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Effects of Brown and Green Propolis on Bond Strength of Fiberglass Posts to Root Canal Dentin

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ABSTRACT

Introduction: The purpose of this study was to evaluate the effect of brown and green propolis on bond strength of the fiberglass posts to root canal dentin, and to compare it with conventional endodontic irrigants. **Methods and Materials**: Sixty bovine teeth were selected, decoronated and randomly distributed into six groups (n=10), according to the irrigation solution: 0.9% saline solution (Control); 2% chlorhexidine (CHX); 5% malic acid (MA); 0.5% ethanolic extract of brown propolis (BP); 0.25% ethanolic extract of green propolis (GP); 2.5% sodium hypochlorite (NaOCl). After root canal treatment, fiber posts were cemented into prepared root canals with a self-adhesive resin cement. The roots were cross-sectioned to obtain two discs from each third and submitted to the micro push-out test. Failure patterns were evaluated under optical microscopy. The influence of irrigants agents was analyzed using one-way ANOVA followed by Games-Howell's test (α=0.05). Failure modes were analyzed using Fischer's exact test (α=0.05). **Results**: There were statistically significant differences among the groups (P<0.05). The control, NaOCl and BP groups showed the highest bond strength with no statistically significant difference between them (P>0.05). Adhesive failure type was the predominant in all groups. **Conclusion**: Based on this *in vitro* study, the use of 0.5% brown propolis did not influence the bond strength of fiberglass posts to root canal dentin, while the use of 0.25% green propolis did affect it negatively.

Keywords: Dental Materials; Propolis; Root Canal Irrigants

Introduction

Fiberglass posts (FGPs) are a viable alternative for rehabilitation of structurally damaged endodontically treated teeth [1-4]. The success and longevity of FGPs restorations depends on long-term effective bonding between dentin, post and cement [5-7].

Endodontic and prosthetic procedures may interfere with bonding of FGPs to root canal dentin. The chemical constitution of irrigant solutions [5, 8], root canal sealers [5], and luting cements and application technique [2], as well as the post features [9] and smear layer removal methods [7] should be considered during dental rehabilitation with FGPs.

When treating infected root canals, cleaning and shaping, together with the action of an irrigant solution, are responsible to reduce the remaining microbiota [10]. Sodium hypochlorite (NaOCl), the most widely used endodontic irrigant, presents effective tissue dissolution ability, antimicrobial activity, and acceptable biological compatibility at lower concentrations [11]. However, the detection of organochlorine compounds (such as chloroform, hexachloroethane, dichloromethylbenzene and benzaldehyde) formed during the contact of NaOCl with pulp and dentin was previously showed [12]. These products are neurotoxic, highly lipophilic, chemically stable and persistent in nature, which have aroused efforts by governmental agencies and environmental groups to control their use [13].

Chlorhexidine (CHX) is also recommended as root canal irrigant due to its potent disinfectant property which is related to its wide-ranging antimicrobial activity [3, 8, 11, 14]. CHX is a protease inhibitor capable of neutralizing matrix metalloproteinases (MMP) and cathepsin cysteine activity, preserving the hybrid layer [15] and reducing adhesive interface degradation [16]. A great concern points to CHX decomposition into reactive by-products such as 4-chloroaniline (pCA), a possible human carcinogen, when used alone or as ointment base of calcium hydroxide paste [17].

Malic acid has been investigated to assess their demineralizing capacity and reduction on dentin microhardness [18]. Previous study has demonstrated the antimicrobial activity by malic acid, a mild organic acid used as an acid conditioner in adhesive dentistry, against *Enterococcus faecalis, Candida albicans, and Staphylococcus aureus* [19]. Moreover, the use of 7% maleic acid was more efficient than 17% EDTA in removal of smear layer from the apical third of the root canal system [20] and exhibited higher bond-strength of FGPs to the root dentin, acting as high-efficiency final irrigant in activation protocols [6].

Due to the known limitations of conventional endodontic irrigants, developing improved irrigating solutions for endodontics remains an area of great interest. The search for alternative products that have satisfactory cleansing lubrication of root canal system, dissolution of inorganic and organic substances, antimicrobial activity, low toxicity, availability, preservation of dental microstructure, and without interference in the adhesion of dental materials to root dentin [7] has driven the growing interest for the derivative agents of natural products and plant extracts [21].

Propolis is a resinous product produced by bees of the species *Apis mellifera*, whose main components are the flavonoids and phenolic acids that are responsible for its biological properties [22-24]. In endodontics, the effect of propolis has been investigated as an intracanal medication [21, 24] and irrigating solution [25, 26]. Previous studies have shown the potential of propolis against resistant microorganisms, as *Enterococcus faecalis,* common in endodontic infections [21, 24]. It was found only one study evaluating the effect of propolis as a root canal irrigant on dentin bond strength [26]. The authors observed that propolis solution had a favorable effect on the dentin bond strength of a resin self-etch adhesive to coronal dentin.

Since the materials used in endodontic therapy may interfere with the adhesion process of FGPs to root dentin, impairing dental rehabilitation [3-5], the purpose of this study was to investigate the influence of brown and green propolis and to compare with conventional endodontic irrigants on the bond strength of FGPs to root dentin. The null hypothesis tested was that there would be no difference among the bond strength value of the FGP to the root dentin regardless of the irrigants agents.

Materials and Methods

Specimen selection and preparation

The sample size was calculated using G* Power 3.1.2 software (Universitat, Düsseldorf, Germany), considering alpha error probability of 0.05 and power of 80% (effect size = 0.50). The software recommended 10 samples per group as the sample size. Sixty recently extracted bovine incisors with roots that were anatomically similar in size and shape, with canals that were less than 1 mm in cervical diameter and with completely developed root apices were selected for this study. Before root canal preparation (RCP), each tooth was decoronated with a double-faced diamond disc (KG Sorensen, Barueri, SP, Brazil) operated perpendicularly to its longitudinal axis to produce standardized roots of 15 mm length. The working length (WL) was established by deducting 1 mm from the root length. Preoperative radiographs in the mesio-distal and bucco-lingual directions were performed in order to confirm the absence of calcifications within the root canal and fractures, as well as the presence of a straight root with a single canal and complete root formation. All roots had their root surfaces evaluated using a stereomicroscope with 20× magnification (Motic K, Quimis, Diadema, SP, Brazil) in order to identify pre-existing cracks and root fractures.

Experimental groups and irrigants preparation

The specimens were randomly divided into six experimental groups (*n*=10), according to different root canal irrigants: Control-0.9% saline solution (Equiplex, Aparecida de Goiânia, GO, Brazil); CHX-2% chlorhexidine (Biológica, Cuiabá, MT, Brazil); MA-5% malic acid (Biológica, Cuiabá, MT, Brazil); BP-0.5% ethanolic extract of brown propolis; GP-0.25% ethanolic extract of green propolis; NaOCl-2.5% sodium hypochlorite (Biológica, Cuiabá, MT, Brazil)

To obtain propolis-based irrigants, the brown propolis was collected in the Cerrado region of the state of Mato Grosso, the second largest biome in Brazil, situated at 19°40' of south latitude. Samples of green propolis were obtained from Minas Gerais state, in the southeast region in Brazil, situated at 19°48′ of south latitude. The preparation of the crude ethanolic extracts of brown and green propolis were previously described [21]. Briefly, the crude ethanolic extracts of propolis were obtained by extraction in 80% cereal alcohol at 60°C and subsequent concentration in a Rota-Evaporator (Rotary evaporator 802, Fisatom, São Paulo, Brazil).

The propolis irrigants were prepared with the solvent dimethylsulfoxide (DMSO), in the following concentrations: BP-500 mg crude brown propolis extract/5 mL DMSO /95 mL saline solution; GP-250 mg crude green propolis extract/5 mL DMSO/95 mL saline solution.

Root specimens instrumentation and obturation

The root specimens were instrumented using a crown-down technique with ProTaper Next rotary nickel-titanium instruments (Dentsply Maillefer, Ballaigues, Switzerland), with apical enlargement performed at a size of 40/0.04, by a single endodontist. During instrumentation, the root canals were irrigated with 3 mL of the tested irrigating solution. After the preparation, the root canals were irrigated with 3 mL of 17% EDTA, pH 7.2 (Biodinâmica, Ibiporã, PR, Brazil) for 3 min to remove the smear layer, and then neutralized with 3 mL of distilled water. The root canals were dried with sterilized paper points (Dentsply Maillefer, Petrópolis, RJ, Brazil) and filled with gutta-percha (Dentsply Maillefer, Petrópolis, RJ, Brazil) and a resin-based sealer (AH-Plus, Dentsply Maillefer, Petrópolis, RJ, Brazil) that was mixed according to the manufacturers' directions, using a cold lateral compaction technique. The excess of gutta-percha and endodontic sealer were removed with a heated instrument, the root canal openings were filled with a temporary restoration (Cimpat, Septodont, Pomerode, SC, Brazil), and all samples were stored at 100% humidity for 4 weeks at 37º C.

Post space preparation and fiberpost cementation

To create a 10 mm post space, the condensed gutta-percha was removed using a heated plugger. The root canals were prepared using #1-4 Largo drills (Dentsply/Maillefer, Ballaigues, Switzerland) with a low-speed hand piece, and then irrigated with 3 mL of distillated water after each bur change. After the post space preparation, the root canals were actively rinsed with 3 mL of 17% EDTA, and distilled water and dried with absorbed paper points. In all groups, size #2 tapered, parallel-sided, and serrated fiberglass posts (Reforpost, Angelus, Londrina, PR, Brazil) with 20 mm length, 1.3 mm cervical diameter, and 0.9 mm apical diameter were used. The fiberglass posts were cleaned with 70% alcohol, and a silane agent (Silano, Angelus, Londrina, PR, Brazil) was applied with a micro brush for 1 min. The self-adhesive resin cement (RelyX-U200, 3M ESPE, Sumaré, SP, Brazil) was prepared following to the manufacturer's instructions, inserted to the root canal with the aid of an endodontic instrument and applied on the post. The post was seated to its full depth with digital pressure. Any excess cement was removed after 1 min. Three min later, the self-adhesive resin cement was light-cured using a 1,200 mW/cm^{-2} (Radii-Cal; SDI, Bayswater, Australia) source for 40 sec each on the cervical face of the specimen, along the long axis of the specimen, and oblique to the buccal and lingual surfaces, for a total of 120 sec. The specimens were stored at 100% humidity for 7 days at 37° C.

Micro push-out test

Seven days later, each specimen was sectioned perpendicularly to its long axis with the aid of a double-faced diamond disc (4" diameter × 0.012" thickness × 1/2"; Arbor, Extec, Enfield, CT, USA) and a precision saw (Isomet 1000, Buehler, Lake Bluff, IL, USA) at low speed with water cooling, obtaining two slices 1-mm in thickness from each third (cervical, middle, and apical) of the root, for a total of six slices per root.

The root specimens were then submitted to a push-out test in a universal testing machine (EMIC DL 2000, Instron, Barueri, SP, Brazil). A compressive load of 500 Kgf was applied at crosshead speed of 0.5 mm/min^{-1} in the apical-coronal direction until failure occurred. Each specimen was carefully positioned over a rigid basis, with the apical surface facing the punch tip with the diameter corresponding with the diameter with the set post/cement (0.8 mm to 1.2 mm). The bond strength in MPa was calculated by dividing the load at failure (N) by the area of the bonded interface. The area of the bonded interface was calculated as follows: $\boldsymbol{A=2\pi r \times h}$, where $\boldsymbol{A}$ is the area of the bonded interface, $\boldsymbol{\pi} = 3.14$, $\boldsymbol{r}$ is the radius of the post segment (mm), and $\boldsymbol{h}$ is the thickness of the post segment (mm) [9].

Failure pattern analysis

The failure pattern was determined after all specimens were air-dried. Both sides of the slices were analyzed using a light microscope at 40× magnification (Carl Zeiss, Jena, Germany). The failure pattern was categorized into three types: (i) adhesive (failure between the post and resin cement or between resin cement and root dentin); (ii) mixed, between post, resin cement and root dentin; and (iii) cohesive (failure in dentin, cement or in post) [4].

Statistics analysis

The Statistical Package for Social Sciences (IBM SPSS 21, IBM Co., New York, NY, USA) was used for statistical analysis. Shapiro-Wilk and Levene tests were used to test normality and homogeneity. According to Shapiro-Wilk test, the push-out bond strength data are in non-normal distribution ($P \leq 0.001$) and Levene test for equality of variances confirmed the inequality of variances for the results ($P \leq 0.001$). The influence of irrigants agents on bond strength was analyzed using the one-way ANOVA (Welch's). The Games-Howell post-hoc test was used for multiple comparisons ($\alpha=0.05$). The distribution of failure modes were analyzed by Fischer's exact test ($\alpha=0.05$).

Results

Table 1 shows the mean bond strength values, the standard deviations, the failure pattern distribution and the differences between the irrigants solution after the micropush-out test. There were statistically significant differences among the groups ($P<0.05$). The control, NaOCl and BP groups showed the highest bond strength with no statistically significant difference between them ($P>0.05$). Adhesive failure type was the predominant in all groups ($P<0.05$).

Discussion

The null hypothesis tested in this study was partially rejected. The bond strength of FGPs to root canal dentin was affected by 0.25% green propolis, 5% malic acid and 2% chlorhexidine.

Previous studies have validated the push-out test, the experimental methodology used in the present study, for the evaluation of bond strength which continues advantageous and required for the screening of new materials/products and the analysis of different experimental variables [3, 27, 28]. Push-out tests present more homogenous stress distribution and less variability in mechanical testing results [2, 3]. In addition, fewer samples are lost in push-out tests, which seems to be more efficient and safer than micro tensile method [2].

In the present study, bovine teeth were used because of the difficulty of obtaining human teeth for academic purposes [9]. Bovine teeth are easier to collect and the tooth age can be standardized [29]. In addition, previous studies have demonstrated that human and bovine root dentin have similar properties [29] confirming the use of the bovine tooth as a substitute for the human tooth for the evaluation of fiberglass posts bonded to root canal dentin [4, 8, 14].

Endodontic therapy aims the cleaning, shaping and disinfection of root canal system [9]. Several irrigant solutions have been proposed as an adjunct to the disinfection process [6-8, 11]. NaOCl is the first choice irrigant in endodontics and despite its advantages, it may negatively influence the bond strength of fiber-reinforced composite post restoration to root canal dentin because it oxidizes some component of the dentin matrix and inhibits the polymerization of the resin cement [30]. In fact, the impact of NaOCl on bond strength is still a controversial subject, since some researches have demonstrated positive results with the use of this solution and justify them by the fact that NaOCl removes the organic portion of the smear layer that turns into a rough root surface [8]. The results of the present study confirm NaOCl has no effect of on the bond strength of fiber post.

Chlorhexidine has also been indicated as an irrigant solution for root canal preparation [3, 8, 11, 14]. Some studies have investigated the role of CHX in the bonding process of fiberglass post to root dentin [31, 32]. However, its mechanism of action and the ability to predict these associations remain unclear [33]. An improvement in the bond strength of fiberglass posts to dentin was observed when the 2% CHX was compared to the 5.25% NaOCl [3], which may have occurred due to the absorption of CHX by the dentin that favors the infiltration of the resin in the dentin tubules and also because it is a non-oxidizing agent [14]. Furthermore, 2% CHX contains surface surfactant in its composition, which increases the surface energy of the dentin. In this way, there is an increase of the dentin wettability, allowing an increase in the bond strength of the resin cements to the root dentin [34]. In addition, recent studies have shown that CHX could act as a non-specific inhibitor of dentine's intrinsic proteolytic enzymes (formation of MMPs) and thus, slowing the degradation of the bonding interface [32]. On the other hand, a previous study has demonstrated that the use of CHX seems not to improve the bond strength of fiberglass posts to root canal dentin [33]. The formation of precipitates arising from the reaction between phosphate and CHX was reported to be responsible for decreasing bond strength because among other actions, these precipitates created a physical barrier, thereby reducing the interaction of the self-adhesive resin cement with the surface [31]. In addition, CHX is not able to completely remove the smear layer [3], which may interfere with the bond strength of resin cements to the surface [15]. In the present study, the lowest values of bond strength were observed in teeth irrigated with CHX.

Table 1. Mean and standard deviationof bond strength values in MPa and failure pattern distribution after micro push-out test according to groups

Irrigant agents*	Bond strength	Failure pattern [N (%)**]			
		i	ii	iii	Total
Control	9.97 (5.04)^{A***}	35 (66.0)^{Aa***}	16 (30.2)^{Ab}	2 (3.8)^{Ac}	53 (100)
CHX	4.02 (3.27)^{C}	42 (79.2)^{BCa}	10 (18.9)^{ACb}	1 (1.9)^{Ac}	53 (100)
MA	6.43 (4.70)^{B}	49 (92.5)^{Ba}	2 (3.8)^{Bb}	2 (3.8)^{Ab}	53 (100)
BP	7.95 (7.55)^{AB}	49 (90.7)^{Ba}	1 (1.9)^{Bb}	4 (7.4)^{Ab}	54 (100)
GP	3.87 (3.59)^{C}	44 (86.3)^{Ba}	5 (9.8)^{BCDb}	2 (3.9)^{Ab}	51 (100)
NaOCl	6.71 (7.39)^{ABC}	36 (65.5)^{ACa}	13 (23.6)^{ADb}	6 (10.9)^{Ab}	55 (100)

**0.9% saline solution (Control); 2% chlorhexidine (CHX); 5% malic acid (MA); 0.5% brown propolis extract (BP); 0.25% green propolis extract (GP); 2.5% sodium hypochlorite (NaOCl); **Adhesive (i); Mixed, between post, resin cement and root dentin (ii); Cohesive (iii); ***Capital letters compare groups in vertical lines. Lower letters compare groups in horizontal lines*

MA is a chelating agent, responsible for removing the smear layer deposited during the instrumentation of root canals [35]. However, when comparing to others irrigants such as EDTA and NaOCl, MA performs a lower removal of the smear layer [35], which could interfere in the bond strength process of dentin posts, as observed in the present study. However, Fan *et al.* [6] showed positive results with the use of MA after post space preparation. Additional studies should be conducted to define the best use of MA in Endodontics.

In the present study, it was evaluated the effect of ethanolic extracts of 0.5% BP and 0.25% GP, used as root canal irrigants, on the bond strength of FGPs to root dentin. It showed opposite effects, with 0.5% BP presenting the best results. Unfortunately, no previous published study on the effects of propolis on bond strength of FGPs to root canal dentin have been found. This makes it difficult to compare results. However, it was showed that 1% Amazonian propolis extract was responsible for partial removal of the smear layer, without exposure of the dentin tubules [36], which could explain the low bond strength values observed in the 0.25% GP group. Kalyoncuoglu *et al.* [27] observed that when a 20% Propolis ethanol extract from Turkey was used as a final irrigant after treatment with NaOCl, a high bond strength value of an adhesive system to dentin was registered. For the authors, the result could be attributed to the antioxidant capacity of flavonoids in propolis, which should have eliminated the sodium hypochlorite adverse effect of inhibition of the polymerization of resinous monomers. The effect of propolis as a cavity disinfection agent on the bond strength of a silorane-based resin composite was evaluated and no adverse effect was observed [37].

It is important to note that the different types of propolis are characterized and classified according to their chemical composition, which depends on the bee species, on the climate of the region, on the flora and on the season of the year in which it is collected [23, 24]. The biological activity of propolis is frequently associated with the presence of phenolic compounds, mainly flavonoids, such as flavone (rutin, luteolin), isoflavone (formononetine, daidzein), dihydroflavonol (pinobanksin, pinobanksin-3-acetate), and others [22, 24]. It is interesting to point out that the synergism between the components of Propolis, apart from the effects of the individual components, is not yet very clear and is believed to be a key factor of importance in determining the properties of propolis [22].

The analysis of failure patterns in the present study revealed that the more frequent failures were adhesive, which is in agreement with previous studies, that demonstrated that FGPs cemented with resin cements are weakest at the post-resin cement-root dentin interfaces [4, 28].

Although the samples in this study were not submitted to thermal and mechanical influences, which may occur in the oral cavity, the results can predict the clinical behavior of the tested post system, irrigant agents and the investigated self-adhesive resin cement. Future studies are necessary to analyze the effect of new chemical and mechanical protocols of the disinfection of root canals on the long-term stability of fiberglass posts.

Conclusion

Based on this animal study, the use of 0.5% brown propolis did not influence the bond strength of fiberglass posts to root canal dentin, while the use of 0.25% green propolis did affect it negatively.

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