

Chlorhexidine and proanthocyanidin enhance the long-term bond strength of resin-based endodontic sealer

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Abstract: The aim of this study was to evaluate the effects of proanthocyanidin (PA) and chlorhexidine (CHX) on the bond strength (BS), failure pattern, and resin-dentin interface morphology of the endodontic sealers EndoREZ and AH Plus after 24 h and 6 months of water storage. A total of 120 prepared bovine roots were divided into six groups: AH Plus, CHX+AH Plus, PA+AH Plus, EndoREZ, CHX+EndoREZ, and PA+EndoREZ. Dentin was treated for 1 or 5 min with 2% CHX or 15% PA, respectively. Roots were filled and stored in water for 24 h or 6 months (n = 10). Root slices were subjected to push-out test and scanning electron microscopy (SEM). Data were compared using two-way ANOVA and student's *t*-test ($\alpha = 5\%$). BS decreased over time for AH Plus and untreated EndoREZ ($p < 0.05$). At 24 h, AH Plus had higher BS than EndoREZ ($p < 0.001$), with no differences among treatments for both sealers ($p > 0.05$). At 6 months, EndoREZ had higher BS values for CHX and PA than control ($p < 0.05$). AH Plus had higher BS than EndoREZ ($p < 0.001$), while with CHX or PA, similar BS was observed in both sealers ($p > 0.05$). Cohesive and mixed failures were observed in all groups. SEM revealed sealer tags in the root dentin. In conclusion, BS decreased with time and AH Plus had higher BS than EndoREZ in untreated dentin; however, CHX or PA enhanced long-term BS of EndoREZ. Overall, dentin treatment affected failure pattern and resin-dentin interface morphology, particularly for EndoREZ.

Keywords: Endodontics; Dental Bonding; Root Canal Obturation.

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Introduction

Significant clinical and scientific evidence supports the use of resin-based sealers in root canal filling, such as their ability to reduce microleakage in the root canal because of their adhesive potential and penetrating ability in the dentinal tubules.¹ Endodontic sealers based on epoxy resin, such as AH Plus (Dentsply Sirona, Ballaigues, Switzerland), have high apical sealing capacity, biocompatibility, retention capacity to the root dentin, low solubility, and dimensional stability.² Methacrylate resin-based sealers, such as EndoREZ (Ultradent, South Jordan, USA), were specifically developed to adhere to the walls of the root canal through the formation of a hybrid layer, thereby reducing infiltration and increasing the seal

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between the root canal and the sealing material. However, methacrylate resin-based sealers may exhibit technical sensitivity, preventing complete hybridization of the tissue. In addition, contraction during the polymerization process can negatively interfere with the sealing and adhesive capacities.³ To overcome these limitations which prevent stable dentin adhesion, there has been an intense search for protocols and techniques that favor bonding with the root dentin.^{4,5,6,7,8}

The difficulty in achieving a stable hybrid layer with dentin tissue is related to the hydrophilic nature and organic structure of the sealer,^{9,10} which can interfere with the sealer–dentin interaction.¹¹ In fact, incomplete infiltration of resinous monomers into collagen fibers within the hybrid layer favors hydrolysis and degradation of collagen by metalloproteinase (MMP), thus reducing the bonding longevity.¹² MMPs are calcium- and zinc-dependent endopeptidases trapped within the mineralized dentinal matrix during tooth development in an inactive form. The activation of these enzymes can occur after exposure to collagen because of the breakdown of zinc and calcium bonds and is considered one of the main causes of bond failure between resinous materials and dentin.¹³

Self-etch sealers were developed to reduce the sensitivity of the technique, particularly in the apical third, where primer or adhesive is often improperly applied.¹⁴ The formation of an adhesive interface between the filling material and the dentin occurs after the removal and/or alteration of the smear layer and the demineralization of the underlying dentin.¹⁵ The process of dentin demineralization using this sealer is directly related to the effects of MMPs on collagen degradation and reduction of bond strength (BS).⁵ However some sealers, such as EndoREZ, possess a hydrophilic primer and do not require the use of acid etching, thereby allowing its application in moist root canals, forming tags for retention in the dentinal tubules.¹⁶ All manufacturers of methacrylate-based sealers recommend the use of ethylene diamine tetra acetic acid (EDTA) in the final cleaning protocol to expose dentin collagen fibers and to improve infiltration of the resinous monomers in the matrix. Regardless of the sealer composition, this process increases collagen matrix degradation over time.⁵ Thus, the use of MMP inhibition solutions is essential to neutralize

the lytic activity of dentin on gelatin and collagen, thus increasing the stability of the hybrid layer.^{13,17}

For example, in addition to its well-known antimicrobial properties, chlorhexidine (CHX) is a protease inhibitor capable of neutralizing MMP and cathepsin cysteine activity, thereby preserving the hybrid layer.¹² Liu et al.¹⁸ reported that CHX is the only proven effective substance that reduces adhesive interface degradation by inhibiting MMPs; however they stated that its action lasts for only a few months and weakens over time. Additionally, proanthocyanidin (PA), a flavonoid compound found in fruits, vegetables, seeds, and flowers, has shown antioxidant capacity⁵ as well as inhibition of the catalytic activity of MMPs, such as MMP-1 and MMP-9,^{13,19} thus, PA can be used as an alternative to improve and stabilize collagen resistance against long-term degradation.^{12,13}

Considering that the chosen procedure for cleaning the root canal before filling can compromise the longevity and retention of resin sealers, this study was aimed at investigating the use of MMP-inhibitor and crosslinking solutions that neutralize these enzymes while maintaining the integrity of the dentin matrix to improve short- and long-term BS of resin-based endodontic sealers. The null hypothesis was that no differences would be detected in the BS of sealer and/or treatment groups during the experimental periods.

Methodology

Selection and preparation of specimens

Bovine incisors stored in 0.1% thymol solution at 4°C were cleaned using periodontal curettes and subjected to prophylaxis with pumice stone (SS White, New Jersey, USA) and water, applied with mounted Robinson brushes at low rotation (Intramatic 2068, Kavo Ind. Com. Ltda., Joinville, Brazil). The teeth were radiographed to measure the cervical, middle, and apical diameters. Averages were obtained and teeth with discrepant measurements were discarded. A stereoscopic magnifying glass (ZEISS, Stemi 2000-C, Germany) at 10× magnification was used to analyze the teeth and those that had cracks or structural anomalies were excluded from measurement. The teeth were transversely sectioned near the cementum–enamel junction using a

precision cutting machine set at 375 rpm (IsoMet 1000, Buehler, Germany). A double-faced diamond disc was used to obtain roots with a standardized length of 17 mm under constant water irrigation. Mesiodistal and buccolingual root canal diameters were measured in the cervical region and samples with mean diameters of 1.5 and 2.5 mm were selected. One hundred and twenty bovine incisors were used.

Biomechanical preparation of specimens

A size 15 K-file (Dentsply Sirona, Ballaigues, Switzerland) was passively introduced into each root canal to confirm the working length (16 mm). Teeth with laterally displaced foramina and/or canal length < 17 mm were replaced. The root apex was sealed using utility wax and the canals were instrumented using the ProTaper Universal (Dentsply Sirona, Ballaigues, Switzerland) according to the following protocol. Specimens were stabilized in a bench vice. The cervical third was prepared using the SX instrument and the middle and apical thirds were prepared using the S1, S2, F1, F2, F3, F4, and F5 instruments. The root canals were irrigated with 2 mL 2.5% NaOCl at each file change using NaviTip needles (Ultradent Products Inc.) attached to plastic syringes (Ultradent Products Inc. South Jordan, USA). A VDW Silver (VDW GmbH, Munich, Germany) was used to operate all files. For the final rinse, the root canals were irrigated with 5 mL 17% EDTA (Da Terra, Ribeirão Preto, Brazil) for 3 min, irrigated with 5 mL of distilled water, and dried using sterile absorbent paper cones (# 50) (Dentsply Sirona, Ballaigues, Switzerland).

Dentin treatment

After biomechanical preparation, the roots were separated into groups according to the solutions used for dentin treatment. For the groups treated with 2% CHX (Da Terra, Ribeirão Preto, Brazil), 1 mL of the solution was applied for 1 min. For groups treated with PA, a 15% solution was obtained from grape seed extract capsules (Luna Ervas, Caieiras, Brazil) diluted by simple solubilization. One milliliter of PA solution was stirred with a magnetic stirrer (Biovera, Rio de Janeiro, Brazil), filtered, and used immediately after solubilization for 5 min. The roots were aspirated and dried using sterile absorbent paper (# 50) (Dentsply Sirona,

Ballaigues, Switzerland). Untreated dentin specimens were maintained at relative humidity and 37°C.

Specimen filling

Roots were filled using cold lateral compaction with gutta-percha F5 master cones (Dentsply Sirona, Ballaigues, Switzerland) and L accessory gutta-percha cones (Dentsply Sirona, Ballaigues, Switzerland) with AH Plus or EndoREZ sealers. The base and catalyst pastes for the AH Plus sealer were dispensed onto a glass plate and mixed until a homogeneous mixture at an ideal consistency for filling was obtained. The gutta-percha cone was loaded with the sealer and inserted into the root canal. In groups filled with EndoREZ, 30 g sealer was injected through a self-mixing tip into a syringe applicator and inserted into the canal using 21-mm yellow NaviTip tips until reaching the apex. The gutta-percha cone was inserted into the root canal after filling it with the sealer. The cone was then cut using a heated instrument and vertically condensed with a plugger (Duflex, SS White, New Jersey, USA). Excess sealer was removed using cotton pellets. After filling, specimens filled with EndoREZ were photopolymerized for 40 s and all specimens were filled with temporary restorative material without eugenol (Villevie, Nova Brasília, Joinville, SC, Brazil). The teeth were immediately placed at 37°C and relative humidity for 3 times longer duration than the regular setting duration for the sealer.

Water storage

Specimens were further separated into two subgroups based on storage time in water (24 h and 6 months). The roots were maintained in Eppendorf tubes with 1.5 mL distilled water at 37°C; water was changed on a weekly basis for the 6-month group.²⁰

Bond strength test (Push-out)

The roots were fixed onto acrylic plates using wax (Kota-Import, São Paulo, Brazil) and sectioned using a precision cutting machine set at 375 rpm and equipped with a water-cooling system. Nine 1.5 mm-thick slices were obtained from each root (3 per root-third), resulting in a total of 1,080 specimens. The middle slice from each third canal was selected

and mounted on a stainless steel support used to hold the specimens in an Instron 3345 Universal Testing Machine (Instron Corporation, Canton, USA) in such a way that the side with the smaller root canal diameter faced upward and was aligned to the shaft that would exert pressure on the sealer (apex-crown direction) until debonding occurred. Six-millimeter-long shafts with tip diameters of 0.6 mm for the apical third, 0.8 mm for the middle third, and 1 mm for the coronal third were used. This method assured the alignment of the specimen in an accurate and reproducible manner and also enabled the shaft to remain centralized and avoid contact with dentin during testing. The force required to dislodge the filling material (F in kN) was transformed into tension (σ in MPa) by dividing the force by the adhesive area of the filling material (SL in mm²) using the equation $\sigma = F/A$. After the push-out test, the slices were examined using a stereomicroscope (ZEISS, Stemi 2000-C, Germany) at 25 \times magnification to determine the failure pattern. Failure was considered adhesive if the sealer was completely separated from the dentin (dentin surface without sealer), cohesive if the fracture occurred within the sealer (dentin surface completely covered by the sealer), and mixed when a mixture of adhesive and cohesive modes occurred (*i.e.*, the dentin surface was partially covered by the sealer), according to Haragushiku et al.¹⁷

Scanning electron microscopy

The third slice obtained from each canal third was prepared for scanning electron microscopy (SEM) analysis of the filling material/radicular dentin interface. The slices were immersed in 2.5% glutaraldehyde (Merck KGaA, Darmstadt, Germany) with 0.1-M sodium cacodylate buffer solution (pH 7.4) (Merck KGaA) for 12 h at 4°C. After fixation, the specimens were rinsed using 0.1-M sodium cacodylate buffer solution and then sequentially dehydrated in increasing concentrations of ethanol solutions (Labsynth Produtos para Laboratório Ltda., Diadema, Brazil) as follows: 25% for 20 min, 50% for 20 min, 75% for 20 min, 90% for 30 min, and 100% for 60 min, after which they were immersed in a hexamethyldisilane solution (HMDS, Ted Pella, Redding, USA) for 10 min. The specimens were then placed on an absorbent

paper on glass plates and allowed to dry under an exhaust hood for 24 h. The specimens were mounted on metallic stubs, sputter-coated with gold-palladium alloy (SDC 050; Bal-TecAG, Balzers, Liechtenstein) and examined in an SEM (JSM T330A, JEOL Ltd, Tokyo 190-0012, Japan) operating at 20 kV. SEM micrographs at 500 \times magnification were obtained from four distinct points at the perimeter of the root canal. Qualitative analysis of adhesive interfaces included studying the following characteristics: 1) presence of failure at the interface (gaps) and 2) formation of an extension of sealer into the tubules (tags).¹⁸

Statistical analysis

Quantitative data was subjected to normality and variance homogeneity tests. The effects of the sealers and treatments on BS were evaluated using two-way ANOVA, followed by Tukey test when appropriate. The comparison between BS obtained at 24 h and 6 months in each experimental group was conducted using student's *t*-test. The level of significance adopted was 5%.

Results

Push-out test

At 24 h, there was no interaction between the sealer and treatment factors ($p = 0.784$). Overall, a significant difference between the sealers was verified, with the BS of AH Plus > EndoREZ regardless of the treatment ($p < 0.001$), while no significant differences were observed between the treatments used, regardless of the sealer applied ($p = 0.930$). BS results of the 24-hour water storage treatment are summarized in Table 1.

At 6 months, there was interaction between the factors under study, *i.e.*, the effect of the sealers varied according to the treatment used ($p = 0.012$). Comparison among treatments revealed the following: for AH Plus, Control = CHX = PA ($p > 0.05$) and for EndoREZ, CHX = PA > Control ($p < 0.05$). Comparison between sealers showed the following results: for Control, AH Plus > EndoREZ ($p < 0.001$) and for CHX and PA, AH Plus = EndoREZ ($p = 0.332$ and $p = 0.089$, respectively). BS results for 24 h and 6 month water storage are given in Table 1.

Table 1. Mean values \pm standard deviation of Bond Strength (BS) values obtained in root dentin pretreated or not (Control) with Chlorhexidine (CHX) or Proanthocyanidin (PA) and sealed with AH Plus or EndoRez, after 24 h or 6 months of water immersion.

Variable	AH Plus			EndoRez		
	Control	CHX	PA	Control	CHX	PA
24 h	0.54 \pm 0.09 Aa*	0.54 \pm 0.11 Aa*	0.55 \pm 0.08 Aa*	0.33 \pm 0.09 Ab*	0.32 \pm 0.11 Ab	0.30 \pm 0.06 Ab
6 months	0.43 \pm 0.11 Aa	0.36 \pm 0.10 Aa	0.41 \pm 0.10 Aa	0.21 \pm 0.05 Ab	0.32 \pm 0.11 Ba	0.33 \pm 0.10 Ba

Capital letters indicate statistical difference among treatments (Control \times CHX \times PA) in the same sealer and time point (two-way ANOVA, $p < 0.05$). Lowercase letters indicate statistical difference between sealers (AH Plus \times EndoRez) in the same treatment and time point (two-way ANOVA, $p < 0.05$). Asterisks indicate statistical difference between time points (24 h \times 6 months) in the same experimental group (t -test, $p < 0.05$).

Table 2. Failure mode distribution (%) in the specimens of root dentin pretreated or not (Control) with Chlorhexidine (CHX) or Proanthocyanidin (PA) and sealed with AH Plus or EndoRez, after 24 h or 6 months of water immersion

Experimental groups	24 hours			6 months		
	Adhesive	Mixed	Cohesive	Adhesive	Mixed	Cohesive
AH Plus	20	56.7	23.3	0	70	30
AH Plus + CHX	3.3	76.7	20	6.7	63.3	30
AH Plus + PA	3.3	40	56.7	0	60	40
EndoRez	30	20	50	6.7	73.3	20
EndoRez + CHX	16.7	20	63.3	0	63.3	36.7
EndoRez + PA	0	30	70	6.7	83.3	10

Failure mode after the push-out test

The failure pattern analysis revealed lower adhesive and cohesive failures for AH Plus than those for EndoREZ at both time points. CHX and PA affected the sealers' failure pattern; at 24 h, both treatments resulted in lower adhesive failures for AH Plus and EndoREZ. However, AH Plus with CHX had higher mixed failures and with PA higher cohesive failures than those of the control group. EndoREZ with CHX had higher cohesive failures and with PA higher cohesive and mixed failures. At 6 months of water storage, CHX resulted in lower adhesive and mixed failures for EndoREZ than those with control. Also, AH Plus with CHX resulted in higher adhesive failures and PA resulted in higher cohesive failures than those with control. Failure mode distribution in the specimens is displayed in Table 2.

Scanning electron microscopy analysis

Morphological analysis enabled the evaluation of the filling material–root canal interface. SEM micrographs showed the formation of long tags in the adhesive interface for most sealers except for some samples of AH Plus where this characteristic was not observed (Figure).

Discussion

According to the results, the null hypothesis has to be rejected because BS differences were observed between the sealer and/or treatment groups during the evaluated periods. In general, AH Plus sealer exhibited higher BS than EndoREZ when no pretreatment was applied, and CHX or PA improved the BS of EndoREZ after 6 months of storage in water.

It was previously reported that the BS of endodontic filling materials should be as high as possible, not only to ensure efficient root canal sealing but also to ensure long-term endodontic treatment success.^{6,21,22} Furthermore, the bonding interface must remain intact over time and not compromise clinical procedures, such as the removal of part of the sealing material for the installation of root canal posts, because the teeth are subjected to mechanical forces that can displace the sealing material beyond root canal limits.^{23,24} Therefore, in the present study, the BS of the filling material was evaluated after 24 h and 6 months of storage in water.

The push-out test is widely used to simulate clinical conditions and represents a reproducible method with

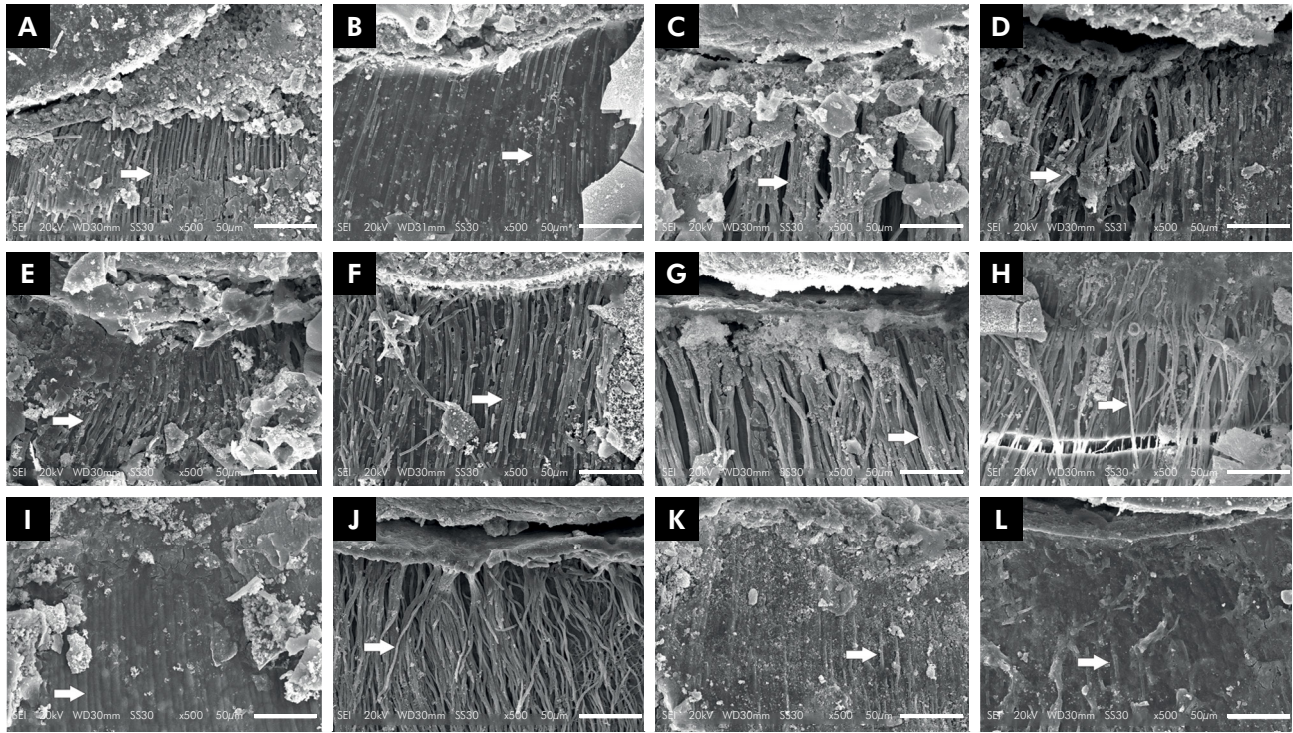


Figure. Scanning electron microscope photomicrograph of resin-dentin interface in AH Plus (A and B), AH Plus+CHX (C and D), AH Plus+PA (E and F), EndoREZ (G and H), EndoREZ+CHX (I and J), and EndoREZ+PA (K and L) after 24 h (A, C, E, G, I and K) or 6 months (B, D, F, H, J and L) of water storage. Note the formation of sealer tags for all experimental groups at both time points (indicated with arrows). Magnification: 500×.

fidelity in results.^{24,25,26,27} However, this method is limited by the standardization of specimen positioning, which is essential for reproducibility.⁸ In the present study, specimens were positioned upright to the dentinal tubules, which act as a retaining force within the root canal.²⁸ In addition, the force application rod was positioned in the center of the filling mass, thus preventing contact with dentin⁷ and favoring a more accurate measurement of material retention.

The results of the 24-hour immediate push-out test showed that the BS of both types of sealers were not influenced by dentin surface pretreatment. Similar results were reported by Kalra et al.,⁵ who pretreated dentin with 6.5% PA and filled the root canals with self-etching sealers; differences in the BS following 1 week of storage in water were not observed. Similarly, Cecchin et al.²⁹ reported that pretreatment of dentin with 2% CHX yielded no significant effect on the BS of the resin sealer at the immediate level. These findings may be related to inadequate activation time of MMPs during this

period because the exposed dentin collagen matrix is slowly degraded by proteolytic enzymes.¹³

In the 6-month water storage group, both CHX and PA treatments resulted in higher BS values only for the EndoREZ sealer as compared to the control group. These results indicate that specimens sealed with EndoREZ and treated with CHX or PA had stable BS over time. In contrast, this benefit was significantly limited to those with 6 months of storage in water from the control group. These findings are in agreement with those of Kalra et al.,⁵ who observed that the use of PA facilitated an increase in the resistance to biodegradation at the interface of the methacrylate-based RealSeal SE sealer. This process promoted the maintenance of BS values, with similar values after 1 week or 3 months of storage in water. Hass et al.³⁰ also evaluated the effects of PA on the degradation of the hybrid layer and observed that PA tightened the collagen polypeptides and inactivated catalytic activity of proteases, creating peptide binding with adjacent peptides. Regarding the positive CHX

results, studies have demonstrated advantages in its application. Cecchin et al.³¹, for instance, observed that the application of 2% CHX prior to fiberglass post filling resulted in maintenance of BS after 12 months of storage in water. Nawareg et al.³² also used 2% CHX and observed stable BS between composite resin and dentin after storage in water for 6 and 12 months. Both authors attribute these results to the inhibition of MMPs by CHX, which favors the stabilization of the hybrid layer.^{12,18,31}

In contrast to the methacrylate sealer, AH Plus showed no effects of CHX or PA on BS after 6 months of storage in water, showing significantly lower values than storage in water for 24 h. These results were not expected because both CHX and PA reduce the effects of dentin collagen biodegradation,^{5,7} which would maintain chemical bond stability of the sealer over time. However, for the AH Plus sealer, variables other than collagen degradation may have interfered with BS, such as sealer solubility and destabilization of the covalent bonds formed between the AH Plus sealer and dentin collagen over time.³³

In the comparison between the sealer groups, significantly higher BS was observed in the specimens filled with AH Plus than those filled with EndoREZ after 24 h of storage in water, regardless of the treatment. After 6 months, these differences were maintained in the control groups; however, the BS of the EndoREZ sealer with CHX and PA were equivalent to that of all AH Plus groups. The stability of the AH Plus sealer^{1,33} may be one of the factors that allows satisfactory resistance values to be obtained, even after storage over a long period. In fact, similar results were described by Haragushiku et al.,¹⁷ who speculated that the lower BS values of EndoREZ are because of its incomplete polymerization and the polymerization contraction inherent in this sealer.

Another factor that has been evaluated for its influence on the BS of endodontic sealers to the root canal is sealer penetration into the dentin tubules³⁴. In the present study, SEM analysis revealed the formation of long tags for both types of sealers regardless of the evaluated period, which could indicate the absence of a relationship between the formation of tags and the BS of the sealers. Corroborating this hypothesis, Neelakantan et al.²⁵ observed that the penetration of the tags had no relationship with the sealing capacity and the BS of the AH Plus. Nevertheless, according to Doyle et al.,⁴ the formation of long resin tags may not provide sufficient strength to resist the polymerization contraction process experienced by EndoREZ, which could explain the lower BS values than those of AH Plus.

Regarding the hypothesis of the current study, it was observed that the root dentin treatment using PA and CHX provided stable BS for the methacrylate sealer EndoREZ and did not affect this evaluation parameter in the resin sealer AH Plus. However, further studies are required to evaluate the chemical stability of the bonds between the filling material and root dentin and the ability of the sealers to seal the root canal system in the long term.

Conclusion

Based on the results of the current study, it was concluded that BS decreased over time and AH Plus exhibited higher BS than EndoREZ in untreated dentin. However, dentin pretreatment with CHX (2%) or PA (15%) stabilized the BS of EndoREZ over time with values similar to those of AH Plus, without interfering with the BS of AH Plus. In addition, dentin treatment protocols affected failure pattern and resin-dentin interface morphology, particularly for EndoREZ.

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