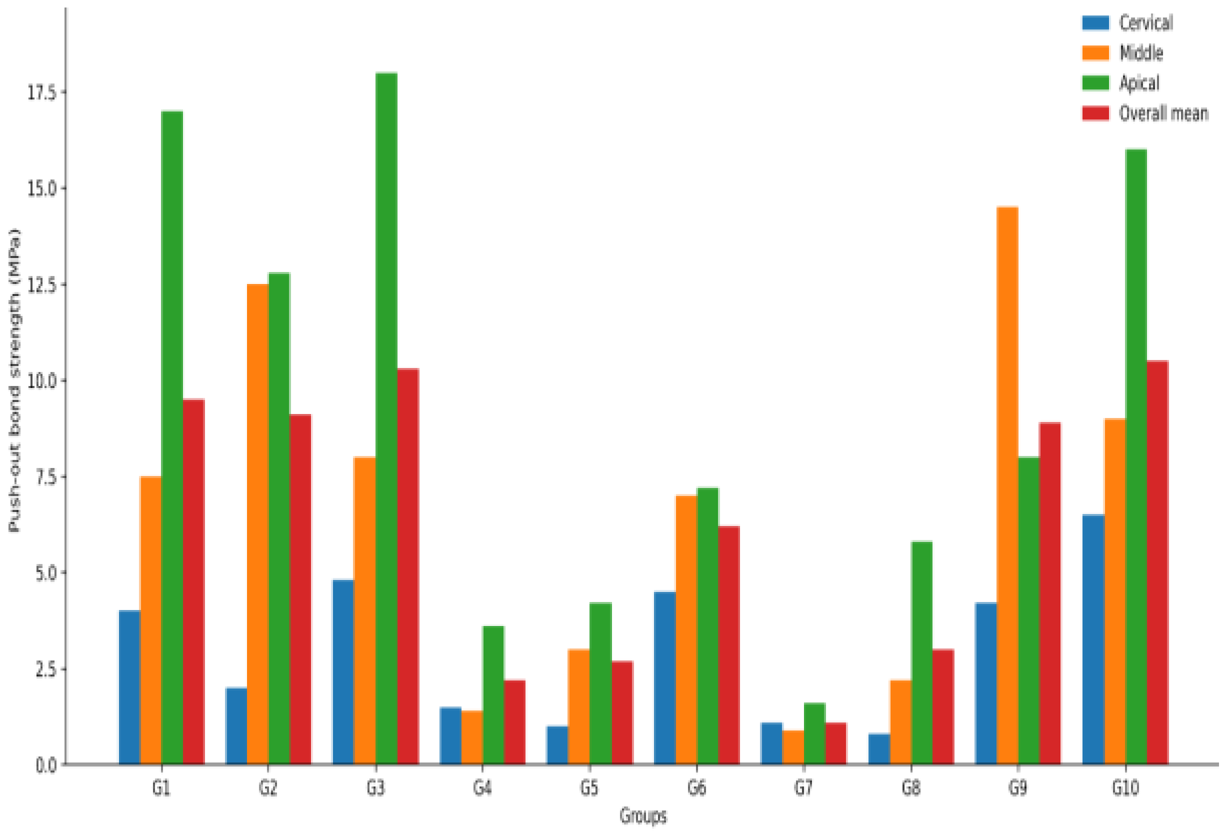
Cohesive failures predominated. Mixed failures were observed. No adhesive failures were detected. SEM of the apical third showed smear layer remnants, partial dentinal tubule occlusion, exposed tubules, and heterogeneous surface morphology.

Table 1: Push-out bond strength (MPa; mean ± SD) and failure mode distribution according to experimental group and root third.

Group	Cervical	Middle	Apical	Overall Mean	Adhesive	Cohesive	Mixed
G1	4.0 ± 1.2 aA	7.5 ± 1.2 bB	17.0 ± 1.2 cA	9.5 ± 1.3 A	0	38	16
G2	2.0 ± 0.6 aB	12.5 ± 2.5 bA	12.8 ± 2.5 bB	9.1 ± 1.5 A	0	40	14
G3	4.8 ± 1.2 aA	8.0 ± 1.2 bB	18.0 ± 1.2 cA	10.3 ± 1.4 A	0	42	12
G4	1.5 ± 0.6 aC	1.4 ± 0.6 aD	3.6 ± 0.6 bD	2.2 ± 0.6 C	0	35	19
G5	1.0 ± 0.4 aC	3.0 ± 0.8 bC	4.2 ± 1.3 cD	2.7 ± 0.7 C	0	33	21
G6	4.5 ± 1.2 aA	7.0 ± 1.2 bB	7.2 ± 1.2 bC	6.2 ± 1.1 B	0	37	17
G7	1.1 ± 0.5 aC	0.9 ± 0.3 aD	1.6 ± 0.5 bD	1.1 ± 0.4 D	0	30	24
G8	0.8 ± 0.3 aC	2.2 ± 0.7 bC	5.8 ± 0.7 cC	3.0 ± 0.6 C	0	32	22
G9	4.2 ± 1.2 aA	14.5 ± 1.2 cA	8.0 ± 1.2 bC	8.9 ± 1.3 A	0	41	13
G10	6.5 ± 1.3 aA	9.0 ± 1.3 bB	16.0 ± 1.3 cA	10.5 ± 1.3 A	0	39	15

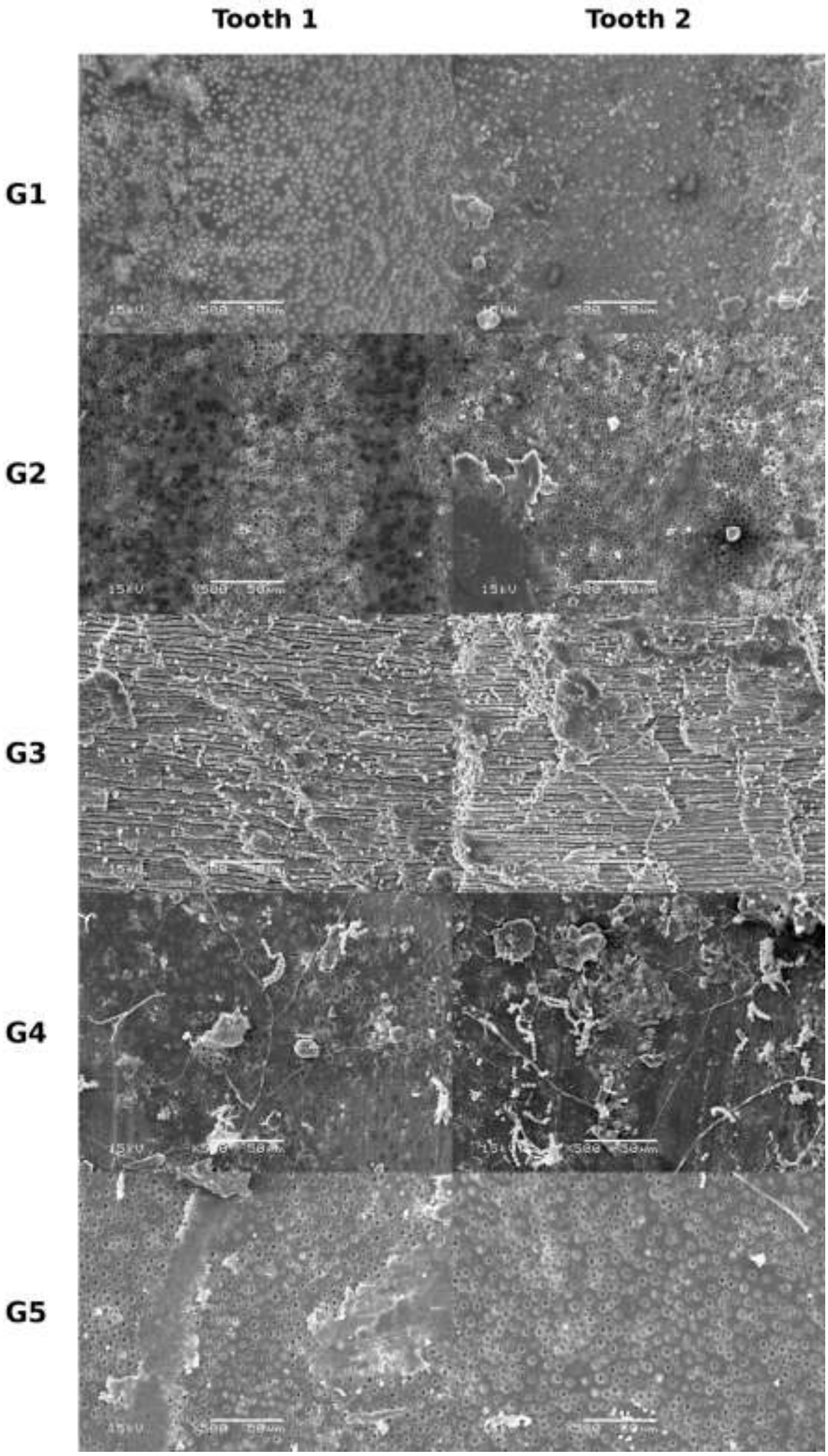
*Lowercase letters indicate intragroup differences among thirds; uppercase letters indicate intergroup differences within each third (two-way ANOVA/Tukey, α = 0.05).
Source: Prepared by the author (2026)

Figure 1: Push-out bond strength (MPa) by root third and overall mean for each experimental group (G1–G10).



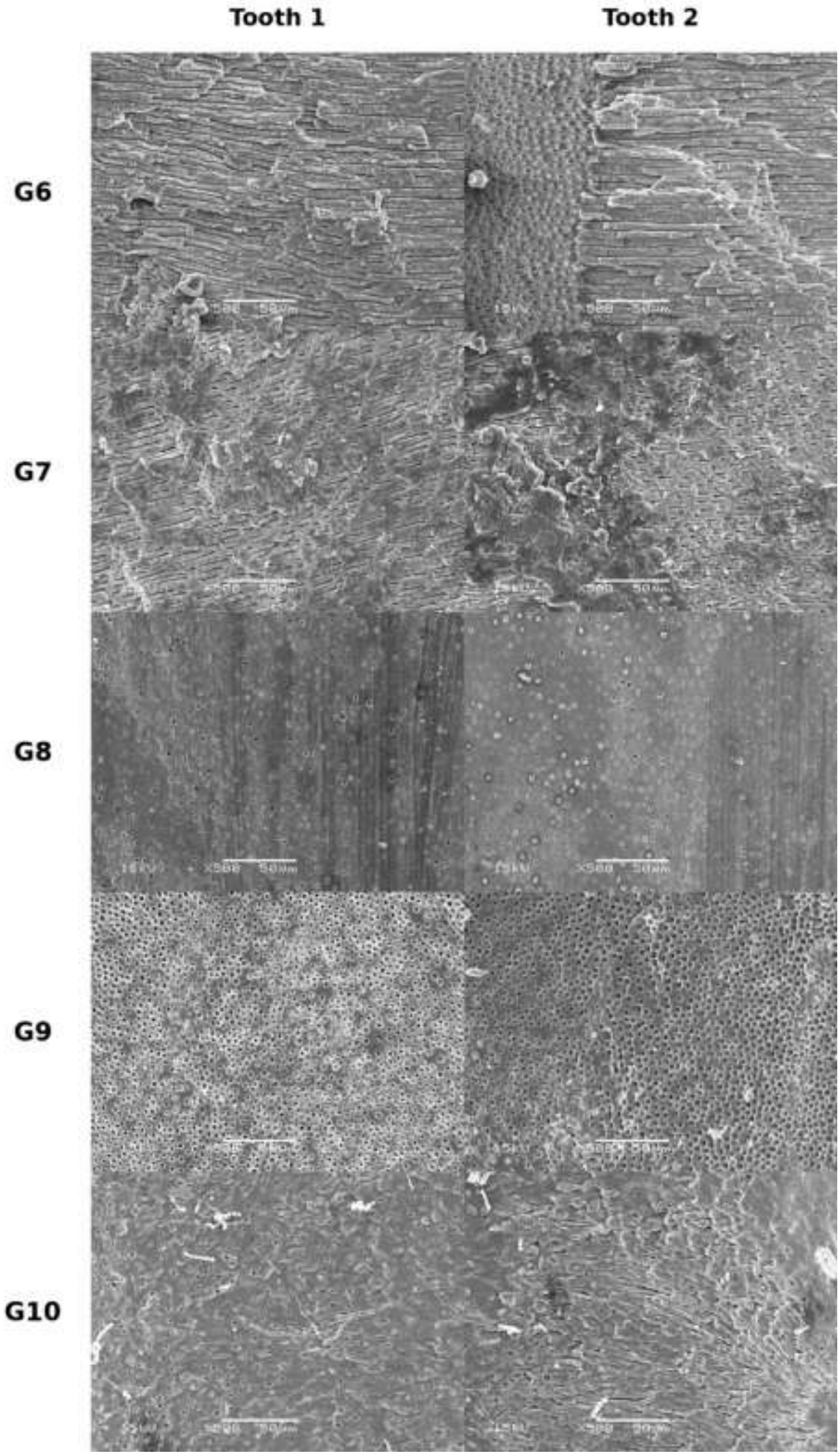
Source: Prepared by the author (2026)

Figure 2: SEM micrographs of dentin surfaces from groups G1–G5 showing two representative specimens per group (Tooth 1 and Tooth 2) (15 kV; ×500; 50 μm), allowing qualitative comparison of surface morphology and smear layer distribution.



Source: Prepared by the author (2026)

Figure 3: SEM micrographs of dentin surfaces from groups G6–G10 with two representative specimens per group obtained under identical imaging conditions for comparative evaluation of tubule exposure and surface morphology.



Source: Prepared by the author (2026)

DISCUSSION

Final irrigation protocols significantly influenced the push-out bond strength of the epoxy resin–based sealer, indicating that dentin conditioning before obturation affects interfacial retention. Differences among groups and root thirds suggest that the chemical environment created during the final rinse influenced adhesion more than smear layer removal alone[19,20].

Bond strength increased toward the apical third, consistent with previous push-out studies[16,17,21]. Higher values in this region may be related to anatomical confinement and prolonged irrigant contact[2].

EDTA and sodium ascorbate yielded the highest bond strength, particularly apically. SEM showed incomplete and heterogeneous smear removal, indicating that improved bonding was not solely associated with surface cleanliness. These findings support the relevance of chemical substrate modification. In contrast, protocols primarily based on sodium hypochlorite showed lower performance, possibly due to interference with resin polymerization[24,25].

The performance of sodium ascorbate may be related to its antioxidant action, which has been associated with improved bonding after hypochlorite exposure[16,26]. Notably, high bond strength values were observed despite persistent smear remnants, reinforcing that chemical conditioning may outweigh purely morphological factors.

Sodium metabisulfite demonstrated intermediate to high bond strength, especially in the middle third. Its reducing effect may minimize oxidant-related interference with resin curing[5,26,27]. Lower apical values without activation may reflect limited irrigant distribution.

Conversely, sodium thiosulfate and apple cider vinegar resulted in lower bond strength across all thirds, indicating limited dentin conditioning. Although apple cider vinegar reduced the smear layer, this did not correspond to higher bonding. These findings indicate that smear removal alone is insufficient and that chemical conditioning governs adhesion[28].

Passive ultrasonic activation did not consistently increase peak bond strength. Instead, activated groups showed reduced variability among thirds, indicating more uniform bonding. Improved irrigant distribution did not translate into stronger adhesion[10-12,29].

Cohesive failures predominated, suggesting that interfacial strength approached or exceeded the intrinsic strength of the sealer. Similar patterns have been reported for epoxy resin–based materials such as AH Plus[16,17,29].

SEM revealed persistent smear remnants and partial tubule occlusion in all groups, confirming the difficulty of achieving complete canal cleanliness. Because SEM analysis was qualitative and limited to representative specimens, these observations should be interpreted cautiously[30-32].

Overall, these findings indicate that modulation of the dentin chemical microenvironment was associated with differences in sealer retention. Chelating and antioxidant protocols showed more consistent bonding than smear removal alone.

CONCLUSIONS

Antioxidant and chelating irrigants improved epoxy resin sealer adhesion, whereas acidic solutions showed lower bond strength. Chemical conditioning of dentin governed sealer retention more than smear layer removal.

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