Ex vivo evaluation of dye penetration associated with various dentine bonding agents in conjunction with different irrigation solutions used within the pulp chamber

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Abstract

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Aim To compare the effect of sodium hypochlorite (NaOCl), chlorhexidine, povidone iodine and sodium ascorbate on dye penetration associated with various dentine adhesives used within the pulp chamber.

Methodology One hundred and sixteen mandibular molar teeth were divided into eight groups of 12 teeth each by irrigation regimen and adhesive system. The roof of pulp chambers and roots were removed under water cooling. The pulp chambers in the experimental groups were irrigated with 5.25% NaO-Cl, 0.2% chlorhexidine, 5% w/v povidone iodine or 5.25% NaOCl followed by sodium ascorbate and restored with Surefil using either Prime & Bond NT or Xeno III with each irrigation regimen. Twenty teeth were used as control specimens in which no disinfectant was used before bonding. Ten teeth from each group were immersed in 2% methylene blue dye and assessed for dye penetration. The data were

statistically analysed by Kruskal–Wallis and Mann– Whitney *U*-tests. Two samples in each group were observed under scanning electron microscopy for interfacial gap evaluation.

Results No significant difference was found in dye leakage between control and groups with 5.25% NaOCl pretreatment with both adhesive systems. Chlorhexidine and povidone iodine pretreatment resulted in significantly less dye penetration with Xeno III as compared with Prime & Bond NT. Sodium ascorbate treatment following NaOCl application significantly reduced microleakage and improved the marginal adaptation with both adhesive systems.

Conclusions The type of irrigant affected the sealing ability of bonding agents inside the pulp chamber and was material specific. Xeno III had less dye penetration with CHX and povidone iodine whilst sodium ascorbate pretreatment after NaOCl reduced dye penetration with both PBNT and Xeno III.

Keywords: bonding agents, microleakage, pulp chamber, root canal irrigants.

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Introduction

950

The restoration of root filled teeth with adhesive systems offers many advantages over the use of

traditional, nonadhesive materials. For example, bonded resins permit transmission of functional stresses across the bonded interface to the tooth, with the potential to reinforce the weakened tooth structure (Eakle 1986, Belli *et al.* 2001a,b). Numerous commercial bonding systems are available and are currently categorized by 'generations' with total etch systems being described as fourth and fifth generation and selfetch systems being described as sixth and seventh generation bonding systems. For the total etch bonding

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agents, the assumption is that the acid etching process followed by rinsing may be capable of removing the dissolved mineral component of dentine and the remaining irrigating solutions or interaction byproducts (Kazemi et al. 2002). With self-etch dentine bonding system, the smear layer and any possible free or interacted residual chemical irrigant components may remain on the bonding site and be included in the hybrid layer (Kazemi et al. 2002). However, the weaker acidic primer in self-etching bonding systems results in less obvious change in the dentinal wall structure than the stronger total etch systems. These bonding strategies might behave differently on dentine surfaces within pulp chambers altered by irrigants used during root canal treatment. Although several studies on adhesion have concentrated on occlusal, proximal and root dentine surfaces with current dentine bonding systems, investigation of adhesion of these materials to the walls of pulp chamber dentine has not been reported extensively. Most studies have considered the effects of these regimens on bond strength but few have considered their effect on dye or fluid penetration, which can be associated with post treatment disease (Belli et al. 2001a,b, Galvan et al. 2002, Ozturk & Ozer 2004).

The application of NaOCl to dentine has been reported to decrease its microhardness because of removal of the organic components and alteration of its mineral profile. This has been shown to adversely affect the bonding of total-etch and self-etch adhesives to dentine (Fuentes et al. 2004. Ari & Erdemir 2005). Erdemir et al. (2004) reported that use of NaOCl, H₂O₂ or combinations of NaOCl and H₂O₂ decreased bond strength to root canal dentine significantly, whereas Hayashi et al. (2005) reported improved bonding with total etch systems after NaOCl application. Sodium ascorbate, a potent anti-oxidant has been reported to alter the oxidizing agents by redox reaction and allows the free-radical polymerization of adhesives to proceed without premature termination and reverse the compromised bonding on NaOCltreated dentine (Lai et al. 2001). Nikaido et al. (1999) reported that with the use of 10% sodium ascorbate, clinicians can acid-etch and bond with total-etching adhesive immediately to endodontically treated teeth that were irrigated with NaOCl without any harmful effect. Chlorhexidine [CHX, N,N 1-Bis(4-chlorophenyl)-3,12-diiamino-2,4,11,13, tetraaza tetradecadediimidamide] has been recommended as a root canal irrigant because of its broad antibacterial spectrum, biocompatibility and substantivity (Menezes et al.

2004, Rosenthal et al. 2004, Ari & Erdemir 2005). CHX also binds with the amino acid moieties of collagen and released slowly; however, the presence of CHX residue in the dentine may interfere with the bonding of hydrophilic resins. The use of CHX as a cavity disinfectant has been reported to increase microleakage when used with two-step self-etch adhesive (Syntac; Ivoclar-Vivadent AG, Schaan, Liechtenstein) and two-step total-etch adhesive (Prime & Bond: Dentsply Caulk, Milford, DE, USA) (Tulunoglu et al. 1998). However, Meiers & Kresin (1996) reported CHX-based irrigants did not significantly affect microleakage when used with two-step self-etch adhesive and two-step total-etch adhesive (Tenure; Den-Mat, Santa Maria, CA, USA). Torneck (1976) advocated the use of povidone iodine (polyvinylpyrrolidone-iodine) solution as an endodontic irrigant, citing its rapid antiseptic action against a wide range of microorganisms, low toxicity, hypoallergenicity and decreased tendency to stain dentine compared with other iodine-containing antiseptics. Buck et al. (1999) found that povidone iodine was capable of permeating throughout the dentinal tubules but its effectiveness was dependent on the type of bacteria. However, the effect of reaction byproducts of CHX and povidone iodine with amino acids of bacteria and collagen molecules on adhesion of composite resins is yet to be determined.

The objective of this study was to evaluate dye penetration of recent dentine bonding agents within the pulp chamber following the application of commonly used root canal irrigants.

Materials and methods

A total of 116 extracted human noncarious first and second mandibular molars with fully developed apices were thoroughly cleansed, stored in sterile water and used within 6 months of extraction. Teeth were examined under the stereomicroscope for visible cracks or defects and defective teeth were excluded from the study. The roof of the pulp chambers was removed using rotary carborundum cutting disc and roots were removed 2 mm cervical to the bifurcation. To avoid touching the pulpal wall dentine, pulp tissue was removed carefully with an excavator and endodontic instruments. The canal orifices were widened with Gates Glidden drill numbers 2-3 (Mani Inc, Tochigiken, Japan). Teeth were randomly divided into eight groups with 12 teeth in each group. A total of 20 teeth were used as controls.

Group1 [Prime & Bond NT (PBNT) with 5.25% NaOCI] Pulp chambers were continuously irrigated with chemically pure 5.25% NaOCl for 1 min followed by washing with distilled water for 1 min; dentine was dried with moist cotton before bonding with PBNT.

Group2 (PBNT with 0.2% CHX) Irrigation with 0.2% CHX solution (Rexidin; Warren Pharmaceuticals, Andheri, Mumbai, India) was carried out for 1 min followed by washing and drying as group 1 before bonding with PBNT.

Group3 (PBNT with 5% w/v povidone iodine) Irrigation with 5% w/v povidone iodine (Betadine; Win-Medicare Nehru Place, New Delhi, India) solution was carried out for 1 min followed by washing and drying as group 1 before bonding with PBNT.

Group4 (Xeno III with 5.25% NaOCl), group 5 (Xeno III with 0.2% CHX) and group 6 (Xeno III with 5% w/v povidone iodine) Pulp chambers were irrigated with 5.25% NaOCl, 0.2% CHX or 5% w/v povidone iodine solution, respectively, following the same protocol as in groups 1, 2 and 3 before bonding with Xeno III.

Group7 (PBNT with 5.25% NaOCl and 10% sodium ascorbate) Pulp chambers were continuously irrigated with 5.25% NaOCl for 1 min followed by washing with chemically pure 10% sodium ascorbate for 1 min and then dentine was dried before bonding with PBNT. Distilled water was not used as a final irrigant after irrigation with sodium ascorbate.

Group8 (Xeno III with 5.25% NaOCl and 10% sodium ascorbate) Pulp chambers were bonded with Xeno III after irrigation with 5.25% NaOCl and 10% sodium ascorbate following the same protocol as in group 7.

Adhesive systems belonging to the same manufacturers were applied to the pulp chamber floor and walls of access cavity according to the manufacturer's directions (Table 1). Pulp chambers in all groups were filled with Surefil (Dentsply Caulk) in two increments and condensed with teflon-coated condenser and light cured for 40 s each. Light curing units with intensitometer (Spectrum 800; Dentsply Caulk) were used at an intensity of 500 mW cm⁻² throughout the study. A total of 20 teeth divided into two groups of 10 teeth each, were used as controls and no irrigation solution was used before bonding with PBNT & Xeno III, respectively. Of 12 samples in each group, 10 from each group were used for dye penetration and two specimens were subjected to scanning electron microscopic evaluation to investigate marginal adaptation and relate that to dye penetration.

Dye penetration

Specimens were dried superficially and coated with two layer of sticky wax, leaving 1 mm window around the cavity margins. Samples were immersed in freshly prepared 2% methylene blue dye for 7 days, rinsed with tap water, left to air dry at room temperature for 24 h and then sectioned mesiodistally using an isomet saw (Buehler Ltd, Evanston, IL, USA). Dye penetration at the tooth restoration interface was assessed by stereomicroscope (Stemi 2000; Carl Zeiss, Göttingen, Germany) at magnification $10\times$ and graded (Table 2; Saunders & Saunders 1990).

Specimen preparation for scanning electron microscopy (SEM)

The specimens were sectioned vertically in a mesiodistal plane through the centre of the restoration and polished with Enhance system disks and paste containing 1 and 0.05 μ m alumina particles (Dentsply Caulk). After acid base treatment, specimens were subjected to dehydration in ascending grades of ethanol up to 100%.

Bonding agent	Components	Bonding procedures
PBNT (Dentsply)	Acid: 36% phosphoric acid	Acid etching15 s, rinse 30 s and blot dry
	Bond: PENTA, UDMA resin R5-62-1,T-resin, D-resin, nanofillers, photoinitiators, stabilizers, cetylamine hydrofluoride, acetone	Apply bond 20 s, air dry 5 s and light cure 15 s
Xeno III (Dentsply)	Liquid A: HEMA, purified water ethanol, BHT, nanofiller Liquid B: Pyro-EMA, PEM-F, UDMA, BHT, Camphorquinone EPD	Mix equal amount of liquid A and B – 5 s Apply bond 10 s, air dry 2 s and light cure 10 s

Table 1 Bonding agents and procedures used in the study

PENTA, di pentaerythritol penta acrylite mono phosphate; UDMA, urethane dimethacrylate; HEMA, 2-hydroxyethyl methacrylate; BHT, 2,6-di-tert-butyl-*p*-hydroxy toluene; Pyro-EMA, tetramethacryloxyethyl pyrophosphate; PEM-F, pentamethacryloxyethyl cyclophosphazen mono fluoride; EPD, *p*-dimethylamino ethyl benzoate.

952

Degree of leakage	Depth of penetration
0	No leakage detected
1	Slight, just reaching the pulp chamber
2	Moderate, penetrating half way into pulp chamber
3	Extensive, with leakage to floor of pulp chamber
4	Gross, extending into root canals

 Table 2
 Scoring system used Saunders & Saunders (1990)

Specimens were mounted on aluminum stubs and further dried in a vacuum before sputter coating with gold. Gold sputter coating was carried out under reduced pressure in an inert argon gas atmosphere in an Agar Sputter Coater P 7340 (Agar Scientific Ltd, Stansted, Essex, UK). The resin-tooth interface was evaluated for gaps under SEM (Leo 435 VP, Cambridge, UK) at 250× magnification.

Statistical analysis

The scores of dye penetration in samples of all the groups were analysed with Kruskal–Wallis nonparametric analysis followed by Mann–Whitney *U*-test to evaluate differences amongst the experimental groups at a significance level of P = 0.05.

Results

Dye leakage study

The dye penetration measured within the experimental groups is shown in Table 3. Extensive dye leakage was observed in all samples in the control group without irrigant with both PBNT and Xeno III. None of the experimental groups prevented dye leakage and there was significant difference in dye penetration amongst all groups (Table 4, P < 0.05). Extensive leakage was observed with both Prime & bond NT and Xeno III after NaOCl irrigation (groups 1 and 4) and in PBNT after CHX and povidone iodine irrigation (groups 2 and 3). No significant difference in dye penetration was observed amongst groups 1, 2, 3 and 4 and in control groups (P > 0.05). Significantly less dye penetration was observed with Xeno III after CHX and povidone iodine irrigation (groups 5 and 6) than NaOCl pretreatment and control groups (P < 0.05). Sodium ascorbate pretreatment after NaOCl allowed significantly less dye penetration in both PBNT and Xeno III group (groups 7 and 8) than all other experimental and

Table 3	Score of l	leakage	around	restorat	ions o	bserve	d in	the
samples	subjected	to 2% r	nethyle	ne blue	dye for	r 7 da	vs	

Restorative groups	No. of samples	Dye leakage scores				
		0	1	2	3	4
NaOPB ^a	10	0	0	0	0	10
CHPB ^a	10	0	0	0	4	6
BePB ^a	10	0	0	0	0	10
NaOXe ^a	10	0	0	0	0	10
CHXe ^c	10	0	2	6	0	2
BeXe ^b	10	0	0	0	7	3
NaAsPB ^c	10	0	3	7	0	0
NaAsXe ^c	10	0	2	8	0	0

NaO, sodium hypochlorite; CH, chlorhexidine; Be, Betadine; NaAs, sodium ascorbate; PB, Prime & Bond NT; Xe, Xeno III. Groups with the same superscript letter indicate no statistically significant difference at P = 0.05.

Table 4 Statistical analysis (Kruskal–Wallis) for dye leakage study amongst all groups

Groups	H tab at 0.05%	H cal
NaOPB, CHPB, BePB, NaOXe, CHXe, BeXe, NaAsPB & NaAsXe	14.07	53.03

Statistically significant at P < 0.05.

control groups excepting group 5 (CHX with Xeno III). No significant difference in dye penetration was observed amongst groups 5, 7 and 8 (P > 0.05).

SEM

Scanning electron microscopic observation of resindentine interfaces demonstrated the presence of gaps along the entire margin of the cavity in NaOCl-treated groups showing poor adaptation at the resin-dentine interface (Figs 1 and 2). Sodium ascorbate application following application of NaOCl with both the adhesive systems revealed excellent adaptation of the resin composite to the cavity walls (Figs 3 and 4). CHXpretreated groups with PBNT and povidone iodinepretreated groups with PBNT and Xeno III revealed presence of continuous gap and poor adaptation of resin-dentine interface (Figs 5, 6 and 7). Specimens with CHX pretreatment with Xeno III revealed almost continuous margin and better adaptation of resin composite to dentine (Fig. 8). Average width of gap and percentage of continuous margin measured is presented in Table 5.

Discussion

Sealing of the pulp chamber is an important factor that may influence the outcome of root canal treatment



Figure 1 SEM image of cross-section of interface obtained *ex vivo* between Prime & Bond NT with NaOCl pretreatment showing gap along whole margin.



Figure 4 Cross-section of interface obtained *ex vivo* with Xeno III with NaOCl and sodium ascorbate pretreatment showing presence of gap along whole margin.



Figure 2 SEM image of cross-section of interface obtained *ex vivo* with Xeno III after NaOCl pretreatment showing gap along whole margin.





Figure 3 Cross-section of interface obtained *ex vivo* between Prime & Bond NT with NaOCl and sodium ascorbate pretreatment showing better marginal adaptation.

Figure 5 Cross-section of interface obtained *ex vivo* between Prime & Bond NT with CHX pretreatment showing gap formation and poor marginal adaptation.

(Hommez *et al.* 2002). Several investigations have assessed the sealing of the pulp chamber floor with different restorative materials; however, none of the materials studied was able to prevent leakage completely (Belli *et al.* 2001a,b, Galvan *et al.* 2002, Wells *et al.* 2002).

Extensive microleakage in control groups without surface pretreatment with both PBNT and Xeno III in this study could be attributed to different characteristics of pulp chamber dentine such as collagen rich predentine, enlarged tubules, small amounts of intertubublar dentine and surface free from smear layer. In addition, presence of high C factor (ratio of bonded walls to that

954



Figure 6 Cross-section of interface obtained *ex vivo* between Prime & Bond NT with Betadine pretreatment showing large gap and poor marginal adaptation.



Figure 7 Cross-section of interface obtained *ex vivo* with Xeno III after Betadine pretreatment showing large gap and poor marginal adaptation.

of nonbonded walls) and high viscosity of packable composite resins might also be responsible for the extensive microleakage reported in this study.

Significantly greater microleakage and poor adaptation of resin to dentine surface with both adhesives observed after 5.25% NaOCl treatment could be related to the oxidizing effects of NaOCl and its reaction byproducts causing inhibition of the interfacial polymerization of adhesives. Some reactive residual-free radicals in NaOCl-treated dentine may compete with the propagating vinyl-free radicals generated during light activation of the adhesive system, resulting in premature chain termination and incomplete polymerization (Nikaido *et al.* 1999, Lai *et al.* 2001, Morris *et al.* 2001, Ozturk & Ozer 2004).



Figure 8 Cross-section of interface obtained *ex vivo* with Xeno III after CHX pretreatment, showing better marginal adaptation and less gap formation with Xeno III as compared with PBNT.

 Table 5
 Scanning electron microscope observations

Average width ofPercentage of continuous margGroupsgap in μmmeasuredNaOPB25.533.34	
NaOPB 25.5 33.34	in
CHPB 24 22.23	
BePB 36 34	
NaOXe 30.67 25	
CHXe 5.34 75	
BeXe 34.6 33.34	
NaAsPB 6 80	
NaAsXe 5.34 90	

Significantly less microleakage after sodium ascorbate treatment of the collagen-depleted dentine could be related to the reversal of the adverse effects of NaOCl on the polymerization of the composite resin by sodium ascorbate. Lai et al. (2001), Morris et al. (2001) and Vongphan et al. (2005), stated that the reduction in bond strength, when NaOCl was used either before or after acid etching could not be attributed to incomplete deproteinization but to the oxidizing effect of NaOCl. Nikaido *et al.* (1999) reported that with the use of 10%sodium ascorbate, clinicians can acid etch and bond with total-etching adhesive immediately to root filled teeth that were irrigated with NaOCl without any harmful effect. These findings suggest that sodium ascorbate treatment after NaOCl could be used to improve the bonding of both total-etch (PBNT) and selfetch (Xeno III) bonding systems with in the pulp chamber.

955

Significantly lower microleakage and better adaptation at the resin-dentine interface with Xeno III after 0.2% CHX pretreatment is consistent with the findings of Turkun *et al.* (2004) and Meiers & Kresin (1996) who reported positive effect of CHX on bonding of selfetch adhesives. Erdemir *et al.* (2004) observed the highest bond strength values of C&B Metabond (etch and rinse self cure composite) to root canal dentine with CHX irrigant and related it to the adsorption of CHX by dentine which may have the positive effect of adsorbing dentine-bonding agents inside the dentinal tubules.

The findings of this study are contrary to the studies of Pappas *et al.* (2005) and Silva *et al.* (2005), who reported better bonding with total etch acetone-based adhesive systems after CHX application. However, these studies had used CHX in combination with other irrigants on smear layer covered coronal dentine and the disinfectants were not rinsed off before bonding.

Better bonding with Xeno III after CHX application in this study could be related to difference in the composition of the solvents in dentine bonding systems, as PBNT is acetone-based total-etch adhesive having relatively small 'window of opportunity' to achieve optimal hybridization (Tay et al. 1998), whereas Van Meerbeek et al. (2003) classified Xeno III, as water/ ethanol-based 'intermediary strong' self-etch adhesive. Whilst total-etch systems completely remove the hydroxyapatite up to $3-5 \,\mu\text{m}$ depth and bonding is primarily diffusion-based, following an 'intermediary strong' self-etch approach, the deepest region of the hybrid layer up to a maximum of 1 µm still contains hydroxyapatite, by which the transition of the hybrid laver to the underlying unaffected dentine is more gradual. This allows better micromechanical interlocking than the mild self etch whilst still allowing similar chemical intermolecular interaction at the base.

Significantly less microleakage after 5% w/v povidone iodine treatment with Xeno III as compared with PBNT were contrary to the results of Turkun *et al.* (2004), Meiers & Kresin (1996) and Meiers & Shook (1996). Meiers & Kresin (1996) found that Ora-5 produced significantly greater gingival microleakage when used with a two-step self-etch system (Syntac; Ivoclar-Vivadent), but did not increase microleakage with a two-step total-etch system (Tenure; Den-mat).The difference in results found in these studies could be attributed to different bonding substrates used (coronal dentine), disinfectants were not rinsed off before bonding and different chemical composition of bonding agents were used (CSEB, PLP, Syntac versus Xeno III).

Conclusion

With in the limits of this laboratory study, it can be postulated that the bonding of composite resin to pulp chamber dentine after root canal treatment is still questionable and optimum dentine surface pretreatments need to be determined. Caution should be exercised in extrapolating conclusion from this study, because the experimental design does not totally mimic actual clinical condition. However, the data provide useful preliminary information on the effects of root canal irrigants on sealing properties of the materials that were tested.

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