Original Research
Endodontic Therapy

Influence of bioceramic intracanal medication on the bond strength of bioceramic root canal sealer

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Declaration of Interests: The authors certify that they have no commercial or associative interest that represents a conflict of interest in connection with the manuscript.

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https://doi.org/10.1590/1807-3107bor-2023.vol37.0056

Submitted: September 28, 2022
Accepted for publication: December 20, 2022
Last revision: February 9, 2023

Abstract: To investigate the influence of the remaining volume of a new intracanal medication based on bioceramic compounds on the bond strength (BS) and formation of an adhesive interface between calcium silicate-based and epoxy resin-based root canal sealers. For this purpose, the specimens were distributed according to the intracanal medication (n = 26): Bio-C Temp (BCT) and Ultracal XS (UXS). The roots were scanned in microCT, and after 7 days, the medication was removed. Then a new scan was performed to evaluate the volume of medication remaining. Subsequently, 40 specimens were redistributed into 2 subgroups (n = 10) and filled according to the sealer used: AH Plus (AHP) and Bio-C Sealer (BCS), to assess the bond strength by using the push-out test, and the adhesive interface by confocal laser fluorescence microscopy (CLSM) and scanning electron microscopy (SEM). The *t* test showed a smaller remainder of BCT (1.77 ± 0.86) compared with UXS (10.47 ± 5.78), irrespective of the root third evaluated. The BS showed that teeth with BCT + BCS had higher bond strength values (3.70 ± 1.22) when compared to the other groups: BCT + AHP (2.15 ± 1.07), UXS + BCS (3.18 ± 1.09) and UXS + AHP (2.11 ± 1.02) ($p<0.001$). The cervical third had higher BS when compared with the middle and apical thirds ($p < 0.001$), and higher number of adhesive failures. The adhesive interface in SEM and CLSM images showed better adaptation for the association between BCT + BCS. Intracanal medication and silicate-based endodontic sealer appeared to interact chemically by forming a biomineralizing layer, allowing for an increase in the bond strength and forming an adhesive interface between the materials, with no or less gap formation.

Keywords: Adhesives; Root Canal Obturation; Calcium Hydroxide.

Introduction

The prime purpose of the cleaning shaping procedures is to manage intracanal infection by achieving a significant reduction in the intracanal bacterial load and infected dentin.[1] However, the condition of suboptimal disinfection of fully shaped canals has been confirmed by the literature, which points out that approximately 40 to 60% of root canals still remain infected.[2,3] Hence, intracanal medication between sessions has been used over the decades as an aid to improving the effectiveness of the

disinfection process after performing full cleaning and shaping procedures.[2-4]

Calcium hydroxide ($Ca(OH)_2$), the traditional intracanal dressing that has been used for decades as the first choice in Endodontic therapy, is a treatment that has been strongly supported in the literature.[2] However, recently, the incorporation of bioceramic compounds,[5,6] has brought new perspectives for the potential improvement of root canal disinfection between appointments. The term bioceramic was created to differentiate tricalcium silicate-based hydraulic cements from Portland cements, since bioceramic cements are purer and have bioactive potential.[7,8]

The intracanal bioceramic dressing Bio-C Temp® (Angelus, Londrina, Brazil) has antimicrobial action and capacity for bioactivity.[9] It is indicated for use as intracanal medicament due to its properties of high alkalinity (pH = 12) and high radiopacity (≥ 9 mm Al) that comply with ISO-6876 specifications (International Organization for Standardization 2012). Furthermore, Bio-C Temp® is a material that has low solubility, thus allowing the medication to remain in contact with the canal walls for a long period of time, continuously and gradually releasing hydroxyl (OH^-) and Ca2+ ions. This results in increasing the pH of the medium, thereby making the environment unsuitable for bacterial growth and favoring tissue repair.[9]

Prior to the filling procedure it is, however, necessary to remove the intracanal dressing as completely as possible. This procedure is well-known to be technically challenging, and largely depends on the canal anatomy; in fact, up to now, no technique has been capable of completely removing intracanal dressing from oval/irregular canals.[8]

This was the background against which the purpose of the present study emerged; namely, to investigate the influence of the remainder of a new intracanal dressing based on bioceramic compounds in the root canal on the quality of root filling, by a series of cascading experiments designed to: a) assess the quality of the adhesive interface; b) determine the filling ability; c) evaluate the sealer penetration into dentinal tubules and, d) measure the bond strength of the root-filling.

In sequence, this study will report the potential correlation of the outcome parameters tested with the amount of intracanal dressing that remained in the root canal.

Methodology

Sample size calculation

Based on a preliminary study, the following parameters were considered for the sample size: probability level of $\alpha = 0.05$ and statistical power of 0.9. The estimated minimum number of specimens was 20 for microCT analysis, 8 for the push-out test and 4 for the adhesive interface analysis.

Sample selection and scanning

After approval of the local Ethics Committee (N°. 43163321.7.0000.5419), 80 mandibular first molars, freshly-extracted for periodontal reasons were obtained and kept in 0.1% thymol solution for no longer than 2 months. The exclusion criteria were teeth with open apices, presence of caries, restorations, or fractures. As a result, 70 teeth were selected and scanned using a micro-CT device (SkyScan 1174; Bruker-microCT, Kontich, Belgium) with an image pixel size of 14.25 μm, operated at 70 kV and 114 mA, 360° rotation around the vertical axis, rotation step of 0.5°, and frame averaging of 5, with a 1.0- mm-thick aluminum filter. After this, the projection images captured were reconstructed (NRecon v.1.6.10; Bruker-microCT) providing axial cross sections of the inner structure of the teeth by using standardized parameters for beam hardening (30%), ring artifact correction of 5, and similar contrast limits. The volume of interest extended from the cemento-enamel junction to the apex of the root, resulting in the acquisition of 800–900 cross-sections per tooth.[9]

Subsequently, using the CTAn v.1.18 (Bruker-microCT) program, two-dimensional morphometric data (area, perimeter, circularity, larger diameter and smaller diameter) were obtained for each millimeter of the root canals, in the apical 10 mm of the root. Then, using the 3D Analysis tool, the three-dimensional parameters of volume (in mm^3), surface area (in mm^2) and 3D geometry (Structure

Model Index; SMI) of the root canals, from the enamel-sealer junction to the apical foramen, were calculated.[10] The shape of the canal was established by calculating the average of the ratio between the largest and smallest diameters of the canal (mean aspect ratio) at each millimeter of the apical 10 mm.[11] Thus, fifty-two distal roots with flattened canals with an average ratio between the largest and smallest diameters equal to or greater than 4 mm were selected.[12]

Root canal preparation

After sample selection, the root canals were irrigated with 2 mL of 2.5% sodium hypochlorite (NaOCl) (Fisher Scientific Company, Ottawa, Canadá) using a disposable plastic syringe (Ultradent Products Inc., South Jordan, USA) and 0.30 mm diameter NaviTip needle (Ultradent). The root canals were passively explored with type K #10 and #15 stainless steel files (Dentsply Sirona Endodontics, Ballaigues, Switzerland) until the tip coincided with the apical foramen, reaching the real length of the teeth. From this measure, 0.5 mm was subtracted to establish the working length (WL).

The biomechanical preparation of the distal canals was performed using the reciprocating motion instrumentation technique with the instruments Wave One Gold Glider (Dentsply Maillefer, Ballaigues, Switzerland) for glide path, and then the canals were prepared using the instruments Wave One Gold Large 45.05 (Dentsply Maillefer, Ballaigues, Switzerland). The canals were prepared in accordance with the manufacturer's recommendations, and the instrument was used passively, with pecking movements and, at every 3 advances, it was removed from the canal and cleaned with gauze, until the (WL) was reached. Each time the instrument was removed, the canals were irrigated with 2.5% NaOCl, aspirated and flooded with the use of a disposable plastic syringe and NaviTip needle.

Once the preparation was completed, the canals were irrigated with 5 mL of 2.5% sodium hypochlorite and then filled with 5 mL of 17% ethylenediaminetetraacetic acid (EDTA) for 3 minutes to remove the smear layer. Subsequently, the canals were irrigated with 10 mL of distilled water to neutralize the residual effects of NaOCl and EDTA. The canals were dried with absorbent paper tips (Dentsply Maillefer, Ballaigues, Switzerland)

Intracanal Medication

After canal preparation, the specimens were distributed homogeneously and divided into 2 balanced experimental groups according to the intracanal medication used, taking into account the three-dimensional parameters of volume and surface area obtained in the initial micro-CT scan.

Group I (n = 26): The specimens were filled with medication based on bioceramic compounds - Bio-C Temp (Angelus, Londrina, Brazil), using the syringe and needle system recommended by the manufacturer.

Group II (n = 26): The specimens were filled with medication based on calcium hydroxide paste - Ultracal XS (Ultradent Products Inc, South Jordan, USA), using the syringe and needle system recommended by the manufacturer.

After inserting the intracanal medication, the specimens were kept in an oven (37°C, 100% relative humidity).

Intracanal Medication Removal

After 14 days, to remove the medication, the final instrument used in the biomechanical preparation was introduced (Wave One Large – 45. /05), in the working length, associated with conventional irrigation with syringe and needle, with 5 mL of 2.5% NaOCl. Then, irrigation, aspiration and flooding with 3 mL of 17% EDTA, at 2 mm from the working length, were performed for 3 minutes.

After this, of the 52 specimens, forty distal canals were filled by the single-cone technique to assess the bond strength by means of the push-out test; the adhesive interface by means of confocal laser fluorescence microscopy and scanning electron microscopy.

Experiment #1: Determining the volume of the intracanal medications remaining in the canal

After removing the intracanal medication, the scan was performed. Images were processed,

reconstructed and analyzed according to the scanning parameters described above. Using the 3D analysis tool (CTAn), the location and volume of the remaining intracanal medications were evaluated. Qualitative analysis of the three-dimensional models was performed using the CTVol (Bruker-microCT).

Filling Procedure

At this time, the specimens were redistributed into 2 subgroups A and B (n = 20), according to the sealer used: Epoxy resin-based sealer (AH Plus, Dentsply Maillefer, Ballaigues, Switzerland) and Bioceramic-based sealer (Bio-C Sealer, Angelus, Londrina, Paraná, Brazil): GIA (n = 10): UltraCal XS + AH Plus; GIB (n = 10): Bio-C Temp + AH Plus; GIIA (n = 10): UltraCal XS + AH Plus; GIIB (n = 10): Bio-C Temp + Bio-C Sealer.

Experiment #2: Bond strength test

After filling the root canals, specimens (n = 40) were sectioned and three slices of dentin 1.0 mm (± 0.1 mm) thick were obtained from each root third. For the push-out test, the first two slices of each third were selected, with the third slice of each third destined for analysis by scanning electron microscopy (SEM).

Metal rods with active tip of 0.6 mm, 0.4 mm and 0.25 mm in diameter were used, compatible with the diameter of the root canal in the cervical, middle and apical thirds, respectively. The Instron machine was activated at a speed of 0.5 mm/min-1 until the sealer of the filling material was displaced. Then the bond strength was calculated in Mpa.

For failure pattern analysis, all slices were evaluated at 25x magnification under an optical stereomicroscope (Leica M165C, Leica Mikrosysteme Vertrieb GmbH, Wetzlar, Germany). Failures were determined in percentages and classified into one of the following subtypes: a) bonding to dentin: if the filling material was dislodged from the dentin; b) adhesive to the filling material: if the gutta-percha had dislodged from the sealer; c) mixed: when gutta-percha was dislodged from both the dentin and the sealer; d) cohesiveness in the dentin: when there was a fracture in the dentin; e) cohesiveness in the sealer: when fracture occurred in the sealer.

Experiment #3: Quality of the adhesive interface

For analysis by SEM, the third dentin slice of each root third (cervical, middle and apical) was used; these were not submitted to the push-out test. The preparation for SEM was performed by polishing the dentin specimens with abrasive paper of decreasing granulation until grammage 1200. Then, the specimens were rinsed in distilled water and superficially decalcified in 6M hydrochloric acid (HCl) for 30 seconds, and deproteinized in 2% NaOCl for 10 minutes.

Subsequently, the specimens were rinsed with deionized water and fixed with 3% glutaraldehyde buffered at a pH of 7.4 with 0.1 M sodium cacodylate at 4°C, for 12 hours. After fixation, the dentin slices were immersed in 0.1 M sodium cacodylate (pH 7.4) for 1 hour, with 3 successive changes, and rinsed with distilled water for 1 minute. After this procedure, they were dehydrated in a series of grades of alcohol at increasing concentrations (25°, 50°, 60°, 70°, 80°, 96°GL) for 20 minutes each, and at a concentration of 100°GL for 1 hour, followed by immersion of the specimens in hexamethyldisilizane for 10 minutes.

Once dehydrated, the specimens were fixed on cylindrical aluminum stubs (10 x 10mm) using double-sided adhesive tape. After vacuum metallization, the specimens were analyzed in a scanning electron microscope (JSM 5410, JEOL Ltd., Tokyo, Japan) operating at 20 KV and representative images were captured at 22, 40 and 120x. At 120x magnification, twelve measurements were taken at equidistant points, at the obturation-dentin interface to identify and quantify the presence of empty spaces (gaps).

To assess the adaptation of the filling material to the root canal wall, after the use of intracanal medication, the following scores were assigned: 0 (most sections did not show gaps between sealer and dentin); 1 (most sections showed some minor flaws,

< 1 μm between sealer and dentin); 2 (most sections showed many gaps, from 1 μm to 10 μm, between sealer and dentin) and 3 (most sections showed no adaptation between sealer and dentin, with gaps > 10 μm). Measurements were performed by a single calibrated examiner (Kappa test - intra-examiner agreement of k = 0.87), according to the methodology described in a previous studies.[13,14]

Experiment #4: Tubular dentinal sealer penetration

For the analysis of confocal laser scanning microscopy with fluorescence, the following dyes were used: Fluo-3-AM (Thermo Fisher Scientific, Waltham, USA) for the different intracanal medications (Bio-C Temp and Ultracal XS), Calcein-AM (Sigma-Aldrich, Merck KGaA, Darmstadt, Germany) for sealer based on bioceramic compounds (Bio-C Temp) and Rhodamine B (Sigma-Aldrich, Merck KGaA, Darmstadt, Germany) for resin sealer (AH Plus), respectively. The remaining 12 samples were used and distributed according to the medication and filling protocol: Group 1 (n=6): Calcium hydroxide paste-based intracanal medication – Ultracal XS (Ultradent Products Inc, South Jordan, USA) with addition of Fluo-3 dye. Of these 6 samples, 3 were filled with AH Plus filling sealer associated with the dye Rhodamine B 0.1% (G1A), and 3 samples were filled with Bio-C Sealer, a sealer associated with the dye Calcein (G1B). Group 2 (n = 6): Intracanal medication based on bioceramic compounds - Bio-C Temp (Angelus, Brazil) associated with Fluo-3 dye. Of these 6 samples, 3 were filled with AH Plus filling sealer associated with the dye Rhodamine B 0.1% (G2A), and 3 samples were filled with Bio-C Sealer, a sealer associated with the dye Calcein (G2B).

Subsequently, the samples were sectioned into 1 mm-thick slices and qualitatively evaluated by means of Leica TCS-SPS inverted fluorescence laser confocal scanning microscopy (Leica, Mannheim, Germany). Images of the filled areas were captured using the epifluorescence mode with absorption and emission wavelengths for Rhodamine B of 553/568 nm, for Fluo-3 of 506/526 nm and for Calcein of 360/449 nm, respectively, with use of the Leica Application Suite-Advanced Fluorescence software (Leica Systems).

The samples were analyzed 10 μm below the sample surface using an objective lens with 10x, 20x, 50x and 100x magnifications, in a field of view of 5x5mm, with a resolution of 512x512 pixels. Qualitative analysis of the slices was performed for each group, subgroup and thirds in which sealer penetration and the density of tags formed were observed.

Statistical analysis

Statistical analyses were described below according to the experiments. All tests were performed using the SigmaStat statistical software (Systat Software Inc., Chicago, USA).

Experiment #1: Determining the volume of the intracanal medications remaining in the root canals

Data on the percentage of volume of intracanal medication remnants were submitted to the t-test.

Experiment #2: Bond strength test

Bond strength data were submitted to two-way Analysis of Variance (ANOVA) to assess the effect of the factors "intracanal medication" (Calcium Hydroxide and Bio C Temp) and "obturator sealer" (AH Plus, and Bio C Sealer).

To evaluate the effect of the "root third" sub-plot on bond strength, the data were submitted to the repeated measures ANOVA test. An analysis was performed for the factor "intracanal medication" and another for "obturator sealer". As the data were not normal (Shapiro-Wil, $p < 0.05$), they were transformed into ranks or log10, for "intracanal medication" and for "obturator sealer", respectively, thereby reaching normality and homogeneity of variance. The Tukey test was used for multiple comparisons (α=0.05). The effect of the factors "intracanal medication" and "obturator sealer" and the sub-plot "root third" on the failure pattern in the push-out test was also evaluated. For this, the Chi-square test of proportions ($\alpha = 0.05$) was used.

Experiment #3: Quality of the adhesive interface and Experiment #4: sealer penetration into dentinal tubules

The non-parametric Kruskal-Wallis test complemented by the Student-Newman-Keuls test ($p < 0.05$) and were used to analyze the data regarding the adaptation of the filling material to the dentinal walls.

Results

Experiment #1: Determining the volume of the remaining intracanal medications

The mean and standard deviation values for the percentage of remnant volume of the different types of intracanal medications within the root canals after 14 days of medication, in the different root thirds are shown in Table 1.

The t-test showed a statistically significant difference in the percentage of remaining volume of intracanal medication ($p < 0.0001$) (Table 1), with a smaller amount of remaining intracanal medication based on bioceramic compounds (Bio-C Temp) (1.77 ± 0.86) when compared with calcium hydroxide-based medication (Ultracal XS) (10.47 ± 5.78), irrespective of the root third evaluated.

Qualitative analysis of three-dimensional models and axial sections of the distal root before and after removal of intracanal medications showed a remnant of medication in the root canal, irrespective of medication composition (Figure 1). When comparing the remaining intracanal medication in relation to chemical composition, it could be observed that the medication based on bioceramic compounds (Bio-C Temp) remained homogeneous and regular, especially in the apical third (Figure 1A).

In contrast, calcium hydroxide-based intracanal medication (Ultracal XS) was more brittle and irregularly arranged along the entire root canal, especially in the middle and cervical thirds and in the polar areas (Figure 1B).

Experiment #2: Bond strength test

The two-factor analysis of variance showed a statistically significant difference for the factors intracanal medication and root third, and for the interaction of the factors intracanal medication x root third ($p<0.0001$). Relative to intracanal medication, it was observed that teeth that received intracanal medication based on bioceramic compounds (Bio-C Temp) and filled with bioceramic sealer (Bio-C Sealer) had higher bond strength values (3.70 ± 1.22) when compared with teeth that received medication based on bioceramic compounds and filled with epoxy resin sealer (AH Plus) (2.15 ± 1.07), medication based on calcium hydroxide (Ultracal XS) and filled with bioceramic sealer (3.18 ± 1.09) or with epoxy resin sealer (AH Plus) (2.11 ± 1.02) ($p < 0.001$) (Table 2).

Relative to the root thirds, irrespective of intracanal medication and sealer, the cervical third had higher bond strength values when compared with the middle third ($p < 0.001$), with bond strength values for the middle third being higher than those of the apical third ($p < 0.001$) (Table 3).

The split-plot analysis of variance showed that bond strength values were influenced by the intracanal medication factor and root third subplot ($p < 0.001$) (Table 3).

Failure pattern analysis

Failure pattern data is shown in Table 4. The chi-square test showed the highest percentage of dentin bonding failures for teeth that received intracanal medication based on bioceramic compounds, irrespective of the sealer used ($p < 0.05$).

For teeth that received medication based on calcium hydroxide, a higher percentage of adhesive

Table 1. Mean ± standard deviation and minimum and maximum percentage value (%) of remaining volume of intracanal medication (Bio-C Temp and Ultracal XS) after using the last instrument, conventional syringe and needle irrigation with 2.5 sodium hypochlorite % and 17% EDTA application for 3 minutes.

	Bio-C Temp	Ultracal XS
Remaining volume (%)	1.77 ± 0.86 A	10.47 ± 5.78 B
	(0.17–2.96)	(3.68–23.91)

Uppercase letters signify statistical difference between columns (t test; $p < 0.0001$).

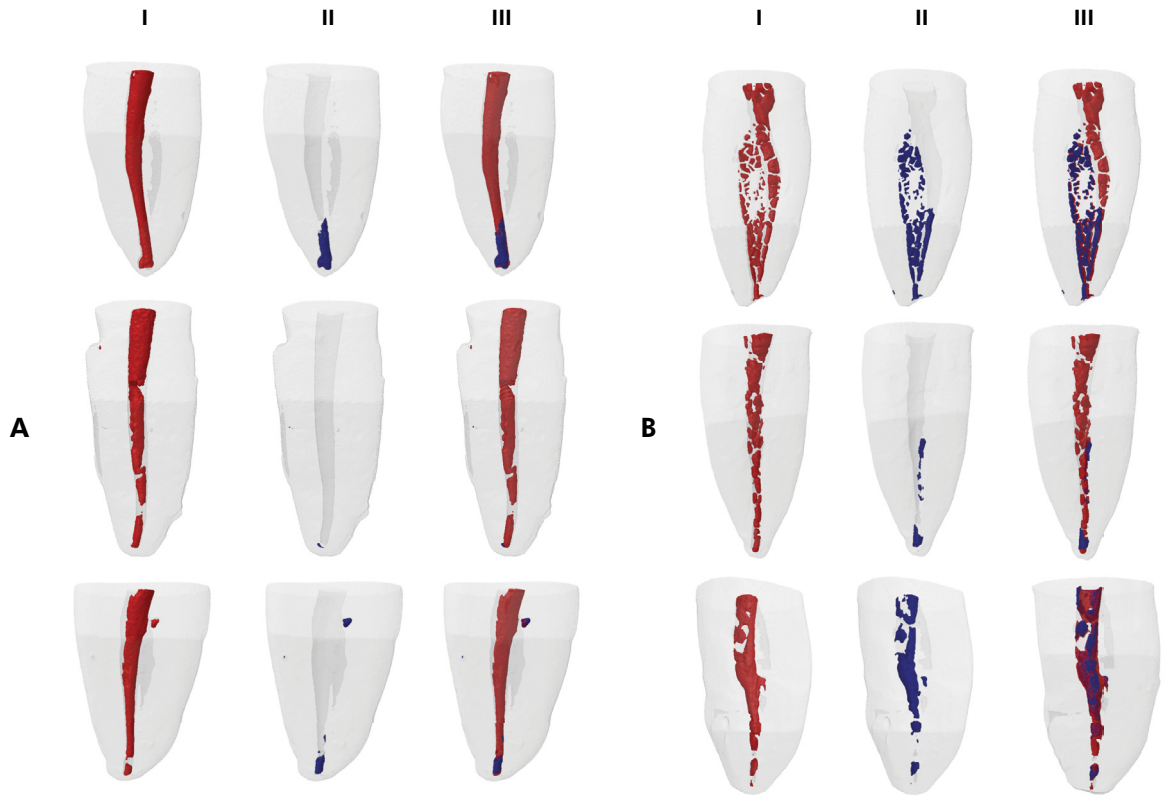

Figure 1. Three-dimensional models in microcomputed tomography images before and after removal of intracanal medication based on: (A) bioceramic compounds – Bio-C Temp (Angelus, Londrina, Paraná, Brazil) and (B): calcium hydroxide – Ultracal XS (Ultradent Products Inc., South Jordan, UT, USA). In red (I): filling the root canal with intracanal medication. In blue (II): remnant of intracanal medication after removal with final instrument and conventional irrigation with sodium hypochlorite. (III) Superimposition of images before and after intracanal medication removal.

Table 2. Mean and standard deviation of the bond strength values (Mpa) found for the push-out test in groups according to intracanal medication (Bio-C Temp or Ultracal XS) and filling sealer (AH Plus and Bio-C Sealer).

Variable	MPa
Bio-C Temp + AH Plus	2.15 + 1.07 A
Ultracal XS + AH Plus	2.11 + 1.02 A
Bio-C Temp + Bio-C Sealer	3.70 + 1.22 C
Ultracal XS + Bio-C Sealer	3.18 + 1.09 B

Uppercase letters mean statistical difference between lines ($p < 0.0001$).

failures to the filling material was observed, followed by mixed failures, irrespective of the sealer used ($p < 0.05$). No cohesive failures were observed in any of the groups evaluated.

Experiment #3: Quality of the adhesive interface

Considering the analysis of the electromicrographs (SEM) of the slices, it was observed that the adaptation showed a uniform pattern in all root thirds, irrespective of the type of medication and/or sealer evaluated. Thus, the data were analyzed taking into account the factors intracanal medication and sealing sealer, as shown in Table 5.

The non-parametric Kruskal-Wallis test showed differences in adaptation to different medications and sealing sealers ($p = 0.01$). Relative to intracanal medication, medication based on bioceramic compounds showed better adaptation when compared with the group that received medication based on calcium hydroxide ($p < 0.001$), irrespective

Table 3. Mean and standard deviation of the bond strength values (Mpa) found for the push-out test of root thirds (cervical, middle and apical) according to the intracanal medication (Bio-C Temp or Ultracal XS) and root canal sealer (AH Plus and Bio-C Sealer).

Variable	Coronal	Middle	Apical
Bio-C Temp + AH Plus	3.35 + 1.02 Aa	2.28 + 0.85 Ab	1.60 + 1.16 Ac
Ultracal XS + AH Plus	3.19 + 0.69 Aa	2.33 + 0.52 Ab	1.26 + 0.24 Ac
Bio-C Temp + Bio-C Sealer	5.00 + 0.37 Ba	3.98 + 0.44 Bb	2.26 + 0.39 Ac
Ultracal XS + Bio-C Sealer	4.69 + 0.54 Ba	3.58 + 0.39 Bb	2.28 + 0.32 Ac

Uppercase letters mean statistical difference between rows, and lowercase letters mean statistical difference between columns ($p < 0.0001$).

Table 4. Percentage values of failure types after push-out test by groups according to intracanal medication (Bio-C Temp or Ultracal XS), sealer (Bio-C Sealer and Ah Plus) and root thirds (cervical, middle and apical).

Variable	Thirds		
Bio-C Temp + AH Plus			
Ad	80	10	10
Af	20	70	10
M	0	20	20
Cd	0	0	0
Cf	0	0	0
Ultracal XS + AH Plus			
Ad	50	25	15
Af	60	25	25
M	40	30	30
Cd	0	0	0
Cf	0	0	0
Bio-C Temp + Bio-C Sealer			
Ad	20	40	20
Af	0	0	30
M	20	20	20
Cd	0	0	0
Cf	0	0	0
Ultracal XS + Bio-C Sealer			
Ad	20	10	40
Af	80	40	30
M	20	20	20
Cd	0	0	0
Cf	0	0	0

Ad: adhesive to dentin; Af: adhesive to the filling material; M: mixed; Cd: cohesive of the dentin; Cf: cohesive of the filling material; C: cervical; M: middle; A: apical.

of the filling sealer used. It is noteworthy that when associated with intracanal medication based on calcium hydroxide with bioceramic filling sealer, a misfit of the filling material-root dentin interface was observed.

The qualitative analysis showed that in the specimens in which the medication based on bioceramic compounds (Bio-C Temp) and epoxy resin sealer (AH Plus) was used, gaps occurred in practically all specimens, not only in the polar areas of the flat canals. Furthermore, gaps were observed at the interface with the dentin and there were also gaps between sealer/cone (Figure 2).

In specimens in which intracanal medication (Bio-C Temp) and bioceramic-based sealer (Bio-C Sealer) were used, better adaptation of the filling material to root dentin was observed, also in polar areas (Figure 3), areas with juxtaposition of the adhesive interface and fewer interfacial flaws between sealer/dentin; and when they occurred, the gaps were smaller than they were in the other groups.

In contrast, in specimens in which calcium hydroxide-based intracanal medication (Utracal XS) and epoxy resin-based sealer (AH Plus) were used, gap formation was observed in practically all specimens, mainly in the polar areas of the flat canals. However, it was also possible to observe areas of interface with good adaptation between sealer/dentin (Figure 4).

In specimens in which calcium hydroxide-based intracanal medication (Utracal XS) and bioceramic sealer (Bio-C Sealer) were used, there was a lack

of adaptation of the material to dentin, with gap formation in practically all specimens, not only in the polar areas of the flattened canals (Figure 5).

It is noteworthy that it was not possible to differentiate between medication and root canal sealer in SEM images in any of the groups studied.

Table 5. Percentage distribution of the types of adaptation of the restorative material to root dentin after the use of different intracanal medications (Bio-C Temp and Ultracal XS).

Variable	Adaptation (%)			
Ultracal XS + AH Plus	10 C	10:00 AM	80 D	12:00 AM
Ultracal XS + Bio-C Sealer	30 B	0 C	60 C	10 B
Bio-C Temp + AH Plus	30 B	40 B	30 B	12:00 AM
Bio-C Temp + Bio-C Sealer	70 A	10:00 AM	20 A	12:00 AM

Adaptation criteria: Good (sections without gaps); Fair (sections with faults < 1μm); Poor (sections with many gaps, between 1 μm and 10 μm); No adaptation (sections with gaps > 10 μm).

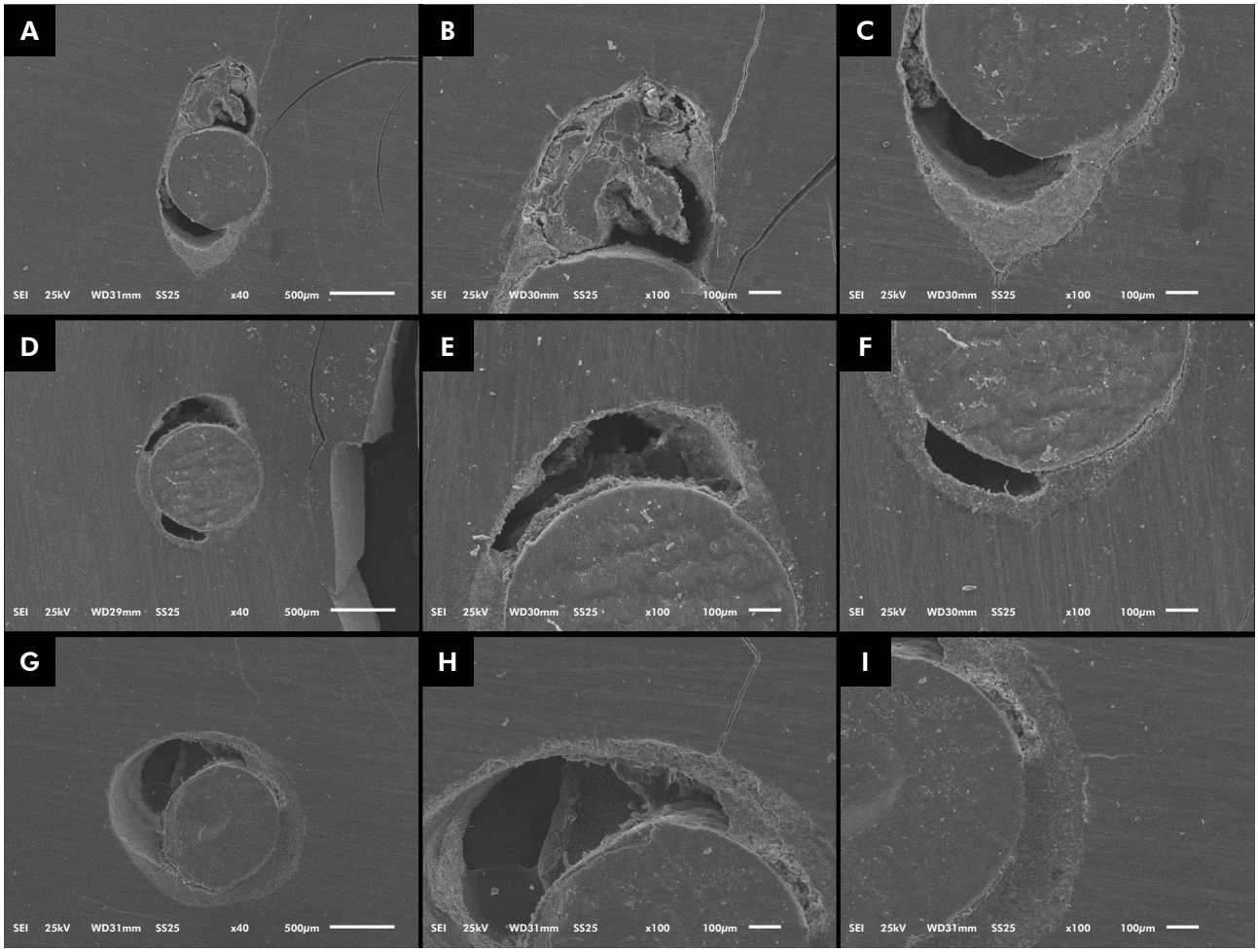

Figure 2. Photomicrographs of the adhesive interface of teeth in which an intracanal medication based on bioceramic compounds (Bio-C Temp) and resinous sealer (AH Plus) were used. The formation of gaps was observed throughout the circumference of the specimens, including in the polar and flat areas. Note the presence of gaps smaller than 1μm at the interface with the dentin as well as gaps between sealer/gutta-percha cone, showing evidence of reasonable adaptation between the filling material and dentin surface.

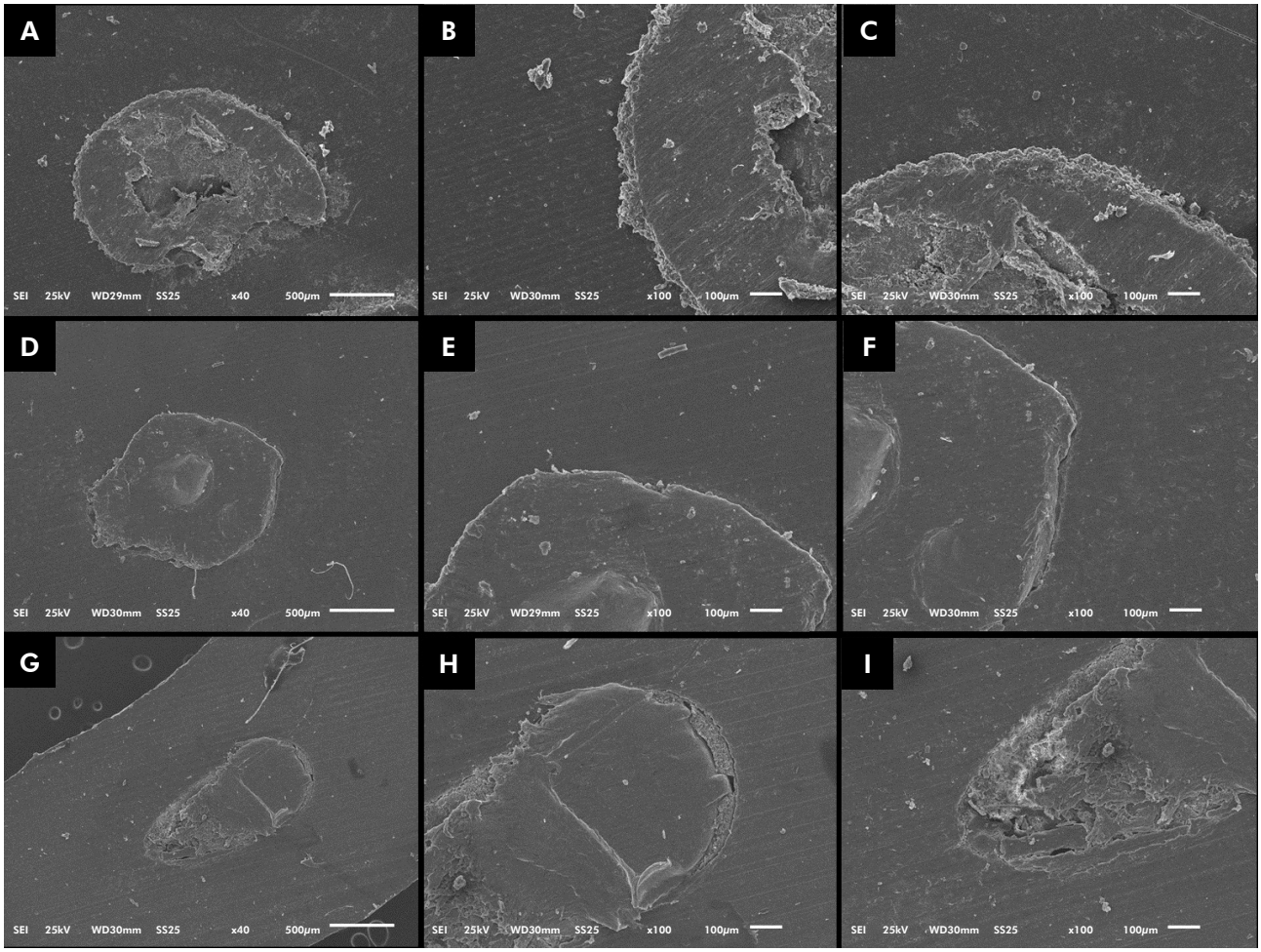

Figure 3. Photomicrographs of the adhesive interface of teeth in which intracanal medication (Bio-C Temp) and bioceramic-based sealer (Bio-C Sealer) were used. In these teeth a better adaptation of the filling material to root dentin was observed, including polar and flattening areas. Also noted, was the presence of areas with juxtaposition of the adhesive interface in the polar areas and a smaller number of interfacial flaws between sealer/dentin. Nevertheless, there was evidence of a smaller number of gaps, with better adaptation of the interface between filling material and dentin surface.

Experiment #4: Tubular dentinal sealer penetration

Analysis of the images in CLSM allowed us to observe that for the group that received medication based on bioceramic compounds and fillings with bioceramic sealer, there was greater penetration of intracanal medication (in fluorescent green) and root canal sealer (in fluorescent blue) into the dentinal tubules, in a regular and homogeneous manner, with the formation of longer tags (Figure 6A). In the analysis of the dentin surface, the formation of a uniform and thick layer was observed, as well as the presence of shorter tags with a product resulting from interaction between the intracanal medication/sealer (in light blue).

For the group that received intracanal medication based on bioceramic compounds and filled with epoxy-resin sealer, there was irregular penetration of the sealer (in fluorescent red) into the dentinal tubules, with the formation of shorter tags without continuity (Figure 6B). On the dentin surface, in parts it was possible to see a thin layer representing the interaction (in orange) of the intracanal medication (in fluorescent green) and the root canal sealer (in fluorescent red) (Figure 6B). In polar areas, it was also possible to note that

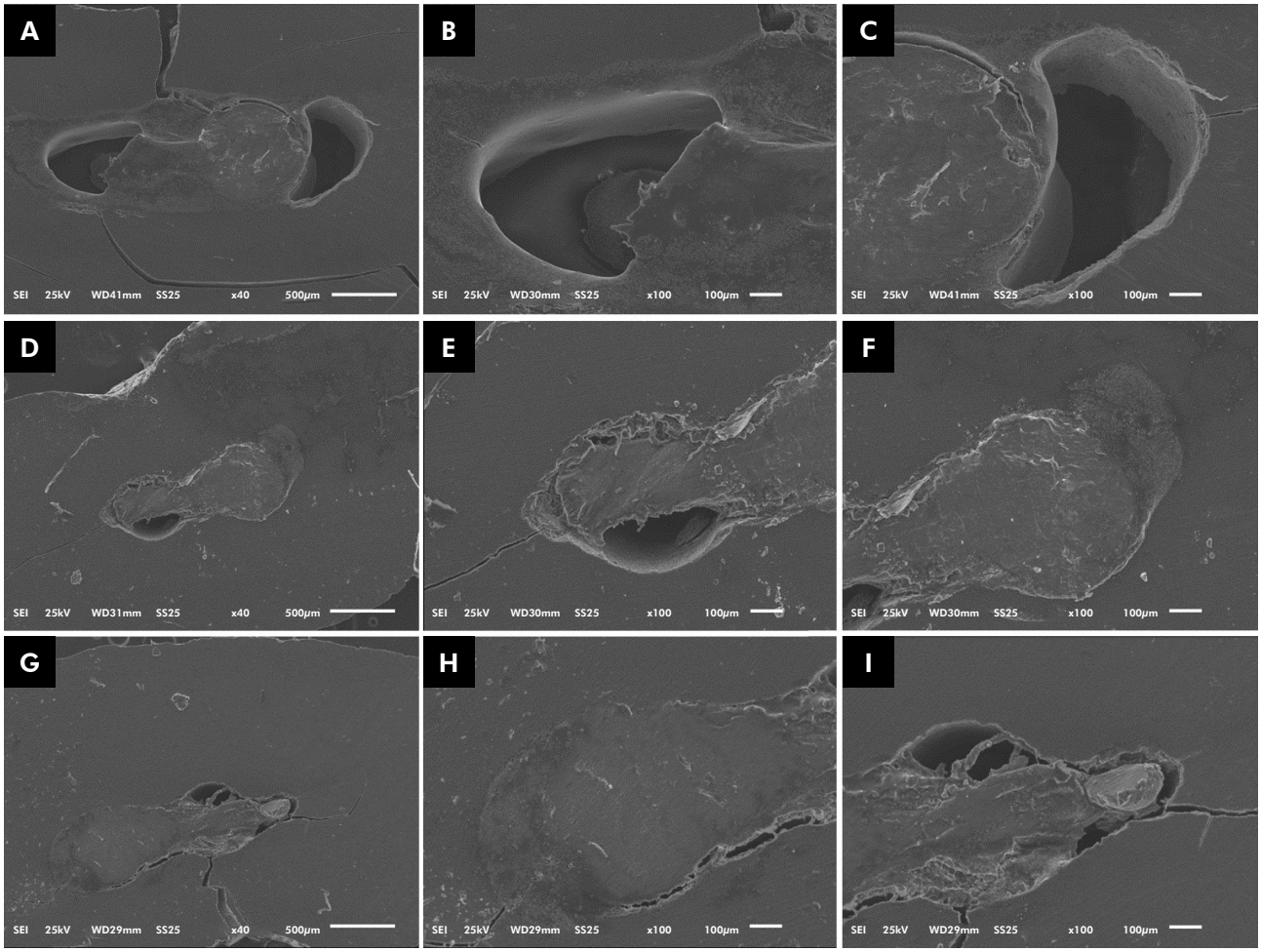

Figure 4. Photomicrographs of the adhesive interface of teeth in which calcium hydroxide-based intracanal medication (Ultracal XS) and epoxy resin-based sealer (AH Plus) were used. The formation of gaps can be noted in practically all specimens, mainly in the polar and flat areas. However, despite the observation of areas of reasonable adaptation at the interface between filling material and dentin surface, there was evidence of the presence of gaps greater than 1 µm, confirming poor adaptation of the adhesive interface.

the intracanal medication (in fluorescent green) acted as a physical barrier between the sealer and the dentin wall, with no chemical interaction between them.

The canals that received intracanal medication based on calcium hydroxide (Ultracal XS) and bioceramic-based sealer (Bio-C Sealer) showed lower penetration of medication (in fluorescent green) and sealer (in fluorescent blue) into the dentinal tubules (Figure 7A). On the dentin surface, there was a predominance of root canal sealer with the presence of short, irregular and misshapen tags, without formation of the layer of interaction between intracanal medication and sealer.

For the group that received intracanal medication based on calcium hydroxide (Ultracal XS) and filling with epoxy resin-based sealer (AH Plus), in most specimens it could be observed that the sealer did not penetrate into the dentinal tubules throughout the entire circumference of the canal, with no formation of tags. Furthermore, it was possible to note that there was no formation of a layer resulting from the interaction between intracanal medication and root canal sealer (Figure 7B). In analysis of the dentin surface, it was noted that the intracanal medication remained homogeneously spread throughout the surface, without interaction with the sealer, preventing sealer penetration.

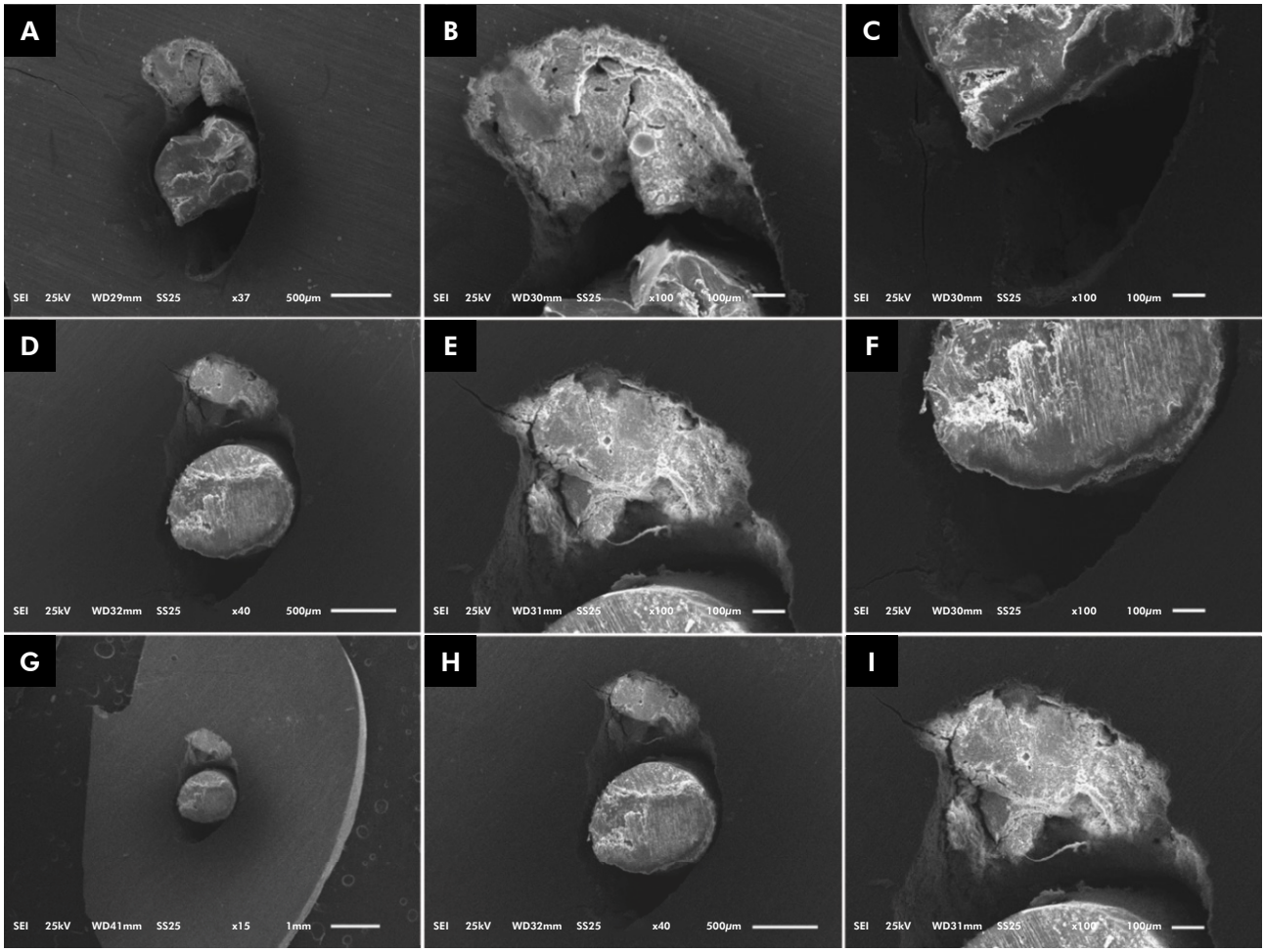

Figure 5. Photomicrographs of the adhesive interface of teeth in which calcium hydroxide-based intracanal medication (Ultracal XS) and bioceramic-based sealer (Bio-C Sealer) were used. There was a lack of adaptation of the material to the dentin, with the formation of gaps, along the entire circumference of the adhesive interface, not only in the polar and flat areas. Note the presence of gaps larger than 1μm, confirming poor adaptation of the adhesive interface.

Furthermore, it was observed that the sealer was limited to the lumen of the root canal.

Discussion

The present study evaluated the influence of the remnant of a bioceramic-based intracanal medication with bioceramics sealers, on the quality of the adhesive interface and presence of gaps, as well as the impact of the remnant of intracanal medication on bond strength in significantly oval root canals.

Micro-CT analysis showed a smaller remaining volume of intracanal medication based on bioceramic compounds, mainly in the apical third, since its formulation has a high concentration of free CaO, low concentration of C_3S and C_2S and a long chain polymer. The low concentration of C_3S and C_2S causes the crystals formed by the hydration reaction to separate due to the amount of material present and the interference of the polymer, making it difficult for these crystals to intertwine and consequently to harden the product,[8,9] which facilitates its removal, especially in flattened areas.[10,15,16]

To the contrary, medication based on calcium hydroxide, in an aqueous solution, dissociates into calcium and hydroxyl ions; reducing its solubility and facilitating its penetration into dentinal tubules and polar areas,[15-18] which may explain the larger

remaining volume, adhering to the walls of root canals acting as a mechanical barrier.[6,10,19] Nevertheless, the qualitative analysis of the three-dimensional models allowed us to observe a brittle aspect of the remnant of the hydroxide-based medication, in comparison with the bioceramic, which had a more homogeneous and uniform appearance.

Whereas the SEM analysis showed greater adaptation of the adhesive interface, especially in the flattened areas, since most sections did not show gaps between the filling material and the dentin, areas with juxtaposition of the adhesive interface and fewer interfacial flaws between sealer/dentin, corroborating the higher number of adhesive failures to dentin, observed after the push-out test.

In contrast, the lowest bond strength values were observed for teeth that received calcium hydroxide-based medication, irrespective of the filling sealer used, since the adhesion of epoxy resin-based

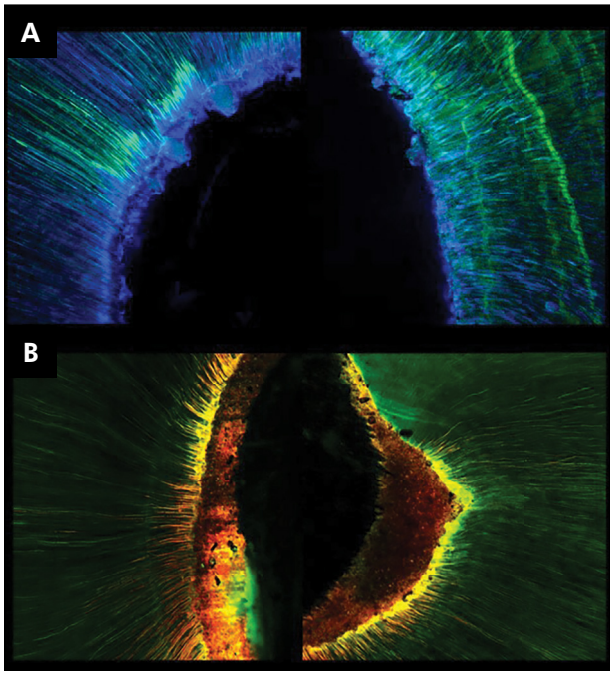

Figure 6. A. Images captured by confocal laser scanning microscopy with fluorescence after the use of intracanal medication based on bioceramic compounds (Bio-C Temp) and filling with bioceramic-based sealer (Bio-C Sealer). In fluorescent green it was possible to observe the presence of intracanal medication remnant inside the dentinal tubules. In fluorescent blue, the penetration of root canal sealer into the dentinal tubules was observed, with the formation of longer and more uniform tags. The formation of tags with product resulting from the interaction of intracanal medication and sealer was observed in light fluorescent blue. B. Images captured by confocal laser scanning microscopy with fluorescence after the use of intracanal medication based on bioceramic compounds (Bio-C Temp) and filling with epoxy resin-based sealer (AH Plus). In fluorescent green it was possible to observe the presence of intracanal medication remnant inside the dentinal tubules. In fluorescent red, the penetration of root canal sealer into the dentinal tubules with the formation of short tags without continuity was observed. In fluorescent orange, the formation of tags with product resulting from the interaction of intracanal medication and sealer was observed.

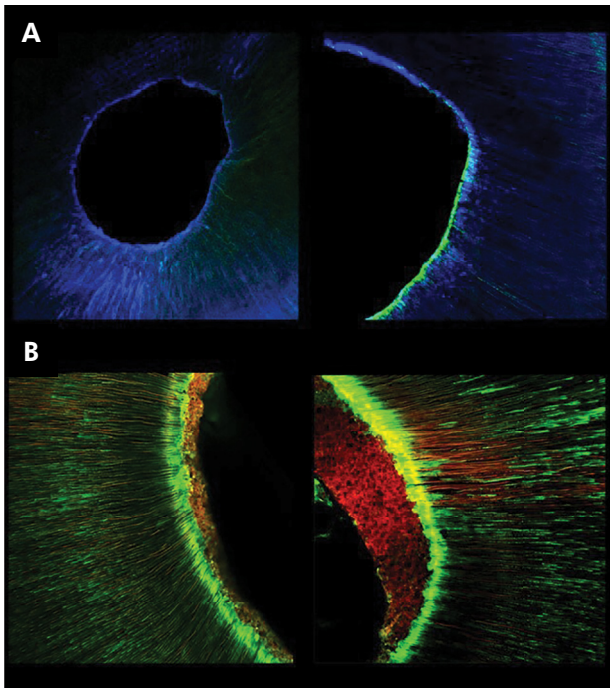

Figure 7. A. Images captured by confocal laser scanning microscopy with fluorescence after the use of intracanal medication based on calcium hydroxide (Ultracal XS) and filling with bioceramic-based sealer (Bio-C Sealer). In fluorescent blue, there was less, non-homogeneous penetration of sealer into the dentinal tubules. It was noteworthy that there was no evident penetration of intracanal medication (in fluorescent green). B. Images captured by confocal laser scanning microscopy with fluorescence after the use of intracanal medication based on calcium hydroxide (Ultracal XS) and filling with epoxy resin-based sealer (AH Plus). In fluorescent green, it was observed that the intracanal medication remained homogeneously spread over the entire length of the surface, without interaction with the sealer, preventing its penetration. In fluorescent red, it was noted that the sealer was found only in the lumen of the canal, with little tag formation, shapeless and without continuity. Furthermore, it was possible to affirm that there was no formation of a layer resulting from the interaction between intracanal medication and root canal sealer.

endodontic sealers to root dentin depends on the formation of a covalent bond with the exposed amino groups of the dentinal collagen and on the anatomy of the dentinal tubules.[15,16,18] Thus it is suggested that after conventional irrigation, a larger amount of calcium hydroxide remnant remained on the walls of the root canals, resulting in a physical barrier between the root dentin and endodontic sealer.[20-23] This could also chemically react with sealer and interfere in its physical chemical properties, reducing flow, working time, film thickness,[24] and sealer penetration into dentinal tubules,[15] consequently affecting the apical sealing capacity,[17,24] thus decreasing the bond strength.[25] It was also possible to observe the formation of gaps throughout the circumference of the root canal, not only in the polar areas of the flattened canals, but also in the areas of mismatch between filling sealer and gutta-percha cone, confirming the higher number of adhesive failures to the filling material.

CLSM images showed that in the majority of specimens, the remaining intracanal medication (fluorescent green) acted as a physical barrier between the filling sealer (red or blue fluorescence) and dentin wall, especially in the polar areas, with no chemical interaction between them. When this interaction occurred, a thin layer was observed representing the interaction (orange) of the intracanal medication (fluorescent green) and the filling sealer (fluorescent red), in addition to penetration of the filling sealer into the dentinal tubules in an irregular manner and formation of shorter and non-continuous tags.

It is noteworthy to emphasize that the resin sealer used in this study is composed of epoxy resins, which initiate the polymerization process through chemical reaction with hardening compounds such as diamines or dicarboxylic acids. Thus, when the sealer is inserted into the root canal, the polymerization reaction between the epoxy resins and the amines (paste A and paste B, respectively) begins, and this reaction will polymerize the entire resin. The compound formed corresponds to a polymer with low affinity for the remaining silicates and oxides of the medication, which in turn are crosslinked, since they have already reacted with water during the period in which they were in contact with the dentin surface.[26] This low affinity acts as a hysterical repulsion factor between sealers and medication remnants, favoring the material mismatch to the dentin surface and influencing the reduction in bond strength.[15,16,18,26,27] Moreover, the use of resin sealer after the use of intracanal medication generates an interaction of low affinity between the biomineralization products that have strongly polarized hydrophilic functional groups, with the hydrophobic or weakly polarized functional groups of the resin sealer. This weak electrostatic interaction can lead to product displacement on the surface of the biomineralized dentin wall, leading to gap formation, before and after the product sets, as seen in the CLSM and SEM images. These findings corroborate the results obtained in the present study, in which higher bond strength values were observed in the group that the teeth received intracanal medication based on bioceramic compounds and then were filled with bioceramic sealer, as well as a higher prevalence of dentin bonding failures.

Relative to the thirds, higher bond strength values were observed in the cervical third, when compared with the middle third, which in turn showed higher values than those in the apical third, irrespective of the intracanal medication and the obturator sealer used.[12,14,28-32] One of the factors that may explain the difference in bond strength between the thirds is the morphological structure of the dentin along the root canal, which has a higher number of dentinal tubules with a larger diameter in the cervical and medial regions, favoring the bond of filling sealers to the dentin walls.[32-34] However, in the present study, the analysis using SEM and CSLM showed that the areas of adaptation or maladaptation of the filling material to root dentin were uniform in all thirds. Thus, the higher bond strength observed in the cervical and middle thirds could be explained by the smaller volume of remaining intracanal medication in these thirds, which provided greater interaction between filling material and dentin.

Considering that the results of the present study showed that the remaining intracanal medication based on bioceramic compounds showed physical-chemical interaction with the bioceramic filling

sealer, this enabled the formation of new by-products, which provided an increase in bond strength and formation of adhesive interface between intracanal medication and filling sealer. Thus, it is necessary to develop laboratory and clinical studies to verify the chemical composition of this by-product generated, and the biological and mechanical behavior of this long-term interaction, with the aim of establishing protocols to reduce the interference of the remaining intracanal medication in the filling of the root canal system.

In the present study, for intracanal medication based on calcium hydroxide or based on bioceramic compounds, Fluo-3 (506/526 nm) was used, which is a selective fluorescent marker for calcium ions.[9,35] This has been indicated for use in materials based on silicates and calcium oxides.[36] Thus, the calcium present in the intracanal medication binds to Fluo 3 and its fluorescence, in a shade of green observed in the confocal images, increases according to the stability of the bonds formed. For the sealer based on bioceramic compounds, calcein (360/449 nm) was used to avoid a confounding factor in the qualitative analysis. Calcein is also a selective fluorescent marker for calcium ions, and it emits increasing fluorescence in shades of blue, according to the stability and strength of the covalent bonds.[37] It is noteworthy that both Fluo-3 and Calcein are appropriate markers for this type of material, since it fluoresces at high pH, is stable as a fluorescent standard and has no residual fluorescence, since the mechanism of exchange of bonds with Ca^{2+} ions, without high variation in pH, makes this method devoid of secondary chemical reactions.[37-39]

Whereas Rhodamine B, a red fluorescent marker, was used for the filling sealer based on epoxy resin (AH Plus) since due to the leaching phenomenon, this marker has a high affinity for moisture, with lower affinity for calcium. This could lead to residual marking and emit fluorescence independent of the material, and present deeper penetration into the dentinal tubules, generating inconclusive result.[36,40] It is noteworthy that due to the physical-chemical interaction between the fluorophore markers, there was fluorescence emission in turquoise blue between Fluo-3 x Calcein, and in orange tones for Fluo-3 x Rhodamine B.

Conclusions

The conclusion reached by the present study was that intracanal medication based on bioceramic compounds chemically interacted with the bioceramic-based root canal sealer to form a biomineralizing layer, which allowed for an increase in the bond strength and formation of an adhesive interface between the materials, with no or less gap formation.

Acknowledgment

We gratefully acknowledge financial supported by the Coordination for the Improvement of Higher Education Personnel (CAPES-Brazil Process nº 33002029032P4) and The São Paulo Research Foundation – FAPESP (Process nº 2018/14450-1).

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