

Research Article



Effect of phytic acid as an endodontic chelator on resin adhesion to sodium hypochlorite-treated dentin

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No potential conflict of interest relevant to this
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ABSTRACT

Objectives: Phytic acid (IP6), a naturally occurring agent, has been previously reported as a potential alternative to ethylenediaminetetraacetic acid (EDTA). However, its effect on adhesion to sodium hypochlorite (NaOCl)-treated dentin and its interactions with NaOCl have not been previously reported. Thus, in this study, the effects of IP6 on resin adhesion to NaOCl-treated dentin and the failure mode were investigated and the interactions between the used agents were analyzed.

Materials and Methods: Micro-tensile bond strength (μ TBS) testing was performed until failure on dentin treated with either distilled water (control), 5% NaOCl, or 5% NaOCl followed with chelators: 17% EDTA for 1 minute or 1% IP6 for 30 seconds or 1 minute. The failed specimens were assessed under a scanning electron microscope. The reaction of NaOCl with EDTA or IP6 was analyzed in terms of temperature, pH, effervescence, and chlorine odor, and the effects of the resulting mixtures on the color of a stained paper were recorded.

Results: The μ TBS values of the control and NaOCl with chelator groups were not significantly different, but were all significantly higher than that of the group treated with NaOCl only. In the failure analysis, a distinctive feature was the presence of resin tags in samples conditioned with IP6 after treatment with NaOCl. The reaction of 1% IP6 with 5% NaOCl was less aggressive than the reaction of the latter with 17% EDTA.

Conclusions: IP6 reversed the adverse effects of NaOCl on resin-dentin adhesion without the chlorine-depleting effect of EDTA.

Keywords: Bond strength; EDTA; Phytic acid; Reaction; Resin tag; Sodium hypochlorite

INTRODUCTION

The importance of good coronal resin-dentin adhesion in endodontically treated teeth cannot be overemphasized. In endodontics, an ideal irrigant should be able to kill microorganisms, dissolve necrotic tissue, remove the smear layer, lubricate the canal [1], and permeate into inaccessible areas with a low surface tension [2,3], all without affecting the

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healthy tissue [1]. Sodium hypochlorite (NaOCl) has been used in endodontics as a root canal irrigant since 1920 [4]. Its numerous favorable properties have led to its use in concentrations ranging from 0.5% to 5.25% [5]. NaOCl has a wide antimicrobial spectrum including spores, viruses, and yeast [6]. It is capable of inhibiting or deactivating some bacterial enzymes through the formation of reactive chlorine, which causes irreversible oxidation of the sulfhydryl group of the bacterial enzymes [7]. Despite the advantages of NaOCl, it is not without its problems and shortcomings; for instance, NaOCl is an oxidizing agent that leaves behind an oxygen-rich layer on the dentin surface [8]. Oxygen inhibits resin polymerization [9] and may interfere with the infiltration of resin into the intertubular dentin [10]; thus, a decrease in dentin-resin bond strength is observed in teeth treated with NaOCl [11,12]. However, this reduction depends on the concentration of NaOCl and the type of the bonding agent [13,14]. Coronal restoration is best accomplished as soon as possible after obturation and the quality of the restoration is important for the long-term outcome of endodontic treatment [15-17]. Therefore, possible ways of inhibiting the negative effect of NaOCl on adhesion have attracted considerable attention from researchers [18,19]. Another problem encountered with the use of NaOCl is its inability to fully remove the smear layer; as a result, the alternate application of NaOCl and a chelating agent is recommended to ensure effective removal of both the organic and inorganic components of the smear layer [20,21]. The most widely used agent for this purpose is ethylenediaminetetraacetic acid (EDTA). However, its negative impact on the environment, concerns about its biocompatibility with the periapical tissues, and its reaction with NaOCl have stimulated interest in finding biocompatible alternatives to EDTA [22-26].

One of the most recently studied agents with the potential to be an alternative to EDTA is phytic acid (IP6) [27]. IP6 is the major storage form of phosphorus in plant seeds and bran, and it plays an important role in a variety of cellular functions [28]. IP6 is a highly negatively charged molecule that has an outstanding ability to chelate with multivalent cations such as calcium, magnesium, and iron [29,30]. In our previous reports, IP6 was shown to effectively remove the smear layer [31], exposing the cross-linked collagen network and thereby improving the bond strength of etch-and-rinse adhesive to dentin [32,33]. Moreover, it has less negative influence on pulpal and osteoblast-like cells than other currently used agents [27,31]. To the best of our knowledge, no research has evaluated the effects of IP6 when used as an endodontic chelating agent on resin bond strength to NaOCl-treated dentin or the reaction between the 2 aforementioned agents. Thus, the aims of this study were 1) to evaluate the effect of IP6 on the bond strength of a self-etch adhesive to NaOCl-treated dentin and 2) to investigate the interactions between IP6 and NaOCl. The null hypotheses tested were 1) IP6 does not affect the bond strength of self-etch adhesive to NaOCl-treated dentin and 2) IP6 does not react with NaOCl.

MATERIALS AND METHODS

Effect of IP6 on resin adhesion to NaOCl-treated dentin

This study was reviewed and approved by the Human Research Ethics Committee of Tokyo Medical and Dental University (approval number: 725). Extracted human sound molars were used in the present study. Under water irrigation, 1-mm-thick flat coronal dentinal surfaces were created using a slow-speed diamond saw (Isomet Low Speed Saw, Buehler, Lake Bluff, IL, USA), followed by smear layer creation using 600-grit silicon carbide paper under water lubrication. Twenty-five specimens were allocated into 5 groups in accordance to the type of

treatment each dentinal surface received. A rinse with distilled water was used for the control specimens. The experimental groups received the following treatment protocols: 1) group I: 5% NaOCl (pH 12) (Wako Pure Chemical Industries, Osaka, Japan) for 5 minutes; 2) group II: 5% NaOCl for 5 minutes followed by 17% EDTA (pH 7.5) (Wako Pure Chemical Industries) for 1 minute; 3) group III: 5% NaOCl for 5 minutes followed by 1% IP6 (pH 1.3) (Wako Pure Chemical Industries) for 1 minute; 4) group IV: 5% NaOCl for 5 minutes followed by 1% IP6 for 30 seconds. A micro-brush was used to agitate the solutions while they were applied to the dentinal surfaces. A final rinse with distilled water was performed for all groups. After blot-drying, a 1-step self-etch adhesive (Scotchbond Universal Adhesive, 3M ESPE, St. Paul, MN, USA) was applied to the treated surfaces according to the instructions provided by the manufacturer, followed by light-curing (Optilux 501, Kerr, Orange, CA, USA) and the incremental placement of resin-based composite (Filtek Z100, 3M ESPE) up to a thickness of 5 mm. The bonded specimens were stored in distilled water at 37°C for 24 hours. Composite-dentin beams (0.85 mm × 0.85 mm) were obtained from the specimens, and each beam was fixed to a testing jig and subjected to micro-tensile bond strength (μ TBS) testing until failure, followed by assessment of the failed specimens under a scanning electron microscope (SEM) operating at 5 kV. The μ TBS data were analyzed in SPSS version 16.0 (SPSS Inc., Chicago, IL, USA) using 1-way analysis of variance, followed by *post hoc* Tukey multiple comparison tests, at $\alpha = 0.05$.

Reaction between NaOCl and EDTA or IP6

NaOCl solution (5%, 1 mL) was mixed with either EDTA (17%, 1 mL) or IP6 (1%, 1 mL) at room temperature, after which changes in temperature, pH, and effervescence (indicating chlorine gas liberation) were recorded. After 1 minute, 200 μ L of each mixed solution was applied to a stained paper to evaluate the whitening effect of these solutions on the aforementioned paper.

RESULTS

Effect on resin adhesion to NaOCl-treated dentin

The mean and standard deviation of the μ TBS values of the groups are presented in **Table 1**. A statistically significant difference in μ TBS values was observed among the groups ($p < 0.05$). NaOCl resulted in a significantly lower μ TBS value than that of the control group ($p < 0.001$). The μ TBS values obtained after the application of either 17% EDTA or 1% IP6 were not significantly different from that of the control group ($P = 0.409$), but were significantly higher than that of the NaOCl group ($p \leq 0.05$). SEM observations of the debonded specimens treated with 1% IP6 for 1 minute (**Figure 1D**) or 30 seconds (data not shown) showed the presence of numerous resin tags protruding from the dentinal tubules.

Table 1. Micro-tensile bond strength values of each tested group

Group	Values
Control	71.6 ± 19.9 ^a
Group I (NaOCl)	51.3 ± 16.4 ^b
Group II (NaOCl/EDTA 1 minute)	68.5 ± 22.5 ^a
Group III (NaOCl/IP6 1 minute)	65.5 ± 19.7 ^a
Group IV (NaOCl/IP6 30 seconds)	64.2 ± 12.5 ^a

Values are presented as mean (MPa) ± standard deviation. Groups identified by different superscript letters indicate statistically significant differences (1-way analysis of variance with the Tukey *post hoc* test, $p < 0.05$). NaOCl, sodium hypochlorite; EDTA, ethylenediaminetetraacetic acid; IP6, phytic acid.

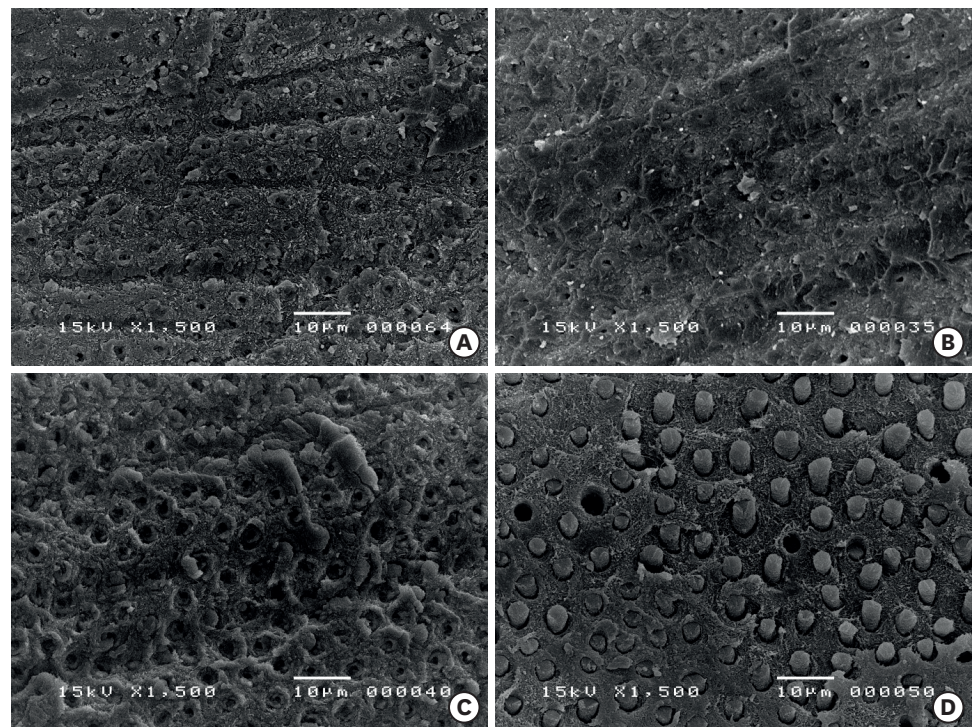


Figure 1. Scanning electron microscopy images of the dentin side of the fractured specimens. (A) Control group, (B) 5% NaOCl treatment for 5 minutes, (C) 1 minute of 17% EDTA treatment of the NaOCl-treated dentin surfaces, and (D) 1 minute of 1% IP6 treatment of the NaOCl-treated dentin surfaces. Resin tags were formed along the surface treated with IP6.

NaOCl, sodium hypochlorite; EDTA, ethylenediaminetetraacetic acid; IP6, phytic acid.

Reaction between NaOCl and EDTA or IP6

The reaction between NaOCl and EDTA was exothermic (43.2°C) with evident effervescence (chlorine gas bubble formation) and a final pH of 7.1, whereas mixing NaOCl with IP6 resulted in a minimal increase in temperature (26°C) with less effervescence (**Figure 2**) and a final pH of 8.0. The NaOCl/EDTA mixture was not able to change the color of the paper from brown to white (**Figure 3B**), reflecting the depletion of chlorine in this mixture, whereas the



Figure 2. The formation of chlorine gas bubbles in the mixed solutions. (A) 17% EDTA and 5% NaOCl, (B) 1% IP6 and 5% NaOCl.

EDTA, ethylenediaminetetraacetic acid; NaOCl, sodium hypochlorite; IP6, phytic acid.

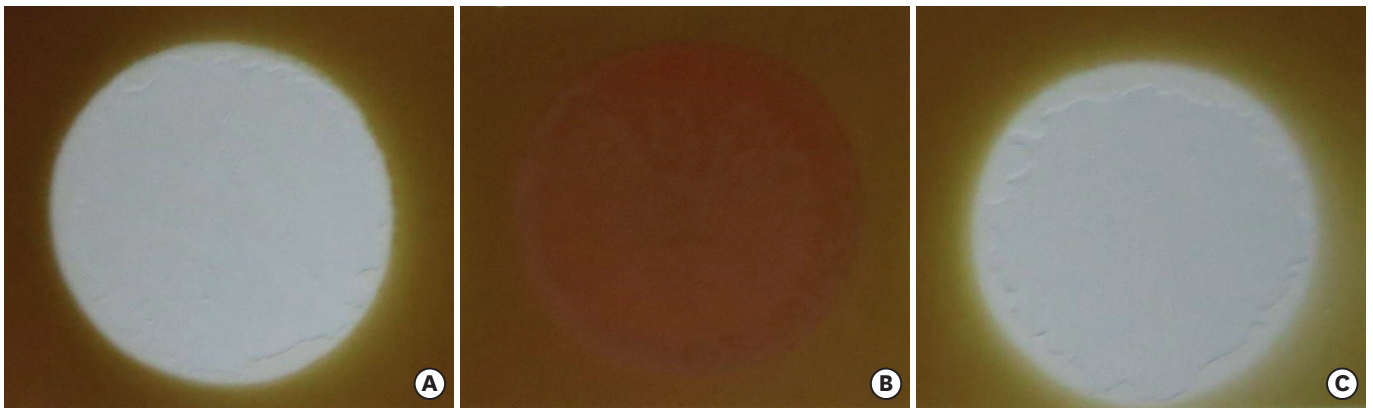


Figure 3. The effect of different solutions on the color of a stained paper. (A) NaOCl, (B) EDTA/NaOCl mixture, and (C) IP6/NaOCl mixture. NaOCl, sodium hypochlorite; EDTA, ethylenediaminetetraacetic acid; IP6, phytic acid.

NaOCl/IP6 mixture showed a bleaching effect (**Figure 3C**) comparable to that of plain NaOCl solution (**Figure 3A**).

DISCUSSION

The problem of resin-polymerization inhibition by NaOCl is not restricted to resin materials that will be in contact with radicular dentin; instead, it is of essential importance to direct more attention to the importance of the coronal seal and leakage prevention [34-36]. The definitive restoration of endodontically treated teeth is better accomplished soon after root canal treatment [15-17]; moreover, it has become more common to restore these teeth with resin composite due to developments in dentin bonding systems [37] and the advantages that are offered by adhesive restorations over non-adhesive restorations [37,38]. This implies that pulp chamber dentin is exposed to NaOCl at the time of composite resin placement, which would adversely affect the bond strength of these restorations to pulp chamber dentin, thereby jeopardizing the coronal seal [37,39]. Chelating agents play an essential role in root canal therapy, and the choice of an agent should take in consideration its effect on the adhesion of resin to dentin and its reaction with other used irrigants. The results of this study require the rejection of the null hypotheses, as IP6 enhanced resin adhesion to NaOCl-treated dentin surfaces and interacted with NaOCl.

The reactions between different irrigants used in endodontics have emerged as a topic of interest [40]. These reactions can happen even after flushing the irrigant out of the canal because the dentinal tubules may still retain a sufficient amount of an irrigant to react with the next irrigant [41]. In this study, the reaction between 5% NaOCl and 17% EDTA was exothermic (43.2°C) with observable effervescence and a final pH of 7.1, and a strong chlorine odor was noticed. In contrast, mixing 5% NaOCl with 1% IP6 did not result in perceivable effervescence; the final pH of the mixture was 8.0, its temperature was 26°C, and the chlorine odor was less strong than that liberated by the EDTA/NaOCl mixture. After 1 minute of mixing, 200 µL of each solution was added on a stained paper to observe the bleaching effect. The NaOCl/EDTA mixture was not able to change the color of the paper from brown to white, whereas the NaOCl/IP6 mixture showed a strong bleaching effect that was comparable to that of NaOCl alone. These findings can be explained by the depletion of active chlorine after mixing NaOCl with EDTA [24].

In this study, the use of NaOCl resulted in decreased μ TBS of the resin adhesive to dentin. Several previous studies have reported negative effects of NaOCl on resin adhesion to dentin, which was mainly attributed to the inhibition of resin polymerization [18,42]. The use of either EDTA or IP6 abolished this deleterious effect, as both resulted in μ TBS values comparable to the control. This finding could have been related to the demineralizing effect of these agents, which may have exposed the underlying dentin on which NaOCl did not have a significant impact, or to the acid-base neutralization reaction between NaOCl and EDTA or IP6, which would minimize the effect of NaOCl on adhesion. EDTA yielded higher bond strength than IP6, but this difference did not reach the level of statistical significance. A possible explanation for this higher value is the better ability of 17% EDTA than 1% IP6 to neutralize the remaining NaOCl on dentin surfaces. Nevertheless, the statistically comparable bond strengths of EDTA and IP6, regardless of the application time, may be due to the higher acidity of 1% IP6 (with a pH of 1.3) than 17% EDTA. This could have contributed to more effective demineralization, as supported by the more evident smear layer removal reported in our previous study [27] and the apparent resin tags in NaOCl/IP6-treated debonded dentin specimens in our current findings. The notable presence of resin tags that formed inside most of the dentinal tubules in specimens treated with IP6, a pattern that was not observed in the other groups when the mode of failure of debonded specimens was examined, reflects the better ability of IP6 to open dentinal tubules. This is a property that could be of importance in root canal treatment, as it may enable the superior penetration of inter-appointment medicament or sealer into root canal dentinal tubules, which would result, respectively, in better disinfection and sealability. However, the formation of resin tags with the use of IP6 did not result in higher bond strength than was observed in the control and EDTA-treated group, which might reflect the minor contribution of resin tags to resin-dentin adhesion with self-etch adhesives [43].

From a restorative vantage point, the significant neutralization of NaOCl and chlorine depletion by EDTA is of great benefit for resin-dentin adhesion. However, this is not the case from an endodontic perspective and is a definite disadvantage of EDTA. Compared to EDTA, IP6 was previously reported to have better biocompatibility and smear layer removal ability on flat dentin surfaces [27]. Overall, based on the endodontic and restorative aspects investigated, 1% IP6 might offer a net advantage when used as an endodontic chelator, as it has more favorable endodontic properties and comparable resin bonding properties to those of 17% EDTA.

CONCLUSIONS

The limitations of this *in vitro* study include, but are not limited to, the use of extracted human teeth from different subjects, the insufficient evidence to support the association between bond strength values and the clinical success of resin-based restorations, and the lack of a chemical analysis of the reaction by-products. Within these limitations, the present study proved IP6 to be an effective chelating agent with a comparable ability to that of EDTA to reverse the deleterious effects of NaOCl on resin bond strength in dentin. Compared to 17% EDTA, 1% IP6 resulted in less chlorine depletion of NaOCl. Considering our previous findings of better biocompatibility and better tubule patency, in addition to the abovementioned results of the present study, IP6 might be considered as a suitable alternative to EDTA for use as an endodontic chelating agent.

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