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SCANNING ELECTRON MICROSCOPIC EVALUATION OF NANOLEAKAGE OF FOUR DIFFERENT DENTIN BONDING ADHESIVES IN CLASS V RESTORATIONS.

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ABSTRACT:

Objective: To evaluate the nanoleakage of self-etch and total-etch dentin bonding adhesives in class V restorations.

Materials and methods: Class V preparations were done in 20 freshly extracted premolars; divided randomly and equally into four groups as follows: Group I: Prime & Bond NT (control); Group II: Clearfil SE Bond; Group III: Xeno III; and Group IV: Adper Prompt. All the cavities were restored with Filtek Z350 composite. Specimens were placed in freshly prepared 50% (w/v) ammonical silver nitrate solution in total darkness for 24 hours, rinsed in running water for 5 minutes, immersed in photodeveloping solution, and exposed to fluorescent light for 8 hours. Longitudinal sections were prepared using a diamond disk. Followed by platinum sputtering, the amount of nanoleakage was calculated directly under the scanning electron microscope using secondary electron images.

Results: No statistical significance was found among the dentin adhesives used ($p>0.05$). Highest nanoleakage values was seen in Prime & Bond NT (total-etch) and the least in Clearfil SE Bond (self-etch).

Conclusion: Self etch adhesives showed promising results as total etch adhesives in Class V restorations. Long term evaluation of the quality and insight of the hybrid layer need to be evaluated for optimal clinical success.

Keywords: Ammonical silver nitrate tracer solution, Dentin bonding adhesives, Nanoleakage , pH, Self-etch, Total –etch, Platinum sputtering, SEM.

INTRODUCTION

The seal of a restorative material against the tooth structure, and the quality of the seal, are major considerations for the longevity of restorations. This may influence the selection of restorative materials in preventing pulpal damage and secondary caries. Thus, the study of resistance to bacterial products and fluid penetration at the interface between the restoration and tooth structure, namely micro leakage, has been of great concern in restorative dentistry.[1] The term 'Microleakage' may be defined as the passage of bacteria, fluids, molecules or ions between a cavity wall and the restorative material applied to it. Consequently it may result in discoloration, post operative sensitivity, recurrent caries and pulpal damage. [2]

The acid-etch technique proposed by Buonocore has proved successful in enamel bonding and has effectively eliminated microleakage at the enamel/

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restoration interface.[3] However, no satisfactory equivalent solution has been found for effectively inhibiting microleakage at dentin and cementum margins of cavities.[4] Dentin bonding is more challenging since the dentin surface is a heterogeneous vital substrate with a low surface energy and outward dentinal fluid flow can occur on to the prepared surface.[5] Gap-free margins at the dentin/restoration interface were achieved with some recent dentin bonding systems. [6]

Sano et al (1994) have described another pattern of leakage by observing the penetration of silver nitrate along the gap free margins with several dentin bonding systems under scanning electron microscope. They described a leakage pattern occurring within the hybrid layer, which they termed 'Nanoleakage'. It represents permeation laterally through the hybrid layer and may be the result of the incomplete infiltration of adhesive resin into the demineralised dentin.[7] This kind of leakage may allow the penetration of bacterial products and dentinal or oral fluid along the interface, which may result in hydrolytic breakdown of either the adhesive resin or collagen within the hybrid layer, thereby compromising the stability of the resin-dentin bond. [8-9]

The development and marketing of newer bonding agents continues to be rapid. The quality of the dentin bond was reported to be material dependent in certain situations and associated with the chemistry of individual materials.[10-11] Nanoleakage tests can provide useful information on the sealing ability of restorations.[12] Despite different classifications of adhesive systems, current adhesion strategies depend on how the adhesive system interacts with the modified dentin surface, total-etch or self etch. [13]

Total-etch adhesives involve a separate etching and rinsing step. Multi-bottle total-etch adhesives have 3 different steps: acid-etching, followed by priming and the application of a fluid resin. Even the most popular "1-bottle" systems, which combine the primer and adhesive resin into 1 solution, usually need more than 1 application to achieve an acceptable micromechanical interlocking of monomers into the micro-retentive collagen network left by etching. [14]

Self-etch adhesives consist of non-rinsing acidic monomers that simultaneously condition and prime dentin and enamel. Self-etch adhesives may be classified according the number of application steps as two-step and all-in-one materials [15] or according to their pH and, consequently, their ability to demineralize dentin and enamel as mild, moderate or aggressive self-etch adhesives. [16]

The quality of the hybrid layer, current knowledge about nanoleakage phenomenon is limited.[17] Hence the purpose of this study was to do a comparative evaluation of the nanoleakage of one total-etch and three self-etching dentin bonding adhesives with ammonical silver nitrate tracer solution under SEM.

MATERIALS AND METHODS

Preparation of Class V tooth preparations:

Freshly extracted premolar teeth due to orthodontic purpose were used and disinfected as per OSHA regulations. 20 Class V cavities were prepared with a No. 330 carbide bur used in a high speed hand piece with copious amount of water. Preparations with occlusal margins in enamel and gingival margins in cementum were prepared on the buccal surface of each tooth.

The dimensions of the preparations (3 X 2 X 2 mm) were as follows: 3.0mm in mesiodistal width, pulpal floor depth of 2mm into dentin; and enamel and cementum margins were placed 1mm from the CEJ. A 0.5 to 1.0mm bevel was placed on the enamel cavosurface margins above the CEJ with a flame shaped NO. 7901 – carbide bur.

Application of Dentin bonding adhesives & restoration:

The prepared teeth were divided randomly and equally into four groups, one control group and three experimental groups. The halogen curing light (QHL75-Dentsply) was used to polymerize the dentin adhesives and composite resin Filtek Z 350.

Control group (Group I):

Conventional etching was carried out with 37% phosphoric acid for 20seconds, rinsed and dried. Prime & Bond NT (pH 2.2) was applied and cured for 20 sec followed by restoration with Filtek Z 350.

Experimental groups:

Group II- Self etching primer & adhesive Clearfil SE Bond (pH-1.9) was applied on the prepared cavities and cured for 20 seconds followed by restoration with Filtek Z 350.

Group III – Self etching adhesive Xeno III (pH-1.4) was applied on the prepared cavities and cured for 20 seconds followed by restoration with Filtek Z350.

Group IV – Self etching adhesive Adper Prompt (pH-0.4) was applied on the prepared cavities and cured for 20 seconds followed by restoration with Filtek Z350.

All procedures were followed strictly as per manufacturer's instructions (Table 1).

Ammoniacal Silver Nitrate Tracer Solution 50% (W/V):

The teeth were immersed in distilled water for 24 hours. After 24 hrs, the specimens were removed then the restorations were finished with an ultrafine low-speed diamond point under water coolant. Specimen apices were sealed cyanoacrylate resin and the entire teeth were coated with nail varnish except for the restoration and a 1 mm circumferential border.

Ammoniacal silver nitrate was prepared by dissolution of 25 grams of silver nitrate crystals (Qualigens) in 25 ml of distilled water. Concentrated (25%) ammonium hydroxide (Qualigens) was used to tritrate the black solution until it became clear as ammonium ions complexed the silver into diamine silver ions ($[Ag(NH_3)_2]^+$). This solution was diluted to 50 ml with distilled water, yielding a 50% solution (pH=9.5). [4, 18-19]

Specimens were placed in freshly prepared 50% (w/v) ammonical silver nitrate solution in total darkness for 24 hours, rinsed in running water for 5 minutes, immersed in photodeveloping solution, and exposed to fluorescent light for 8 hours in order to reduce the silver ions to metallic silver. After removal from photodeveloping solution the teeth were placed in running water for 5 minutes.

Preparation of Tooth Specimens and sputtering:

Longitudinal sections were prepared bucco-lingually across the bonded interface making a total of 40 specimens from 20 teeth (10 specimens in each group) using a diamond disk. The specimens were cleaned ultrasonically, air dried, mounted on brass stubs and placed in a dessicator for 24 hours and platinum sputtering (JEOL – JAPAN) was done.

Observation of nanoleakage under SEM:

The amount of nanoleakage was calculated directly under the scanning electron microscope (JEOL – JAPAN) using secondary electron images. For each specimen, the location where silver penetration terminated was identified at higher magnification (1000 X). The measurements where marked under low magnification (40 X) in the SEM (Fig 1). Nanoleakage scores of each specimen were calculated as the percent of the total cut dentin surface that was penetrated by silver nitrate.

Nanoleakage (N) score = p/L X 100, was calculated where; p= length of silver nitrate penetration along the resin/dentin interface and L= total length of dentinal cavity wall on the cut surface. [7,20]

RESULTS:

Silver particles could be detected at higher magnification (1000X); showed deposition of silver along the resin-dentin interface. No penetration of silver was detected along the enamel-restoration margins in all the specimens. Thus the cementum-restoration margin which showed leakage where photographed. The maximum depth of penetration of the silver ions was marked under higher magnification (1000X) ; and the estimation of the nanoleakage along the resin dentin interface was measured at lower magnification (40X) under SEM (Fig 1).

Penetration of silver nitrate along the dentinal wall was observed in most of the specimens of all the bonding systems used. Silver ion accumulations were often noted at the resin-dentin interface, indicating none of the bonding agents used could prevent the penetration of silver ions; thus resulting in nanoleakage.

The mean nanoleakage scores obtained were as follows; Prime & Bond NT (35.099 mm), Clearfil SE (32.97 mm), Xeno III (34.28 mm) and Adper Prompt (34.12 mm). Statistical analysis done with Kruskal Wallis test (Table 2) and intergroup comparison using Mann Whitney 'U' test (Table 3) showed statistically no significant results. Experimental results (Fig 2) showed that the least nanoleakage was seen in Clearfil SE Bond and highest nanoleakage in Prime & Bond NT.

DISCUSSION :

Class V cavities were used to check the marginal penetration as the restoration of the cervical lesions are always a challenge to the dentist as the margins of the cavity lie on both enamel and cementum.[21] Such a cavity preparation includes three types of dental hard tissue substrates enamel, dentin, cementum and comes in contact with two tissue fluids; saliva and gingival crevicular fluid. Thus the ability of restorative materials to effectively seal cavity margins on dentin is of particular concern in cervical lesions. [22]

Traditional microleakage evaluation methods have been utilized for determining micro leakage, which includes radioactive isotopes, dyes, bacteria and scanning electron microscope. Several silver dyes have been used to test the sealing ability of dentin adhesives- silver nitrate, silver methenamine and, more recently, ammoniacal silver nitrate.[23] Silver nitrate is one of the most commonly used methods for microleakage evaluation. Compared with other staining techniques, silver staining provides a much sharper picture of penetration at tooth-restoration margins. The silver nitrate penetration method, combined with high magnification SEM by means of secondary electron or backscattered electron mode, can provide much better information concerning the sealing ability of the restorations and the quality of the hybrid layer.[24]

Prime & Bond NT is a total-etch self priming adhesive which contains urethane dimethacrylate (UDMA) and dipentaerythritol pentacrylate monophosphate (PENTA) instead of hyroxyethyl methacrylate (HEMA) in acetone with a pH of 2.2. The primary bonding mechanism of etch & rinse adhesives to dentin is diffusion based and depends on hybridization or infiltration of resin within the exposed collagen fibril scaffold, which should be as complete as possible. Most critical in the etch & rinse approach is the priming step. When an acetone-based adhesive is used, the highly technique-sensitive "wet-bonding" technique is mandatory. True chemical bonding is rather unlikely, because the functional groups of monomers may have only weak affinity to the "hydroxyapatite-depleted" collagen. Such challenging monomer-collagen interaction might be the principle reason for what has been documented as manifesting in the form of "nanoleakage" phenomena. [25]

Clearfil SE Bond is a two step "Mild" self-etching adhesive with Methacryloyloxydecyl dihydrogen phosphate (MDP), Hydroxy ethyl methacrylate (HEMA) in water with a pH of 1.9. "Mild" self-etch systems demineralize dentin only to a depth of 1micrometer. This superficial demineralization occurs only partially, keeping the residual hydroxyapatite still attached to the collagen. The preservation of hydroxyapatite within the submicron hybrid layer may serve as a receptor for additional chemical bonding. Methacryloyloxydecyl dihydrogen phosphate (MDP) have a chemical bonding potential to calcium of residual hydroxyapatite. They enable more intimate chemical interaction with the functional monomers on a molecular level and may help prevent/retard marginal leakage. Keeping hydroxyapatite around collagen may also better protect the collagen against hydrolysis and, thus, early degradation of the bond[23]. Nanoleakage associated with Clearfil SE Bond may not be necessarily a result of the failed interdiffusion of hydrophilic monomers within the collagen network, but instead, from areas where residual water competed with the adhesive polymerization. [26]

Xeno III is an "Intermediary strong" self-etch adhesive with HEMA, ethanol, water, has a pH of around 1.4. The deepest region of hybrid layer demineralized is around 1-2 micrometer, but due to their more acid nature they have better micromechanical interlocking to enamel and dentin, compared to mild self-etch adhesives. The residual hydroxyapatite at the hybrid layer base may still allow for chemical intermolecular bonding. [27]

Adper Prompt is an "Strong" self-etch adhesive with methacrylated phosphoric acid esters, water, HEMA, polyalkenoic acid with a pH of 0.4. The resulting acid-etch pattern resembles a phosphoric acid treatment following an etch & rinse approach with a greatest depth of demineralization around 3-4 micrometer. At dentin, collagen is exposed and nearly all hydroxyapatite is dissolved. Consequently, the underlying bonding mechanism of "Strong" self-etch adhesives is primarily diffusion based, similar to the etch & rinse approach. Besides the high initial acidity that appears to dramatically weaken the bonding performance, another concern is the effect of residual solvent (water) that remains within the adhesive interface, which can hardly can be completely removed. [26-27]

The results of the present study was in accordance with previous studies conducted in which self-etching primers tested demonstrated less nanoleakage compared to two total-etch adhesives at the cervical margins. [28-29]

This study was done based on the pH, mode of application and technique of the dentin bonding systems. The SEM pictures were taken under secondary electron images. Further studies require TEM (transmission electron microscope) which could give us insight of the hybrid layer.

CONCLUSIONS:

This study focused on the nanoleakage pattern of dentin adhesives based on technique (total-etch approach or self-etch approach). All dentin adhesives showed silver deposition along the resin-dentin interface with ammonical silver nitrate tracer solution.

Clearfil SE Bond (Self-etch) showed the least leakage while Prime & Bond NT (Total-etch) showed the maximum leakage. Self-etch bonding systems which are less technique sensitive are promising in restoring class V cavities with newer nanocomposite resin (Filtek Z350).

The nanoleakage observed need not necessarily be a result of the failed interdiffusion of the hydrophilic monomers within the collagen network, but instead, from areas of residual water competed with the adhesive polymerization reaction. This finding is of clinical significance because degradation of the bonded surface in all the three self etch adhesives may occur over a period of time as they contain water.

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FIGURES

Fig1: Estimation of depth of penetration of silver ions (white arrow under X 1000). Measurement of nanoleakage scores (X40) under SEM.

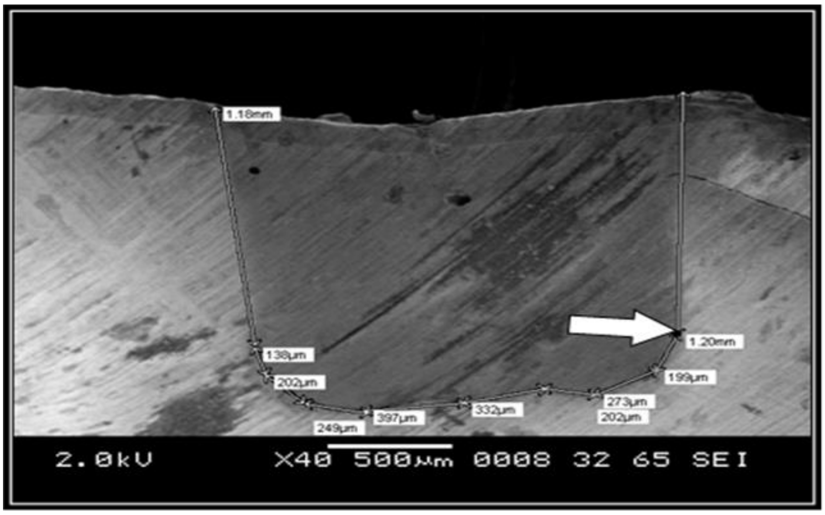
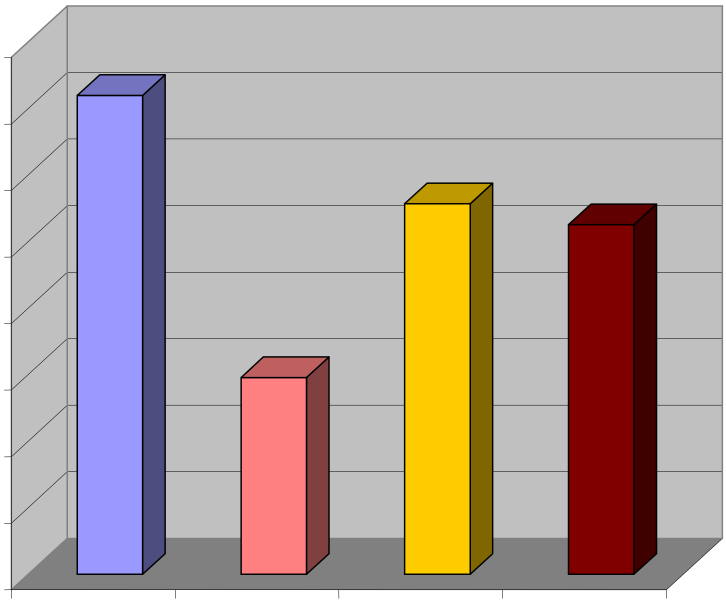


Fig2: Bar chart showing the mean percentage nanoleakage among the four groups



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Group I (Prime & Bond NT), Group II (Clearfil SE), Group III (Xeno III) and Group IV (Adper Prompt)

TABLES:

TABLE 1: pH, composition, application procedure of the dentin adhesives

Bonding Agent	pH	Composition	Mode of application	Manufacturer
Prime & Bond NT (Total –etch Self-Priming)	2.2	PENTA; UDMA resin; Resin R5-62-1;T-resin; D-Resin; nanofiller; initiators; stabilizers; cetylamine hydrofluoride; acetone.	Etch for 15 s. Rinse with water spray for 15 s and remove water with a soft blow of air. Leave a moist surface. Apply ample amounts of the adhesive to saturate the surface. Leave the surface undisturbed for 20 s. Remove the solvent by blowing gently with air for 5 s. Light cure for 10 s.	Dentsply – Germany
Clearfil SE (Two step Self-Etch)	1.9	Primer: MDP, HEMA, dimethacrylates, water, photoinitiator. Bond: MDP, Bis-GMA, HEMA, photoinitiator, colloidal silica.	Apply SE Primer to tooth and leave for 20 s. Dry thoroughly with mild airflow. Apply SE Bond to tooth. Air thin For 3 s. Light cure for 10 s.	Kuraray – Japan
Xeno III (One step Self-Etch)	1.4	Liquid A:HEMA, ethanol, water, highly dispersed silicon dioxide, BHT. Liquid B: phosphoric acid monofluorophosphazene modified poly methacrylate resin , UDMA, BHT, camphorquinone, ethyl-4-dimethyl aminobenzoate.	Equal amounts of liquid A and liquid B was mixed in a clean mixing well for approximately 5 seconds, 2 coats was applied, left undisturbed for atleast 20 s. Air thin For 2 s seconds. Light curing was done for 20 s.	Dentsply– Germany
Adper Prompt (One step Self-Etch)	0.4	Liquid A or compartment 1: methacrylated phosphoric acid esters, photoinitiator, stabilizers. Liquid B or compartment 2: water, HEMA, polyalkenoic acid, stabilizers.	Equal amounts of liquid A and liquid B was mixed in a clean mixing well for approximately 5 seconds, 2 coats was applied, left undisturbed for atleast 20 s. Air thin For 2 s seconds. Light curing was done for 20 s.	3M ESPE – U.S.A

TABLE 2: Intragroup comparison by Kruskal Wallis (H) Test

GROUPS	N (no of samples)	MEAN VALUE (mm)	STD. DEVIATION	H	p
GROUP I	**10**	**35.0990**	**5.923**		
GROUP II	**10**	**32.9792**	**5.741**		
GROUP III	**10**	**34.2836**	**5.854**		
GROUP IV	**10**	**34.1267**	**5.841**	**0.422**	**0.936***

$p<0.05$, * not significant

TABLE 3: Intergroup comparison by Mann Whitney (Z) 'U' Test

GROUP S	Z	p
Group I Vs Group II	- 0. 595	0. 552 *
Group I Vs Group III	- 0. 363	0. 716 *
Group I Vs Group IV	- 0. 040	0. 968 *
Group II Vs Group III	- 0. 357	0. 721 *
Group II Vs Group IV	- 0. 396	0. 692 *
Group III Vs Group IV	- 0. 283	0. 777 *

p<0.05, * not significant