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Effect of Final Irrigation Protocols on Radicular Dentin Permeability and Push-Out bond Strength in Endodontically Treated Teeth

Efecto de distintos protocolos de irrigación final sobre la permeabilidad de la dentina radicular y la resistencia al desplazamiento de un cemento a base de resina en dientes tratados endodónticamente

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ABSTRACT: This study aimed to analyze the effect of several final irrigation protocols on tubular permeability and push-out bond strength of an epoxy resin-based cement to radicular dentin. The crowns of 110 human teeth were removed to standardize at 17mm in length. The root canals were instrumented and irrigated with 5ml of 5% NaOCl and 17% EDTA. The samples were then randomly divided into 5 groups (n=22) according to the final irrigation protocol. Subsequently, the samples were subdivided into two different groups to assess dentin permeability or the evaluation of the push-out bond strength. In Group 1, all samples displayed the formation of brown-like precipitates. Group 2 and Group 5 demonstrated cleaner dentin walls. The push-out bond test after 72 hours denoted a significant difference when comparing Group 1 vs Group 2 and Group 1 vs Group 3. After thermocycling, a significant difference is observed between Group 1 and all remaining groups. The irrigation protocol using distilled water prior to irrigation with chlorhexidine significantly reduces the formation of intracanal precipitates by avoiding interactions between irrigants. The push-out bond strength of epoxy resin-based cement is directly influenced by the precipitates formed in the root canal after irrigation and the time of thermocycling.

KEYWORDS: Chlorhexidine; Push-out bond strength; SEM; Sodium hypochlorite.

RESUMEN: Este estudio tuvo como objetivo analizar el efecto de diferentes protocolos de irrigación final sobre la permeabilidad tubular y la resistencia al desplazamiento de un cemento a base de resina epóxica sobre la dentina radicular. Se removieron las coronas de 110 dientes humanos para estandarizar las muestras a 17mm de longitud. Los conductos radiculares se instrumentaron e irrigaron con 5ml de NaOCl al 5% y EDTA al 17%. Luego, las muestras se dividieron al azar en 5 grupos (n=22) de acuerdo con el protocolo de irrigación final. Posteriormente, las muestras se subdividieron en dos grupos para evaluar la permeabilidad de la dentina o la evaluación de la resistencia al desplazamiento. En el grupo 1 todas las muestras mostraron la formación de precipitados de color marrón, por otro lado, los grupos 2 y 5 revelaron paredes de dentina más limpias. La prueba de push-out después de 72 horas de la obturación mostró una diferencia significativa al comparar el grupo 1 con los grupos 2 y 3. Posterior al termociclado, se observa una diferencia significativa al comparar el grupo 1 con los demás grupos experimentales. El protocolo de irrigación con agua destilada previo al uso de la clorhexidina reduce significativamente la formación de precipitados marrón al evitar interacciones entre irrigantes. La resistencia al desplazamiento del cemento a base de resina epóxica está directamente influenciada por los precipitados formados en el conducto radicular después de la irrigación y por el tiempo de termociclado.

PALABRAS CLAVE: Clorhexidina; Fuerza de unión push-out; SEM; Hipoclorito de sodio.

INTRODUCTION

The main objective of endodontic therapy is the prevention and treatment of pulp and periodontal lesions associated with pulp infection (1). Among the most important phases in endodontic treatment are the biomechanical preparation of the root canal, disinfection, and obturation (2). Moreover, biomechanical preparation and chemical disinfection are fundamental steps for microbial reduction within the root canal system (3,4).

Thus, studies recommend the use of different irrigants, such as sodium hypochlorite, ethylenediaminetetraacetic acid (EDTA), chlorhexidine, calcium hydroxide, distilled water, and even their combinations to take advantage of their different

properties and optimize the cleaning and disinfection of root canals (5-6).

Previously, a study showed that a combination of 2.5% sodium hypochlorite and 2% chlorhexidine was more effective for root canal disinfection, compared to their individual use (7). However, studies demonstrated that the combination of these substances produces a brown-like precipitate characterized as para-chloroaniline (PCA) (7,8). Further investigations corroborated that it is not PCA but the formation of several precipitates of guanidine groups (9-11).

Moreover, the effectiveness of the root canal obturation can be jeopardized due to the presence of the precipitate by acting as a type of chemical

smear layer, decreasing permeability in the apical third of the root canal, and acting as a physical barrier during the obturation (10,12).

Due to the synergy provoked when using the combination of irrigants, such as 2% chlorhexidine and 5% sodium hypochlorite, the main objective of this study was to analyze the effect of different final irrigation protocols in the formation of a brown-like precipitate, and its effects on the bond strength of an epoxy resin-based cement to radicular dentin.

MATERIALS AND METHODS

The investigation project and research protocol were approved by the Ethics Committee of Universidad de Costa Rica. Using an SBT diamond blade (Bay Technology South, San Clemente, CA, USA) on high-precision cutting equipment (Buehler Ltda., USA), the crowns of one hundred and ten unradicular teeth were removed 1mm below the cemento-enamel junction (CEJ), standardizing the specimen at 17mm in length. Subsequently, the specimens were inserted into flasks with sponges moistened with distilled water to simulate *ex vivo* conditions.

The root canals were instrumented with the Wave One Gold® Primary rotary system (Dentsply, Maillefer, Ballaigues, Switzerland). The canals were irrigated with 5ml of 5% NaOCl using a 27-gauge needle (Ultradent, South Jordan, UT, USA) at a 1mm working length. During the last irrigation with NaOCl, ultrasonic vibration was performed for 30 seconds. Patency was maintained with the aid of the #15 K-FILE file (Dentsply Maillefer, Ballaigues, Switzerland). Subsequently, all the samples were irrigated with 5ml of 17% EDTA solution for 3min, followed by 1min in distilled water. Then, the canals were dried.

The specimens were randomly divided into five groups (n=22), and the final irrigation was performed as described below: Group 1: 5ml of

5% NaOCl followed by 5ml of 2% Chlorhexidine (Ultradent, South Jordan, UT, USA), Group 2: 5ml of saline water, Group 3: 5ml of 5% NaOCl (the University of Costa Rica, Endodontics section protocol), Group 4: 5ml of 2% Chlorhexidine (Ultradent), Group 5: 5ml of saline water followed by 5ml of 2% Chlorhexidine (Ultradent).

Subsequently, the samples were subdivided into two different subgroups according to the evaluation: Subgroup 1 (n=10): Assessment of dentin permeability, Subgroup 2 (n=12): Resistance to displacement (Push-out bond strength).

ASSESSMENT OF DENTIN PERMEABILITY

The specimens were separated into two halves to evaluate the dentinal walls of the root canal. Five samples from each group were evaluated under a stereo microscope (Nikon, Melville, NY, USA). The cervical, middle, and apical thirds were evaluated. Representative microphotographs (15X) of each area were taken. Subsequently, the microphotographs were analyzed by two previously calibrated observers (kappa: 0.84) evaluating the presence or not of precipitates on the root canal walls. The groups received scores according to Arslan *et al.* (13) as described below: Score 0: no precipitates, dentin wall completely clean and free of debris; Score 1: precipitates or debris are present on less than half of the dentin surface (a slight coloration is observed); Score 2: precipitates or debris cover more than half of the dentin surface (slight staining covering more than half of the surface); Score 3: precipitates or debris completely cover the surface.

Then, the remaining five samples from each group were coated with a 300 Å gold-palladium layer, and examined by SEM-Scanning Electron Microscope (HITACHI, S-570, Japan), operating with 15Kv at different magnifications (500-3000X). Representative microphotographs of each root canal third were recorded, and subsequently,

analyzed by two previously calibrated observers (kappa: 0.91) evaluating the presence or obliteration of precipitates/debris in the dentinal tubules. The scoring system for microphotographs (500X) used was: Score 0: no presence of precipitates/debris, dentinal tubules clean and free of debris; Score 1: precipitates/debris is present on less than half of the dentin surface, partially obstructing the entrance to the dentinal tubules; Score 2: precipitates/debris densely cover the dentin surface, more than $\frac{3}{4}$ of the surface or entrance to the dentinal tubules is obstructed by the presence of debris/precipitates. The statistical analysis was performed using the Kruskal-Wallis and Mann-Whitney U test ($p=0.05$).

RESISTANCE TO DISPLACEMENT (PUSH-OUT BOND STRENGTH)

The root canals of 50 specimens were obturated with Wave One Gold® Primary gutta-percha cones (Dentsply) and Top Seal resin cement (Dentsply) using the single-cone filling technique according to the manufacturer's instructions. The samples were stored in PBS at 37°C for 72h. Subsequently, the push-out test was performed in five samples (72 hours after root canal obturation) from each group using a Universal Testing Machine (Tinius Olsen, Horsham, PA, USA) that applied a constant compressive force at 0.5mm/min. The remaining five samples per group were placed in the thermocycling equipment with 10,000 cycles, and then, the push-out test was performed.

To achieve the push-out test, each specimen was taken to an ISOMET precision machine (Buelher, NY, USA), in which 2mm thick dentin discs were prepared, providing a disc of the cervical, middle, and apical third. The discs were positioned in the universal testing machine applying a constant compressive force at 0.5mm min. The adhesive surface area was calculated individually. The push-out force value in MPa was calcu-

lated from the force kgf (maximum force applied before the displacement was recorded) converted to Newton (N) divided by the area in mm². Data were subjected to ANOVA, and the Bonferroni posthoc test ($p<0.05$). For the analysis of the type of failure that occurred, the specimens were subjected to visual analysis with the aid of a 10X magnification lens. The failures were classified as 1-Adhesive between dentin and cementum; 2-Adhesive between cementum and gutta-percha cone; 3-Cohesive of cement; and 4-Mixed failure.

RESULTS

DENTIN PERMEABILITY

The results of the data obtained for the analysis of the formation of brown-like precipitates on the dentin walls of the root canal are summarized in Table 1. In all samples of Group 1 (Positive Control), it was possible to observe the formation of brown-like precipitates covering all the surfaces (Figure 1). The statistical analysis indicates that there is a significant difference when comparing Group 1 vs Group 2 ($p=0.0195$). There was no significant difference between the other groups ($p>0.05$). Moreover, there is no significant statistical difference when comparing the different root thirds ($p>0.05$). In Group 2, however, the descriptive analysis allows us to observe that in Group 3, despite not forming a brown precipitate, it was possible to observe the presence of white precipitates in three samples, which could suggest the crystallization of hypochlorite from sodium remaining on the dentin walls. It is also important to highlight that in two samples from Group 4, specifically in the cervical third, a slight brown coloration was observed, and in one of the samples, a slight formation of white precipitates was observed.

The data obtained for the analysis of the permeability of the dentinal tubules in SEM are summarized in Table 2. In Group 1 (Positive

Control) (Figure 2), it was possible to observe the homogeneous formation of precipitates covering all the surfaces and obstructing more than $\frac{3}{4}$ parts of the root canal. The statistical analysis demonstrated that there is a significant difference when comparing Group 1 with Group 2 ($p=0.0184$). It also indicates a significant difference between Group 1 and Group 5 ($p=0.0006$). It is important

to highlight that there is a significant difference between Group 4 and Group 5 ($p=0.0464$).

In Group 2 the dentin canal walls were clean, and debris free (Figure 3). In Groups 3, 4, 5 and 6, the dentin surface can be observed with areas partially obstructed by precipitates and/or debris (Figure 4, Figure 5, Figure 6).

Table 1. Mean of the scores in the stereomicroscope analysis.

Group	G1	G2	G3	G4	G5
Cervical	3	0	0.6	0.6	0.2
Middle	3	0	0.6	0.6	0.2
Apical	2.3	0	0.6	0.4	0.2
Mean	2.767	0	0.6	0.533	0.2
SD	0.4041	0	0	0.115	0

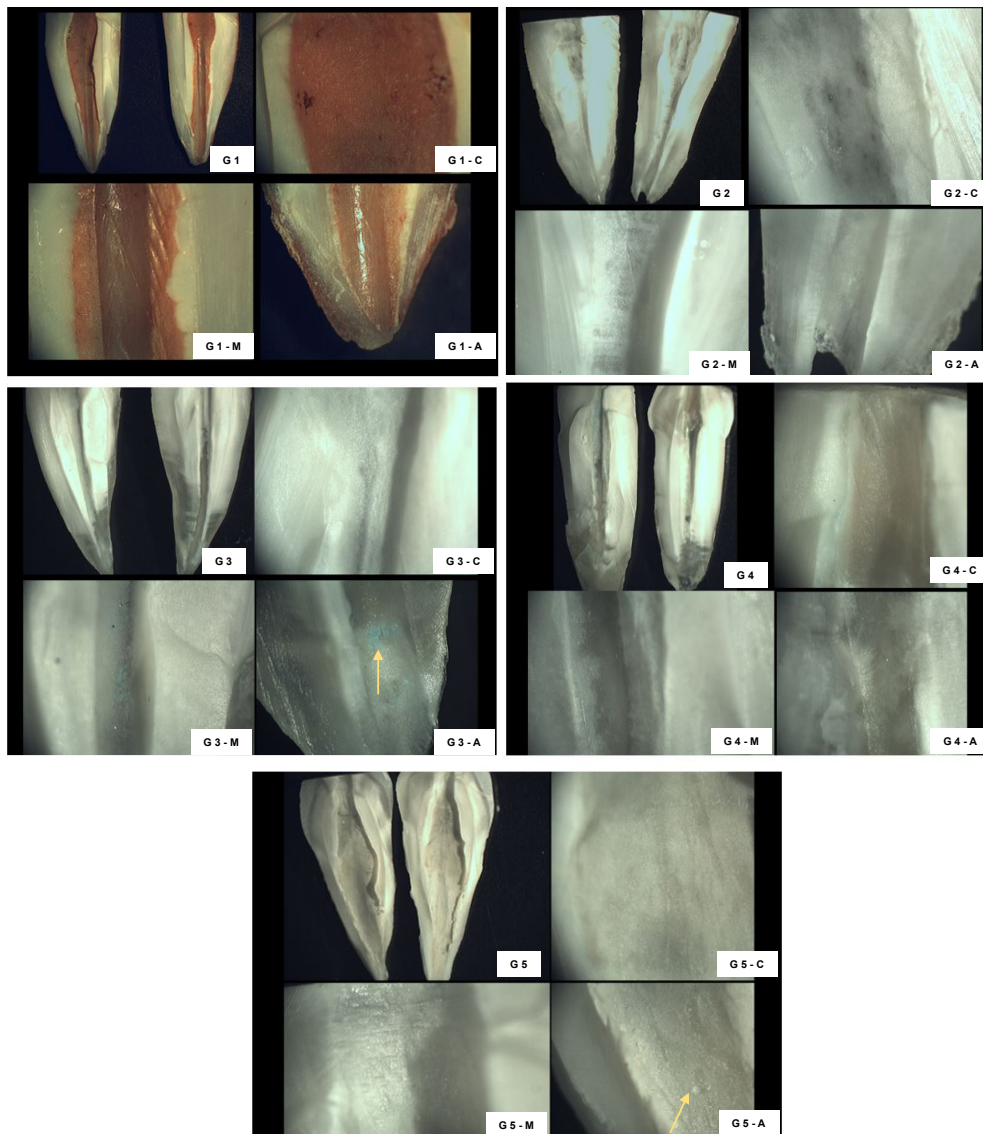


Figure 1. Representative photomicrographs of each group at Cervical (C), Middle (M) and Apical (A) thirds (5X-15X). In Group 1 was observed the formation of a brown-orange precipitate homogeneously covers the entire root dentin surface. In Group 2, the entire root dentin surface is observed clean with no precipitates. The formation of white precipitates (yellow arrow) with a slight blue hue is observed in the middle and apical (15X) of Group 3. A slight brown coloration is observed in the cervical area of Group 4 (15X). Group 5 samples are debris-free, just in one sample the presence of a small amount of white precipitate (yellow arrow) was observed at the apical third.

Table 2. Mean of the scores of the MEV analysis.

Group	G1	G2	G3	G4	G5
Cervical	3	0.2	1	1	0.6
Middle	3	0.2		1	0.4
Apical	3	0.2	0.6	1	0.4
Mean	3	0.2	0.866	1	0.466
SD	0	0	0.2309	0	0.115

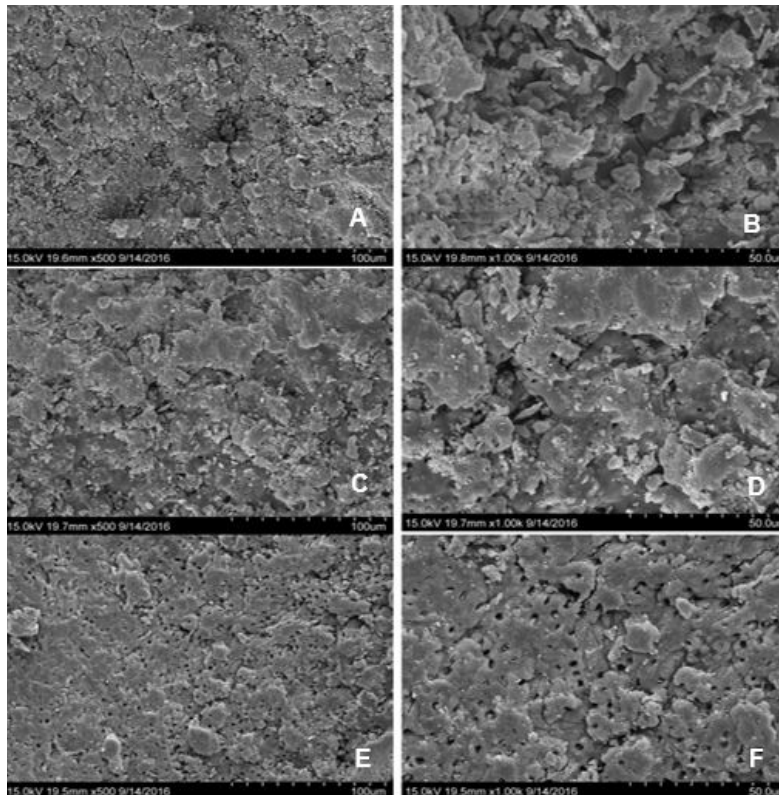


Figure 2. Representative SEM photomicrographs of Group 1. A. Cervical third. C Middle Third. E. Apical third. B, D, and F represent areas of higher magnification (1000X). It can be observed that more than $\frac{3}{4}$ parts of its surface are with precipitates that obstruct the entrance of the dentinal tubules.

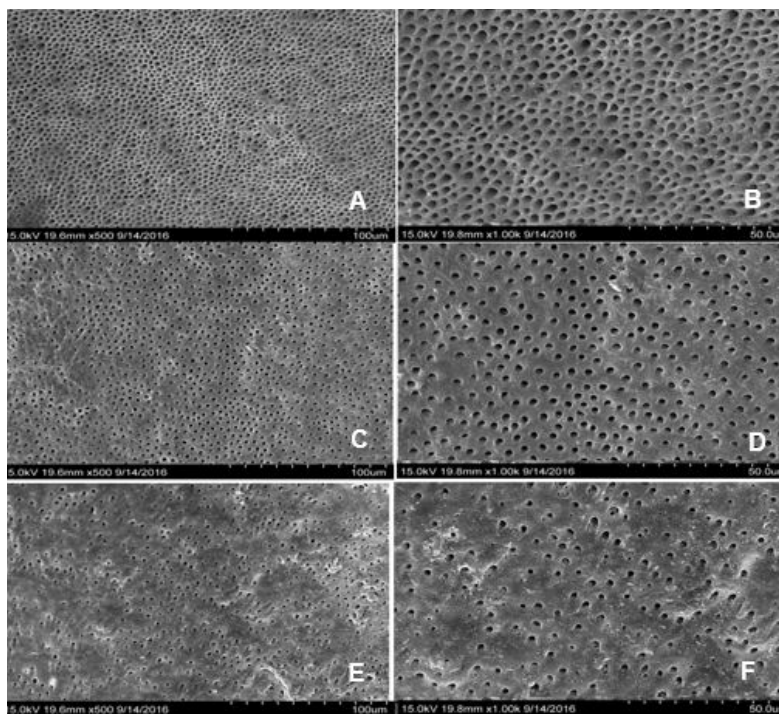


Figure 3. Representative SEM photomicrographs of Group 2. A. Cervical third. C Middle Third. E. Apical third. B, D, and F represent areas of higher magnification (1000X). The dentin surface is free of precipitates and/or debris.

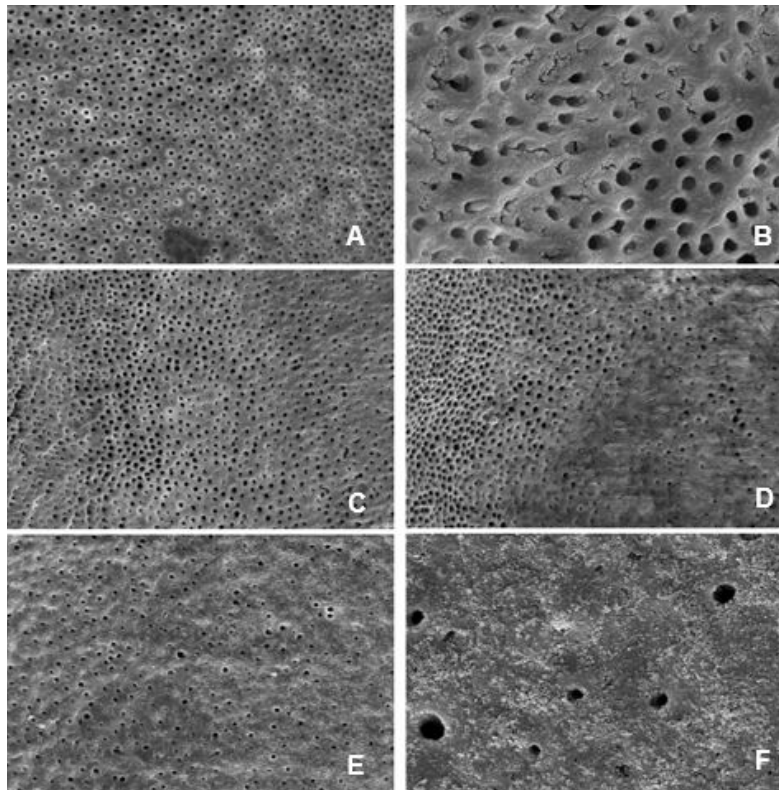


Figure 4. Representative SEM microphotographs of Group 3. A. Cervical third. C Middle Third. E. Apical third (500X). B, D, and F represent areas of higher magnification (1000X). The dentin surface can be observed with areas partially obstructed by precipitates and/or debris (less than half the surface).

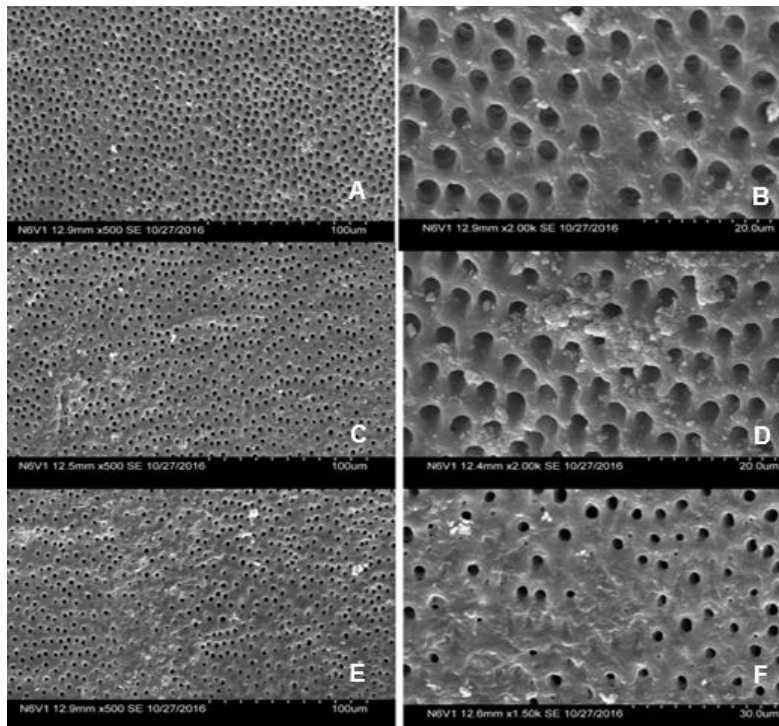


Figure 5. Representative SEM photomicrographs of Group 4. A. Cervical third. C Middle Third. E. Apical third. B, D, and F represent areas of higher magnification (1500-2000X). Small areas with partially obstructed dentinal tubules (less than half the surface) can be seen in D.

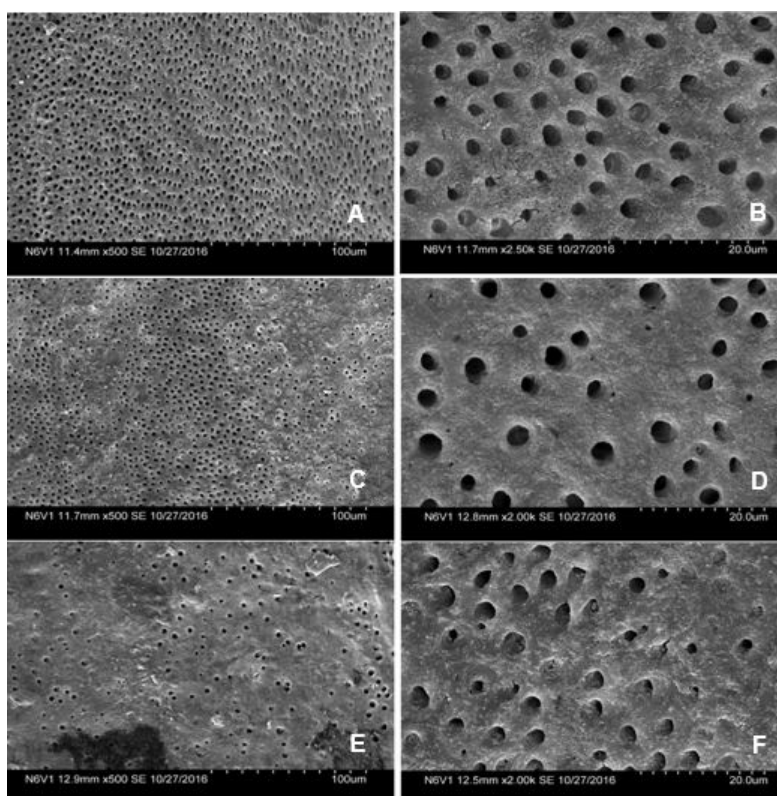


Figure 6. Representative SEM photomicrographs of Group 5. A. Cervical third. C Middle Third. E. Apical third. B, D, and F represent areas of higher magnification (2000-2500X). Small areas with partially obstructed dentinal tubules (less than half the surface) can be seen in D. However, it is observed that the dentin walls are cleaner and more permeable.

PUSH-OUT BOND STRENGTH

The results obtained from the push-out test are summarized in Table 3. After 72 hours, there was a significant difference when comparing Group 1 vs Group 2 ($p=0.009$) and between Group 1 vs Group 3 ($p=0.017$). When analyzing the data after thermocycling, a significant difference is observed when comparing Group 1 vs Group 2 ($p=0.02$), Group 1 vs Group 3 ($p=0.016$), and Group 1 vs Group 5 ($p=0.013$). In all groups, the statistical analysis showed that there is a significant difference

in the push-out bond strength when evaluating the root third factor, with groups 2, 3, 4, and 5 presenting statistical significance between the cervical vs middle and apical third ($p=0.009$). Moreover, no significant difference was observed between the middle vs apical thirds. In Group 1, the radicular third factor did not show a significant difference ($p\geq 0.05$). When evaluating the effect of thermocycling on resistance to displacement, it is observed that there is a statistically significant difference in all groups when evaluating the time factor (72h vs. After thermocycling) ($p\leq 0.05$).

Table 3. Mean values of push-out bond strength (MPa) for all groups in the different periods of time.

Group	G1	G2	G3	G4	G5
After 72h	3.956	7.639	7.100	6.259	5.712
After Termociclagem (10.000 ciclos)	1.312	5.021	3.998	3.001	3.988

DISCUSSION

The chemical and physical reactions promoted by the different solutions used within the endodontic irrigation protocol may generate adverse effects within the root canal system (14, 15). Specifically, the reaction between sodium hypochlorite with Chlorhexidine has generated controversy in the field of endodontics, due to the formation of brown-like precipitates (11,13,16). Initially, it was determined that these precipitates were PCA and that they could be carcinogenic, so it was recommended to avoid their intracanal combined use (17). However, recent investigations have questioned these results and showed that it is not a PCA precipitate, but a mixture of precipitates made up of guanidine groups attached to the Chlorhexidine aromatic system (9,10,16). Regardless of the chemical composition of the precipitates formed, the literature demonstrated that the formation of these debris and/or precipitates could act as an agent that affects dentin permeability, and thus, interfere with the adhesion of sealants used during obturation of root canals (12,17-19).

Our results confirmed the formation of brown-like precipitates covering the entire dentin surface along the root canal when the canal is irrigated with sodium hypochlorite followed by Chlorhexidine. Moreover, when analyzing the microphotographs of Group 4, it is worth noting the presence of a slight brown coloration in the cervical third. This could be because, in the cervical third, there is a greater surface area in which traces of sodium hypochlorite may remain, which when interacting with Chlorhexidine produces this coloration on a lesser scale. It should be noted that this finding did not occur in Group 5, since distilled water was used as an intermediate irrigant in this protocol. Despite not being statistically significant, this data is of clinical interest, since based on these results, it is recommended that in cases where Chlorhexidine is to be used as the final irrigant, irrigation with distilled water should

be carried out previously. Moreover, in Group 3 the presence of bluish-white precipitates along the root dentin surface was observed, probably the remains of sodium hypochlorite that crystallized when dried. Observing this detail, it can be seen that irrigation with hypochlorite leaves remnants in the dentin, which when dried are observed as crystals that partially obstruct the entrance of the dentinal tubules.

Dentin permeability is essential to achieve a mechanical interlocking of the sealer with the dentin (15-18). In theory, the presence of permeable dentinal tubules improves the penetration of the sealer and, therefore, the sealing of the root canal obturation (19). Our findings demonstrated that the resistance to dislodgement of an epoxy resin-based cement to radicular dentin is directly influenced by the presence of precipitates that obstruct dentin permeability. This statement is verified by comparing the bond strength of Group 1 (positive control) with Group 2 (negative control). However, it is observed that even though the samples of Group 3 presented white precipitates on the dentin surface, this did not directly affect their performance. In general, all groups presented greater bond strength at 72h compared to the groups after thermocycling. The thermocycling used in this study was for a duration of 10,000 cycles, which is similar to the restoration aging of 1 year (20). Although there was no statistically significant difference between groups 4 and 5, it was possible to detect a significant difference between Group 1 and 5, suggesting that the irrigation with distilled water between irrigating solutions has a positive effect on dentin permeability, and therefore, the sealing of the obturation material. This data is of clinical relevance since one of the fundamental objectives of endodontic therapy is to maintain an effective seal over time. Taking our results into consideration, we concluded that in cases where greater antimicrobial action is required and it is desired to implement the irrigation with Chlorhexidine, a final irrigation protocol

using distilled water is suggested to reduce the formation of intracanal precipitates by avoiding interactions between irrigants, and to promote a greater push-out bond strength of epoxy resin-based cement to radicular dentin.

AUTHOR CONTRIBUTION STATEMENT

Conceptualization and design, J.R-C.

Literature review: M.B.

Methodology and validation: J.R-C., E.H.M, and M.B.

Investigation and data collection, J.R-C., E.H.M, and M.B.

Resources: J.R-C.

Data analysis and interpretation: J.R-C., E.H.M, and M.B.

Writing-original draft preparation: J.R-C. and M.B.

Writing-review & editing: JJ.R-C., E.H.M, and M.B.

Supervision: J.R-C., M.B.

Project administration: J.R-C.

Funding acquisition: J.R-C.

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