The effects of endodontic substances and naturally reducing agents on the bond strength of epoxy resin-based sealer to root dentin

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Abstract

Aim: To evaluate the effects of sodium hypochlorite (NaOCl), chlorhexidine (CHX), and two naturally derived reducing agents on the bond strength of epoxy resin-based sealer to root dentin.

Materials and Methods: A total of 60 single-rooted human teeth were prepared using ProTaper (Dentsply Tulsa Dental Specialties, Johnson City, TN, USA) and an irrigation protocol including 5\% NaOCl or 2\% CHX gel, and ethylenediaminetetraacetic acid (EDTA), for smear layer removal. The following subgroups \((n = 10)\) were also assessed according to the naturally derived reducing agent used: no agent (control group); grape seed extract (GSE); and green tea. Root canals were filled with gutta-percha and AH Plus (Dentsply DeTrey, Konstanz, Germany). Bond strength was measured using the push-out test, and statistical analyses were performed using ANOVA; failure patterns (modes) were classified as adhesive, cohesive, or mixed. The types of failure modes were evaluated using the Chi-squared test at \(\alpha = 0.05\).

Results: The irrigation protocols demonstrated similar bond strength values \((P > 0.05)\). However, the Chi-squared test revealed significant differences in failure mode among the groups \((P < 0.05)\). An increase in the incidence of adhesive failures was observed for the NaOCl and EDTA groups. The other groups demonstrated a prevalence of mixed and cohesive failures.

Conclusion: The irrigation protocols and use of naturally derived reducing agents had no effect on the bond strength of the resin-based sealer to dentin; however, improvement was evident in the adhesion quality of AH Plus to NaOCl-treated root dentin, due to the prevalence of cohesive failure.

Keywords: Adhesion; compressive strength; root canal irrigants; root canal filling material

INTRODUCTION

Root canal disinfection is achieved through chemomechanical preparation using manual, rotary, and/or reciprocation instruments that are associated with endodontic auxiliary chemical agents.\textsuperscript{[1]} Sodium hypochlorite (NaOCl) is commonly used as an endodontic irrigant due to its ability to dissolve organic matter and its antimicrobial properties.\textsuperscript{[3]} The disadvantages of NaOCl include toxicity,\textsuperscript{[3]} promotion of structural changes in organic dentin components (mainly collagen),\textsuperscript{[4]} and its effects on the mechanical properties of dentin, such as reducing flexural strength and the elastic modulus.\textsuperscript{[5,6]} Furthermore, NaOCl lacks residual antimicrobial activity.\textsuperscript{[7,8]} Chlorhexidine digluconate (CHX) has been used as a chemical auxiliary agent in root canal treatment because of its broad-spectrum antibacterial properties\textsuperscript{[1,2,9]} and its ability to be adsorbed by hard dental tissues. It exhibits gradual and prolonged release at therapeutic levels, which is referred to as the residual effect or substantivity.\textsuperscript{[7,8,10]} Moreover, CHX affects neither the mechanical properties of dentin\textsuperscript{[6]} nor the

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integrity of dentin collagen.[14] In addition, it has potent inhibitory matrix metalloproteinase (MMP)\textsuperscript{[11]} and cysteine cathepsin\textsuperscript{[12]} activities, resulting in the preservation of adhesive restoration over time.[13]

AH Plus (Dentsply DeTrey, Konstanz, Germany) is a hydrophobic, epoxy resin-based sealer that is widely used because of its particular physical properties.[14] The adhesiveness of AH Plus to root dentin is based on covalent bonding between the open epoxide ring and the exposed side-chain amine groups of the collagen network.[15] Dentin surface treatments with different irrigation protocols predominantly cause alterations to the collagen fibrils of dentin, which may compromise the adhesiveness of endodontic sealers to the dentin surfaces. Many studies have demonstrated that NaOCl reduces the bond strength between adhesive materials and dentin.[16-20] Furthermore, it potentially reduces the bonding strength of AH Plus sealer.[19,20] This may be attributable to the presence of residual oxygen species on the dentin, which affects the setting time of the adhesive material.[16,17]

The compromised bond strength of NaOCl-treated dentin could be restored by the application of antioxidants before the adhesive procedure;\textsuperscript{[21]} these agents interact with the by-products of NaOCl,[17] resulting in the neutralization and reversal of the oxidizing effects of NaOCl on the dentin surface.\textsuperscript{[16,17,21]} Sodium ascorbate is the most studied antioxidant agent.\textsuperscript{[16,17]} However, Erhardt et al.\textsuperscript{[22]} reported that some materials benefited from its protective effects, whereas others yielded negligible results. Naturally derived reducing agents have the ability to chemically modify collagen without damaging biological tissues and moreover, improve dentin matrix properties.\textsuperscript{[23,24]} Examples include proanthocyanidin (PAC)-rich plant extracts from grape seed extract (GSE)\textsuperscript{[23,25]} and green tea (GT).\textsuperscript{[23,26]} The application of these provided redox potential as reducing agents for NaOCl-treated dentin, and can potentially improve the quality of collagen modified by NaOCl.

Given the importance of providing an adequate seal during endodontic treatment, the aim of this study was to evaluate the effects of NaOCl, CHX, and two naturally derived reducing agents on the bond strength of epoxy resin-based sealer to root dentin. The hypotheses were that an irrigant could be restored by the application of antioxidants before the adhesive procedure;\textsuperscript{[21]} these agents interact with the by-products of NaOCl,[17] resulting in the neutralization and reversal of the oxidizing effects of NaOCl on the dentin surface.\textsuperscript{[16,17,21]} Sodium ascorbate is the most studied antioxidant agent.\textsuperscript{[16,17]} However, Erhardt et al.\textsuperscript{[22]} reported that some materials benefited from its protective effects, whereas others yielded negligible results. Naturally derived reducing agents have the ability to chemically modify collagen without damaging biological tissues and moreover, improve dentin matrix properties.\textsuperscript{[23,24]} Examples include proanthocyanidin (PAC)-rich plant extracts from grape seed extract (GSE)\textsuperscript{[23,25]} and green tea (GT).\textsuperscript{[23,26]} The application of these provided redox potential as reducing agents for NaOCl-treated dentin, and can potentially improve the quality of collagen modified by NaOCl.

**MATERIALS AND METHODS**

**Specimen preparation**

Sixty single-rooted human teeth with anatomically similar root segments and fully developed apices were selected in a protocol approved by the institutional review board and ethics committee of the local University (\#434100). No teeth had undergone previous endodontic treatment. Each tooth was decoronated below the cementoenamel junction perpendicular to the longitudinal axis using a slow-speed, water-cooled diamond disc (Isomet 2000; Buehler Ltd, Lake Bluff, IL, USA). The roots were cut to a uniform length of 15 mm from the apical end. The working length was determined to be 15 mm. Subsequently, the root canals were irrigated with distilled water, and the pulp tissue was removed using a \#10 K-file (Dentsply Maillefer, Ballaigues, Switzerland). A \#20 K-file was used to standardize the foramenal size.

**Root canal preparation**

A ProTaper instrument (Dentsply Tulsa Dental Specialties, Johnson City, TN, USA) was used in a 16:1 gear reduction handpiece powered by a torque-controlled electric motor (X-Smart Plus; Dentsply Maillefer). A consistent rotation speed of 300 rpm was used in a crown-down manner involving a gentle in-and-out motion according to manufacturer’s instructions. A shaping file (S1) was passed apically to within 2 mm of the working length, and SX files were then used until resistance was felt (4–5 mm from working length), followed by the use of the ProTaper instrument to carry the S1 and S2 until the full working length was achieved. The apical one-third was finished by passing the F1, F2, and F3 until the working length was reached. The instruments were used in a pecking motion and were regularly cleaned to remove debris from the flutes. Once the instrument had been passed to the end of the canal and could be rotated freely, it was subsequently removed. At this point, the next instrument in the sequence was used.\textsuperscript{[27]}

**Experimental groups**

The specimens were randomly assigned to two experimental groups (\(n = 30\) each) according to the chemical auxiliary agent.

In Group 1 (5% NaOCl solution, Sigma-Aldrich, St. Louis, MO, USA), the root canal was filled with 5% NaOCl using a 5-mL syringe and a 19-gauge needle before the use of the instrument. The needle was centered within the canal, 3 mm short of the working length. Each instrument was used on the root canal for 3 min. After the use of each instrument, 5 mL of NaOCl was used as an irrigating solution.

In Group 2 (2% CHX gel), the root canal was filled with CHX using a 3 mL syringe and a 19G needle before insertion of the instrument into the root canal. The needle was centered within the canal, 3 mm short of the working length. After the use of each instrument, 5 mL of distilled water was used as an irrigating solution with a 5-mL syringe and a
30G needle placed 3 mm short of the working length. A chemical auxiliary agent (i.e., CHX) was used with the endodontic instrument to prepare the root canal. Distilled water was used as an irrigating solution to liberate CHX and instrumentation-related debris from the root canal.[10]

The smear layer was removed from both groups through irrigation of the root canal using 3 mL of 17% EDTA for 1 min, followed by further irrigation with 5 mL of distilled water.

The specimens from each group were randomly subdivided into three subgroups \((n = 10)\) according to the naturally derived reducing agent used as follows: control group (without a naturally derived reducing agent); and two treatment groups GSE (irrigation with 5 mL of 10% GSE [Vitis vinifera, Mega-Natural gold GSE, Polyphenolics Madera, CA, USA]) and GT (irrigation with 5 mL of 10% GT [Sigma-Aldrich]). The canal was infused with the naturally derived reducing agent for 1 min and was then aspirated using a capillary tip (size 0.014; Ultradent, South Jordan, UT, USA) at low vacuum with a gentle up-and-down motion for 5 s. The root canals were subsequently dried with sterile paper points.

### Root filling procedures

All root canals were filled with gutta-percha cones and AH Plus sealer (Dentsply Maillefer). An F3 master cone was coated with the sealer and inserted into the canal up to the working length. Additional cones were used to complete the canal obturation. The cold lateral compaction technique was used in the present study. Excess gutta-percha was removed using a heated instrument. The root filling material was allowed to set for 2 weeks before the push-out assessment.

### Push-out test: Specimen preparation, root filling dislodgment, and failure pattern analysis

Each root was cut horizontally using a slow-speed water-cooled diamond saw (Isomet 2000) to produce three slices, approximately 1 mm thick. The apical slice from the top was excluded. Thus, two slices from the cervical and medium thirds from each root canal \((n = 20)\) were considered. The push-out test was performed by applying a load at 0.5 mm/min to the apex in the direction of the crown until the root filling was dislodged from the root slice. Care was also taken to ensure that the contact between the punch tip and the root filling section occurred over an extended area to avoid a notching effect of the punch tip on the root filling surface. Furthermore, the punch tip was centralized in the root canal and positioned to contact only the root filling without stressing the surrounding root canal walls.

The push-out bond strength was measured using a universal testing machine (Instron Corp, Norwood, MA, USA). The load at failure, recorded in Newtons, was divided by the area \((\text{mm}^2)\) of the postdentin interface to yield the bond strength in megapascals. To calculate the bonding area, the following formula was used: \(\pi (R + r) [(h/2) + |R - r|]^{0.5}\), in which \(R\) represents the coronal root canal radius, \(r\) the apical root canal radius, and \(h\) the thickness of the slice. The thickness of each slice was measured using a digital caliper.

Following the debonding test, both sides of the failed bond were evaluated at \(\times 30\) magnification to determine the mode of failure. Each sample was evaluated and assigned to one of three failure modes: adhesive failure at the sealer-dentin; cohesive failure within the sealer; and mixed failure in both the sealer and dentin.

### Statistical analysis

Normal distribution of bond strength data was confirmed using the Kolmogorov–Smirnov test \((P > 0.05)\). Bond strength values were statistically analyzed using one-way analysis of variance. Failure mode distribution was evaluated using the Chi-squared test \((\alpha = 0.05)\). Data were analyzed using Stat Plus version 6.0 (AnalystSoft Inc., Vancouver, BC, Canada).

### RESULTS

The mean \((\pm\) standard deviation) push-out bond strength values and the modes of failure are summarized in Table 1. The irrigation protocol yielded similar bond strength values \((P > 0.05)\). However, the Chi-squared test revealed significant differences in failure mode among the groups \((P < 0.05)\). An increase in the incidence of adhesive failures was observed in the NaOCl and EDTA groups \((P < 0.05)\). The other groups demonstrated a prevalence of mixed and cohesive failures \((P > 0.05)\).

### DISCUSSION

The goal of root obturation is to obtain an adequate seal; thus, there appears to be no direct clinical correlation...
between sealer bond strength and marginal leakage.\[29\] Moreover, the use of resin-based obturation material to fill root canals has been reported to increase the fracture resistance of endodontically treated teeth.\[29,30\] Several studies have described the adverse effects of NaOCl on dentin.\[4,6,16,17,19\] Nonetheless, in this study, the bond strength of AH Plus to root dentin was unaffected by the irrigation protocols including NaOCl and CHX; consequently, our first hypothesis was rejected. This may have been attributable to the use of NaOCl as only the initial irrigant and not as the final irrigant following the use of EDTA. During the preparation of a root canal, there is deposition of debris and a smear layer on the dentin walls, preventing the direct contact of NaOCl with dentinal collagen; this also prevents the removal of loose organic remnants from the canal system. AH Plus can bond to the organic components, predominantly the collagen network, of root dentin.\[31\] Therefore, direct contact between AH Plus and the dentinal structures is possible only after the use of EDTA. Neelakantan et al.\[31\] have reported that an irrigation protocol using NaOCl, EDTA, and NaOCl (as the final irrigant), significantly reduced the bond strength of AH Plus to dentin; conversely, the use of NaOCl and EDTA– without the use of NaOCl as a final irrigant– had no effect on bond strength. It has been reported that the use of NaOCl as a final irrigant, after the use of demineralization agents, resulted in the notable erosion of root canal dentin.\[32\]

Fracture analysis revealed that adhesive failures occurred predominantly in the NaOCl and EDTA groups. This suggested that the resin cement and root canal dentin bonded weakly,\[33\] and that the use of NaOCl may have degraded the collagen fibrils of the dentin surface.\[4,6,16,17,19\] PACs had no effect on the bond strength of AH Plus to dentin; consequently, our second hypothesis was rejected. Conversely, the prevalence of the mixed and cohesive modes of failure of the root canal sealer used in the PAC groups revealed a distinct adhesive interface.\[33\] As such, the use of antioxidants can restore the redox potential of the oxidized dentin substrate, thereby facilitating normal setting of the adhesive materials.\[19\] Furthermore, bioactive compounds derived from PAC (including GSE and GT) improved the mechanical properties of the dentin matrix, impaired biodegradation, and inhibited the action of proteases associated with extracellular matrix breakdown.\[23-25\] The interaction between PAC and collagen fibrils involves the formation of complexes that are primarily stabilized by hydrogen bonding between the carbonyl and hydroxyl functional groups of amide linkages and phenols, respectively,\[24,25\] in addition to covalent and hydrophobic interactions.\[24\] The prevalence of mixed and cohesive failures that were evident following the use of the PAC-containing agents may have been due to the combined effects of antioxidant use and the enhanced mechanical tissue properties of dentin collagen. Root canal preparations with CHX did not reduce the bond strength values of AH Plus to dentin. The 2% CHX gel used in the present study was a cationic bisguanide containing a natrosol gel (hydroxyethylcellulose), which is viscous, highly inert, and water-soluble agent that leaves less of a smear layer than the use of NaOCl during chemomechanical preparation. This facilitates removal using a final flush of distilled water or physiological solution.\[29\] Furthermore, the exposure of root canals to 2% CHX, regardless of the follow-up use of 17% EDTA, did not promote morphological or structural alterations to the dentin organic matrix.\[4\] CHX has potent inhibitory effects on MMP-2, MMP-8 and MMP-9,\[11\] and can also inhibit cysteine cathepsins,\[12\] conferring beneficial and ongoing preservation to adhesives.

Although different irrigation protocols failed to affect the bond strength values of AH Plus to dentin, long-term studies will be necessary to evaluate the effects of endodontic irrigation solutions, such as NaOCl, on the bond formation of adhesive procedures. The treatment of root dentin with PAC could reduce the biodegradability of the demineralized dentin matrix,\[22,24\] and enhance the durability between the resin-based sealer and root dentin.\[36\] PAC-rich extracts are also able to inhibit the activities of MMP and cysteine cathepsins to a greater extent than CHX.\[37\] In addition, the anti-bacterial\[6\] and anti-inflammatory properties of PAC\[30\] are favorable characteristics that could be beneficial in root canal therapies.

**CONCLUSION**

Within the limitations of this study, the irrigation protocols and naturally derived reducing agents had no effect on the push-out bond strength of the resin-based sealer to root dentin; however, an improvement in the adhesion quality of AH Plus to NaOCl-treated root dentin was observed due to the prevalence of cohesive failure.

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**Conflicts of interest**

There are no conflicts of interest.

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