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Influence of a propolis-based irrigant solution on gap formation and bond strength of posts bonded to root canal dentin using different resin cements

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This study evaluated the influence of an aqueous propolis-based solution (PROP) on gap formation and bond strength of posts bonded to root canal dentin using resin cements. Endodontically-treated bovine incisors received different irrigation protocols: 1) 2.5% sodium hypochlorite (NaOCl)/17% EDTA/NaOCl; 2) saline solution (NaCl)/EDTA/0.12% chlorhexidine (CHX); 3) NaOCl/PROP/NaOCl; 4) NaCl/PROP/CHX; 5) NaCl/PROP/NaCl. Posts were then bonded with cements: RelyX ARC; Panavia F2.0; or RelyX U200 (n=10). The specimens were cross-sectioned. Gaps were assessed and performed the push-out bond strength test. Surface roughness of dentin fragments was also evaluated. Statistical analysis was performed (5%). RelyX U200 exhibited greater gap-free interfaces. Bond strength varied as a function of cements and irrigation protocols. PROP irrigation had no negative effect on the bond strength (p>0.05). Roughness increased significantly after NaOCl/EDTA/NaOCl, but remained unaltered after PROP irrigation protocols. Propolis-based irrigation protocols do not interfere in the bonding performance of posts cemented to root canal dentin.

Keywords: Push-out bond strength, Post and core technique, Resin cements, Gaps, Propolis

INTRODUCTION

Complete elimination of microorganisms in infected root canals represents one of the most difficult task in endodontics[1]. No evidence in the literature can clearly support that mechanical instrumentation can achieve a bacteria-free root canal system[2]. Thus, numerous endodontic techniques have been advocated to reduce root canal microorganisms such as various instrumentation techniques, irrigation regimens, and intra-canal medications. Clinicians usually rely on an adequate accomplishment of the working length, mechanical instrumentation, antimicrobial irrigation, and removal of the smear layer to eliminate intracanal bacteria[3]. Considering the complexity of the root canals' anatomy (such as lateral/accessory canals and apical ramifications), a complete root canal disinfection would be only achieved if some sort of irrigation/disinfection procedures removed completely the remaining pulpal tissue and microorganisms[4].

Sodium hypochlorite (NaOCl) is the most widely used irrigation agent, as it is able to dissolve necrotic tissues due to its proteolytic action[5]. Associated with a specific calcium chelator agent for (ethylenediamine tetraacetic acid - EDTA), NaOCl is capable of dissolving the inorganic component of the smear layer and, thus removing it from the dentin surface[6]. However, NaOCl has some disadvantages such as high cytotoxicity[7] and peritubular and intertubular erosion being enhanced by the association with EDTA[8]. A further problem related to the use of EDTA is that it reduces the availability of chlorine in solution, rendering sodium hypochlorite ineffective on bacteria and necrotic tissue[9]; hence, EDTA should not be mixed with sodium hypochlorite. It is also well known that NaOCl can cause structural changes of dentin collagen, which leads to the formation of a porous and brittle dentin surface[10]. Indeed, this can reduce tooth resistance over time, especially in clinical cases in which the remaining tooth structure has been drastically compromised by all sorts of cavity preparations and endodontic instrumentation[11]. NaOCl can also cause serious complications when apically extruded[12], such as chemical burning and severe cytotoxicity[13].

Because conventional endodontic irrigants present potential side effects and safety concerns, a trend exists toward the use of biological medications extracted from the roots, leaves, seeds, stem, and flowers of natural plants[14]. The interest in using propolis has increased in the last decades in dentistry due to its antimicrobial activity against a wide range of pathogenic microorganisms[15]. Such potent natural therapeutic agent is composed of resin and balsams (50–60%), pollen (5–10%), and other constituents such as amino acids, minerals, vitamins A and B complex, flavonoids, phenols, and aromatic compounds[16]. In an *in vitro* study[17], the antimicrobial effects of propolis against *Enterococcus*

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faecalis and *Candida albicans* were evaluated in comparison to those of other commercial irrigants (5% NaOCl, BioPure MTAD, and 2% CHX). It was found that propolis was effective in eradicating *E. faecalis* and *C. albicans*, so that it is nowadays considered propolis as a promising intracanal irrigant. Propolis also showed effective antimicrobial activity against *S. mutans* and *Lactobacillus*, thus decreasing dental caries in a rat model system[18]. A further study[19] proved that propolis was as efficient as sodium hypochlorite when used as root canal irrigation agent, but without the unfavorable properties that characterize NaOCl[19].

Considering the immense potential of propolis as an antimicrobial agent evidenced in the literature, the present study aims to evaluate the influence of a standardized propolis aqueous solution comprising of 1% green propolis extract[20], 5% EDTA and 10% of an anionic surfactant, used as an intracanal irrigation agent for endodontic therapy. This aim was accomplished by evaluating the influence of such an aqueous propolis-based irrigant solution on the bond strength of fiberglass posts bonded using different types of resin-based cements to root canal dentin; results were compared to those obtained when using conventional irrigation protocols. Furthermore, the presence of gaps within the interface cement-post-dentin was evaluated at different root thirds. The surface roughness was also evaluated in dentin specimens treated with the same chemical irrigant combinations. The following research hypotheses were tested: (i) a propolis solution would induce no negative influence on gap formation in the different root thirds, regardless the cement employed for bonding procedures; (ii) the resin-dentin bond strength would not be affected by the application of the propolis-based solution, irrespective of the cement and root thirds.

MATERIALS AND METHODS

Experimental design

In this *in vitro* study, the push-out bond strength of glass-fiber posts bonded to root canal dentin using different types of resin-based cements was evaluated, according to the following factors: (1) resin cements —in 3 levels (total-etch cement RelyX ARC [ARC], self-etch cement Panavia F2.0 [PAN], and self-adhesive resin cement RelyX U200 [U200]); (2) root thirds —in 2 levels (cervical, and middle thirds) and (3) root canal treatment, in 5 levels (described below). The characteristics of the cements used in this study are described in Table 1. The luting cements were applied into the root canals following the manufacturers' instructions (Table 2).

Specimen preparations

A total of 150 recently extracted sound bovine teeth were cleaned with slurry of pumice/water and then stored in 0.1% thymol solution at room temperature no longer than 1 month. The tooth crowns were sectioned below the cement-enamel junction to obtain 21 mm roots. Endodontic access was achieved with the working length established at 20 mm. The canal space was mechanically

Table 1 Restorative materials used in the present study*

Material	Lot No/Exp. date	Composition	Working time (min)	Setting time (min)	Curing time (s)
RelyX ARC 3M ESPE, St. Paul, MN, USA	1715300541 2019-02	Paste A: Bis-GMA, TEGDMA, silane treated silica, functionalized dimethacrylate polymer, 2-benzotriazolyl-4-methylphenol, 4-(Dimethylamino)-Benzene ethanol. Paste B: Silane treated ceramic, TEGDMA, Bis-GMA, silane treated silica, functionalized dimethacrylate polymer, 2-benzotriazolyl-4-methylphenol, benzoyl peroxide (72/wt). Clicker delivery system	3–5	10	40
Panavia F 2.0 Kuraray, Tokyo, Japan	Paste A: 330158 Paste B: 370038 2019-02	Paste A: Bis-GMA, TEGDMA, 10-MDP, DMA, hydrophobic aromatic dimethacrylate, silanated barium glass filler, silanated colloidal silica. Paste B: Bis-GMA, DMA, hydrophobic aromatic dimethacrylate, silanated barium glass filler, silanated colloidal silica, surface treated sodium fluoride. Hand mixing.	1	2–4	20
RelyX U200 3M ESPE	659007 2018-08	Base: Methacrylate monomers containing phosphoric acid groups, methacrylate monomers, initiators, stabilizers, rheological additives. Catalyst: Methacrylate monomers, alkaline fillers, silanated fillers, initiator components, stabilizers, pigments, rheological additives. Zirconia/silica fillers. Automix delivery system	2	6	20

*Manufacturer's information

Table 2 Application modes of the cements investigated

Cement	Instructions to use*
RelyX ARC	1. Etch preparation and apply Adper Single Bond Plus adhesive according to instructions. Trial fit the post after applying and light curing the adhesive. 2. Dispense appropriate amount of cement onto a mixing pad and mix for 10 s. 3. Apply cement to the bonding surface of the preparation in and around canal using an instrument such as a spiral paste filler. Place a thin layer of mixed cement on post. 4. Seat and hold the post in place. Begin clean-up of excess cement approximately 3–5 min after seating. 5. Light cure for 40 s from the occlusal surface.
Panavia F 2.0	1. Mix equal amounts of ED Primer II A&B. Apply to the tooth. Then, wait 30 s. Gently air dry. Remove the excess primer with paper points. 2. Dispense equal amounts of paste A&B. Mix Paste A&B and coat posts with mixed cement. 3. Seat posts and apply the excess cement to coronal tooth structure. 4. Light cure for 20 s from the occlusal surface.
RelyX U200	1. Mix base paste and catalyst paste into a homogenous paste within 20 s using a spatula. Avoid incorporating air bubbles. Do not use Lentulo-Spirals to insert the cement in the root canal as this can excessively accelerate setting. 2. Spread cement to the post and place the post in the pretreated root canal; apply moderate pressure to hold it in position. Recommendation: rotating the post slightly during insertion avoids the inclusion of air bubbles. 3. Light cure for 20 s from the occlusal surface.

*Manufacturers' information

enlarged using K-files (up to #70) and Gates Glidden burs (#2, #3, and #4, sequentially) (L.D. Caulk Division, Dentsply International, Milford, DE, USA) in a slow-speed contra-angle handpiece. The teeth were randomly distributed into 15 experimental groups having 10 teeth each.

Propolis solution preparation

A standardized ethanolic propolis solution was prepared (Green Propolis, Apis Brasil, SP, Brazil) and characterized, including its total phenols and flavonoids by UV-spectrophotometer (Varian Cary 50 UV-Vis spectrophotometer, Agilent Technology, Santa Clara, CA, USA), antioxidant activity by the DPPH method and Artepillin C content by HPLC[20].

Irrigant solution preparation

Propolis extract diluted in water was solubilized in hydrogenated castor oil (Cremophor-RH 40, Basf Latin America, São Paulo, Brazil). Subsequently, the anionic surfactant sodium PEG-7 olive oil carboxylate (5%-Olivem 400, The Hallstar, Chicago, IL, USA) was added to the formulation. Finally, a 5% EDTA solution was added to the final formulation.

Experimental irrigant treatments

Treatment 1 (NaOCl /EDTA/ NaOCl): Teeth were irrigated with 2.5% NaOCl during root canal instrumentation (a total estimated time of 15 min each tooth) as in a typically clinical scenario. The specimens were then irrigated with 5 mL of 17% EDTA for 5 min. After being rinsed with 10 mL of NaOCl, the root canals were rinsed with 10 mL of physiologic saline solution.

Treatment 2 (NaCl/EDTA/CHX): Root canals were prepared using 0.9% physiologic saline solution (NaCl) as an irrigant for 15 min. The specimens were then irrigated with 5 mL of 17% EDTA for 5 min. Subsequently, the canals were rinsed with 10 mL of 0.12% CHX solution, and then rinsed with 10 mL of physiologic saline solution.

Treatment 3 (NaOCl/PROP/NaOCl): Teeth were irrigated with 2.5% NaOCl during the mechanical instrumentation of the root canals for 15 min. The specimens were then irrigated with 10 mL of 1.0% propolis-based aqueous solution for 5 min. After being rinsed with 10 mL of NaOCl, the canal was rinsed with 10 mL of physiologic saline solution.

Treatment 4 (NaCl/PROP/CHX): Teeth were irrigated with 0.9% NaCl during the cleaning and shaping of the root canal. The canals were then rinsed with 10 mL of 1.0% propolis aqueous solution for 5 min, followed by 10 mL of 0.12% CHX solution, and 10 mL of physiologic saline solution.

Treatment 5 (NaCl/PROP/NaCl): Teeth were irrigated with 0.9% physiologic saline solution for 15 to 20 min during the preparation of the root canals. After being rinsed with 10 mL of the same propolis solution for 5 min, the canals were rinsed with 10 mL of physiologic saline solution.

All the canals were finally dried with paper points.

Post cementation procedures

All of the roots received a glass fiber post of 1.8 mm in diameter (lot #420717, Glass Fiber Post #2, Maquira Dental Products, Maringá, PR, Brazil). Before cementation, the posts were placed into the root canal to confirm their position and adaptation. Afterward, the posts were cleaned with 70% ethanol, dried with absorbent paper towels, and silanized (Silane Angelus, Londrina, PR, Brazil) for 1 min. The posts were then bonded to root canal dentin using different resin-based luting cements applied for manufacturer's instructions (Table 2). The cements were light-cured with an LED curing light (Bluephase, Ivoclar Vivadent, Schaan, Liechtenstein), with a radiant emittance of >1,000 mW/cm^2. Subsequently, the apical foramen of all the specimens was sealed with U200 and stored in deionized water at 37°C for 24 h.

The specimens were subsequently embedded in epoxy resin (EpoThin Epoxy Resin, Buehler, Lake Bluff, IL, USA) and sectioned using a diamond blade (#11-4245, Diamond Wafering Blade, Buehler) mounted on a low-speed microtome (Isomet, Buehler). The root specimens were sliced perpendicularly to the long axis under water-cooling to obtain six 1-mm-thick slabs (3 coronal and 3 middle). The thickness of the slabs was measured using a digital caliper (Absolute Digimatic, Mitutoyo, Tokyo, Japan). The first slab was not included, but all the others were codified to identify the region of the root the slabs were obtained (C1, C2, C3, and M1, M2, M3 for the cervical and middle thirds, respectively)[21].

Percentage of gap at the cement layer

Prior to the push-out bond strength test, digital images of both sides of the slices were captured using a digital camera connected to a dissecting microscope (Stereozoom, Bausch & Lomb, Rochester, NY, USA), using 40× magnification. The photomicrographs obtained were analyzed using the ImageJ software application (National Institute of Health, Bethesda, MD, USA). For this, the surface area of the root canal and the surface area of the post were defined. Subsequently, the surface area of the cement was calculated by subtracting the root's surface area from the post's surface area. Using these measurements, the uniformity of the cement layer was evaluated through the percentage of the areas with and without gaps[22].

Push-out compressive bond strength test

The push-out bond strength was tested in a universal testing machine (#3342, Instron Universal Testing Machine, Instron, Canton, MA, USA), with a crosshead speed of 0.5 mm/min at the apical–coronal direction. The post segments were loaded with the punch pin (Ø 0.9–1.1 mm) centered on the post segment, with no contact with the surrounding dentin surface. The force of the post's dislocation was registered at the moment of displacement of the post fragment from the canal. The maximum failure load was recorded in Kgf and converted into MPa. The bonding surface area was calculated for each slice using the following formula (1):

$$A=\pi(R2+R1)[h^2+(R2-R1)^2]^{0.5}, \quad (1)$$

where π=3.14, R2=the fragment coronal radius, R1=the fragment apical radius, and h=the slice thickness.

Failure mode analysis

Subsequent to the bonding test, the failure mode of each slab was assessed using a stereomicroscope microscope (Stereozoom, Bausch & Lomb), and classified as[23]:

- Type 1 —Adhesive;
- Type 2 —Mixed 1: Adhesive/cohesive in the cement;
- Type 3 —Mixed 2: Adhesive/cohesive in the cement and dentin;
- Type 4 —Cohesive in the cement; and
- Type 5 —Cohesive in dentin.

Topographical surface characterization using surface micro-roughness test

Dentin fragments were obtained (3.5 mm in height×2.5 mm in width) from along the root canal dentin portion of freshly extracted bovine teeth. The fragments were cut under running water. All of the fragments were then immersed in 5.25% NaOCl to dissolve the organic tissue[24]. The root canal dentin side of each fragment was ground for 5 s at a designated constant speed using #600 SiC paper to produce a flat surface with a smear layer[24]. All of the specimens were finally rinsed with distilled water for 20 s. The specimens were then treated using the irrigation protocols as previously described. The surface micro-roughness of the flat side of the dentin specimens was then assessed with a surface roughness tester (Surftest–SJ 301 Surface Roughness Tester, Mitutoyo), using a diamond stylus tip of 5 µm, with a measuring force of 4 mN at a speed of 0.25 mm/s. Sequentially, the treated specimens were first acid etched with a 37% phosphoric acid gel and finally rinsed with distilled water for 15 s. Three consecutive measurements were performed in different positions of the specimens were recorded for and on each surface of every single specimen (n=4). Prior to testing, and throughout the experiment, the surface roughness tester was monitored using a calibration block (Mitutoyo Precision Reference Specimen, Mitutoyo). The arithmetic surface height parameter (R_a) was obtained at a scan length of 1.25 mm. Furthermore, the dentin surface roughness of the control group with smear layer-covered dentin was also analyzed; these were then acid-etched using a 37% phosphoric acid gel and water-rinsed for 15 s. The variations in the different surface roughness parameters evaluated after the irrigant treatments and after acid etching were then calculated.

Statistical analysis

The data on push-out bond strength and gap formation were initially submitted to normality tests (Shapiro-Wilk and Anderson-Darling), and normal distribution was rejected for both datasets (p>0.05). The data were then submitted to a Kruskal-Wallis test, with a 5% of significance level. The surface roughness data were analyzed using two-way ANOVA (factors: dentin treatment associated or not associated with posterior application of a 37% phosphoric acid gel/water-rinsing for 15 s). All statistical analyses were performed at a pre-set alpha of 0.05.

RESULTS

The results of the percentage of gaps encountered in the different groups are depicted in Table 3 displays. Significant lower gap percentage was observed when using U200, regardless the irrigation protocol employed ($p<0.05$). The same situation was observed for the root canals that received the irrigation protocol 5 and PAN cement ($p<0.05$). The percentage of gaps in treatment 1 ranged from 2.9% (U200, middle third) to 16.7% (PAN, middle third); from 0.0% (U200, middle third) to 18.7% (PAN, middle third) for treatment 2; from 1.6% (U200, middle third) to 14.4% (PAN, middle third) for treatment 3; from 1.4% (U200, cervical third) to 17.9% (PAN, middle third) for treatment 4; and from 0.0% (U200, cervical third) to 9.9% (ARC, middle third) for treatment 5 (Table 3). Higher percentage of gap-free was found when U200 was used for luting procedures of the posts to dentin (80–100%) in comparison to ARC (10–70%) and PAN (10–80%) (Table 4).

The mean bond strength varied as a function of the cement type and irrigant treatment. For treatment 1, the mean bond strength ranged from 16.1 (PAN, middle third) to 37.2 MPa (U200, cervical third); for treatment 2, from 23.6 (PAN, middle third) to 48.8 MPa (U200, cervical third); for treatment 3, from 21.3 (ARC, middle third) to 35.2 MPa (U200, cervical third); for treatment 4, from 16.0 (PAN, middle third) to 35.0 MPa (ARC, middle third); and for treatment 5, from 19.8 (ARC, cervical third) to 36.0 MPa (U200, cervical third) (Table 5). The fiberglass posts cemented using U200 had the greatest mean bond strength; from 30.8 to 48.8 MPa in the cervical third and from 28.9 to 42.3 MPa in the middle third (Table 5). The root treatment performed using the aqueous propolis-based solution in combination with saline solution (treatment 5) exhibited comparable mean bond strength to those of most of the irrigation protocols used in this study. In addition, when comparing the mean bond strength obtained with treatment 5 to those obtained using the conventional irrigation protocol, NaOCl/EDTA/NaOCl (treatment 1), no significant difference was observed, regardless of the cement employed ($p>0.05$). In general, the variable 'root third' was not significant ($p<0.05$), except for treatments 3 and 4, in which the posts were bonded with PAN; the mean bond strength of the cervical third (33.8 and 30.5 MPa, respectively) were significantly higher

Table 3 Means and standard deviation of marginal gap formation

Resin Cements	Thirds	Treatment 1 NaOCl/EDTA/ NaOCl	Treatment 2 NaCl/EDTA/ CHX	Treatment 3 NaOCl/PROP/ NaOCl	Treatment 4 NaCl/PROP/ CHX	Treatment 5 NaCl/PROP/ NaCl
RelyX ARC	Cervical	5.9 (10.4) ABb	6.2 (9.6) ABab	2.5 (4.5) Bbc	5.9 (9.3) ABb	7.0 (5.4) Aa
	Middle	16.3 (11.7) Aa	12.6 (13.8) ABa	12.3 (12.3) ABa	5.8 (5.9) Bb	9.9 (10.4) ABa
Panavia F2.0	Cervical	12.8 (8.9) Aab	7.0 (9.7) Aab	7.9 (7.0) Aab	7.8 (8.0) Aab	5.4 (9.3) Aab
	Middle	16.7 (13.1) Aa	18.7 (15.8) Aa	14.4 (10.5) Aa	17.9 (11.7) Aa	2.6 (7.0) Bb
RelyX U200	Cervical	3.1 (4.9) Abc	1.3 (2.8) Abc	3.5 (8.2) Abc	1.4 (4.3) Ac	0.0 (0.0) Ab
	Middle	2.9 (8.7) Ac	0.0 (0.0) Ac	1.6 (4.9) Ac	2.3 (5.3) Ac	2.6 (7.7) Ab

Means followed by different capital letter in row and small letter in column show statistical difference according to Kruskal-Wallis test.
n=10, Unit: %
NaOCl: 2.5% sodium hypochlorite, EDTA: 17.0% ethylenediaminetetraacetic acid, NaCl: 0.9% sodium chloride (saline) solution, CHX: 0.12% chlorhexidine digluconate, PROP: 1.0% aqueous propolis-based solution

Table 4 Percentage of specimens with perfectly sealed margins per group

Materials	Thirds	Treatment 1 NaOCl/EDTA/ NaOCl	Treatment 2 NaCl/EDTA/ CHX	Treatment 3 NaOCl/PROP/ NaOCl	Treatment 4 NaCl/PROP/ CHX	Treatment 5 NaCl/PROP/ NaCl
RelyX ARC	Cervical	30%	30%	70%	50%	10%
	Middle	10%	40%	40%	40%	30%
Panavia F2.0	Cervical	20%	50%	30%	40%	70%
	Middle	20%	20%	20%	10%	80%
RelyX U200	Cervical	80%	80%	80%	90%	100%
	Middle	90%	100%	90%	80%	90%

n=10.
Abbreviations: NaOCl–2.5% sodium hypochlorite; EDTA–17.0% ethylenediaminetetraacetic acid; NaCl–0.9% sodium chloride (saline) solution; CHX–0.12% chlorhexidine digluconate; PROP–1% aqueous propolis-based solution.

Table 5 Means and standard deviation of the push out bond strength

Materials	Thirds	Treatment 1 NaOCl/EDTA/ NaOCl	Treatment 2 NaCl/EDTA/ CHX	Treatment 3 NaOCl/PROP/ NaOCl	Treatment 4 NaCl/PROP/ CHX	Treatment 5 NaCl/PROP/ NaCl
RelyX ARC	Cervical	17.5 (4.9) Bc	32.4 (23.9) ABb	22.4 (13.9) ABb	31.8 (15.2) Aa	19.8 (5.2) ABb
	Middle	21.2 (9.6) Bbc	28.8 (17.5) ABb	21.3 (12.8) Bb	35.0 (13.4) Aa	20.7 (7.0) Bb
Panavia F2.0	Cervical	19.1 (6.5) Bc	28.0 (10.7) ABb	33.8 (7.4) Aa	30.5 (13.3) ABa	30.3 (20.5) ABa
	Middle	16.1 (6.5) Ac	23.6 (9.2) Ab	23.3 (9.8) Ab	16.0 (7.8) Ab	29.4 (19.0) Aab
RelyX U200	Cervical	37.2 (17.3) ABa	48.8 (15.6) Aa	35.2 (7.1) Ba	30.8 (9.0) Ba	36.0 (9.5) Ba
	Middle	33.8 (22.1) ABab	42.3 (11.7) Aab	31.7 (15.3) ABab	28.9 (12.0) Ba	31.1 (14.3) ABab

Means followed by different capital letter in row and small letter in column: significant (p<0.05).
n=10, Unit: MPa.
Abbreviations: NaOCl–2.5% sodium hypochlorite; EDTA–17.0% ethylenediaminetetraacetic acid; NaCl–0.9% sodium chloride (saline) solution; CHX–0.12% chlorhexidine digluconate; PROP–1.0% aqueous propolis-based solution.

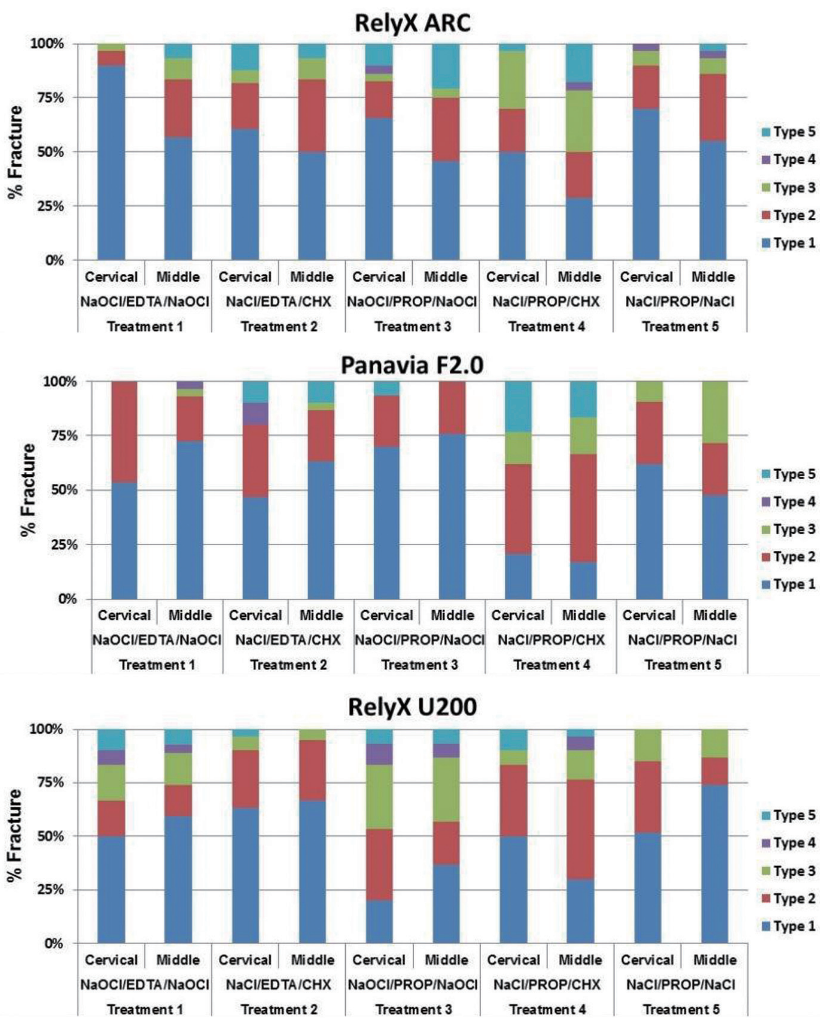

Fig. 1 Incidence of failure modes (%) after the push-out compressive bond strength test as functions of the irrigant treatments and resin cements used.
Type 1: adhesive, Type 2: Mixed 1; adhesive/cohesive in the cement, Type 3: Mixed 2; adhesive/cohesive in the cement and dentin, Type 4: cohesive in the cement, Type 5: cohesive in dentin[23)]

Table 6 Results of percentage of increase in surface roughness after dentin treatment, after acid etching and the total increase for the parameter Ra

Treatments	Ra		
	after treatment	after acid etching	total increase
Control (NaCl)	11.1 (8.6) B	31.9 (56.5) A	46.0 (8.9) B
NaOCl/EDTA/NaOCl	196.7 (141.5) A	7.3 (42.9) A	180.3 (98.3) A
NaCl/EDTA/CHX	21.4 (12.1) B	19.9 (21.4) A	46.3 (35.7) B
NaOCl/PROP/NaOCl	33.8 (35.2) B	14.5 (28.8) A	46.5 (16.4) B
NaCl/PROP/CHX	5.2 (8.8) B	2.2 (32.5) A	8.4 (38.2) B
NaCl/PROP/ NaCl	15.5 (23.8) B	1.1 (10.5) A	15.1 (14.7) B

Means followed by different capital letter in column: significant ($p<0.05$).
$n=10$, Unit: %.
Abbreviations: NaOCl–2.5% sodium hypochlorite; EDTA–17.0% ethylenediaminetetraacetic acid; NaCl–0.9% sodium chloride (saline) solution; CHX–0.12% chlorhexidine digluconate; PROP–1.0% aqueous propolis-based solution.

for the middle third (23.3 and 16.0 MPa, respectively) ($p<0.05$). The type 1 (adhesive) and type 2 (mixed 1: adhesive/cohesive in the cement) mode of failures were predominant in all the experimental groups (Fig. 1). The surfaces of the treated dentin specimens exhibited topographic variations when evaluating the different surface roughness parameters (Table 6).

Surface roughness significantly increased in the dentin specimens treated with NaOCl /EDTA/ NaOCl (treatment 1) in comparison to all the other irrigation protocols and to the untreated group (#600 SiC paper, $p<0.05$). After acid-etching with 37% phosphoric acid gel (Control group), the surface roughness increased significantly for the specimens that received the treatments 2 (NaCl/EDTA/CHX) and 3 (NaOCl/PROP/ NaOCl). The surface roughness remained unaltered in the experimental groups treated with the propolis-based aqueous solution, treatments 4 (NaCl/PROP/CHX) and 5 (NaCl/PROP/NaCl), even after the acid-etching/ water-rinsing procedures. Conversely, although the dentin surface roughness significantly increased after the treatment 1 (NaOCl /EDTA/ NaOCl), the roughness values were significantly reduced after acid etching ($p<0.05$, Table 6).

DISCUSSION

The green propolis used in this study was collected from the plant *Baccharis dracunculiforia* in Southeastern Brazil[25,26]. Such type of propolis contains several different types of phenolic compounds, such as Artepillin C (3,5-diprenyl-4-hydroxycinnamic acid); the most biologically active phenolic component found in nature. Artepillin C has antioxidant, antimicrobial, anti-inflammatory, antigenotoxic, anti-angiogenic, and anticancer properties[20]. Other phenolic compounds, such as caffeic acid, *p*-coumaric acid, and 3-prenyl-4-hydroxycinnamic acid, are also found in this type of propolis extract (data not published). Green propolis also has an antiradical action against 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals (EC_{50} 20.7 μg/mL). It also exhibits a wide spectrum of action, possibly related to the synergic effects of phenolic type compounds such as ferulic acid, caffeic acid, and *p*-coumaric acid in the extract[27]. Most of the studies and registered patents about endodontic irrigation solutions have similar chemical compositions; these all containing propolis in alcohol solutions collected from different countries around the world. Conversely, the endodontic solution proposed here contains no ethanol, thus it is advocated to maintain the characteristics of a natural product.

In order to benefit of all the properties previously mentioned, an endodontic irrigation solution was developed in this study with careful selection of raw materials to meet all of the pharmacotechnical requirements. Extract of green propolis were incorporated into a formulated aqueous solution containing 1 % ethanol extract of green propolis and 5 % of EDTA. In order to evaluate synergism, ethanol extract and EDTA were added to formulations also containing pharmaceutical auxiliaries and an anionic surfactant (Olivem 400); this latter was an extract of olive oil fatty acids, which presents mild deterging properties. In this way, stock solutions of the propolis-based irrigant were formulated, and characterized for stabilization, presence of flavonoids and phenolic compounds. This solution resulted efficient in terms of biocompatibility, antioxidant, and antimicrobial activity, mainly against *Enterococcus faecalis* and *Staphylococcus aureus* (unpublished data).

The first research hypothesis that our propolis solution would induce no negative effect on gap formation in different regions of the root canal dentin, regardless of the cement tested, was accepted. Indeed, the percentage of gaps observed when the aqueous propolis-based solution was used, presented similar or better results in terms of the presence of gaps compared to the other tested combinations. Moreover, the second hypothesis that the resin-dentin bond strength would not be affected by the application of the propolis-based solution, irrespective of the cement and root thirds, was also accepted. In general, the bond strength of posts bonded root canal dentin varied as functions of the cement type and irrigant treatments. In addition, when the root canals were treatment with the aqueous propolis-based solution associated with other solutions, comparable bond strength to most of combination treatments was observed. Indeed, the bond strength seems to be also correlated to both the cement type and bonding approach/technique.

Based on their bonding approach to tooth substrates, the cements selected in the present study represent different categories of materials used nowadays. The reason to possibly may explain the differences in interfacial gaps and bond strength obtained in this study can rely on the bonding approach of such luting cements. For instance, RelyX ARC is classified as a conventional resin luting cement, associated with a two-step, total-etch adhesive system (Adper Single Bond 2, 3M ESPE, St. Paul, MN, USA)[28]. The self-etch approach of Panavia F2.0 is based on the conditioning of root canal dentin and its smear layer without a subsequent removal of the dissolved calcium phosphates, as there is no rinsing step[29]. This cement contains functional monomers named 10-MDP, which is well known to interact with residual hydroxyapatite through primary ionic bond to calcium ions[30]. Alternatively, the self-adhesive cement RelyX U200 requires no acid-etching, priming, and adhesive application[31], due to the presence of multifunctional acidic methacrylate monomers that are able to bind straightly the calcium ions in hydroxyapatite and promote self-adhesion to root canal dentin[32,33].

Since self-adhesive cements require greater and longer contact with the dental hard tissues in order to react with hydroxyapatite, their application is facilitated by automix delivery system; this helps to diffuse and to decalcify the underlying dentin, thus allowing a better monomer dentinal interaction with the dental tissues[34,35]. It has also been stated that such procedures allow the formation of secondary chemical reactions between the material and hydroxyapatite[36]. This may explain in part the results of RelyX U200, which exhibited higher percentages of gap-free cementation areas compared to other cements tested in this study. Conventional application technique represents the placement of the adhesive luting material into the root canal, accessed using a dental probe, followed by the insertion of a post previously covered with the same material[37]. In the present study, both cements RelyX ARC and Panavia F2.0 were dispensed onto a mixing pad and then mixed into a homogenous paste as per manufacturer's instructions. Subsequently, the cements were spread onto the post and then placed into the root canal. RelyX U200 was dispensed using an automix dispenser device directly onto the post, or directly into the root canal using a specific application tip. According to the literature, there is a trend of a decrease in the bond strength as a function of coronal-apical direction when dual-cured cements are used for post cementation[38,39]. This was particularly true only for the cement Panavia F2.0. For both 3M ESPE products (ARC and U200), no significant difference was observed in terms of bond strength when the results obtained in the cervical and middle thirds were compared.

Pulpal-infected teeth can be successfully treated in endodontics if the smear layer created during instrumentation is totally removed along with bacteria and pulpal tissue[40,41]. The smear layer produced by various instrumentation techniques represents a barrier for the intimate contact between restorative materials and the root canal dentin. For this reason, various types of irrigation protocols have been advocated for smear layer removal and root canal disinfection. It is true that immediate sealing of endodontically-treated teeth through restorative materials represents a key procedure to prevent early coronal leakage[42]. However, clinicians should pay special attention on the that chemical irrigants used during root canal preparation may alter the chemical and mechanical properties of dentin surface and affect with the performance and longevity of all those materials used for endo-restorations[43]. The percentage of the chelating agent (EDTA) used in the propolis-based solution tested in this study favored the bonding of cements to root canal dentin, although irrigation with 17 % EDTA (60 s) followed by a final irrigation with NaOCl is the commonly recommended method to remove the smear layer[44,45].

The surface roughness significantly increased after treatment 1 (NaOCl /EDTA/ NaOCl) in comparison to other irrigation protocols as well as to the control, untreated group (#600 SiC paper, Table 6). The results of the present study are in accordance with those of previous studies[8,46], which advocated that the sequential use of EDTA and NaOCl can lead to a sort of erosion effect on the root canal dentin surfaces. In another study[47], it was demonstrated that the superficial erosive effect of NaOCl on root canal dentin surfaces was considered irreversible, irrespective of the association or not with the sequential application of EDTA as a final irrigant. Regarding the EDTA, according to these authors, it removes the collagen-depleted apatite phase, achieving a sort of dentin erosion extending from 10 to 15 µm in the subsurface[47]. On the other hand, the aqueous propolis-based solution was found to be less aggressive to the dentin root canal walls as the surface roughness in the experimental groups treated with treatments 4 (NaCl/PROP/CHX) and 5 (NaCl/PROP/NaCl) remained unaltered, even after the acid-etching/water-rinsing procedures.

The reasons that explain the results of the bond strength test in which the bond strength of fiberglass posts to the root canal dentin was not affect by the aqueous-based propolis solution is mainly due to its formulation. The propolis extract was diluted in water and then solubilized in hydrogenated castor oil (Cremophor-RH 40). Subsequently, the anionic surfactant sodium PEG-7 olive oil carboxylate (5%-Olivem 400) was added to the formulation. Finally, a 5% EDTA solution was added to the final formulation. Cremophor RH 40 is a non-ionic solubilizer and emulsifying agent obtained by reacting hydrogenated castor oil with ethylene oxide. It is used to solubilize ethereal oils and hydrophobic active substances in purely aqueous solutions. The finished preparations are particularly stable. PEG-7 olive oil carboxylate is also water soluble, and it is an anionic surfactant derived from olive oil with foaming, cleansing and solubilizing properties. Finally, even with in a lower concentration, EDTA is a versatile chelating agent. EDTA is frequently used associated with detergents, because it forms complexes with calcium and magnesium ions. The final appearance of the aqueous-based propolis solution is a clear and precipitate-free liquid. In this way, it can be speculated that the aqueous-based propolis solution possibly removed the smear layer from the dentin surface, and from inside the dentinal tubules. It is also important to remember that, although the cements tested present different bonding approach, all of them present acidic components in the composition that would somehow help to completely remove the remaining smear layer and debris left by the root canal mechanical treatment and/or incorporate the smear layer in the interfacial area: for the cement RelyX ARC, acid etching and water-rinsing may remove the remaining smear layer; for Panavia F2.0, conditioning of root canal dentin with a primer containing functional acidic monomers (no rinsing) may dissolve or incorporate the remaining smear layer in the hybrid layer; for RelyX U200, may incorporate the smear layer in the hybrid layer due to the presence of multifunctional acidic methacrylate monomers in the composition that are able to bind straightly the calcium ions in hydroxyapatite. The synergic effect between irrigant treatments and bonding approach seems to explain the reasons that the propolis based irrigant solution had no negative effect in the bond strength of fiberglass posts to the root canal dentin.

The development of an aqueous propolis-based endodontic irrigant is in agreement with what has been advocated in the literature in which an irrigating solution should ideally have a strong antibacterial effect, dissolve the necrotic tissues, and still be innocuous to the periapical tissues[48]. Overall, the results of the present study demonstrated that the fullness of the aqueous propolis-based solution in terms of chemical compounds (especially the phenolic contents) confer exceptional properties that allow it for being applied as an irrigant endodontic solution.

CONCLUSIONS

- The presence of gaps in the cementation interfacial areas seems to be more related to the cement type, bonding approach, and the cement application technique;
- In general, when cemented with the self-adhesive resin cement RelyX U200, higher bond strength and lower percentage of gaps in the cementation area were observed;
- Irrigant sequence sodium hypochlorite/EDTA/sodium hypochlorite is more aggressive for the root canal dentin in comparison to other irrigation protocols;
- The aqueous propolis-based solution used as an endodontic irrigant is less aggressive to the dentinal root surfaces without affecting the bonding of posts, proving to be a promising endodontic irrigant.

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