Effect of Proanthocyanidin Treatment on the Bonding Effectiveness of Adhesive Restorations in Pulp Chamber

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Objective: *To analyze the effect of proanthocyanidin (PA) treatment of the pulp chamber dentin after NaOCl and EDTA irrigation on the microleakage and interfacial morphology of adhesive restorations.* ***Study Design****: Pulp chambers of 66 extracted permanent molars were exposed. In half of the samples, pulp chamber dentin was bonded with Clearfil S3 after irrigation with normal saline(Group1a); 1% NaOCl and 17% EDTA(Group1b) or 1% NaOCl, 17% EDTA and 30% PA (Group1c) and in other half samples, pulp chamber dentin was bonded with Futurabond NR after irrigation with normal saline (Group 2a); 1% NaOCl and 17% EDTA (Group 2b) or 1% NaOCl, 17% EDTA and 30% PA(Group 2c). After adhesive procedures, Filtek Z 250 restorations were placed in the pulp chambers. Microleakage assessment was done in ten samples from each group and scanning electron microscopic examination was done in one sample from each group. Statistical analysis was done using Mann–Whitney and Kruskal Wallis tests at a significance level of P<0.05.* ***Results****: Both the adhesives showed extensive microleakage. NaOCl and EDTA irrigation had no significant effect on the microleakage of both the adhesives. PA treatment of the pulp chamber dentin after NaOCl and EDTA irrigation significantly reduced microleakage in both the adhesives.* ***Conclusion****: It was concluded that 1 minute application of 30% proanthocyanidin solution in the pulp chamber after NaOCl and EDTA irrigation improved the subsequent bonding of self-etch adhesives to pulp chamber dentin.*

Keywords: *Proanthocyanidin, Bonding, Pulp chamber, Microleakage, Self-etch adhesive.*

INTRODUCTION

Although, effective cleaning, shaping and formation of a fluid-tight apical seal are fundamental goals of endodontic therapy, the overall success of endodontic treatment depends upon creation of a reliable coronal seal.[1-3] Endodontic treatment and subsequent final restoration represent the two elements of a endodontic-restorative continuum. Lack of adhesion and sealing between final restoration and tooth structure can permit movement of microorganisms or their toxins along canal walls or through voids in root canal filling to periapical tissue reducing the prognosis of nonsurgical root canal treatment.[4]

Restoration of endodontically treated teeth with adhesive restoration permits transmission of functional stresses across the bonded interface to the tooth, with the potential to reinforce weakened tooth structure.[5,6] One-step self-etch adhesives involve the application of non-rinse acidic primer and adhesive in a single application step without the use of conventional phosphoric acid etchant thereby simplifying the bonding procedure. However, achieving predictable dentinal adhesion in endodontically treated teeth is more difficult when compared with bonding to coronal vital dentin. This is because the root canal system presents a unique environment with interplay of various factors like use of irrigants, medicaments and differences in dentin substrate, all of which can affect the success of adhesive procedures.

The irrigants used during biomechanical preparation can potentially affect bonding of subsequently placed adhesive restorations either by directly affecting the bonding procedure or by affecting the structural and mechanical properties of bonding substrate i.e., pulp chamber dentin.

Although sodium hypochlorite continues to be the main irrigant which is widely used in endodontic therapy to provide gross debridement, disinfection, lubrication, and dissolution of tissues,[7] there is no single irrigant that alone sufficiently covers all of the functions required from an irrigant. Zehnder recommends that a hypochlorite solution should be employed throughout instrumentation, without altering it with EDTA or citric acid. Once the shaping procedure is completed, canals should be thoroughly rinsed using aqueous EDTA or citric acid.[8]

Reductions in calcium and phosphorus levels and in mechanical properties of dentin, such as elastic modulus, flexural strength, and microhardness, reported after irrigation of root canals with 5% sodium hypochlorite, are supposed to contribute to a decrease in the micromechanical interaction between adhesive resins and NaOCl-treated dentin.[9,10]

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Table 1. Composition and manufacturer's directions of adhesives

Adhesive	Composition	pH	Manufacturer's directions
Clearfil S^3 Bond (Kuraray Medical Inc, Tokyo, Japan)	10-MDP, Bis-GMA, HEMA, dicamphorquinone, ethanol, water, silanated colloidal silica	2.7	Apply adhesive with gentle agitation for 20 seconds. Air dry for 5 seconds. Light cure for 10 seconds.
Futurabond NR (VOCO, Cuxhaven, Germany)	Liquid A: Polyfunctional adhesive monomers (Methacroyl-Phosphorus-Acid-Ester, Methacroyl-Carbon-Acid-Ester), Dimethacrylates, Functionalized SiO2-nano-particles, Initiators. Liquid B: Ethanol, Water, Hydrophilic adhesive monomers, Fluorides	1.4	Mix 1 drop of liquid A and 1 drop of liquid B on a mixing palette for 5 seconds. Apply the adhesive and massage into tooth substrate for 20 seconds. Air dry for 5 seconds. Light cure for 10 seconds.

Erdemir and others indicated that NaOCl significantly decreased bond strength of C&B Metabond to root canal dentin.[11] However, when Wachlarowicz and others examined the effects of commonly employed endodontic irrigants on Epiphany (root canal sealer)-dentine bond strengths, they found that only NaOCl improved the bond strengths.[12]

Ari and others evaluated effect of NaOCl on the regional bond strengths of four adhesive systems to root canal dentin. They found that, depending on the adhesive system, NaOCl enhanced the bond strength.[13] Therefore it appears that the effect of endodontic irrigants on contemporary adhesive systems is controversial as it varies with the specific adhesive system, time, concentration and sequence of irrigants used and still needs to be investigated.

Some researchers have demonstrated that applying an antioxidant/reducing agent (e.g., sodium ascorbate) had a reversal effect on dentin bonding, compromised by NaOCl treatment.[14,15,16,17] Similarly, due to their anti-oxidant property use of proanthocyanidins (PA) may also help in neutralizing or overcoming the oxidizing effect of NaOCl irrigation before adhesive restoration of the pulp chamber. PA are a class of bioflavonoids that are naturally occurring plant metabolites available in fruits, vegetables, nuts, seeds, flowers and barks.[18-20] Recently, PA have gained popularity in the fields of nutrition, health and medicine due to their physiological activities such as antioxidant, anti-microbial, anti-inflammatory properties, effects on cardiovascular diseases, anti-allergic and enzyme inhibitory activity against phospholipase A2, cyclooxygenase and lipooxygenase.[21-24]

Hence, the aim of this study was to analyze the effect of PA application after NaOCl and EDTA irrigation of the pulp chamber on the microleakage of subsequently placed adhesive restorations using one-step self-etch adhesives.

MATERIALS AND METHOD

Study was performed in sixty-six caries free extracted human mandibular molars which were cleaned and stored in distilled water and used within 6 months of extraction. Roof of the pulp chamber was removed 1.5mm coronal to the cementoenamel junction using a diamond disc. Roots 2 mm apical to the bifurcation were resected. Pulp tissue was extirpated with the help of excavator and endodontic instruments. Canal orifices were widened with Gates Glidden drill no. 2-3(Gates drills, Mani,Inc., Tochigi, Japan). Root ends were sealed with IRM.

Solution of 30% PA was prepared from grape seed extract proanthocyanidin powder (Xena BioHerbals, India). Teeth were randomly divided into six groups (n=11) according to two self-etch adhesives used (i.e. Clearfil S^3 [CS^3] and Futurabond NR [FB]) and three different irrigation regimens:

Group 1a: CS^3 with normal saline. Pulp chambers were continuously irrigated with normal saline solution for 1 min.

Group 1b: CS^3 with 1% NaOCl and 17% EDTA. Pulp chambers were continuously irrigated with 1% NaOCl for 10 min followed by 17% EDTA for 1 min.

Group 1c: CS^3 with 1% NaOCl, 17% EDTA and 30% PA. Pulp chambers were continuously irrigated with 1% NaOCl for 10 min followed by 17% EDTA for 1 min further followed by 30% PA for 1 min.

Group 2a: FB with normal saline. Pulp chambers were continuously irrigated with normal saline solution for 1 min.

Group 2b: FB with 1% NaOCl and 17% EDTA. Pulp chambers were continuously irrigated with 1% NaOCl for10 min followed by 17% EDTA for 1 min.

Group 2c: FB with 1% NaOCl, 17% EDTA and 30% PA. Pulp chambers were continuously irrigated with 1% NaOCl for 10 min followed by 17% EDTA for 1 min, further followed by 30% PA for 1 min.

Distilled water for 1 min was used as final flush in all the groups. Excess water was blot dried with cotton pellet. Adhesives were applied according to manufacturer's directions on the dentin surface [Table 1]. Pulp chambers in all the groups were restored with composite resin Filtek Z250 (3M,ESPE, St. Paul, USA) in one to two increments and were light cured at 600 mw/cm^2 by Spectrum 800 (Dentsply, Caulk,Milford,USA) for 40s. Ten teeth from each group were used for microleakage analysis and one specimen was subjected to scanning electron microscopic examination.

Microleakage analysis

Samples were dried superficially and coated with two layers of sticky wax leaving 1 mm window around restoration margin. Samples were then immersed in 2% methylene blue for 48 hours and were rinsed under running water. Samples were air dried at room temperature for 24 h and were sectioned to evaluate for dye penetration under stereomicroscope at magnification 10x according to the following scoring system:

Microleakage Score	Degree of leakage
0	No leakage
1	Leakage extending into pulp chamber
2	Leakage involving pulp floor
3	Leakage involving root canal

Table 2. Microleakage scores in all the groups (n=10)

Study Groups	Dye Leakage Scores				Mean
	0	1	2	3	
Group 1a$^{\alpha}$ (CS^3-NS)	0	1	5	4	2.3
Group1b$^{\alpha}$ (CS^3- NaOCl -EDTA)	0	1	2	7	2.6
Group 1c$^{\beta}$ (CS^3- NaOCl-EDTA + PA)	5	3	2	0	0.7
Group 2a$^{\alpha}$ (FB-NS)	0	4	1	5	2.1
Group2b$^{\alpha}$ (FB-NaOCl -EDTA)	0	1	1	8	2.7
Group 2c$^{\beta}$ (FB-NaOCl -EDTA +PA)	5	4	1	0	0.6

Same superscript letters indicate no statistically significant differences

Scanning Electron Microscopy

One sample per group was used for SEM analysis. Restored samples were sectioned vertically through the centre of the restoration and polished. Acid base treatment was done and then samples were dehydrated in ascending ethanol concentration (50%, 75%, and 95% for 20 min each and 100% for 1 h), then transferred to a critical point dryer for 30 min. The specimens were gold sputter coated and the interface was examined under scanning electron microscope.

Statistical Analysis

Microleakage scores were statistically analyzed by Kruskal–Wallis non-parametric analysis and Mann–Whitney U-tests using SPSS Base 15.0 software at significance level of $P<0.05$.

RESULTS

Microleakage scores and statistical analysis results are presented in Table 2. None of the groups completely prevented dye leakage. Extensive dye leakage was observed in both the adhesives when normal saline was used as an irrigant. NaOCl and EDTA irrigation had no significant effect on the microleakage of both adhesives. PA treatment after NaOCl and EDTA irrigation significantly decreased microleakage in both the adhesive systems.

Scanning electron microscopic observation of resin dentin interfaces of all groups are summarized in figures:1-6. With normal saline irrigation, both FB and CS^3 demonstrated presence of gap along the entire interface with poor interfacial seal (Figures 1 and 4). With conventional NaOCl and EDTA irrigation also, poor interfacial adaptation was observed in both the adhesives (Figures 2 and 5). When PA treatment followed the conventional NaOCl and EDTA irrigation regimen, an excellent interfacial adaptation without gap was observed for both the adhesives with pulpal dentin. (Figures 3 and 6).

DISCUSSION

Optimal irrigation is based on the combined use of two or several irrigating solutions, in a specific sequence, to predictably obtain the goals of safe and effective irrigation. The reduction of intracanal microbiota, is not any greater when 5% sodium hypochlorite is used as an irrigant as compared to 0.5%.[25,26] Zehnder stated that there is no rationale for using hypochlorite solutions at concentrations over 1% wt/vol.[8] Accordingly, in the current study 1% NaOCl irrigation was followed by 17% EDTA irrigation.

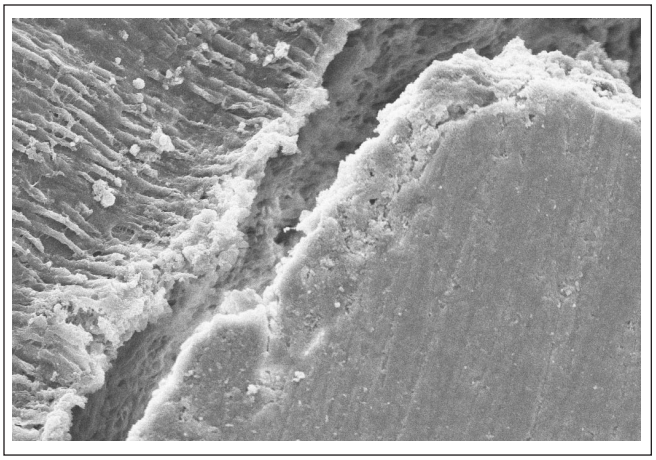

Figure 1. SEM photomicrograph of resin dentin interface between CS^3 and pulp chamber dentin after irrigation with normal saline depicting generalized gap.

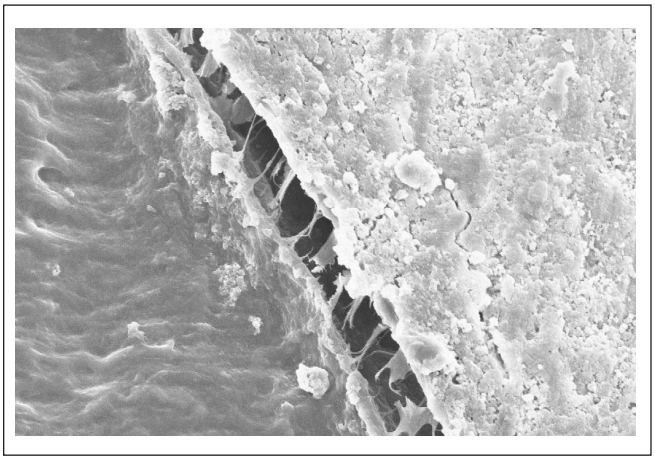

Figure 2. SEM photomicrograph of resin dentin interface between CS^3 and pulp chamber dentin after NaOCl and EDTA irrigation shows gap along the entire interface.

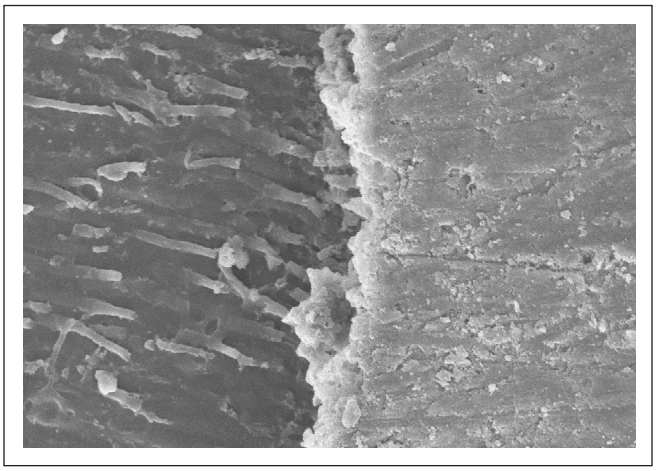

Figure 3. SEM photomicrograph of resin dentin interface obtained after 1-min application of 30% PA to pulp chamber dentin following NaOCl and EDTA irrigation and bonded with CS^3 shows excellent interfacial adaptation.

In the current study, both self-etch adhesives showed extensive leakage when normal saline was used as an irrigant. Bonding to deep dentin, as it is found on the pulp chamber walls, can be occasionally more difficult to achieve than to superficial dentin,

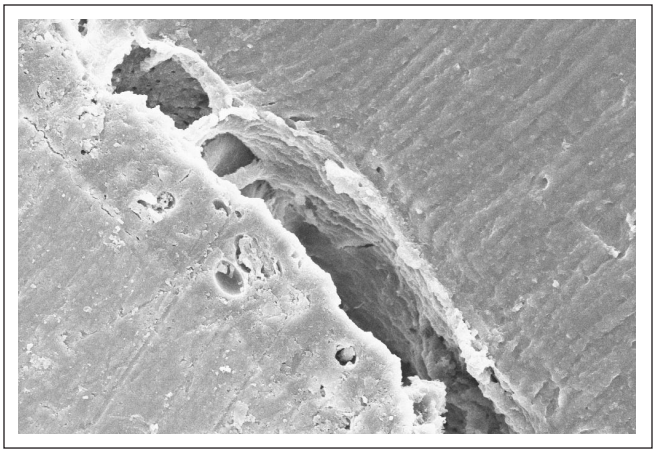

Figure 4. SEM photomicrograph of resin dentin interface between FB and pulp chamber dentin after normal saline irrigation depicts presence of generalized gap.

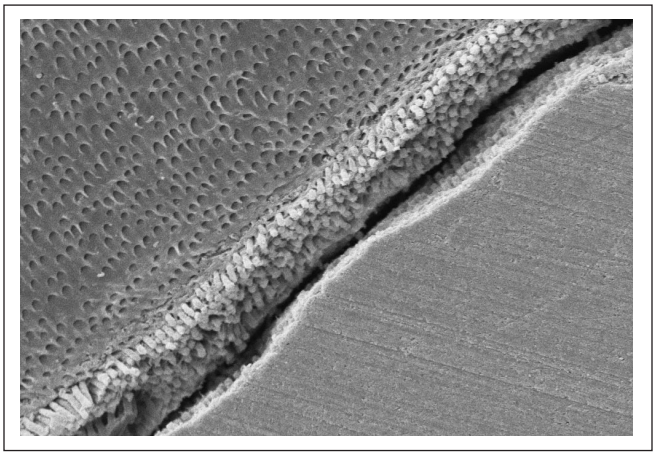

Figure 5. SEM photomicrograph of resin dentin interface between FB and pulp chamber dentin after NaOCl and EDTA irrigation still shows interfacial gap.

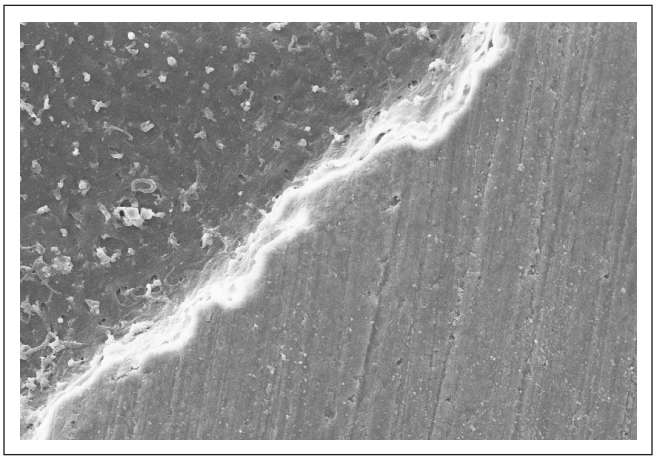

Figure 6. SEM photomicrograph of resin dentin interface obtained after 1-min application of 30% PA to pulp chamber dentin following NaOCl and EDTA irrigation and bonded with FB shows excellent interfacial seal.

since deeper dentin surfaces contain large tubule diameters and high tubule density, making them a more challenging bonding substrate.[27] The cavity configuration factor (C-factor), showing a ratio of five bonded walls to one unbonded wall, may also adversely affect the resin-dentin bonding.[28]

It was observed that NaOCl and EDTA irrigation had no detrimental effect on the microleakage of the adhesives when compared with normal saline irrigation. Bansal and Tewari also reported no significant difference in dye leakage between control and groups with 5.25% NaOCl pretreatment with both adhesive systems bonded to pulp chamber dentin.[14] Contrary to our results, NaOCl has been previously shown to jeopardize the polymerization of adhesive resins.[29-31] This compromised bonding has been attributed to the fact that NaOCl leads to biological oxidation of some component in the dentin matrix, forming protein-derived radicals that would compete with the propagating vinyl free radicals generated by the light-activation of resin adhesives, resulting in premature chain termination and incomplete polymerization.[32-34] However lower concentration of NaOCl (1%) and sequential use of EDTA as used in the current study may account for the different results. Endogenous dentin matrix metalloproteinases (MMPs) have been reported to contribute to extracellular collagen matrix degradation in hybrid layers after adhesive dentin bonding procedures. Thompson and others suggested that endodontic irrigants, including chlorhexidine and EDTA, might help protect the hybrid layer from this process. They demonstrated that 17% EDTA significantly inhibits endogenous MMP activity of human dentin within 1-2 minutes. This might minimize hybrid layer degradation after resin bonding procedures in the root canal space.[35]

Traditionally PAs have been used in nutritive medicine as potent antioxidants. Antioxidant agents have three main antioxidant mechanisms to control oxidation, which include free radical chain-breaking, metal-chelating and free radical quenching mechanisms. In the case of the free radical quenching mechanism, antioxidants can react with oxidants to neutralize unpaired electrons and form a stable product, which limits the activity of oxidant agents.[36] In other words, treatment with an antioxidant agent can restore the redox potential of the oxidized dentin substrate, leading to optimal polymerization of the resin composite.[16]

PA also called condensed tannins, are oligomers and polymers of monomeric flavanoids. These secondary plant metabolites have substantial antioxidant activity.[37,38] They are prevalent in some foods and dietary supplements including several berries, pine bark, red grapes and their wines, and seeds, baking chocolate and cinnamon. The unique polyhydroxy phenolic nature of PAs and the resulting electronic configuration allows relatively easy release of protons that accounts for their substantial antioxidant activity. Investigators have shown grape seed extract PA is a better free radical scavenger and inhibitor of oxidative tissue damage than vitamin C, vitamin E succinate.[38-41] Clearly, due to their antioxidant activity, the therapeutic potential of proanthocyanidins is quite broad. Proanthocyanidins lack toxicity and are known to stabilize and increase the cross-linkage of type-1 collagen fibrils. Grape seed extract (PA) has been reported to induce exogenous cross-links.[18] The proposed mechanisms for interaction between PA and proteins include covalent, ionic, hydrogen bonding, and hydrophobic interactions.[18]

In the current study, sequential irrigation with NaOCl, EDTA and PA significantly reduced the microleakage of both the adhesive systems. This may be attributed to its antioxidant effect which can theoretically also counter the oxygen inhibition layer and thus ensure complete polymerization. Grape seed extract as used in the current study contains OPCs (oligomeric proanthocyanidin complexes) made up of dimers or trimers of (+)-catechin and (-)-epicatechin.[42,43] Esterification of (-)- epicatechin and procyanidin B2 by gallic acid

increases their free radical scavenging ability. These gallate esters are only found in the grape seed extract form.[20]

As indicated by Green and others, inclusion of PA in dental adhesives may inhibit the biodegradation of unprotected collagen fibrils within the hybrid layer.[44] It is hoped that the prospective development of adhesive resin systems and bonding techniques take into consideration the specific features of the pulp chamber substrate and irrigation regimens to achieve better defense against microleakage. Long-term clinical studies will also enhance our knowledge regarding effect of endodontic irrigation on bonding of adhesive restorations.

CONCLUSION

One minute application of grape seed extract proanthocyanidin after NaOCl and EDTA irrigation significantly improved the adhesion of self-etch adhesives to pulp chamber dentin.

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