Effect of local anesthesia containing vasoconstrictor on sealing ability of dentin with two adhesive systems: Dye leakage and scanning electron microscopy study

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Abstract

Objectives: To evaluate the effect of 2% lignocaine containing 1:80,000 adrenaline on the microleakage and ultrastructure of resin tooth interface using an acetone based total etch adhesive (Prime and Bond NT) and an ethanol water based self etch adhesive system (Xeno III).

Materials and method: Class V cavities were prepared on buccal surfaces of 72 maxillary first premolars scheduled for orthodontic extraction. In 36 premolars procedure was performed under local anaesthesia (LA) and in other 36 teeth without LA. Restorations were done with hybrid composite Spectrum TPH using either Prime and Bond NT or Xeno III bonding agents.

Results: Administration of 2% lignocaine with 1:80,000 adrenaline significantly reduced microleakage and improved quality of hybrid layer and tubular penetration in Prime and Bond NT specimens; but no significant effect was observed in Xeno III specimens.

Conclusion: Under clinical conditions, administration of LA reduced microleakage and improved quality of hybrid layer and tubular penetration in total etch adhesive systems.

Keywords: Dentin bonding; local anesthesia; sealing; self-etch; total etch

INTRODUCTION

A central goal in adhesive dentistry has been securing an intimate adaptation between the restorative material and prepared margins to prevent microleakage and influx of microbes or their metabolites, which may lead to postoperative sensitivity and recurrent caries. Vital dentin is an intrinsically hydrated tissue, penetrated by a network of fluid-filled dentinal tubules. The flow of fluid from the pulp to the dentinoenamel junction is the result of a slight but constant pulpal pressure, which is estimated to be 25–30 mm of Hg or 34–40 cm of H₂O. Under clinical conditions, this outward fluid flow across exposed dentin interferes with adhesion of composite resins to dentinal surface.

Local anesthetics with vasoconstrictor have been widely used in dentistry for pain control. The purpose of adding the vasoconstrictor to local anesthesia (LA) is to potentiate and prolong the anesthetic effect by reducing the blood flow in the area in which the anesthetic is administered. Although...
this enhances anesthesia, various studies have shown that it significantly reduces pulpal blood flow.\cite{8,10} Theoretically, this reduced pulpal blood flow should decrease the outward dentinal fluid flow and may affect dentin bonding. Clinical studies conducted so far under LA with vasoconstrictor using different adhesive systems have reported variable results. While some of these studies demonstrated good resin-dentin adaptation\cite{11,12} and increased bond strength,\cite{11,12} others have found no significant effect of LA on bonding.\cite{11,13,14}

Therefore, the purpose of this in vivo study was to investigate the effect of LA containing vasoconstrictor on the microleakage and ultrastructure of resin-tooth interface using total etch and self-etch adhesives. The null hypothesis to be tested was that there is no effect of LA on resin-dentin bonding using total etch and self-etch bonding systems.

**MATERIALS AND METHODS**

This in vivo study was performed on 36 pairs of vital, noncarious maxillary first premolars scheduled for orthodontic extraction from 36 healthy individuals of age 18–25 years. Informed consent of each patient was obtained after explaining the clinical procedures and clarifying all the questions raised by the patients who agreed to participate in the study. The protocol was reviewed and accepted by the institutional ethics and review board.

All the tooth preparations and restorations were performed under rubber dam by the principal investigator (R. S). In each patient, the procedure was done with LA containing vasoconstrictor in the first premolar in one quadrant and without anesthesia in the premolar of other quadrant.

In the LA group, 2 ml of 2% lignocaine containing 1:80,000 adrenaline (Xicaine, ICPA Health Products) was infiltrated in the mucobuccal fold adjacent to the apex of the tooth selected. Class V cavities were prepared on the buccal surfaces of the teeth using an ISO 012 straight fissure diamond bur (Dentsply Detrey) in an air water-cooled high-speed handpiece. A new bur was used for each patient. The cavity preparations were standardized with a width of 3 mm mesiodistally, 2 mm occluso-gingivally, and 2.0 mm deep measured at the gingival level, ensuring the axial wall to be always in dentin. The occlusal and gingival cavosurface margins were prepared butt joint in enamel without any bevel. No additional mechanical retention was placed. Teeth were divided into four groups, with 18 teeth each according to the LA administered and type of adhesive used.

**Group I (Prime and Bond NT without local anesthesia)**

Cavities were prepared without administration of LA and etched with 37% phosphoric acid (Scotchbond Universal Etchant, 3M) for 15 s, rinsed with water for 20 s. The etched dentin surface was blot dried with a dry sterile cotton pellet, leaving it visibly moist. Prime and Bond NT bonding agent (PBNT) was applied for 20 s with gentle agitation, slightly air blown for 5 s and then light cured for 10 s at a light intensity of 750 mW/cm² (Translux Power Blue-Hareaus Kulzer, Germany).

**Group II (Prime and Bond NT with local anesthesia)**

Cavities were prepared under LA and PBNT was applied by following the same protocol as in Group I.

**Group III (Xeno III without local anesthesia)**

No LA was administered before cavity preparation. An equal amount of liquid A and liquid B was dispensed into a clean mixing well and mixed thoroughly for 5 s with an applicator tip. A generous amount of Xeno III was applied to wet all cavity surfaces thoroughly, left undisturbed for 20 s and was spread using gentle stream of air for 2 s and then light cured for 10 s at 750 mW/cm².

**Group IV (Xeno III with LA)**

Cavities were prepared under LA followed by application of Xeno III bonding agent in the same manner as in Group III.

All the cavities were bulk filled with hybrid composite, Spectrum TPH (Dentsply Detrey) and light cured. Restorations were polished using enhance system disks (Dentsply Detrey). After 1 week, the teeth were extracted by means of initial elevation, followed by careful application of forceps to the root surfaces, so as to minimize damage to the restorations. Teeth were rinsed and stored in distilled water for between 2 and 4 weeks. From each group, 12 premolars were used for evaluation of dye leakage and 6 premolars were subjected to scanning electron microscopic examination.

**Dye leakage test**

In preparation for the dye penetration test, the specimens were dried superficially and coated with two layers of sticky wax, leaving a 1 mm window around the cavity margins. The samples were then immersed in an inverted fashion in a freshly prepared 2% methylene blue dye for 5 days, individually in small bottles so as only the crown portions were dipped in the dye. After dye submersion, the teeth were rinsed with water, sticky wax removed, and teeth were left to air dry at room temperature for 24 h. Carborundum disk at slow speed without water spray was used to section the teeth longitudinally in a buccolingual direction by a cut through the center of the restoration. The degree of marginal leakage was determined by the penetration of the tracer agent, starting from the gingival margin of the restoration and moving toward the axial wall. Dye penetration at the tooth restoration interface was
assessed by stereomicroscope at magnification 10× by an independent examiner who was unaware of the treatment groups. The following scoring system was used.

**Degree of leakage depth of dye penetration**
- 0 no evidence of microleakage
- 1 dye penetration up to half the cavity depth
- 2 dye penetration of more than half the cavity depth
- 3 dye penetration along the axial wall.

**Specimen preparation for scanning electron microscopy**
For scanning electron microscopic evaluation, the specimens were sectioned vertically in a buccolingual plane through the center of the restoration and polished with Enhance system disks (Dentsply Detrey). Sections were fixed in 10% formalin for 24 h and decalcified in 6N HCl for 30 s, rinsed in distilled water, deproteinized by 10 min immersion in 5.25% NaOCl and rinsed in distilled water. After acid-based treatment, specimens were subjected to dehydration in ascending grades of ethanol up to 100% (25% for 20 min, 50% for 20 min, 75% for 20 min, 95% for 30 min, and 100% for 60 min). Specimens were mounted on aluminum stubs, and further dried in vacuum before sputter coating with gold. Gold Sputter Coating was carried out under reduced pressure in an inert argon gas atmosphere in Agar Sputter Coater P7340 (Agar Scientific Ltd, Essex, England). The gold-coated samples were examined under scanning electron microscope (Leo 435 VP, Cambridge, UK) operated at 15 kV. Micrographs of the axial resin-dentin interface were taken at 1000x to observe the quality of bonding between the restorations and dentinal tissue.

**Statistical analysis**
The results of dye penetration were analyzed with Kruskal–Wallis nonparametric analysis followed by Mann–Whitney U test to evaluate differences among the experimental groups at a significance level of \( P = 0.05 \).

**RESULTS**

**Dye leakage study**
Leakage scores observed in different treatment groups after immersion in 2% methylene blue are presented in Table 1.

Statistical analysis revealed significant difference between Group I and the other three groups \((P < 0.05)\). In Group I, most of the specimens showed extensive leakage up to the axial wall. In group II, significant reduction in microleakage was observed as compared to Group I \((P < 0.05)\). No significant difference in dye leakage scores was observed in Group III and Group IV specimens.

**Scanning electron microscopy**
Results of the qualitative SEM analysis are presented in Figures 1 and 2. For specimens bonded with PBNT without administering LA, hybrid layer appeared irregular and discontinuous along the interface; fewer resin tags were also observed [Figure 1a]. Specimens treated with PBNT with LA revealed uniform hybrid layer with numerous resin tags [Figure 1b]. No difference in the interfacial structure was seen in Xeno III specimens bonded without [Figure 2a] or with LA [Figure 2b]. Hybrid layer appeared uniform with the absence of resin tags in both groups.

**DISCUSSION**
Marginal leakage and consequent marginal discoloration remains the frequent reason to replace or repair an adhesive restoration. A composite restoration might be retained in a class V restoration; without being entirely bonded at the resin-dentin interface. Therefore, beside bond strength, marginal sealing effectiveness of different adhesives was evaluated in the present study. Microleakage evaluation is the most common method to assess the sealing efficiency of a restorative material.[15]

Results of the current study have shown a significant difference in dye leakage between Group I and Group II. This difference could be attributed to the etch and rinse phase employed in PBNT adhesive system; which completely removed the smear layer, thereby increasing dentinal permeability and causing enhanced outward fluid movement. This outflow of dentinal fluid under intrapulpal pressure might have affected the intimate interaction of PBNT with intrinsically wet dentinal tissue as is evident by reduced resin tag penetration and discontinuous hybrid layer along with increased leakage in Group I. Moreover, moist bonding technique used in the present study might have produced over wet conditions in Group I. Over-wetting may lead to dilution or deterioration of the monomers, reduced final degree of cure and formation of water-containing defects within the adhesive layer.[12] On the other hand, prior use of lignocaine with adrenaline in Group II significantly reduced pulpal blood flow; thereby decreasing intrapulpal pressure and consequently reducing the outward fluid flow. This permitted better resin tag penetration and good interfacial adaptation with uniform hybrid layer formation and decreased dye leakage. Over-wetting could not be present in Group II, as intrinsic moisture has been taken care of with the aid of lignocaine containing adrenaline. These findings are similar to the clinical studies that demonstrated gap-free resin-dentin

| Table 1: Dye Leakage scores in different groups |
|-----------------------------------------------|
| Restorative groups | No. of samples | Dye leakage scores (mean ± SD) |
|---------------------|----------------|-----------------------------|
| Group I             | 12             | 0, 0, 1, 11, 11              |
| Group II            | 12             | 1, 1, 1, 4, 4                |
| Group III           | 12             | 2, 2, 3, 3, 3                |
| Group IV            | 12             | 2, 3, 3, 3, 4                |

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Results of our investigation are also supported by the studies that observed no morphologic differences in the interfacial surface obtained in vivo or in vitro placed restorations when LA was administered in vivo. Results of this in vivo study presented no significant difference in dye leakage scores in Group III (Xeno III without LA) and Group IV (Xeno III with LA). SEM micrographs also appeared similar; revealing a distinctly thin, uniform hybrid layer with the absence of resin tags in both groups. Xeno III is an “intermediate strength” self-etch adhesive (pH = 1.4) which partially dissolves smear layer without much-affecting dentin permeability. Since dentin permeability is not much altered, effect of LA is not significant unlike the case of total-etch adhesive PBNT.

In the present study, specimens bonded with Xeno III, irrespective of the use of LA, demonstrated less microleakage scores than PBNT specimens bonded without LA. These results can be explained on the basis of bonding mechanism of the two adhesives. In Xeno III specimens, in addition to micromechanical bonding, chemical intermolecular interaction also takes place with the residual hydroxyapatite at the base of hybrid layer;[19] resulting in less microleakage. Findings of the present study support the observations of Ozok and others who reported better sealing ability of Prompt L-Pop, as compared to Scotch bond 1 under 15 cm dentin perfusion.[20] However, these findings contradict the study of Koumpia and others who revealed higher dye leakage scores in Prompt-L-Pop, a self-etching adhesive as compared to Bond 1 and Stae; 2-step total etch adhesives in class V restorations in dog teeth.[21] They attributed these results to the low pH and incomplete polymerization of hybrid layer in case of Prompt-L-Pop adhesive system.

Other studies found no significant differences in dye leakage scores at dentin margin in class V cavities restored with Xeno III or PBNT.[22,23] However, these studies were conducted in vitro without maintaining pulpal pressure.

Results of our investigation indicate that dentin perfusion might decrease the efficacy of total-etch adhesives, but has no significant effect on self-etch adhesives. However, microleakage cannot be completely eliminated by any of the methods employed. This could have been attributed to high C-factor in class V cavities prepared in the study.

Although standard of care involves routine use of LA before tooth preparation; in the present study, to actually evaluate the effect of LA; specimens with and without LA were selected, with the patient’s informed consent. The procedure could be terminated at any time if the patient felt discomfort. The present study used adrenaline in a concentration of 1:80,000 because at this concentration, there is significant reduction in pulpal blood flow while preserving pulp vitality.[10] To remove the inter-individual variability from the estimates of the treatment effect, split-mouth design was employed in the study.

The use of organic dyes as tracers is one of the oldest and most common methods of studying microleakage. In the present study, methylene blue dye was used for the dye
leakage test. Methylene blue is a small molecular weight dye which has high penetration ability. Kersten and Moorer found that leakage of the commonly used dye methylene blue was comparable with that of a small bacterial metabolic product of similar molecular size.[24]

Results of this investigation partially rejected the null hypothesis that there is no effect of LA on resin-dentin bonding using total etch and self-etch bonding systems. Administration of LA significantly affected the bonding of total-etch adhesive system but had no effect in case of self-etch adhesive. The current study did not include mandibular teeth, block anesthesia, caries-affected dentin, effect of polishing, and the long-term clinical evaluation of the restorations. Further studies are required to illustrate the effect of various techniques of LA containing different available concentrations of adrenaline; using different type of bonding agents on different teeth. Furthermore, long-term evaluation of such effects is required for more predictable and definitive clinical conclusions.

CONCLUSIONS

Within the limits of this in vivo study, it can be concluded that administration of LA containing vasoconstrictor has a positive effect on resin-dentin interfacial adaptation using total-etch adhesives and no effect in case of self-etch adhesives.

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Conflicts of interest
There are no conflicts of interest.

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