



Evidence of improved bond strength of resin-based sealer with the use of natural antioxidants on hypochlorite treated dentin: an *in vitro* study

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Abstract

Aim. To evaluate the effect of natural antioxidants as final irrigants on the push-out bond strength of epoxy resin-based sealer to the hypochlorite treated dentin.

Methods. Eighty single-rooted human mandibular incisors were prepared using ProTaper Gold (Dentsply, TN, USA) and an irrigation protocol including 3% NaOCl, followed by ethylenediaminetetraacetic acid (EDTA), and 3% NaOCl. The groups (n =20) were divided according to the final irrigant used: Group 1: None (Control); Group 2: 5% sodium ascorbate, Group 3: 5% grape seed extract (GSE); and Group 4: 5% pine bark extract. The obturation of root canals was performed using gutta-percha and AH Plus (Dentsply DeTrey, Germany). Bond strength was evaluated using the push-out test under the universal testing machine at a crosshead speed of 0.5 mm/min, and statistical analyses were performed using one-way ANOVA. The p-value significance was evaluated by Tukey's post hoc test (p<0.01).

Results. Mean push-out bond strength values were compared in all the groups, and there was a statistically significant improvement in the experimental final irrigating groups. 5% pine bark extract had improved bond strength than the other groups, and the least bond strength was observed in the control group.

Conclusion. The irrigation protocols and naturally derived antioxidants affected the resin-based sealer's bond strength to root dentin. It was observed that the use of antioxidants effectively reversed the compromised bond strength of resin-based sealers to root dentin. 5% pine bark extract application showed better bond strength.

Keywords: antioxidant, epoxy resin-based sealers, grape seed extract, pine bark extract, sodium ascorbate

Introduction

Endodontic therapy success depends on the canal disinfection using proper irrigation solutions and subsequent fluid-tight seal between the obturation material and the root dentin. AH Plus is a hydrophobic, epoxy resin-based sealer that is widely used because of its physical properties [1]. The adhesive property of AH Plus to root dentin depends on covalent bonding between the exposed side-chain amine groups and the open

epoxide ring of the collagen network [2]. Dentin surface treated with different irrigation protocols predominantly causes alteration to dentin's collagen fibrils; this may compromise endodontic sealers' adhesiveness to the dentin surfaces [3].

AH Plus can bond to the organic components, principally the collagen network, of root dentin. An irrigation protocol using NaOCl as the final irrigant significantly reduced the bond strength of AH Plus to root dentin (Neelakantan et

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al.) [4]. Many other studies have demonstrated that the commonly used irrigating solution NaOCl reduces the bond strength between adhesive materials and dentin [5]. Moreover, it potentially reduces the bond strength of the AH Plus sealer. This may be attributed to residual oxygen species' presence on the dentin, which affects the adhesive material's setting time [6].

The compromised bond strength of NaOCl-treated dentin might be restored by applying antioxidants before the adhesive procedure [7]. These agents interact with the by-products of NaOCl, resulting in the reversal of the oxidizing effects of NaOCl and neutralization of the dentin surface [5-7].

However, Erhardt et al. [8] reported that some materials benefited from its protective effects, whereas others yielded negligible results. Naturally derived oxidizing agents can chemically modify collagen without damaging biological tissues and improve dentin matrix properties [9].

The aim of the study is to evaluate the effect of 5% sodium ascorbate (SA), 5% grape seed extract (GSE), and 5% pine bark extract (PBE) on the push-out bond strength of epoxy resin sealer to hypochlorite treated root dentin. The null hypothesis is that antioxidants, as a final irrigant, would not reverse the compromised bond strength of epoxy resin-based sealer and root dentin caused by NaOCl.

Methods

Specimen preparation

After obtaining ethical clearance from the institutional Review Board (238/IRB/SIBAR/2020), eighty non-carious permanent human mandibular incisors extracted due to periodontal reasons with fully developed root apices were selected. All the samples were investigated for the root canal morphology, and teeth with a single canal were included in the study. Each sample was decoronated at the level of cemento-enamel junction perpendicular to the tooth's long axis using a diamond disc at a low speed along with water coolant. The root lengths were standardized to 15 mm. Working length (WL) was established with a size 10 K-file and was set at 1 mm from the apex. A #25 K-file was used to standardize the foramina size. The roots were mounted on the self-cure acrylic resin.

Root canal preparation

ProTaper gold instruments (Dentsply Tulsa Dental Specialties, Johnson City, TN, USA) were used in a 16:1 gear handpiece and torque-controlled electric motor (X-Smart Plus; Dentsply). According to the manufacturer's instructions, a consistent rotation speed of 300 rpm was used in a crown-down manner involving a gentle in-and-out motion. Initially, the orifice was enlarged using orifice opener SX. A shaping file (S1) was passed apically within 2 mm of the working length. Later S1 and S2 files were used till the full working length.

The finishing of the apical one third was done by passing the F1 and F2 till the entire working length was reached. Pecking motion was used for instrumentation, and flutes were regularly cleaned to remove debris.

Irrigation protocol

The irrigation protocols used for all the groups were:

1. 2 ml of 3% NaOCl (prime dental products Pvt Ltd, Maharashtra, India) after each instrument change.
2. Irrigation using 2 ml of 17% EDTA (MD-Cleanser, META Biomed, Korea) in all groups.
3. 1 ml of 0.9% of saline solution.
4. Irrigation for 3 minutes using 5ml of NaOCl and experimental antioxidant solutions as final irrigation.

The root samples were divided into four groups ($n = 20$ each) according to the final irrigation solution.

Group 1: None (Control).

Group 2: 5 ml of 5% sodium ascorbate solution.

Group 3: 5 ml of 5% of grape seed extract solution.

Group 4: 5 ml of 5% of pine bark extract solution.

Preparation of solutions

1.5% sodium ascorbate solution.

5 g of sodium ascorbate (All pure organics, India) was dissolved in 100 ml of distilled water to make a 5% sodium ascorbate solution.

2.5% grape seed extract solution.

5 g of grape seed extract (All pure organics, India) was dissolved in 100 ml of distilled water to make a 5% grape seed extract solution.

3.5% pine bark extract solution.

5 g of pine bark extract (All pure organics, India) was dissolved in 100 ml of distilled water to make a 5% pine bark extract solution.

Root filling procedures

The samples were dried using 25 size paper points (Dentsply Konstanz, Germany). After root canal preparation, all samples were obturated using the single cone technique with 25/.06 gutta-percha points (Dentsply Konstanz, Germany) and AH Plus sealer (Dentsply DeTrey, Konstanz, Germany) according to the manufacturer's instructions. The obturating material was allowed to set for 2 weeks.

Push-out test

A 2 mm slice was obtained from the middle third of the root using a microtome from each root. The push-out test was evaluated by applying a load at 0.5 mm/min, which was applied in an apical to coronal direction until the root filling was dislodged from the root slice. The push-out bond strength was measured under a universal testing machine (Instron Corp, Norwood, MA, USA).

Statistical analysis

The obtained results were statistically analyzed using computer software SPSS version 21.0. One-way analysis of variance (ANOVA) followed by Mann Tukey's Post hoc test was used to analyze the data. Significance was established at $p < 0.01$ level.

Table I. Comparison of mean push-out bond strength of different groups (MPa) using a one-way ANOVA test and Tukey's post hoc test.

Group	N	Minimum	Maximum	Mean	SD	Comparison	p-value
Group-1 (Control)	20	18.809	44.666	31.761	8.334	G-1 VS G-3, G-4	p<0.01*
Group-2 (SA)	20	32.142	45.762	41.116	4.203	G-2 VS G-1	p<0.05*
Group-3 (GSE)	20	31.843	54.456	42.251	8.077	G-3 VS G-4	p<0.05*
Group-4 (PBE)	20	41.559	60.929	51.572	6.452	G-4 VS G-2	p<0.01*

* P- value significant, SD- Standard deviation, N- no of samples.

Results

The one-way ANOVA revealed that the push-out bond strength was significantly affected by antioxidants as final irrigating solutions ($p<0.01$). The mean push-out bond strength values, standard deviation, and p-value significance are shown in table I and figure 1.

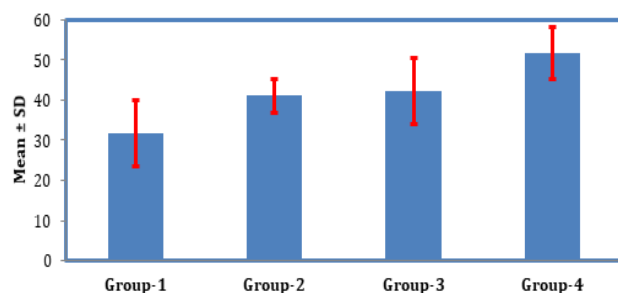


Figure 1. Graph representing the comparison of mean push-out bond strength among the groups (MPa).

When comparing the groups, Group 4 pine bark extract showed a statistically significant highest push-out bond strength compared to all other groups. The mean push-out bond strength value of Group 3 was statistically significant with that of Group 4 ($p<0.05$) and Group 1 ($p<0.01$) but not with that of Group 2 ($p>0.05$). The mean push-out bond strength value of Group 2 was statistically significant with that of Group 4 ($p<0.01$) and Group 1 ($p<0.05$) but not with that of Group 3 ($p>0.05$). Group 1 showed the least mean push-out bond strength values than all other groups and was statistically significant ($p<0.01$).

Discussion

Failure in the endodontic therapy occurs due to inadequate fluid-tight seal between the obturating material and the root dentin. Microleakage occurs due to bond failure between the epoxy resin-based sealer and the root dentin. Several studies described the adverse effects of NaOCl on dentin, which includes promoting structural changes in organic dentin components (mainly collagen) [10], and its impact on the mechanical properties, such as reducing the flexural strength and elastic modulus of dentin [11]. Similar reduction in the AH Plus sealer bond strength was

observed in Group 1 of the present study, which may be attributable to the release of residual oxygen species from NaOCl on the root dentin [6]. To overcome this problem, different techniques have been proposed to treat root dentin [12]. Antioxidants such as sodium ascorbate (SA) and oligomeric proanthocyanidin complexes (OPC) have been demonstrated to reverse oxidants' adverse effects and preserve resin-dentin bond integrity.

SA is a biocompatible and nontoxic antioxidant (salt of ascorbic acid-vitamin C) able to reduce a wide variety of oxidizing compounds, mainly free radicals [13]. In the present study, SA (Group 2) showed statistical significance compared to the control group. SA solution reduces the residual free radicals from the interior of the dentinal tubules [14] and dentin matrix [15]. SA reverses the denaturing effect of acid etching, NaOCl, or H_2O_2 on dentin collagen. It is a potent inhibitor of matrix metalloproteinases (MMPs). Hence, it protects against the long-term degradation of the resin-based materials and dentin interface [16]. Khoroushi et al. [17] also indicated that the treatment with SA solution improved the bond strength of fiber posts to dentin.

Group 2 specimens could not reverse the push-out bond strength as much as that of Group 4, which was in accordance with the studies that confirmed the antioxidant potential and the free radical scavenging ability of OPCs are 20 times greater than those of vitamin E and 50 times greater than those of vitamin C (Fine AM [18] and Stokes et al. [19]).

OPCs exhibited 78-81% inhibition of superoxide anion and hydroxyl radical. Under similar conditions, vitamin C inhibited these two oxygen free radicals by approximately 12-19%, while Vitamin E inhibited the two radicals by 36-44% (Bagchi et al. [20]). OPCs present in natural antioxidants like grape seed extract and pine bark extract have free radical scavenging activity [21]. OPCs are a class of polyphenolic bioflavonoids most commonly found in fruits and vegetables, free radical scavenging and antioxidant activity. They also have antibacterial, antiviral, anti-inflammatory, antiallergic, anticarcinogenic, and vasodilatory actions [18].

Grape seed extract consists of OPCs in the form of monomeric phenolic compounds such as catechin, epicatechin, and epicatechin-3-0-gallate and free flavanol

monomers [22]. Pine bark extract consists of phenolic compounds generally divided into monomers like catechin, epicatechin, taxifolin, and compacted flavonoids like oligomeric to polymeric proanthocyanidins [23]. Group 4 showed a statistically higher significant mean POBS value than Group 3. The difference in the antioxidant activity might be attributed to their different phenolic compositions, which is in accordance with a study conducted by Subramonian et al. [24]. OPCs (grape seed extract and pine bark extract) reacts with free radicals (e.g., oxygen) generated by the degradation of NaOCl, thereby neutralizing them within the dentin in which they are trapped. Oligomeric proanthocyanidin complex (OPC) contains multiple electron donor sites (hydroxyl sites) that bind to unstable molecules called free radicals by donating its hydrogen atoms [18]. The presence of gallic acid also increases the free radical scavenging activity by esterification of epicatechin [25].

Antioxidants can return the oxidized dentin substrate's redox potential, thereby facilitating the usual setting of the adhesive materials [26]. Furthermore, bioactive compounds derived from OPC (including GSE and PBE) improved the dentin matrix's mechanical properties, impaired biodegradation. It inhibited the action of proteases associated with extracellular matrix breakdown [9]. The interaction between OPC and collagen fibrils involves the formation of complexes that are predominantly stabilized by hydrogen bonding between carbonyl and hydroxyl functional groups of phenols and amide linkages [27].

The treatment of root dentin with OPC could reduce the demineralized dentin matrix's biodegradability and increase the durability between the resin-based sealer and root dentin. OPC rich extracts can also inhibit the activities of MMP and cysteine cathepsins to a greater extent [28]. Besides, the anti-inflammatory and antibacterial properties of OPC are favorable characteristics that could be beneficial in root canal therapies [29].

Limitations

As it was an *in vitro* study, the direct estimation of these results to the clinical application requires further *in vivo* studies.

Conclusion

Within the limitations of this *in vitro* study, it can be concluded that the use of antioxidants as final irrigating solutions can reverse the reduced bond strength of epoxy resin-based sealers to root dentin. The use of 5% pine bark extracts significantly increases epoxy resin-based sealers' bond strength to root dentin compared to no antioxidant therapy.

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