Influence of Irrigation Sequence on the Adhesion of Root Canal Sealers to Dentin: A Fourier Transform Infrared Spectroscopy and Push-out Bond Strength Analysis

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Abstract

Introduction: There is a lack of evidence on the chemical interaction between sealers and dentin. The influence of irrigation on the chemical interaction between root canal sealers and dentin was analyzed by using Fourier transform infrared spectroscopy (FTIRS) and measurement of dislocation resistance. **Methods:** Single-rooted teeth (n = 120) were instrumented with 3% NaOCl as the irrigant and divided into 4 groups (n = 30) on the basis of irrigation protocol: group 1, 3% NaOCl, 17% EDTA, water; group 2, 17% EDTA, 3% NaOCl, water; group 3, 3% NaOCl, QMix, water; group 4, 3% NaOCl, water. Each group was divided into 3 subgroups (n = 10) on the basis of the root canal sealer: A, epoxy resin (AH Plus); B, silicone (RoekoSeal); C, calcium hydroxide (Sealapex). The dislocation resistance was assessed by using push-out bond strength test. The data were statistically analyzed by three-way analysis of variance and Holm-Sidak tests (P = .05). Dentin powder treated as per the conditioning protocols mentioned was mixed with the sealers and analyzed by FTIRS. **Results:** A significant interaction was observed between irrigation protocol, type of sealer, and root segment (P < .001) for AH Plus but not for RoekoSeal and Sealapex (P > .05). AH Plus showed the highest bond strength (P < .05). FTIRS showed chemical bonding between AH Plus and dentinal collagen. In groups 2 and 4, no chemical bonding was observed. **Conclusions:** Bond strength of sealers is differentially affected by the irrigation protocol. The epoxy resin sealer AH Plus chemically bonds to dentinal collagen. This interaction is influenced by the irrigation protocols. *(J Endod 2015;41:1108–1111)*

Key Words

AH Plus, bond strength, dislocation resistance, FTIR spectroscopy

Root canal irrigants play a key role in chemomechanical preparation of the root canal system. In addition to their antibacterial action, irrigants serve as lubricants during instrumentation (1) and influence the sealing ability and adhesion of root-filling materials (2–4).

One commonly used irrigation protocol involves the use of sodium hypochlorite (NaOCl), followed by 17% EDTA and a final flush of NaOCl. Logically, the use of a proteolytic agent such as NaOCl after the use of a demineralizing agent such as EDTA would impose undesired consequences on the dentin substrate (5). The peptide chains of the dentinal collagen are broken down in addition to chlorination of the terminal protein groups. The collagen that is encapsulated by hydroxyapatite is not subject to destruction. When a sequence of NaOCl-EDTA-NaOCl is used, collagen fibrils lose the hydroxyapatite encapsulation. This protocol was shown to negatively influence the bonding of an epoxy resin sealer, AH Plus, to dentin (4). It has been hypothesized that the epoxide rings of the sealer bonded chemically to the amino groups of dentinal collagen (4, 6). Such an assertion has not been drawn for other root canal sealers.

An irrigant combining chlorhexidine, EDTA, and a detergent (QMix; Dentsply Tulsa Dental Specialties, Tulsa, OK) has been introduced to be used as a final rinse. The smear layer removal by QMix has been shown to be comparable with that of 17% EDTA (7). The use of chlorhexidine (4) or QMix (8) as a final rinse appears to increase the dentin bond strength of epoxy resin root canal sealers. The effect of these irrigation protocols on the dislocation resistance of other sealers is still unknown.

The aim of this study was to determine the influence of irrigation protocols on the chemical interaction of 3 root canal sealers (epoxy resin, silicone, and calcium hydroxide) to dentin by using Fourier transform infrared spectroscopy (FTIRS) and measurement of the dislocation resistance (measured by the push-out bond strength test). The null hypothesis tested was that the irrigation protocols had no influence on the dislocation resistance of the sealers.

Materials and Methods

Specimen Preparation

Human single-rooted mandibular first premolars (n = 120) were collected and thoroughly cleaned by removing the hard deposits by using curettes and the soft deposits by soaking in 5.25% NaOCl for 10 minutes under a protocol approved by the Institutional Review Board and Ethical Committee of the university. The teeth were decoronated at the cementoenamel junction by using a diamond disk under water-cooling. The root lengths were standardized to 15 mm and radiographed (DSX 730; Owandy Dental Imaging, Champs sur Marne, France; Kodak 2100 X ray

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unit; Kodak Dental Systems, Atlanta, GA) at 2 angulations to confirm the presence of a single canal. The root canals were instrumented by using ProTaper nickel-titanium rotary instruments (Dentsply Maillefer, Ballaigues, Switzerland) up to size F4. During instrumentation, irrigation was performed with 3% NaOCl (Parcan; Septodont, Saint-Maur-des-fossés, France) by using a 5-mL disposable plastic syringe (Ultradent Products Inc, South Jordan, UT), with an irrigator tip (Navitip 31-gauge double side-port; Ultradent) placed passively into the canal, up to 2 mm from the apical foramen without binding. This was done to simulate the clinical scenario wherein irrigating needles are placed short of the apex to prevent irrigant extrusion. This step was performed in accordance with previous studies (4, 9, 10). A total of 5 mL NaOCl was used for each canal for duration of 15 minutes.

The samples were divided into 4 groups (n = 30) according to the final irrigation regimen, during which 5 mL of the following irrigants was used: group 1, 3% NaOCl (Parcan; Septodont) followed by 17% EDTA (Pulpdent, Watertown, MA); group 2, 17% EDTA followed by 3% NaOCl; group 3, 3% NaOCl followed by QMix; group 4, 3% NaOCl followed by saline. Each irrigant was allowed to remain in the canal for 2 minutes. Five milliliters distilled water was used between the irrigants in groups 1–3.

The canals were rinsed with 5 mL distilled water and dried by using paper points (Dentsply Maillefer), and samples of each group were allotted to one of the subgroups that were based on the root-filling material: A, epoxy resin root canal sealer (AH Plus; Dentsply DeTrey, Konstanz, Germany); B, silicone root canal sealer (RoekoSeal; Coltène/Whaledent AG, Altstätten, Switzerland); and C, calcium hydroxide root canal sealer (Sealapex; Sybron Endo, Orange, CA). Sealers were applied by using a lentulo spiral (Dentsply Maillefer). The teeth were radiographed (DSX 730; Owandy Dental Imaging) at 2 angulations. Samples with voids or bubbles were discarded. Specimens were placed in 100% humidity for 48 hours to ensure complete setting of the sealer.

Measurement of Dislocation Resistance by Push-out Bond Strength Test

Each root was embedded in epoxy resin in a custom-made split-ring copper mold. After setting of the epoxy resin, 1-mm-thick slices were obtained from each root by using a water-cooled precision saw (Ernst-Leitz, Wetzlar, Germany). The first slice of each third (n = 10 slices per root third) was selected for the push-out test. Each specimen was marked on its coronal surface with an indelible marker, and the exact thickness of each slice was measured by using digital calipers to 0.04-mm accuracy (Mitutoyo, Tokyo, Japan). The apical and coronal diameters of the obturated area were determined by using Olympus Camedia C-5060 digital camera (Tokyo, Japan) attached to a stereomicroscope (Global G6, St Louis, MO).

Each root section was subjected to a compressive load via a universal testing machine (Lloyd LRX-plus; Lloyd Instruments Ltd, Fareham, United Kingdom) at a crosshead speed of 1 mm/min by using stainless steel plungers of different diameters, 1.10 mm (coronal), 0.8 mm (middle), and 0.5 mm (apical), positioned so that it contacted only the filling material (11, 12). Push-out force was applied in an apico-coronal direction until bond failure occurred, manifested by extrusion of the filling material and a sudden drop in load deflection. The force was recorded by using Nexygen data analysis software (Lloyd Instruments Ltd). The maximum failure load was recorded in newtons, and push-out bond strength was calculated in megapascals (MPa).

FTIRS

Pure collagen (Type I) was mixed with equal quantity (15 mg) of each of the sealers and allowed to set for 24 hours in 100% humidity, after which it was rinsed thoroughly with distilled water to remove any chemically unbound molecules. The samples were dried and analyzed in triplicate by FTIRS (Perkin Elmer FTIR Spectrometer, Waltham, MA) to identify the chemical interaction between the sealer and collagen.

To study the effect of irrigation protocols on the chemical interaction between the sealers and dentinal collagen, dentin powder was obtained on the basis of a protocol described previously (13). Dentin powder was immersed in 3% NaOCl for 15 minutes, filtered, and divided into 4 groups (150 mg each) on the basis of the chemical treatment as mentioned in the push-out bond strength section. Untreated dentin powder served as the control. The dentin powder (50 mg) was dried and mixed with an equal quantity (50 mg) of each of the sealers and analyzed in triplicate by FTIRS. Spectra were obtained between 400 and 4000/cm at 4/cm resolution by using 32 scans.

Data Presentation and Analysis

The main outcome variable in this study was push-out bond strength (in MPa). Data analysis by Shapiro-Wilk test showed normal distribution of data, and parametric statistical tests were applied. Three-way analysis of variance was performed to weigh the impact of sealer, root third, and group (ie, irrigating protocol) as the 3 independent variables on the outcome (dependent variable). Holm-Sidak procedure was used for multiple testing. The alpha-type error was set at 0.05 for all statistical analyses.

Results

Push-out Bond Strength

There was a statistically significant interaction between the irrigation protocol, type of sealer, and root segment ($P < .001$) for AH Plus but not RoekoSeal and Sealapex ($P > .05$). Roekoseal showed no measurable bond to the canal wall, and hence data for this material have not been presented. The highest bond strength was demonstrated by AH Plus in all 3 root thirds (Table 1). Specifically, the highest bond strength was demonstrated by AH Plus in the coronal third, when specimens were irrigated with NaOCl-EDTA (4.8 ± 0.2 MPa). However, this was not significantly different from the bond strength of AH Plus in the coronal thirds of specimens irrigated with NaOCl-QMix (3.61 ± 0.55 MPa). With regard to the irrigation protocol, group 1 (NaOCl-EDTA) showed the highest bond strength values for all sealers at all root thirds. The mean bond strength values were significantly higher than samples in groups 2 (EDTA-NaOCl) and 4 (NaOCl) ($P < .05$) but not from group

TABLE 1. Push-out Bond Strength (MPa, mean ± standard deviation) of 2 Sealers (AH Plus and Sealapex) after 4 Irrigation Protocols According to Root Third (N = 10)

Group	Coronal third	Middle third	Apical third
NaOCl-EDTA-water			
AH Plus	$4.8 \pm 0.2^{a,A}$	$2.4 \pm 0.09^{a,A}$	$2.1 \pm 0.29^{a,A}$
Sealapex	$0.52 \pm 0.05^{b,A}$	$0.44 \pm 0.05^{c,A}$	$0.39 \pm 0.07^{c,A}$
EDTA-NaOCl-water			
AH Plus	$2.8 \pm 0.26^{a,B}$	$1.7 \pm 0.19^{a,B}$	$1.3 \pm 0.16^{a,B}$
Sealapex	$0.39 \pm 0.19^{b,A}$	$0.29 \pm 0.03^{c,A}$	$0.21 \pm 0.03^{c,A}$
NaOCl-QMix-water			
AH Plus	$3.61 \pm 0.55^{a,C}$	$1.89 \pm 0.22^{a,B}$	$1.27 \pm 0.15^{a,B}$
Sealapex	$0.48 \pm 0.10^{b,A}$	$0.38 \pm 0.02^{c,A}$	$0.30 \pm 0.02^{c,A}$
NaOCl-water			
AH Plus	$2.80 \pm 0.58^{a,B}$	$1.5 \pm 0.34^{a,C}$	$0.80 \pm 0.19^{a,C}$
Sealapex	$0.42 \pm 0.12^{b,A}$	$0.26 \pm 0.07^{c,A}$	$0.12 \pm 0.04^{c,A}$

Within each group, values with identical lowercase superscript letters indicate no significant difference ($P > .05$); between groups for the same subgroup, values with identical uppercase superscript letters indicate no significant difference ($P > .05$). RoekoSeal showed no measurable bond strength, and hence the data are not shown.