

Scientific article

**Impact of final rinse with saline
or alcohol solution on root canal
sealability**

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Keywords

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Abstract

Endodontic irrigation protocols are not only used to clean and disinfect the root canal system, but also to condition the canal wall dentine for subsequent root filling. In this study we tested whether a final irrigation step with saline solution or 80% ethanol improved root canal sealability by two popular sealers, an epoxy resin (AH Plus) and a hydraulic calcium silicate cement-based product (BioRoot RCS). Root canals in extracted single-rooted human teeth were instrumented and filled with a matched gutta-percha cone and sealer. During instrumentation and prior to root filling, sealer-specific irrigation protocols were applied. These involved a combined sodium hypochlorite/1-hydroxyethylidene-1,1-diphosphonic acid application, which was followed by irrigation with ethylenediaminetetraacetic acid (EDTA) for AH Plus. Protocols were followed by a 5-ml ultimate rinse with saline solution or 80% ethanol. No such final rinse was the control ($N = 9$). Canals were then dried with matched paper points. One week after root filling and storage of the teeth at 37°C in a humid environment, Rhodamine B was used to trace leakage. Two-way ANOVA revealed that the type of sealer had a significant ($P < 0.05$) impact on apical dye penetration while the final rinse did not ($P > 0.05$). AH Plus provided the slightly better seal ($P < 0.05$). Leakage occurred between the sealer and the dentin with AH Plus, and between the sealer-to-dentin as well as the sealer-to-gutta-percha interface with BioRoot RCS. In summary and under current conditions, there was no benefit from applying saline or ethanol as an ultimate rinsing solution prior to drying the canal with matched paper points.

Introduction

Sealing root canals after an endodontic procedure is an essential step in root canal treatments. In this context, matching the irrigation protocol to the obturation materials that are used is recommended (1, 2). The conditioning of the canal wall for subsequent root filling should not be neglected. However, the question of the possible role of chemical remnants when clinical protocols are used, and also the impact of chemically (using alcohol) versus just physically drying the root canal has not been investigated. This may be important, as research has shown that there are diverging mechanisms by which contemporary root canal sealers bind to the root canal wall (2, 3). In essence, the most popular epoxy resin sealers bind to exposed collagen fibrils in the canal wall (4), while hydraulic calcium silicate (CaSi) sealers bind directly to the calcium in the hard tissue phase (2). In this context, not only chemical remnants that interfere with the setting of these sealers may play a role, but also the wetness of the canal wall and the hydration of the collagen.

On a more practical note, there is an obvious incongruence between studies on root canal irrigation regimens and what is done in clinical practice. In scientific studies, carry-over or continued effects of the chemicals that are employed have to be controlled for to single out the effect that is studied. Authors administered either a stop solution to discontinue antimicrobial effects, or water/physiological saline to dilute the chemical remnants that may interfere with the outcomes to be studied (5, 6). In clinical practice, however, that is not normally done. Instead, most clinicians appear to not follow their disinfection and chelation protocol by anything (7), and therefore just use paper points to dry the canals before filling the root canal system. However, some practitioners outside the US appear to strongly believe in alcohol as a final irrigant (8), because it used to be of value in controlled drying ("dry bonding") with traditional methacrylate-based adhesive systems (9).

It has been suggested that a moist canal may interfere with the obturation quality of traditional root canal sealers (10), and also with the adhesion of contemporary epoxy resin and the hydraulic CaSi sealers (11). Moreover, the use of phosphate-buffered saline as a final irrigation solution which was suggested for use with hydraulic cement sealers to enhance the bond strength by biomineralization (12) has resulted in a reduction in antimicrobial activity of the sealer (13).

Based on the issues raised above, the current study addressed this basic question: after using sealer-specific irrigation protocols, what is the effect of washing the root canal with a saline solution, and that of chemical drying with 80% ethanol, on root canal sealability by either an epoxy resin-based (AH Plus) or a hydraulic CaSi (BioRoot RCS) sealer? The null hypothesis of the study tested was that neither a final rinse with saline solution nor with 80% ethanol affect the sealability of root canals subsequently filled with AH Plus or BioRoot RCS over drying the canal directly with paper points after the sealer-specific irrigation protocol.

Materials and methods

Materials

Two sealers with different chemistries were investigated and used with irrigation protocols during and after instrumentation that have resulted in the best sealer adhesion in previous studies (1, 4). The sealers were:

- AH Plus (Dentsply Sirona Endodontics, Ballaigues, Switzerland), an epoxy resin sealer;
- BioRoot RCS (Septodont, Saint Maur des Fosses, France), a hydraulic CaSi cement sealer.

The focus of this study was on the *last step* of irrigation before root filling, and how that affected dye movement into the filled root canal. Technical details of the irrigation process are listed below (subchapter *Simulated clinical protocols*). After instrumentation and irrigation, the canals either received a final rinse with 5 ml of physiological saline solution (Otsuka Pharmaceuticals India Pvt. Ltd., Gujrat, India), or 80% ethanol (Sri Durga Laboratory Equipment Supplies, Karnataka, India). No final rinse was applied in the third irrigation group. Canals were dried with matched paper points (F3, Dentsply Sirona) and then filled.

Sample size calculation

Longitudinal dye movement along the root filling was the main outcome that was assessed. An effect size of 0.3 was considered for two-way ANOVA analysis with global effects. With 6 groups (2 sealers times 3 irrigation protocols), an alpha error probability of 0.05, and 95% power (G*Power 3.1, Heinrich Heine Universität Düsseldorf, Düsseldorf, Germany), a total sample size of 48 was calculated. Nine teeth per group ($N = 9$) instead of 8 were then used. Six extra teeth were used as positive and negative controls ($N = 3$) in the dye movement assessment and 6 for cross-sectional interface analysis. Therefore, a total of $6 \times 9 + 6 + 6 = 66$ single-rooted human teeth were selected for this study.

Sample selection and ethics

Ethical clearance for the use of anonymized extracted human teeth was obtained from the institutional review board (IEC 27/2022). These selected teeth were extracted for reasons not related to this study, and all clinical protocols adhered to the declaration of Helsinki. In the selection process, radiographs of the specimens were taken from buccal and mesial aspect to confirm a straight, single canal with mature apices and without any calcifications or resorption. Canals were narrow enough to allow shaping to a standardized instrument size (see below). Soft tissue fragments and calcified debris on the specimens were removed using ultrasonic scalers. Necessary hygiene precautions were observed by personnel handling the teeth. Great care was exercised that all the human tooth specimens were *fully hydrated*, i.e. stored in physiological saline solution with 0.2% sodium azide (Sigma-Aldrich, Steinheim, Germany) at 4°C, prior to the experiments described below.

Simulated clinical protocols

The teeth were decoronated at the cemento-enamel junction using a diamond disc (Horico, Berlin, Germany). Working length was established by inserting a size 10K file (Mani Inc.,

Tochigi Ken, Japan) into each root canal until it became visible at the apical foramen (observed using magnifying loupes (2.5x), EyeMag Smart, Carl Zeiss, Oberkochen, Germany) then subtracting 1 mm from the recorded length. The apices of all the teeth were sealed with sticky wax to prevent apical extrusion of the irrigants, and to allow their reverse flow. The specimens were then randomly divided into three groups ($N = 9$) per sealer.

In all protocols, a fresh 3% NaOCl (Vista Dental Products, Racine, WI, USA) & 9% HEDP (Dual Rinse HEDP, Medcem, Weinfelden, Switzerland) mixture was used during the instrumentation and subsequently (14). Root canals were cleaned and shaped using rotary ProTaper Universal system (Dentsply Sirona) with Tri Auto Mini endo motor (J. Morita Corp, Kyoto, Japan), according to the manufacturer's instructions up to a size of F3. All the irrigation was performed using a 27-gauge side vented needle (Vista Dental) which was inserted 1 mm short of the working length. Teeth were irrigated using a "bathtub technique", i.e. canals were always flooded with the irrigant when instrumented (15). Before and between the 5 instruments in the sequence, 2 ml of the irrigant was administered for 1 min each, and 10 ml subsequently during 10 min, resulting in a total of 20 ml per tooth. In the AH Plus group, this was followed by 5 ml of 17% EDTA (Vista Dental) administered for 1 min to expose to collagen (16), while in the teeth filled with BioRoot RCS, no extra decalcification step was undertaken (2). Subsequently, the root canal was either dried with matched paper points (F3, Dentsply Sirona) directly (control protocol), or rinsed for 1 min with 5 ml of either a physiological saline solution (Otsuka Pharmaceuticals India Pvt. Ltd.) or 80% ethanol (Sri Durga Laboratory Equipment Supplies) prior to that.

Roots were then filled using the epoxy resin root canal sealer (AH Plus; Dentsply Sirona) or the hydraulic CaSi counterpart (BioRoot RCS, Septodont) in conjunction with a matched gutta-percha cone (F3, Dentsply Sirona). Sealers were mixed according to manufacturer's instructions and applied into the root canal by using a #25 (red) lentulo spiral (Dentsply Sirona) until the sealer extruded the opening of the root canal orifice. The matched cone was then inserted using a gentle pumping motion and coronally cut off using a heat plugger (Gutta-Smart, Dentsply Sirona). The coronal portion of the specimen was then sealed with flowable composite resin (Brilliant Flow, Coltène, Altstätten, Switzerland) after conditioning with etchant (Eco-Etch, Ivoclar Vivadent, Schaan, Liechtenstein) and adhesive agent (One Coat Bond SL, Coltène). Specimens were then kept in an incubator at 100% humidity for 7 days at 37°C to ensure complete setting of the sealers.

Assessment of sealability

Each root was embedded in cold-cure acrylic (Lucitone, Dentsply Sirona) and sectioned horizontally in the middle third using a hard-tissue microtome (Leica Biosystems, Wetzlar, Germany) under continuous water cooling. The apical halves of roots of teeth were used for this experiment. The coronally-directed dissection surface including the root canal was sealed using flowable resin composite (Brilliant NG Flow, Coltène). Then the roots were coated with two layers of nail varnish except in the apical 2-3 mm. Six separate single rooted teeth were taken and divided into two groups ($N = 3$) of positive and negative controls. Root canals of these teeth were prepared and irrigated as in the AH Plus group (as mentioned above), with a final rinse with 5 ml of saline solution for 1 min. In the positive control group, canals were

obturated with a matched gutta percha point (ProTaper, Dentsply Sirona) without any sealer. In negative control group, the apices were sealed with flowable resin composite (Coltène, Switzerland).

All samples were then immersed in a tracer dye solution (2% Rhodamine B, Merck KGaA, Darmstadt, Germany) and placed under negative pressure of 75 torr for 30 min, and then allowed to remain in the dye for seven days. After exposure to the dye, samples were rinsed with running water to remove the dye and the nail varnish was gently removed with a #15 disposable scalpel blade (Lister, Kanpur, India). The roots were then longitudinally grooved using a diamond disk (Horico) and split into two halves using a chisel (HuFriedy, Chicago, IL). Following the separation of the roots, the gutta-percha was removed and dye movement was measured by an examiner who was unaware of the experimental groups using a 10 x stereomicroscope (Zeiss, Thornwood, NY, USA) in a straight line from the apical canal terminus to the coronal extent of the apparent dye movement using the built-in micrometer scale. The length values between the two root halves were added and divided by 2 to get the average dye movement per root (in mm), which was used for the statistical analysis.

Following dye movement measurements, 6 additional specimens were prepared to assess the sealer/dentine and sealer/gutta-percha interfaces. Cross-sectional cuts of the roots were performed at levels where dye movement was present, and the specimens were microscopically inspected using a digital microscope (VHX-2000, Keyence, Mechelen, Belgium).

Data presentation and analysis

Dye movement was assessed to 0.1 mm, and data is reported to that level of accuracy. Data was statistically analyzed using the jmp 15 software (SAS, Cary, NC, USA). Values were evenly distributed (Shapiro-Wilk test), and therefore mean values were compared between groups using two-way ANOVA and Bonferroni's *post hoc* correction. The alpha-type error was set at 5% ($P < 0.05$).

Results

Positive and negative controls were run with the AH Plus irrigation protocol, i.e. a penultimate rinse with EDTA and then saline solution. With 8.7 ± 0.6 mm, the Rhodamine B dye penetrated roughly 3 times deeper into the canal space when there was no sealer (positive control) used with the matched gutta-percha cone, while there was zero (0.0 ± 0.0) Rhodamine B leakage when the apex was sealed in the negative controls.

Two-way ANOVA revealed that there was a significant ($P < 0.05$) impact of the sealer on apical dye leakage. AH Plus provided a slightly but significantly better seal than BioRoot RCS. On the other hand, there was no significant influence by the final irrigation protocol ($P > 0.05$, Table 1). The interaction between sealer and final irrigant was also not significant, ($P > 0.05$, Table 1), indicating that the effects of final irrigation were statistically similar for the two sealers under investigation (Fig. 1).

With AH Plus, dye movement occurred mainly at the sealer/dentine interface, whereas in BioRoot RCS, it occurred both at the sealer/dentine and sealer/gutta-percha interfaces (Fig. 2).

Table 1. Two-way ANOVA assessing the impact of the sealer and final irrigation on apical dye penetration.

	Sum of squares	df	F ratio	P
Sealer	7.78	1	5.46	0.024
Final irrigant	3.86	2	1.35	0.268
Sealer*final irrigant	1.68	2	0.59	0.559

Note. N = 9. df = degrees of freedom.

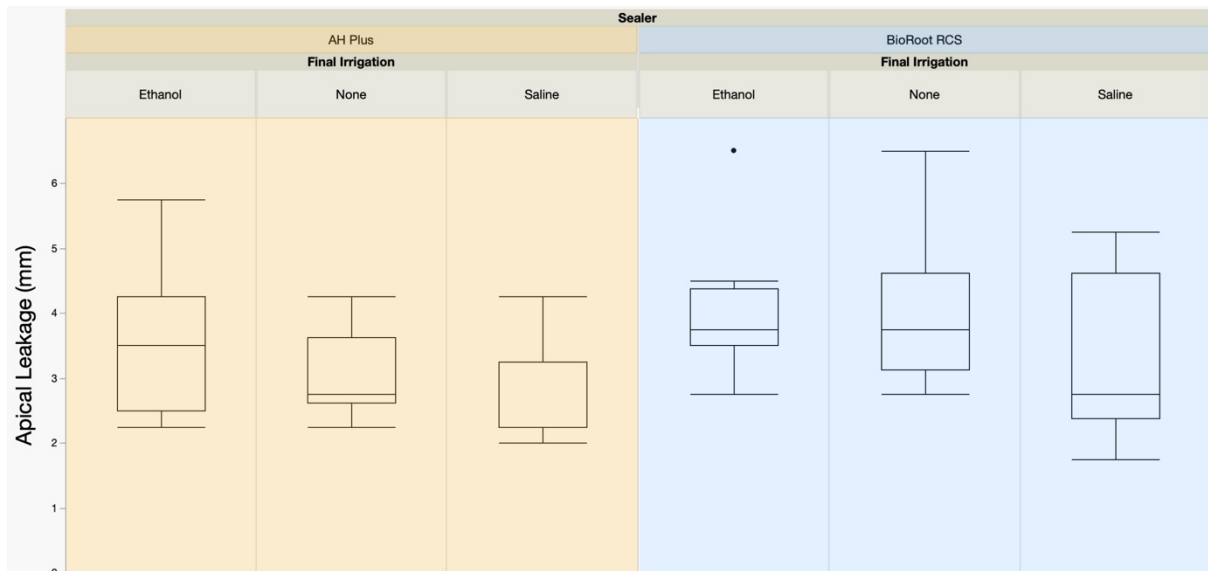


Figure 1. Box and whisker plots representing apical Rhodamine B dye leakage according to the sealer that was used and the final irrigant that was applied before drying the canal with matched paper points. Boxes: inter-quartile ranges; horizontal lines: medians, whiskers: minima and maxima; dot: outlier.



Figure 2. Cross sectional microscopical images of the sealability assessment. The arrows highlight the Rhodamine B movement at the sealer/dentine interface for AH Plus, and both at the sealer/dentine and sealer/gutta-percha interfaces for BioRoot RCS (arrows).

Discussion

The current study investigated two sealers of different chemistries used in single-cone obturation technique. Due to the different chemistries the clinical protocols used were not the same. In the current study, two different irrigation protocols were used, one tailored for the epoxy resin sealer using EDTA to expose the collagen in the canal wall (16), and no such final erosive step for the hydraulic CaSi sealer under investigation (3). This enabled the optimized use of the sealers and to get a broader idea regarding the necessity of the washing/drying steps under investigation.

The focus of this study was on the final step before applying the paper points, which is an issue that is vividly discussed among clinicians, yet not in the literature. Former studies assessed the impact of canal dryness on sealer adhesion (11, 17). However, these studies did not discern between the effects of dryness and washing out chemical remnants from the previously administered irrigants.

This study showed that, under current conditions, root canal sealability was neither significantly improved when a saline solution was used to wash out chemical remnants from the previously applied irrigants, nor when 80% ethanol was administered as a washing/chemical drying step. The current results were obtained in an ex-vivo experiment in extracted human teeth. The methodologies that were applied are not undisputed (18, 19), and their clinical value remains uncertain. On the other hand, at least with AH Plus, there is a clear within-specimen correlation between sealability in the root filling as assessed by fluid filtration and adhesion of the sealer to the canal wall (1). Moreover, as implied in its name, a sealer is supposed to seal. Failure to do so may lead to catastrophic clinical outcomes (20, 21).

The sealability assessment performed here was not modern (22), and needs to be considered in conjunction with complementary observations (23). Dye leakage does not necessarily correspond to microbial leakage, especially with sealers that exert anti-microbial properties such as the two sealers in this study (24). Moreover, in clinics teeth are subjected to masticatory forces. These were not simulated in the current study. Some incongruence between results in previous dye leakage studies has been attributed to air bubbles that prevented dye leakage

when no vacuum was applied (25). A pilot study was carried out prior to the experiments presented here, and indeed there was significantly more dye movement when negative pressure was applied. Hence, in the present study dye leakage assessment was performed under vacuum. Rhodamine B is acidic and has the tendency to interact preferentially with hydraulic cements as has been shown in previous research (26). In the current study the Rhodamine B was detected with a light microscope rather than a confocal microscope. This was done because the fluorescence of Rhodamine B is pH-dependent (27), and BioRoot RCS is strongly alkaline (28). Simple light microscopy in conjunction with this dye, however, gives reliable results. Rhodamine B can even be directly mixed with a strong alkali such as calcium hydroxide and does not lose its color, as was tested in pilot experiments.

The finding that AH Plus prevented leakage at the sealer-to-gutta-percha interface should not surprise, as epoxy resins bind directly to the gutta-percha. This is in contrast to all other types of "classic" sealer materials (29). Hydraulic CaSi sealers such as BioRoot RCS are inherently wet, and therefore unable to bind to a hydrophobic material such as gutta-percha. However, whether leakage along that interface is clinically important remains to be seen. The first hydraulic CaSi sealers have appeared about a decade ago on the dental market, and clinical problems, such as those that were seen with polycaprolactone-based materials in conjunction with a methacrylate-based sealer within a similar time frame (21), have not emerged thus far.

It can be stated that under the conditions of this study, washing out chemical remnants and/or alcohol-drying did not further improve root canal sealability or sealer adhesion in conjunction with AH Plus and BioRoot RCS when sealer-specific canal wall conditioning steps had been undertaken priorly. However, it needs to be considered that BioRoot RCS contains an aqueous phase and therefore sets without the need to draw water from the tooth and/or the periapical tissues. This may explain why its performance under current conditions was not affected by chemical drying with 80% ethanol prior to administering the paper points. Future studies should therefore assess the (potentially negative) effect of chemical drying with alcohol on the seal and dentin adhesion obtained with syringe-based hydraulic CaSi sealers, which contain glycol rather than a second aqueous component that secures their hydration and setting.

Conclusions

Washing out chemical remnants of standard root canal irrigation protocols with a physiological saline solution and/or drying with alcohol are frequently recommended. Our data obtained in a controlled *ex vivo* environment do not support these concepts in terms of improving root canal sealability with the sealers under investigation.

Acknowledgements

Matthias Zehnder declares a conflict of interest because of a patent related to a product that was used in this study: Dual Rinse HEDP (EP3284456A1; US10434038B2). The other authors report no conflicting interest related to this study.

Zusammenfassung

Einleitung

Die möglichst hermetische Abdichtung von Wurzelkanälen ist der letzte Schritt bei einer Wurzelkanalbehandlung. Jüngere Studien haben gezeigt, dass es hierbei eine Rolle spielt, wie man das Dentin in der Kanalwand konditioniert. Epoxidharz-basierte Sealer dichten besser ab, wenn die Kanalwand entkalkt wird. Hydraulische «biokeramische» Kalziumsilikatsealer hingegen binden direkt ans Kalzium im Dentin. Was unklar bleibt ist, ob man nach der Verwendung der entsprechenden Spüllösungen noch verbleibende Chemikalien mit physiologischer Kochsalzlösung ausspülen, oder gar das Dentin mittels Alkoholspülung chemisch trocknen soll. Dies wurde in dieser Studie untersucht.

Material und Methoden

Die Wurzelkanäle von einwurzeligen extrahierten menschlichen Zähnen wurden chemomechanisch aufbereitet, mit rotierenden Nickel-Titan-Instrumenten und einer kombinierten 3% NaOCl & 9% HEDP-Spüllösung. Danach wurde das Kanalsystem entweder nur mit NaOCl & HEDP nachgespült, oder für die Füllung mit Epoxidharzsealer (AH Plus) noch mit EDTA zusätzlich entkalkt. Vor der Wurzelkanalfüllung mit AH Plus oder einem hydraulischen CaSi-Sealer (BioRoot RCS) wurde dann entweder mit 5 ml physiologischer Kochsalzlösung, 5 ml 80% Ethanol, oder gar nicht nachgespült, und die Kanäle wurden mit auf das letzte rotierende Instrument passenden Papierspitzen getrocknet. Die Wurzelfüllung erfolgte mittels Guttapercha-Einstifttechnik und dem entsprechenden Sealer ($N = 9$ pro Spülprotokoll/Sealer). Nach Lagerung der Zähne für 7 Tage bei 37°C in 100% Feuchtigkeit wurde die Dichtigkeit der Wurzelfüllungen mittels Rhodamin B optisch untersucht, und zwar in Längsfragmenten der Wurzel zur Quantifizierung der Undichtigkeit, und in zusätzlichen Querschliffen.

Resultate

AH Plus dichtete leicht aber statistisch signifikant ($P < 0.05$) besser ab als der hydraulische CaSi-Sealer. Die Abschlusspülung hatte hierbei keinen Effekt auf die Dichtigkeit der Wurzelfüllung. Die undichten Stellen waren zwischen Sealer und Dentin bei AH Plus, und sowohl zwischen Sealer und Dentin als auch zwischen Guttapercha und Sealer bei BioRoot RCS.

Diskussion

In unserer Studie konnte kein positiver Effekt einer Abschluss-Spülung mittels physiologischer Kochsalzlösung oder 80% Ethanol auf die Dichtigkeit von sealerbasierten Wurzelkanalfüllungen gefunden werden.

Résumé

Introduction

L'obturation la plus hermétique possible des canaux radiculaires est la dernière étape d'un traitement canalaire. Des études récentes ont montré que la manière dont on conditionne la dentine des parois du canal joue un rôle crucial. Les ciments de scellement canalaire à base de résine époxy assurent une meilleure étanchéité lorsque la paroi du canal est décalcifiée. En revanche, les scellants hydrauliques "biocéramiques" à base de silicate de calcium se lient directement au calcium de la dentine. Ce qui reste incertain, c'est de savoir si, après l'application des solutions de rinçage correspondantes, il faut rincer les produits chimiques restants avec une solution saline physiologique ou même sécher chimiquement la dentine par rinçage à l'alcool. Ce point a été l'objet de notre investigation dans cette étude.

Matériels et méthodes

Les canaux radiculaires de dents humaines extraites à une racine ont été préparés par chimio-mécanique, en utilisant des instruments rotatifs en nickel-titane et une solution d'irrigation combinée de NaOCl 3% & HEDP 9%. Ensuite, le système canalaire a été soit rincé uniquement avec du NaOCl/HEDP, soit détartré avec de l'EDTA pour l'obturation avec du scellant de résine époxy (AH Plus). Avant l'obturation du canal radiculaire avec l'AH Plus ou avec un scellant hydraulique CaSi (BioRoot RCS), le rinçage a été effectué soit avec 5 ml de solution saline physiologique, soit avec 5 ml d'éthanol à 80%, soit sans rinçage du tout, et les canaux ont été séchés avec une pointe de papier adaptée au dernier instrument rotatif. L'obturation radiculaire a été réalisée à l'aide de la technique d'une pointe de gutta-percha et du scellant correspondant (N = 9 par protocole de rinçage/scellant). Après avoir conservé les dents pendant 7 jours à 37°C dans une humidité de 100%, l'étanchéité des obturations radiculaires a été examinée optiquement à l'aide de rhodamine B, sur des fragments longitudinaux de la racine pour quantifier les fuites, ainsi que sur des coupes transversales supplémentaires.

Résultats

AH Plus a scellé légèrement mais statistiquement significativement ($P < 0,05$) mieux que le scellant hydraulique à base de CaSi. Le rinçage final n'a pas eu d'effet sur l'étanchéité de l'obturation radiculaire. Les zones de fuite étaient situées entre le scellant et la dentine pour AH Plus, et à la fois entre le scellant et la dentine ainsi qu'entre la gutta-percha et le scellant pour BioRoot RCS.

Discussion

Dans notre étude, aucun effet positif d'un rinçage final avec une solution saline physiologique ou de l'éthanol à 80% sur l'étanchéité des obturations canalaires à base de scellant n'a été trouvé.

References

1. Neelakantan P, Subbarao C, Subbarao CV, De-Deus G, Zehnder M. The impact of root dentine conditioning on sealing ability and push-out bond strength of an epoxy resin root canal sealer. *Int Endod J.* 2011;44:491-498.
2. Fernandes Zancan R, Hadis M, Burgess D et al. A matched irrigation and obturation strategy for root canal therapy. *Sci Rep.* 2021;11:4666.
3. Ballal NV, Roy A, Zehnder M. Effect of sodium hypochlorite concentration in continuous chelation on dislodgement resistance of an epoxy resin and hydraulic calcium silicate sealer. *Polymers (Basel).* 2021;13
4. Neelakantan P, Sharma S, Shemesh H, Wesselink PR. Influence of irrigation sequence on the adhesion of root canal sealers to dentin: a fourier transform infrared spectroscopy and push-out bond strength analysis. *J Endod.* 2015;41:1108-1111.
5. Buchbinder M, Bartels HA. Criticism of the use of root canal cultures in evaluating antibiotic therapy. *Oral Surgery.* 1951
6. Zamany A, Spångberg LS. An effective method of inactivating chlorhexidine. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod.* 2002;93:617-620.
7. Dutner J, Mines P, Anderson A. Irrigation trends among American Association of Endodontists members: a web-based survey. *J Endod.* 2012;38:37-40.
8. Willershausen I, Wolf TG, Schmidtman I et al. Survey of root canal irrigating solutions used in dental practices within Germany. *Int Endod J.* 2015;48:654-660.
9. Pashley DH, Tay FR, Carvalho RM et al. From dry bonding to water-wet bonding to ethanol-wet bonding. A review of the interactions between dentin matrix and solvated resins using a macromodel of the hybrid layer. *Am J Dent.* 2007;20:7-20.
10. Hosoya N, Nomura M, Yoshikubo A, Arai T, Nakamura J, Cox CF. Effect of canal drying methods on the apical seal. *J Endod.* 2000;26:292-294.
11. Razmi H, Bolhari B, Karamzadeh Dashti N, Fazlyab M. The Effect of canal dryness on bond strength of bioceramic and epoxy-resin sealers after irrigation with sodium hypochlorite or chlorhexidine. *Iran Endod J.* 2016;11:129-133.
12. Reyes-Carmona JF, Felipe MS, Felipe WT. A phosphate-buffered saline intracanal dressing improves the biomineralization ability of mineral trioxide aggregate apical plugs. *J Endod.* 2010;36:1648-1652.
13. Arias-Moliz MT, Camilleri J. The effect of the final irrigant on the antimicrobial activity of root canal sealers. *J Dent.* 2016;52:30-36.
14. Zollinger A, Mohn D, Zeltner M, Zehnder M. Short-term storage stability of NaOCl solutions when combined with Dual Rinse HEDP. *Int Endod J.* 2018;51:691-696.
15. Zehnder M. Root canal irrigants. *J Endod.* 2006;32:389-398.
16. Neelakantan P, Varughese AA, Sharma S, Subbarao CV, Zehnder M, De-Deus G. Continuous chelation irrigation improves the adhesion of epoxy resin-based root canal sealer to root dentine. *Int Endod J.* 2012;45:1097-1102.
17. Wang JS, Bai W, Wang Y, Liang YH. Effect of different dentin moisture on the push-out strength of bioceramic root canal sealer. *J Dent Sci.* 2023;18:129-134.
18. Editorial Board of the Journal of Endodontics. Wanted: a base of evidence. *J Endod.* 2007;33:1401-1402.
19. Rechenberg DK, De-Deus G, Zehnder M. Potential systematic error in laboratory experiments on microbial leakage through filled root canals: review of published articles. *Int Endod J.* 2011;44:183-194.
20. Paque F, Sirtes G. Apical sealing ability of Resilon/Epiphany versus gutta-percha/AH Plus: immediate and 16-months leakage. *Int Endod J.* 2007;40:722-729.

21. Barborka BJ, Woodmansey KF, Glickman GN, Schneiderman E, He J. Long-term clinical outcome of teeth obturated with resilon. *J Endod.* 2017;43:556-560.
22. De-Deus G. Research that matters - root canal filling and leakage studies. *Int Endod J.* 2012;45:1063-1064.
23. Viapiana R, Moizadeh AT, Camilleri L, Wesselink PR, Tanomaru Filho M, Camilleri J. Porosity and sealing ability of root fillings with gutta-percha and BioRoot RCS or AH Plus sealers. Evaluation by three ex vivo methods. *Int Endod J.* 2016;49:774-782.
24. Saavedra FM, Pelepenko LE, Boyle WS, Zhang A, Staley C, Herzberg MC, Marciano MA, Lima BP. In vitro physicochemical characterization of five root canal sealers and their influence on an ex vivo oral multi-species biofilm community. *Int Endod J.* 2022;55:772-783.
25. Spångberg LS, Acierno TG, Yongbum Cha B. Influence of entrapped air on the accuracy of leakage studies using dye penetration methods. *J Endod.* 1989;15:548-551.
26. Camilleri J, Pitt Ford TR. Evaluation of the effect of tracer pH on the sealing ability of glass ionomer cement and mineral trioxide aggregate. *Journal of Materials Science: Materials in Medicine.* 2008;19:2941-2948.
27. Jorge J, Castro GR, Martines Utrera MA. Comparison among different pH values of Rhodamine B solution impregnated into mesoporous silica. *Orbital: The Electronic Journal of Chemistry.* 2013:23-29.
28. Khalil I, Naaman A, Camilleri J. Properties of tricalcium silicate sealers. *J Endod.* 2016;42:1529-1535.
29. Lee KW, Williams MC, Camps JJ, Pashley DH. Adhesion of endodontic sealers to dentin and gutta-percha. *J Endod.* 2002;28:684-688.