

## Epoxy Resin-Based Root Canal Sealer Penetration into Dentin Tubules Does not Improve Root Filling Dislodgement Resistance

 Gustavo DE-DEUS,  Maria Claudia BRANDÃO,  Erick Miranda SOUZA,  Claudia REIS,  Kátia REIS,  Ricardo MACHADO,  Prasanna NEELAKANTAN

### ABSTRACT

**Objective:** This study sought to evaluate the effect of the penetration of an epoxy resin-based root canal sealer into dentin tubules on the force required to dislodge the root canal filling.

**Methods:** Sixty extracted human central incisors with single canals were decoronated, instrumented, and filled with gutta-percha and AH Plus sealer labeled with 0.1% rhodamine B dye. The roots were further sectioned horizontally at 3, 6, and 8 mm from the apex. The coronal surfaces of the resulting 180 slices were evaluated using confocal laser scanning microscopy to measure the amount of sealer that penetrated into the dentin tubules. To quantify the force required to dislodge the root filling material, the root fillings of the slices were subjected to a dislodgement resistance test (push-out). Spearman's rho correlation test was further used to test the correlation between the push-out bond strength and sealer penetration into the dentin tubules ( $P < 0.05$ ).

**Results:** No significant correlation was observed between sealer penetration into the dentin tubules and the force required to dislodge the root canal filling ( $P = 0.626$ ).

**Conclusion:** Following the results of this study, the penetrating ability of the AH Plus sealer into dentin tubules has no correlation with the force required to dislodge the root canal filling.

**Keywords:** Dentin tubules, dislodgement resistance, epoxy sealer

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From the Department of Endodontics (G.D.), Fluminense Federal University, Rio de Janeiro, Brazil; Department of Periodontology (M.C.B.), Central Airforce Hospital, Rio de Janeiro, Brazil; Department of Endodontics (E.M.S.), Federal University of Maranhão, Maranhão, Brazil; Department of Dental Clinic (C.R.), Federal University of Espírito Santo, Espírito Santo, Brazil; Department of Prosthodontics and Dental Materials (K.R.), Federal University of Rio de Janeiro, Rio de Janeiro, Brazil; Clinical Practice Limited to Endodontics (R.M. ✉ ricardo.machado.endo@gmail.com), Navegantes, Brazil; Department of Endodontics (P.N.), University of Hong Kong, Hong Kong.

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### HIGHLIGHTS

- Root canal sealers have been shown to extensively penetrate into the dentin tubules of root-filled teeth.
- To date, no strong evidence exists proving that tubular dentin sealer penetration influences intracanal resistance to the dislodgement of root fillings.
- The ability of AH Plus sealer in penetrating dentin tubules has no correlation to the force required to dislodge the root canal filling.

### INTRODUCTION

Most well-established root canal filling techniques employ a core material and a root canal sealer. This is based on the rationale that root canal sealers improve the overall quality of root fillings; in other words, without a root canal sealer, root fillings can easily allow the passage of oral and/or tissue fluids, which may become a possible nutritional source for remaining bacteria. This may primarily be attributed to the ability of the root canal sealers to enhance the interfacial adaptation between the gutta-percha and dentin walls (1, 2).

Regarding the adaptation to root canal walls, it has been hypothesized that sealer penetration into the dentin tubules can improve the force required to dislodge a root canal filling (3). Indeed, root canal sealers have been shown to extensively penetrate into the dentin tubules of root-filled teeth (4, 5). During the last few decades, sealer penetration inside dentin tissue has been recognized as a feature that might improve the sealability and dislodgement resistance of the filling material because of a close adaptation between the interfaces (3, 6, 7). Tao and Pashley (8) used an adhesive system to bond to coronal dentin and found that the dislodgement resistance and resin tags inside the dentin tubules were poorly correlated. However, the total volume of the inner tubular dentin is much greater than that of the coronal outer dentin (9). Therefore, in endodontics, there is a common belief that there is a meaningful contribution of dentin tubular sealer penetration to

the force required to dislodge the root canal filling materials. Theoretically speaking, this potential correlation might be due to the mechanical interlock between the sealer and the dentin structure. However, to date, no strong evidence exists proving that tubular dentin sealer penetration influences intracanal resistance to the dislodgement of root fillings.

Despite the lack of evidence, sealer penetration into dentin has been largely emphasized as a required feature of root canal sealers that can improve the retention of root canal fillings (3-5). This study was designed to assess the potential correlation between the epoxy resin-based root canal sealer penetration into dentin tubules and the dislodgement resistance of the root filling from the dentin walls. The null hypothesis was that there is no correlation between the sealer penetration and the force required to dislodge a root canal filling using gutta-percha and an epoxy resin-based root canal sealer.

## MATERIALS AND METHODS

### Sample size calculation

The ideal sample size for the study was determined using a Bivariate Normal Distribution Model and Correlation (Exact family, G\*Power 3.1.9 for Mac OS). The following values were used:  $r=0.5$ ,  $\alpha=0.05$ ,  $\beta=0.95$ , and correlation P for  $H_0=0$ . The results indicated a minimum sample size of 16 teeth and a critical  $r$  of 0.4259 as the upper limit for accepting  $H_0$ .

### Specimen selection

Firstly, extracted human maxillary central incisors ( $n=150$ ) were collected. Then, buccolingual and mesiodistal radiographs were taken to prove the existence of single and straight canals without anatomical complexities or previous endodontic treatments, ultimately resulting in 60 included teeth. The crowns were then sectioned to prepare samples with 13-mm standardized root lengths.

### Canal preparation

The working length was set at the apical foramen. The coronal and middle thirds were prepared using Gates Glidden drills (Dentsply Maillefer, Ballaigues, Switzerland); while the apical third was sequentially prepared in a crown-down sequence with sizes 100, 90, 80, and 70 hand K-files (Dentsply Maillefer). The canals were irrigated with 3 mL of 2.5% NaOCl (Fórmula & Ação, São Paulo, Brazil) after each instrument and then flushed with 3 mL of 17% EDTA (Fórmula & Ação) for 3 min. The canals were finally flushed with 3 mL of distilled water (Fórmula & Ação).

### Canal filling

The root canals were dried with paper points, and a size 70 master gutta-percha cone (Dentsply Maillefer) was fit into the root canal. To allow for the analysis of the sealer through a confocal laser scanning microscope (CLSM), the AH Plus sealer (Dentsply De Trey, Konstanz, Switzerland) was labeled with a 0.1% rhodamine B dye (Sigma-Aldrich, Saint Louis, USA). An ISO size 40 reamer (Dentsply Maillefer) was used to place 20  $\mu$ L of dyed AH Plus sealer into the canal in a counter-clockwise rotation. This quantity was determined using a micropipette. Then, the canals were filled by a lateral compaction technique

using B8 accessory cones (Tanari, São Paulo, Brazil). The roots were stored at 37 °C and 100% humidity for 7 days to allow complete setting of the sealer.

### Sample preparation for analyses

Three cross sectional dentin slices ( $1\pm 0.1$  mm thick) were obtained from each root at 3, 6, and 8 mm from the apex using a low-speed saw under continuous water irrigation, resulting in a total of 180 slices. The apical and coronal aspects of the specimens were microscopically examined before testing in order to confirm a circular canal shape.

The microscopic procedures to determine the percentage of sealer penetration into the dentin tubules followed the same routine in a previously published article (10). The coronal facing surface of each slice was microscopically examined after performing a standard metallographic preparation. A high-resolution stereomicroscope was used to obtain an image of the entire root surface from each slice. The external outline of each root canal and the internal circumference of the root canal walls were measured using AxioVision software (Carl Zeiss, MicroImaging GmbH, Munich, Germany). The total canal wall area was calculated for each section, and the data were stored. CLSM (Carl Zeiss, Jena, Germany) was used to further examine all the slices, and the images were recorded using the fluorescent mode. The areas along the canal walls in which the sealer penetrated the dentin tubules (i.e., rhodamine B dyed sealer tags) were outlined and measured using the AxioVision software. The percentage of sealer penetration into the dentin tubules in each section was calculated using the total canal wall area obtained from the high-resolution stereomicroscopic images. The operator was blinded, and all measurements were double checked. The second evaluation was performed 2 weeks later. This step was performed to ensure reproducibility.

### Assessing the dislodgement resistance of the root fillings

The root canal filling of each slice was loaded with a 0.5-mm diameter cylindrical plunger, which touched only the root filling. The load was applied at the apical-coronal direction to avoid any constriction interference due to the root canal taper. Loading was performed on a universal testing machine (EMIC DL200MF, São José dos Pinhais, Brazil) at a crosshead speed of 0.5 mm/min until dislocation occurred. To express the dislodgement resistance in MPa, the failure load was recorded in Newton and divided by the area of the interface between the inner root canal wall and the filling material (3, 11-13).

### Confocal laser scanning microscope control

To ensure that the rhodamine extension observed in the CLSM was equivalent to the sealer penetration, five maxillary central incisors were subjected to the same instrumentation as the experimental teeth with no attempt to remove the smear layer. Thus, the existing smear layer should prevent the diffusion of the rhodamine deep into the root canal walls, thus obstructing any dye pigmentation from appearing in the dentin tubules. The roots were filled with gutta-percha and labeled with AH Plus. After storage, these samples were sectioned and prepared for CLSM analysis (5, 14, 15). The analysis of these specimens showed very little or no sealer penetration in the three

root thirds (Figure 1a, b, c). All procedures were conducted according to the principles of the Declaration of Helsinki.

### Statistical analysis

The preliminary analysis of the raw data was indicative of non-adherence to a Gaussian distribution (D'Agostino & Pearson omnibus normality test). Spearman's rho correlation test was further used to test the correlation between the push-out bond strength and the tubular sealer penetration data. The significance level was set at 0.05.

### RESULTS

For the CLSM control group, no penetration of rhodamine B was observed in the dentin tubules, indicating that the diffusion of the dye from the AH Plus sealer did not occur in the smear-covered samples. All specimens showed variable but measurable sealer penetration into the dentin tubules (Figure 1d, l) ranging from 1.45% to 45.98% with an average of  $13.67 \pm 7.55\%$ . Data from the dislodgement resistance test varied from 0.28 to 10.77 MPa with a mean of  $3.41 \pm 1.76$  MPa. The central tendency values for this experiment are shown in Table 1. Spearman's rho correlation test could not determine a best-fit line between the two analyzed outcome variables ( $P=0.626$ ; Spearman's rho correlation coefficient,  $r_s=-0.037$ ).

### DISCUSSION

This study showed that sealer penetration into the dentin tubules was unable to improve the force required to dislodge

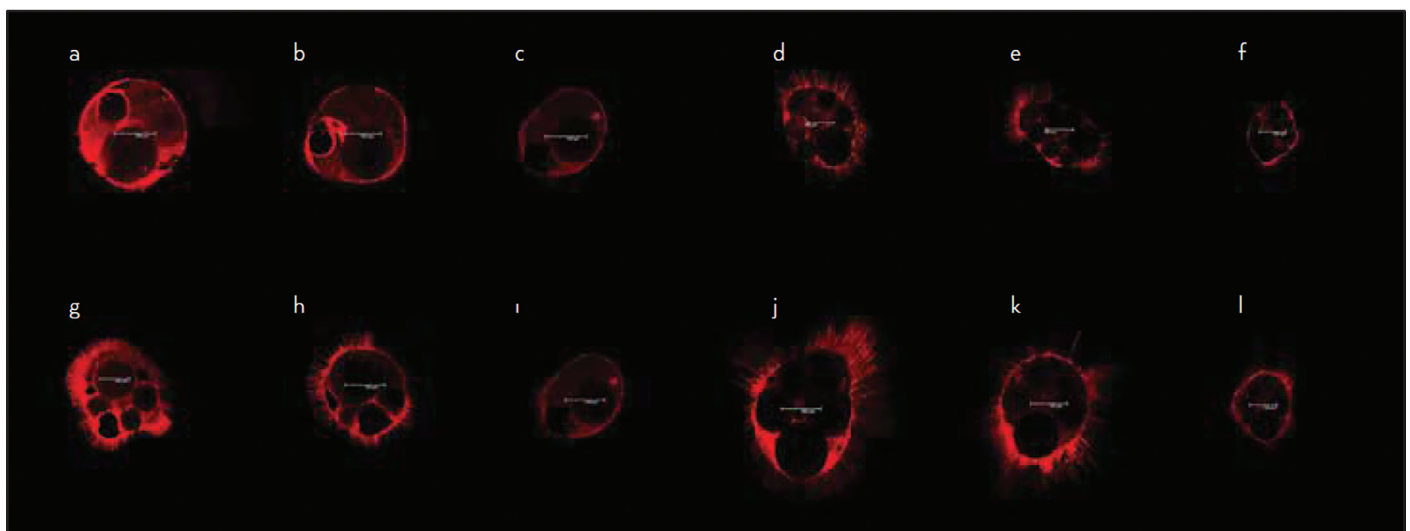
a root canal filling. Thus, the tested null hypothesis was confirmed. This means that sealer penetration into the dentin tubules is unable to explain the variation in the root filling dislodgement resistance ( $r_s=0.037$ ), and it does not support the concept that dentin penetration of a root canal sealer can enhance the intracanal retention of a root filling.

Tao and Pashley (11) used an adhesive system (Scotchbond Multi-Purpose Adhesive, 3M ESPE Adper, Saint Paul, USA) and calculated that 85% of the bond strength between the coronal resin restorations and dentin resulted from the hybrid layer and only 15% from the resin tags deep in the dentin tubules. Similarly, Lohbauer et al. (12) studied a self-etching adhesive system (G-Bond, GC corporations, Saint Alsip, USA) and a resin composite (Gradia direct GC corporations, Saint Alsip, USA) and observed that the resin tags did not improve dentin adhesion. Bitter et al. (13) did not find a correlation between the sealer projections of five different resin-based sealers on the dislodgement resistance of cemented glass-fiber posts following endodontic treatment within the root canal space. They used similar evaluation approaches and arrived at similar conclusions; however, their grouping set-up was different as they tested five different sealers at the same time. Statistically speaking, a standard correlation test accepts that any random factor is capable of affecting a single sample but not the others. Therefore, the condition to test for a potential correlation is violated when more than one experimental group is created, which possibly disturbs the correlation procedure

**TABLE 1.** Mean values (%) and standard deviation ( $\pm$ SD) of remaining filling material in root canal walls after retreatment

	Level 8 mm	Level 6 mm	Level 3 mm	All levels
Sealer penetration mean ( $\pm$ SD)	11.84 ( $\pm$ 7.90)	14.72 ( $\pm$ 7.39)	14.44 ( $\pm$ 7.13)	13.67 (7.55)
Median (IR)	10.82 (9.89)	14.63 (7.39)	14.07 (9.45)	13.73 (9.67)
Push-out mean ( $\pm$ SD)	3.45 ( $\pm$ 1.70)	3.64 ( $\pm$ 2.03)	3.13 ( $\pm$ 1.49)	3.41 (1.76)
Median (IR)	3.38 (2.26)	3.23 (2.58)	3.27 (1.91)	3.31 (1.98)

IR: Interquartile range



**Figure 1.** a-l. Confocal laser scanning microscopic that display the images from the control group (cervical third - (a) middle third - (b) and apical third - (c)) and the great variability in the tubular dentin sealer. Small penetration (cervical third - (d), middle third - (e), and apical third - (f)); medium penetration (cervical third - (g), middle third - (h), and apical third - (i)) and major penetration (cervical third - (j), middle third - (k), and apical third - (l))

itself. In fact, when attempting to verify a potential cause-and-effect correlation, the use of two or more experimental groups is not justifiable. In the present study, a large single group was chosen based on the statistical calculation.

Even in relation to the analysis of the force required to dislodge the root canal filling, the use of a single diameter stainless steel cylindrical plunger for all slices allows for the consideration of the hypothesis that, mainly in the cervical and middle thirds, some parts of the fillings may have been displaced in isolation, characterizing the existence of cohesive failure. However, some previous studies that analyzed the same variable also using a single diameter stainless steel cylindrical plungers for all slices and similar materials, pointed to a higher incidence of adhesive compared to cohesive failures (16, 17). Thus, it seems fair to say that isolated displacement of the filling parts (cohesive failure) may have occurred, albeit at a minimum frequency without the potential for changing the results of the present study.

Sealer penetration into the dentin tubules is generally accepted as a suitable consequence of the root filling; it is expected to improve the sealing ability and the force required to dislodge a root canal filling by mechanical interlocking, thereby preventing reinfection of the dentin tubules and the root canal (7). However, very recently, no correlation has been found between the penetration ability of a sealer into the dentin tubules and the sealability in non-bonded root fillings (10). The present results point out that the force required to dislodge a root canal filling is not dependent on the tubular dentin sealer penetration. Thus, it appears that the claim for the superiority of a root canal sealer in terms of both sealability and mechanical interlock due to its tubular dentin penetration should be considered a hypothesis.

Regardless of this, sealer penetration into the dentin tubules can be influenced by a number of other factors, including dentin permeability, smear layer removal and filling technique (18, 19). For instance, the permeability of radicular dentin is much lower than that of coronal dentin because of the decrease in the density of the dentin tubules from approximately 42,000 to 8,000 per mm<sup>2</sup> (7). In the present study, the results for sealer penetration into dentin varied from 1.45% to 45.98%, which can be regarded as a consequence of uncontrolled dentin permeability (Figure 1). Enhanced sclerotic dentin in the apical third, especially in older adults, may negatively impact sealer penetration into the dentin tissue (10, 18, 20). Even though dentin permeability was not controlled in this study and could have been responsible for the large variation in the results, the reliability of the correlation was not affected since the data regarding the variability of the force required to dislodge a root canal filling should agree with the variability in the sealer penetration. Moreover, a well-indicated correlation test assumes that a large variation in the raw data should be present in order to allow the test to detect any possible correlation.

In the present study, it was possible to clearly observe the amount of Rhodamine labeled sealer inside the dentin using the CLSM model rather than the commonly used scanning electron microscopy (SEM) evaluation. This improves upon the

traditional SEM analysis since it is possible to determine the total amount of sealer penetration into the dentin tubules (5, 20-23) while avoiding the drying process and alcohol dehydration required when using the conventional high-vacuum SEM, which might result in loss of the root canal sealer (20). CLSM provides observations under environmental conditions with no special specimen processing, resulting in a lower production of technique artifacts (24-26). Furthermore, the non-destructive characteristic of the CLSM evaluation allows for use of the same sample for other evaluations. Various previous reports used the terms push-out or shear-bond strength test to classify the method used in the present study (27-29). These terminologies were not used here since a non-adhesive root canal sealer was tested, and it does not make sense to call it an adhesion strength test. In addition, adhesion is only one aspect, among others, that influences the dislodgement resistance of a root canal filling.

Based on the present results, it is possible to question whether dentin sealer penetration is really tied to the quality of root fillings. Preventing the colonization of oral pathogens and reinfection of the root canal space in order to maintain long-term periapical health remains the ultimate goal of the treatment. However, to date, there is no evidence showing that sealer penetration into dentin tubules can improve root canal treatment outcomes. The same is true for the force required to dislodge a root canal filling and microleakage. Therefore, it should be questioned if i) sealer penetration into dentin tubules, ii) the force required to dislodge a root canal filling, and iii) microleakage are indeed desirable experimental outcome variables to rank the root filling materials and techniques. Considering the present results plus the findings obtained by De-Deus et al. (10), the use of sealer penetration into dentin can lead to experimental ranking of the root filling materials and techniques.

## CONCLUSION

Under the limitations of this in vitro study, the ability of AH Plus sealer in penetrating dentin tubules has no correlation to the force required to dislodge the root canal filling.

## Disclosures

**Ethical Approval:** Authors declared that the research was conducted according to the principles of the World Medical Association Declaration of Helsinki "Ethical Principles for Medical Research Involving Human Subjects", (amended in October 2013).

**Informed Consent:** N/A.

**Peer-review:** Externally peer-reviewed.

**Authorship Contributions:** Concept - G.D., E.M.S.; Design - G.D., E.M.S.; Supervision - G.D.; Resource - M.C.B.; Materials - M.C.B.; Data Collection and/or Processing - C.R., K.R.; Analysis and/or Interpretation - E.M.S.; Literature Review - M.C.B., C.R., K.R.; Writer - M.C.B., R.M., P.N.; Critical Review - G.D., E.M.S.

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