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The impact of root dentine conditioning on sealing ability and push-out bond strength of an epoxy resin root canal sealer

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Abstract

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Aim To investigate the impact of dentine conditioning on sealing ability and dentine bond strength of an epoxy resin sealer.

Methodology Root canals in 90 single-rooted teeth were instrumented using a rotary Ni-Ti system. Fifty canals were irrigated with water during instrumentation, 40 with 3% NaOCl. A final flush was performed in the water-irrigated specimens with water (negative control), 3% NaOCl, 17% EDTA, 7% maleic acid (MA) or 2% chlorhexidine. The hypochlorite irrigated specimens received a final flush with a decalcifying agent (EDTA or MA) and then 3% NaOCl or 3% NaOCl and then the decalcifying agent ($n = 10$, each). Canals were all filled with AH Plus. Fluid transport was measured on day 3 and 30. Roots were then sectioned, and push-out tests were performed in coronal, middle and apical root thirds. Results were analysed using analysis of variance (ANOVA) with Bonferroni's adjustment. Spearman's rank correlation was computed between fluid transport and push-out bond strength.

Results Leakage decreased over time ($P < 0.05$). Push-out bond strength was highest in coronal and lowest in apical root thirds ($P < 0.05$). Irrigating protocols with final application of a decalcifying agent greatly decreased the leakage and increased push-out bond strength values, in contrast to groups where NaOCl was applied last ($P < 0.05$), wherein the effect of the decalcifying agent was abolished. Chlorhexidine had no impact on the outcomes. Fluid transport and push-out bond strength correlated strongly ($\rho = -0.83$).

Conclusions AH Plus appears to bond to the organic phase of dentine. This bond influences its sealing ability.

Keywords: AH Plus, bond strength, fluid transport, leakage.

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Introduction

Adhesion is a clinically desirable property of root canal sealers. Ideally, these materials should not shrink and bond effectively to both the surrounding substrates: the root canal walls and the core root filling material (Grossman 1976). Upon introduction of adhesive root filling materials, it was claimed that methacrylate-based sealers, which are based on adhesive technology developed for restorative dentistry and thus designed to adhere to coronal dentine, could minimize leakage by increasing the seal between the core root filling material and the root canal walls (Schwartz 2006). However, it has been shown that epoxy resin cements such as AH Plus (Dentsply DeTrey, Konstanz,

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Germany) have higher bond strength to root dentine than methacrylate sealers (Gesi *et al.* 2005, De-Deus *et al.* 2009). Furthermore, epoxy resin sealers have higher bond strength to the core filling material than other types of sealers (Lee *et al.* 2002). Methacrylate-based root canal sealers undergo significant volumetric shrinkage during the polymerization process (Souza *et al.* 2009). Epoxy resin sealers, in contrast, do not shrink when they cure (Ørstavik *et al.* 2001, Souza *et al.* 2009).

Interestingly, there appears to be an impact of irrigating protocols on the adhesion of sealers to root dentine (De-Deus *et al.* 2008, Nunes *et al.* 2008, Pinna *et al.* 2009). It has been theorized that the adhesiveness of AH Plus to root dentine is related to covalent bonds between epoxide rings and the exposed amino groups in the collagen network (Fisher *et al.* 2007). Consequently, it could be so that the collagen network needs to be exposed and minimally preserved to improve bond strength (Nunes *et al.* 2008). Root dentine is differentially affected by calcium chelating agents and the proteolytic sodium hypochlorite (Mai *et al.* 2010, Zhang *et al.* 2010). The type of decalcifying agent has a significant impact on the root dentine wall: EDTA will cause a complete demineralization of the exposed wall, whilst organic acids cause a mineral gradient (Lottanti *et al.* 2009). The latter dentine condition could yield itself better to resin infiltration (Prati *et al.* 1990). Furthermore, it has been speculated that chlorhexidine could have a positive effect on dentine bonding because of its inhibitory effect on matrix metalloproteinases (Hebling *et al.* 2005). However, hitherto, little information is available regarding the effect of chemical root dentine conditioning on sealer adhesion. In addition, it remains unknown whether the adhesion of root canal sealers to dentine or to the core root filling material is related to any other outcome variable, such as sealing ability of a given root filling system.

This study was designed to examine the effect of dentine chemical pre-treatment using 3% NaOCl, 17% EDTA, 7% maleic acid and 2% chlorhexidene on the sealing ability and push-out bond strength of an epoxy resin root canal sealer to root dentine. Moreover, the effect of the sequential usage of NaOCl and decalcifying agents was studied. The null hypotheses tested were (i) the irrigation protocols had no influence on the sealing ability measured by fluid transport, (ii) these protocols had no influence on the sealer–dentine bond strength and, (iii) there was no significant correlation between sealer–dentine push-out bond strength and sealing ability of the root canal sealer.

Materials and methods

Human single-rooted maxillary canines ($N = 90$) were collected and thoroughly cleaned by removing the hard deposits using curettes and the soft deposits by soaking in 5.25% NaOCl for 10 min. The teeth were decoronated at the cemento-enamel junction using a diamond disc, under water-cooling. The root lengths were standardized to 15 mm. The teeth were radiographed (DSX 730; Owandy Dental Imaging, Champs sur Marne, France; Kodak 2100 X ray unit; Kodak Dental Systems, Atlanta, GA, USA) at different angulations to confirm the presence of a single canal. Working length was established using a size 10 K-file (Mani Inc, Tochigi, Japan) to the root canal terminus and subtracting 0.5 mm from this measurement. The roots were randomly divided into nine groups ($n = 10$) with the aid of a computer algorithm (http://www.random.org).

The root canals were instrumented using Mtwo nickel titanium rotary instruments (VDW GmbH, Munich, Germany) up to size 35, 0.06 taper. Irrigation was performed using a 5-mL disposable plastic syringe (Ultradent Products Inc., South Jordan, UT, USA) with a polypropylene capillary tip (Ultradent) placed passively into the canal, up to 2 mm from the apical foramen without binding. During instrumentation, irrigation was performed with distilled water in fifty roots. These samples were divided randomly into five groups ($n = 10$) based on the final irrigant (5 mL) used: Group 1, distilled water; group 2, 3% NaOCl (Prime Dental Products, Mumbai, India); group 3, 17% EDTA (Pulpdent, Watertown, MA, USA); group 4, 7% maleic acid (Sigma Aldrich, St.Louis, MO, USA); group 5, 2% chlorhexidine (Asep RC; Stedman Pharmaceuticals, Chennai, India). All final irrigants were allowed to remain in the canal for 2 min.

In the remaining forty roots, irrigation was performed applying 5 mL of 3% sodium hypochlorite during instrumentation. The samples were then divided into four groups ($n = 10$) according to the final irrigation regimen, during which 5 mL of the following irrigants, each, were used: group 6, 17% EDTA followed by 3% NaOCl; group 7, 3% NaOCl followed by 17% EDTA; group 8, 7% maleic acid followed by 3% NaOCl and group 9, 3% NaOCl followed by 7% maleic acid. Each irrigant was allowed to remain in the canal for 2 min.

After test and control irrigating procedures, canals were rinsed with 5 mL of distilled water, dried using paper points (Dentsply Maillefer, Ballaigues, Switzerland) and filled with an epoxy resin root canal sealer

(AH Plus, Dentsply DeTrey, Konstaz, Germany) using a lentulo spiral (Dentsply Maillefer). The teeth were radiographed (DSX 730; Owandy Dental Imaging) at different angulations to verify the quality of filling procedure. The specimens were coated with nail varnish except for the root canal orifices and apical foramina. The roots were coded and placed in 100% humidity for 48 h to ensure complete setting of the sealer.

Fluid transport model

The sealing qualities of the test materials were recorded following the progress of a tiny air bubble travelling within a 25-μL glass capillary tube (Microcaps; Fisher Scientific, Philadelphia, PA, USA). The methodology and set-up for the fluid transport model have been described elsewhere (Kececi *et al.* 2010). To estimate the fluid movement through the root fillings, 0.2 bar water pressure was applied to the roots for 24 h during the third day after the root filling procedure, and fluid flow was measured continuously. At the end of the 24 h, specimens were stored in a humid atmosphere for 27 days at 37 °C. During storage, the specimens were stored in hermetically sealed jars containing 0.2% sodium azide, to prevent any dehydration and contamination. Thirty days after root filling, the specimens were immersed in distilled water for 6 h before recording fluid flow rates for 2 h. Fluid movement during the fluid penetration test was observed under loop magnification to differentiate the 0.5-mm intervals on the scale, in μL h^{-1}. The fluid movement was recorded as 0 in the samples where no air bubble movement was observed.

Push-out bond strength

Each root was embedded in epoxy resin in a custom-made split-ring copper mould. After setting of the epoxy resin, twelve slices (1 mm thick) were obtained from each root (four per root third) using a water-cooled precision saw (Ernst-Leitz, Wetzlar, Germany). The first slice of each third was selected for the push-out test. Each specimen was marked on its coronal surface with an indelible marker, and the exact thickness of each slice was measured using a digital calliper to 0.04 mm accuracy (Mitutoyo, Tokyo, Japan).

Each section was coded and measured for the apical and coronal diameters of the obturated area using an Olympus Camedia C-5060 digital camera (Tokyo, Japan) attached to a stereomicroscope (Global G6, St Louis, MO, USA). Each root section was then subjected to a compressive load via a universal testing machine (LIoyd LRX-plus; LIoyd Instruments Ltd, Fareham, UK) at a crosshead speed of 1 mm min^{-1} using a 0.8-mm diameter stainless steel cylindrical plunger. The plunger tip was positioned, so that it only contacted the filling material. The push-out force was applied in an apico-coronal direction until bond failure occurred, which was manifested by extrusion of the epoxy resin obturation material and a sudden drop along the load deflection. The force was recorded using Nexygen data analysis software (Lloyd Instruments Ltd). The maximum failure load was recorded in Newtons and was used to calculate the push-out bond strength in megapascals (MPa) according to the following formula (Nagas *et al.* 2007):

Push-out bond strength (MPa) = N/A; where, N = Maximum load (N), A = Adhesion area of root canal filling (mm^2).

The adhesion (bonding) surface area of each section was calculated as: $(\pi r_1 + \pi r_2) \times L$, where $L = \sqrt{(r_1 - r_2)^2 + h^2}$; where π is the constant 3.14, r_1 and r_2 are the smaller and larger radii, respectively, and h is the thickness of the section in mm.

Data presentation and analysis

The two main outcome variables in this study were fluid movement (in μL h^{-1}) and push-out bond strength (in MPa). Data analysis by D'Agostino & Pearson omnibus normality test showed normal distribution except when the grouping variable was disregarded. Consequently, parametric statistical tests were applied.

To assess the impact of time after root filling (3 vs. 30 days) and group (i.e. treatment protocols) on fluid movement through filled root canals, repeated measures analysis of variance (ANOVA) was performed. Subsequently, one-way ANOVA was applied to compare values between groups at each time-point. Regarding push-out bond strength, two-way ANOVA was performed to weigh the impact of root third and group, i.e. irrigating protocol, and root thirds as the two independent variables on the outcome (dependent variable). To compare the impact of the different irrigating protocols on push-out bond strength within each root third, one-way ANOVA was applied. Bonferroni's correction for multiple testing was used in one-way analysis of variance.

The correlation between the average push-out bond strength values per root and average leakage (averaged between 3 and 30 days for each root) was viewed on a bivariate scatter plot (Fig. 1). When the grouping variable (irrigation protocol) was disregarded, the two data sets were skewed. Consequently, nonparametric statistics were applied to test for correlation (Spearman's rank correlation).

The alpha-type error was set at 0.05 for all statistical analyses.

Results

Fluid transport

All groups showed some degree of leakage in the fluid transport assay. Time had a significant impact ($P < 0.05$) on fluid flow rate. Leakage was higher on day 3 than on day 30 in all treatment groups (Table 1). This difference was more pronounced if one of the two decalcifying agents (EDTA or maleic acid) were applied last. At both time-points, it was apparent that groups treated with a decalcifying agent last performed significantly ($P < 0.05$) better than the other groups. Furthermore, irrigation with sodium hypochlorite during and after root canal instrumentation reduced fluid transport values in groups with a final application of a decalcifying agent. However, when a sodium hypochlorite flush was applied after the decalcifying agent, this effect was abolished, and leakage values were statistically similar ($P > 0.05$) to the water control. Chlorhexidine had no impact on fluid transport; values were essentially identical to those obtained with mere water application. After 30 days, the effects observed immediately after setting of the sealer became more pronounced (Table 1). The protocols involving NaOCl during and after instrumentation followed by a final application of a decalcifying agent (groups 7 & 9) now allowed less than one-third of the fluid transport observed with the water control group. Chlorhexidine still had no effect.

Table 1 Fluid filtration ($\mu L\ h^{-1}$, means ± standard deviations) through filled roots after the epoxy resin sealer (AH Plus) had set and one month subsequently according to the type of dentine conditioning prior to sealer application ($n = 10$)

Group	Leakage day 3	Leakage day 30
1: Water – water	13.2 ± 0.6^{A}	8.3 ± 0.6^{a}
2: Water – 3% NaOCl	12.9 ± 0.9^{A}	8.6 ± 1.3^{a}
3: Water – 17% EDTA	11.4 ± 0.7^{B}	4.4 ± 1.0^{b}
4: Water – 7% maleic acid (MA)	11.3 ± 0.7^{B}	3.6 ± 0.6^{c}
5: Water – 2% CHX	13.2 ± 0.9^{A}	8.3 ± 1.0^{a}
6: 3% NaOCl – 17% EDTA – 3% NaOCl	12.7 ± 0.5^{A}	8.2 ± 0.8^{a}
7: 3% NaOCl – 3% NaOCl – 17% EDTA	10.8 ± 0.8^{BC}	2.6 ± 0.6^{d}
8: 3% NaOCl – 7% MA – 3% NaOCl	12.1 ± 0.6^{AB}	8.2 ± 0.7^{a}
9: 3% NaOCl – 3% NaOCl – 7% MA	10.2 ± 0.8^{C}	2.2 ± 0.4^{d}

Mean values that share a superscript letter were not significantly different at the 5% level within the same time-point (one-way ANOVA, Bonferroni).

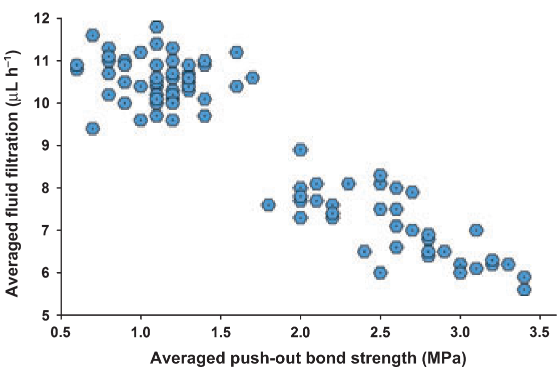

Figure 1 Bivariate scatter plot depicting the correlation between fluid transport (averaged between day 3 and 30) and push-out bond strength (averaged per tooth). There was a high negative rank correlation between the two measurements (Spearman's $\rho = -0.83$).

Push-out bond strength

Two-way ANOVA revealed that both the type of treatment protocol and the root third had a significant ($P < 0.05$) impact on push-out bond strength values. Push-out bond strength was highest in the coronal and lowest in the apical third (Table 2). Irrigation with sodium hypochlorite during instrumentation and a final rinse with maleic acid resulted in the highest bond strengths in all root thirds, significantly ($P < 0.05$) different from all other groups. A similar treatment using EDTA as a final irrigant resulted in the second highest values (Table 2). Following the same pattern of the leakage assessment, a final rinse with 3% NaOCl abolished the desired effects of the decalcifying agents, and push-out bond strength became similar to those observed with the water control. Sodium hypochlorite *per se* lowered the push-out bond strength values compared to those of water significantly ($P < 0.05$) in coronal and middle root thirds. Chlorhexidine had no effect on push-out bond strength.

Table 2 Push-out bond strength (MPa, means ± standard deviations) of epoxy resin sealer (AH Plus) one month after application according to root third and group (n = 10)

Group	Coronal third	Middle third	Apical third
1: Water – water	1.9 ± 0.5^{A}	1.2 ± 0.3^{a}	$0.6 \pm 0.2^{\alpha}$
2: Water – 3% NaOCl	1.1 ± 0.2^{B}	0.8 ± 0.2^{b}	$0.5 \pm 0.2^{\alpha}$
3: Water – 17% EDTA	3.1 ± 0.4^{C}	2.0 ± 0.2^{c}	$1.1 \pm 0.3^{\beta}$
4: Water – 7% maleic acid (MA)	3.3 ± 0.3^{C}	2.6 ± 0.3^{d}	$1.6 \pm 0.3^{\chi}$
5: Water – 2% CHX	1.9 ± 0.5^{A}	1.3 ± 0.3^{a}	$0.6 \pm 0.2^{\alpha}$
6: 3% NaOCl – 17% EDTA – 3% NaOCl	1.5 ± 0.3^{A}	1.1 ± 0.2^{a}	$0.8 \pm 0.1^{\delta}$
7: 3% NaOCl – 3% NaOCl – 17% EDTA	3.5 ± 0.2^{C}	2.7 ± 0.2^{d}	$2.0 \pm 0.2^{\varepsilon}$
8: 3% NaOCl – 7% MA – 3% NaOCl	1.5 ± 0.2^{A}	1.2 ± 0.2^{a}	$0.7 \pm 0.2^{\alpha,\delta}$
9: 3% NaOCl – 3% NaOCl – 7% MA	4.1 ± 0.2^{D}	3.0 ± 0.2^{e}	$2.6 \pm 0.2^{\Phi}$

Mean values that share a superscript letter were not significantly different at the 5% level within the same root third (one-way ANOVA, Bonferroni).

Correlation between outcomes

Analysis of correlation between the averaged leakage values between the two observation times and averaged push-out bond strength values per root showed very high negative correlation (Spearman's rank correlation coefficient $\rho = -0.83$, $P < 0.001$, Fig. 1). This means that treatments that resulted in low leakage (Table 1) also caused the highest bond strength to dentine (Table 2).

Discussion

The current study revealed a significant impact by the chemicals contained in irrigating solutions on both, sealing ability assessed by the fluid transport method and bond strength to dentine in a push-out test of an epoxy resin sealer. Furthermore, sealing ability and bond strength strongly correlated with each other. Consequently, the three null hypotheses tested were rejected.

This study would appear to be the first to correlate sealing ability with bonding to dentine of a root canal sealer. Hitherto, this correlation has been established for adhesive cements used for fibre-post bonding (Zicari *et al.* 2008). As indicated earlier, it still remains questionable whether either of these two outcome variables, whilst being connected to each other for the epoxy resin material under investigation, is related to clinical outcomes. The treatment goal remains to prevent oral pathogens from colonising and re-infecting the root and periapical tissues and to thereby maintain long-term periapical health. To this end, it is questionable whether bonding to dentine is truly necessary for a root filling material. Silicone-based materials, for instance, have good sealing ability (De-Deus *et al.* 2007), but no bonding capacity. However, at least from a clinical standpoint, some adhesion of the root filling is desirable. It is a commonly observed problem with silicone-based sealers that the whole root filling is removed during the attempt to drill a post-space, for instance.

The current study produced results that merit discussion. First and foremost, application of a decalcifying agent improved bond strength and sealing ability of the epoxy resin root canal sealer under investigation – AH Plus. There is but one logical explanation for this result: AH Plus is able to bond to the organic phase of the root dentine, most likely in the collagen network. However, this effect was abolished when sodium hypochlorite was applied subsequently as the final irrigating solution. The point is that a final NaOCl flush after EDTA has been recommended based on the appearance of root canal walls after cleaning and shaping, and final irrigation (Yamada *et al.* 1983, Baumgartner & Mader 1987). However, according to the present observations, this irrigation regimen should be reconsidered, at least if an epoxy resin–based sealer is used. A final sodium hypochlorite flush has two effects, which may be responsible for the decrease in epoxy resin bond strength to dentine and the resulting increase in leakage. First, sodium hypochlorite will remove organic material from the exposed dentine surface (Marending *et al.* 2007). Second, sodium hypochlorite breaks down to sodium chloride and oxygen. Oxygen causes strong inhibition of the interfacial polymerization of methacrylate resins (Munksgaard *et al.* 1985). The polyaddition reaction during the curing of epoxy resins, however, is not affected by oxygen. On the other hand, the generation of oxygen bubbles at the resin–dentine interface may directly interfere with resin infiltration into the tubules and inter-tubular dentine (Rueggeberg & Margeson 1990). Nonetheless, it should be mentioned that using NaOCl during instrumentation and as a flush before application of a decalcifying agent actually had a desired effect on sealing ability (Table 1) and push-out bond strength (Table 2) of AH Plus. The reason for this positive effect may be that NaOCl can remove loose organic remnants from the canal system, which may interfere with sealer bonding to the canal wall.

Maleic acid, an organic acid contained in dentine bonding agents such as Syntac Classic (Vivadent, Schaan, Liechtenstein), appears to provide significantly higher bond strength than EDTA. Based on observations of trans-sectioned roots after different irrigation protocols, it would appear that the complete demineralization of the exposed dentine wall caused by EDTA is less ideal for bonding than the structure created by a final application of maleic acid. Maleic acid is a weak acid, and it is likely that it will affect the dentine similar to other organic acids such as acetic or lactic acid: it may cause a mineral gradient in the exposed dentine rather than the complete surface demineralization observed with strong acids such as phosphoric acid or strong chelators such as EDTA (Kawasaki *et al.* 2000). However, this needs to be investigated further. A recent study showed that maleic acid reduced the microhardness of root dentine similar to EDTA but was able to increase dentine surface roughness markedly more than EDTA (Ballal *et al.* 2010). Thus, the potential relationship between surface roughness, bond strength and sealing ability deserves further investigation.

Chlorhexidine in a concentration of 2% has been recommended as a final antimicrobial rinse for canal disinfection because of its sustained antimicrobial activity (White *et al.* 1999), and the fact that chlorhexidine can enhance the durability of resin–dentine bonds (Carrilho *et al.* 2007). Whilst no positive effect of chlorhexidine on the bond of the epoxy resin material to dentine was found, there was no negative effect either. Chlorhexidine resulted in leakage and push-out bond strength values that were almost identical to those obtained with the water control treatment.

Another notable finding regarding bond strength was that this value decreased in a coronal to apical direction. The explanation for this may be that apical dentine contains less patent tubules than coronal dentine (Paque *et al.* 2006, Lottanti *et al.* 2009). The more complex structure of tubular dentine apparently yields itself better to infiltration with epoxy resin compared to the sclerotic apical counterpart. However, this does not necessarily mean that sclerotic dentine cannot be sealed as well as tubular dentine. Most likely, the opposite is the case (unpublished observations). What the current results show is that overall (averaged) bonding to dentine had an effect on fluid transport. It was not differentiated between different root thirds and dentine structure in this regard.

A recent study found similar effects of irrigating protocols on AH Plus push-out bond strength in root canals (Hashem *et al.* 2009). However, in contrast to the current study, individual chemicals were not singled out in that study. Furthermore, these authors filled canals with gutta-percha and AH Plus. Consequently, it remains unclear whether the failure modes in their push-out test were adhesive, i.e. between sealer and dentine, or cohesive, i.e. between the sealer and the core material. In the current study, only AH Plus sealer was used to fill the entire root canal space. This does not introduce bias, because AH Plus does not shrink. In fact, AH Plus expands over time in a humid environment (Ørstavik *et al.* 2001). This can explain why leakage values decreased over time. Filling the canals just with the epoxy resin material had the advantage that all the failures were adhesive, and thus, only the sealer/dentine interface was studied rather than other confounding factors. Clinically, however, filling with mere AH Plus is not advisable, because the material sets to a hard consistency and thus makes retreating almost impossible. The current results are in accordance with a published report on push-out bond strength of sealers to dentine without a core material in that the bond strength of AH Plus to dentine treated with NaOCl followed by EDTA were higher than specimens treated with NaOCl or distilled water (Nunes *et al.* 2008).

Future studies should look at the effect of alternative irrigating protocols on leakage and dentine bond strength in conjunction with different root filling systems. From a microbiological point of view, it would make sense to flush a strong disinfectant such as NaOCl in the canal system prior to root filling. However, extended exposure to NaOCl may deproteinise dentine to a point that cannot be reversed by the subsequent application of a decalcifying agent.

Conclusions

- Chemical treatment of dentine with commonly used irrigants had a significant impact on sealing ability and dentine bond strength of AH Plus;

- a final flush with a decalcifying agent appears advisable, whilst a final flush with NaOCl caused untoward effects on bond strength;
- conditioning of canal walls by maleic acid, which is a weak acid, resulted in superior sealing ability and higher epoxy resin bond strength compared to EDTA, which is a strong calcium-complexing agent;
- the two outcomes investigated, fluid transport and dentine bond strength, were strongly negatively correlated to each other.

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