Influence of Infected Root Dentin on the Bond Strength of a Self-adhesive Resin Cement

Abstract

Aim: The aim of this study was to determine the bond strength (BS) of a self-adhesive resin cement to the contaminated root dentin. **Materials and Methods:** The crown and apical third of twenty single-rooted teeth were removed. The root canals were flared and 1-mm-thick root sections were obtained. The sections were rinsed, dried, and sterilized. The control group (n=20) was composed of one section of each third, which remained immersed in sterile trypticase soy broth (TSB) for 2 months. The other sections comprised the experimental group ($n = 40$) and were immersed in a suspension of Enterococcus faecalis. The culture medium was changed at every 4 days for 2 months. The sections were rinsed with distilled water, dried, and the root canal space was filled with the self-adhesive resin cement RelyX™ U200. After 24 h, the push-out test was performed and the types of interface failure were observed on a stereo microscope. **Statistical Analysis:** Data were statistically analyzed by the nonparametric Mann–Whitney test (α=5%). **Results:** A significant reduction was observed in the BS of resin cement to the contaminated dentin compared to the healthy dentin, for both thirds analyzed ($P < 0.05$). The BS was significantly greater at the cervical third compared to the middle third for specimens in the experimental group ($P < 0.05$). Adhesive and mixed failures were observed more frequently in specimens contaminated with E. faecalis. **Conclusion:** Bacterial contamination negatively influenced the BS of the self-adhesive resin cement to the root dentin, and there was a predominance of adhesive and mixed failures.

Keywords: *Adhesive systems, bond strength, contaminated dentin, push-out test*

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Introduction

Adhesion is defined as the force joining two substances, when placed in close contact.[1] In dentistry, it is characterized as the interaction between a material and organic and inorganic components of enamel and dentin.[2] The adhesion to dental enamel is well established in contemporary restorative dentistry. However, adequate adhesion to dentin is more hardly achieved due to its biological characteristics and due to the high concentration of organic components and the tubular structure, with the presence of odontoblastic processes.[3] The different dentin structures, with a predominance of hydroxyapatite but also containing collagen Type I, glycoproteins, and water,[4] define different types of adhesion. Chemical adhesion usually occurs by bonding of phosphate monomers present in some dental materials with the calcium in dentin. Mechanical adhesion occurs mostly by entanglement of restorative materials within the collagen matrix[5] and inside the dentinal tubules.[6] Adequate retention of the material to the tooth structure assures good fit, reducing the marginal leakage and allowing good sealing[7] and treatment longevity. However, alterations in the tooth structure may negatively influence the adhesion, such as the continuous physiological mineral deposition, which reduces the diameter of dentinal tubuli; formation of tertiary dentin, which presents fewer tubuli and more irregular structure;[8] and the presence of contamination.[9-11]

Due to the great loss of coronal structure by caries, abrasion, previous restorations, trauma, or even the access to the root canal itself, the restoration of endodontically treated teeth may require the placement of a root canal post, combined with adhesive techniques, aiming to reinforce the remaining tooth structure and provide sufficient retention to the restoration.[12] During root canal preparation for the placement of posts, it is fundamental to perform adequate isolation

of the operative field. However, neglect to follow the recommended procedures may expose the root canal to microorganisms from the oral cavity, with imminent risk of recontamination.[13] Also, in previously contaminated teeth, even after endodontic therapy, microorganisms may remain inside the dentinal tubuli, which may recontaminate the root canal and form a biofilm.[14] Thus, material adhesion will occur on a structurally altered and contaminated dentin, which may possibly impair the bond strength (BS).

From a clinical standpoint, the marginal fit and BS of the material to dentin are important properties for the success of restorative and endodontic procedures. Thus, this study evaluated the BS of a self-adhesive resin cement to the contaminated root dentin, by the push-out test.

Materials and Methods

Teeth selection and preparation

This study was approved by the Institutional Review Board under protocol no. 1.013.887. Twenty single-rooted human teeth were radiographically selected from individuals aged 18 to 30 years, with single-root canals and similar anatomy, extracted for reasons unrelated to this study and donated by the individuals after signing an informed consent form. The teeth were cleaned and stored in 0.9% saline pH 7 until utilization in the study. The crown and apical third of each tooth were removed using a diamond disc at low speed under constant cooling to achieve root segments with approximately 8-mm length. The root canals were flared with a post bur no. 2 at low speed (Exacto, Angelus, Londrina, PR, Brazil), calibrated at 7 mm.

Five sections perpendicular to the root long axis were performed using a precision-sectioning machine Isomet (Isomet 1000, Buehler, Lake Bluff, IL) and a diamond disc with 75-g weight at a speed of 100 rpm, under constant cooling. Six transverse sections with approximately 1-mm thickness were obtained (three from the cervical third and three from the middle third). The sections were examined using a stereo microscope (×4) to check the absence of cracks and defects. The surface corresponding to the apical region was delimited to allow differentiation during testing. Next, the sections were individually inserted in identified Eppendorf tubes and sequentially immersed in 17% EDTA solution for 3 min and in 1% NaOCl solution for 3 min[15] under mild shaking, for removal of debris and smear layer. Thereafter, the sections were rinsed in distilled water, dried with gauze, and sterilized.

Contamination of root sections

From each root, two root sections corresponding to the cervical and middle thirds were immersed in sterile trypticase soy broth (TSB) (Merck, Cotia, São Paulo, Brazil) and were taken as control groups ($n = 20$). The other sections pertaining to the cervical and middle thirds ($n = 40$) were immersed in 1 ml of suspension of *Enterococcus faecalis* (ATCC® 29212) ($\approx 10^8$ UFC/ml). For inoculum formation, an overnight culture of *E. faecalis* was diluted in 1:100 in fresh TSB fresco, supplemented with 0.4% sucrose. The root sections were incubated in a bacteriological oven at 37°C for 60 days, and the culture medium (900 μl) was changed at every 4 days.

Push-out test: Preparation of specimens and analysis of failure types

After the incubation period, the root sections were removed from the Eppendorf tubes using sterile tweezers and the excess inoculum was thoroughly rinsed with distilled water and dried with gauze and sterile absorbent paper points. Next, the root canal space was filled with the self-adhesive resin cement RelyX™ U200 (3M™/ESPE™, St. Paul, MN, USA). The excess cement was removed with spatulas. To avoid the formation of bubbles and allow achievement of a smooth surface, a glass coverslip was positioned under mild pressure on the root section. Light curing was performed using a LED unit (1200 mW/cm²; Elipar™ FreeLight 2, 3M™/ESPE™), following the manufacturer's recommendations. The specimens were individually stored in an environment at 100% humidity, for 24 h, to allow for cement setting.

Each specimen was positioned on a stainless steel metallic base with a 1-mm diameter orifice at the central region, connected to the lower portion of the universal testing machine Instron (model 444, Instron, Canton, MA, USA) [Figure 1]. The specimen was positioned at the same direction as the orifice on the metallic base with the cervical aspect turned downward so as the load was applied in apical-coronal direction of the specimen. A metallic rod with 0.6-mm active tip fixated on the upper portion of the testing machine was used at a crosshead speed of 0.5 mm/min until the occurrence of cement displacement. The force required for cement displacement was measured
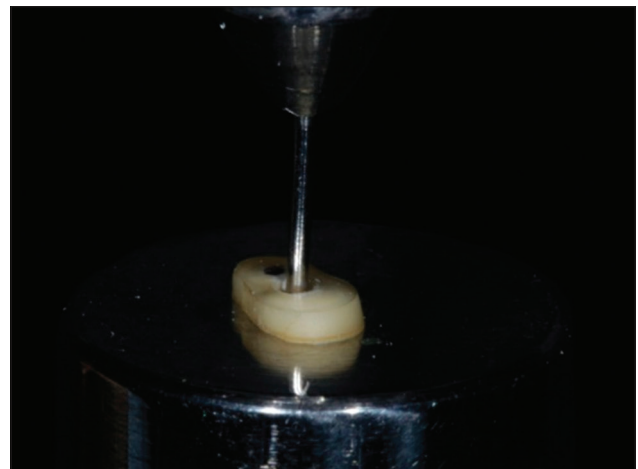

Figure 1: Push-out test design

in kilonewtons, and then converted into Newtons and divided by the cement area in mm², to calculate the BS in megapascals (1 MPa = 1 N/mm²).

After the push-out test, the specimens were analyzed on a stereo microscope (×63) (SZ-CTV, Olympus, Japan) and photographed (Panasonic digital GPKR 222) to determine the type of failure at the cement/dentin interface, according to Teixeira *et al*. 2009 [Figure 2]: (a) adhesive failure, when the cement was completely separated from dentin; (b) cohesive failure of cement, when fracture occurred within the material and dentin remained covered by cement; (c) mixed failure, when adhesive and cohesive failures of cement occurred concomitantly; and (d) cohesive failure of dentin, when fracture occurred in dentin.

Statistical analysis

The Shapiro–Wilk normality test demonstrated nonnormal distribution of data. The set of data, represented by BS values, was statistically analyzed by the nonparametric Mann–Whitney test. The tests were performed using the software SPSS version 17.0 (SPSS Inc., Chicago, IL, USA), at a significance level of 5%.

Results

During the study, there was a loss of one specimen in the control group and eight specimens in the experimental group, due to the laboratory handling.

The mean BS values are presented in Table 1. Specimens contaminated with *E. faecalis* presented significantly lower BS values compared to specimens in the control group, for both thirds analyzed ($P < 0.05$). The BS of resin cement to the contaminated dentin was greater for specimens of the cervical third, compared to specimens of the middle third ($P < 0.05$).

Adhesive and mixed failures were observed more frequently in specimens contaminated with *E. faecalis*. In the control group, varied failures occurred, with predominance of cohesive failures in dentin, mixed, and cohesive failures in cement, for specimens of the cervical third, and predominantly cohesive in dentin for specimens of the middle third [Table 2].

Discussion

There is an increasing interest in the process of adhesion of dental materials to dentin, since this property is very important for treatment longevity.[16] The factors influencing the quality of adhesion of dental materials to the crown dentin, including the presence of microorganisms, are widely investigated.[9,11,17] However, no study has evaluated the influence of the presence of microorganisms and their byproducts on the quality of adhesion to the root dentin.

In this study, the BS of self-adhesive resin cement RelyX™ U200 to the healthy and contaminated root dentin was analyzed by the push-out test. This test is considered an
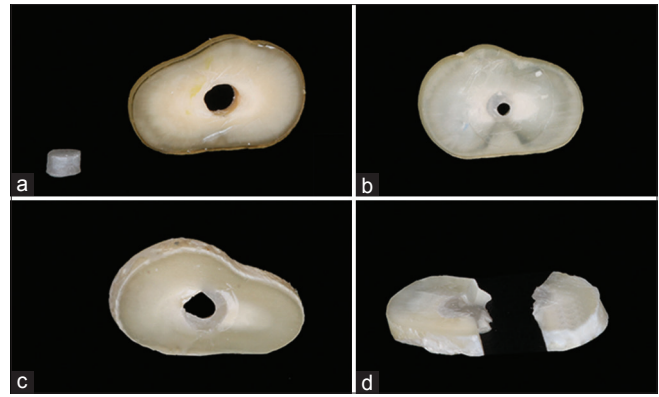
Figure 2: Types of failure occurring after the push-out test: (a) Adhesive failure, (b) cohesive/cement failure, (c) mixed failure, (d) cohesive/dentin failure

Table 1: Mean bond strength values (Megapascals) and standard deviation observed in samples from control and experimental groups in cervical and middle thirds

Groups	Cervical third		Middle third	
	BS (MPa)	SD	BS (MPa)	SD
Control	6.35[b]	2.24	7.13[b]	2.21
Experimental	2.71[aA]	1.69	1.21[aB]	1.06

Different lower case letters in the same column and different capital letters in the same row indicate statistical difference between means (*P*<0.05). BS: Bond strength; SD: Standard deviation; MPa: Megapascals

effective and reliable method,[18] which is very useful to analyze the BS of dental materials,[19] since it allows the identification of regional differences of BS along the root dentin.[20]

Considering the need of root canal posts, several types are commercially available, including nonmetallic posts, which allow adhesive cementation. Even though the multistep acid etching system presented better performance and is considered the gold standard,[21] the single-step adhesive system is attractive and widely used, since it does not require pretreatment of the dentin surface, simplifying and reducing the time of procedures.[22]

The BS values obtained for specimens in the control group ranged from 6.35 MPa to 7.13 MPa, in agreement with previous studies that reported values between 4.56 MPa[23] and 9.3 MPa.[24] The presence of an intact dentin, clean and with unaltered structure, allows adequate entanglement of materials, which is fundamental for formation of the hybrid layer, characterized as the space corresponding to resin infiltration within the collagen matrix.[7]

Conversely, contamination of the root sections with *E. faecalis* significantly reduced the BS of the resin cement to the root dentin. Studies analyzing the adhesion of other cementation systems to the contaminated coronal dentin, by push-out tests, also revealed significant reduction in values compared to the intact dentin.[9,11] The results

Table 2: Distribution of types of failures in the control and experimental groups in the cervical and middle thirds, after the push-out test (%)

Groups	Third (*n*)	Failure types (%)				
		Adhesive/dentin	Cohesive/cement	Mixed	Cohesive/dentin	Lost
Control	Cervical	5.2	31.6	31.6	31.6	5
	Middle	10.0	5.0	20.0	65.0	0
Experimental	Cervical	73.7	7.9	18.4	0	5
	Middle	85.3	2.9	11.8	0	15

are explained by the partial demineralization observed for contaminated dentin, whose structure is altered with higher degree of porosity, which negatively influences the formation of hybrid layer. The metalloproteinases, enzymes released by microorganisms with collagenolytic activity, contribute to hydrolytic degradation of the organic dentin matrix,[25] impairing the entanglement of adhesive materials and endodontic cements.[9] In addition, the permeability of infected dentin is significantly lower compared to the healthy dentin, due to the occlusion of dentinal tubuli by the presence of smear layer, which is composed of denatured collagen and carious crystals.[26] These variables may negatively influence both the chemical interactions between structures involved in the process of adhesion[11] and the hybrid layer thickness.[10]

In specimens from the control group, even though several types of failure were observed, cohesive failure in dentin (65%) was observed in a greater number of specimens. This result suggests that the BS of cement to dentin was greater than the cohesive resistance of dentin. Thus, the dentin fractured after force application. Conversely, for specimens contaminated with *E. faecalis*, adhesive failure was the most frequent, both at the cervical and middle thirds. Probably, due to the weak BS to dentin, total displacement of the resin cement was facilitated.

The presence of dentin contamination and consequent alteration of the dentin structure caused by the action of microorganisms have direct clinical implication, since they impair the process of adhesion of resin cement to root dentin. Thus, careful asepsis and isolation of the operative field during preparation for posts is mandatory, as well as care with the integrity of coronal sealing, since the root canals may be recontaminated due to rupture and/or loss of the temporary restoration.[27] Adequate disinfection of the root canal system is also fundamental, in an attempt to avoid the permanence of microorganisms within the dentinal tubuli and at the deepest dentin layers.[28]

Conclusion

Based on the present results and considering the limitations of an *ex vivo* laboratory study, it was concluded that bacterial contamination negatively influenced the BS of the self-adhesive resin cement RelyX™ U200 to the root dentin, with predominance of adhesive failures.

Conflicts of interest

There are no conflicts of interest.

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