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Research Paper

Comparative Evaluation of Perimarginal Enamel Demineralization among Total Etch, Two Step Self-Etch and One Step Self-Etch Bonding Agents with Nanofilled and Microhybrid Composites - A Scanning Electron Microscopic Study.

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ABSTRACT: Aim: *The aim of this study was to perform an in-vitro evaluation of the demineralization on perimarginal enamel of composite resin restorations and to compare width of perimarginal enamel demineralization zone after artificial cariogenic challenge.*
Materials and Methods: *Class V cavities were prepared in 72 extracted human third molars with margins in enamel on both buccal and lingual surfaces. Cavities were restored with two composite materials and three adhesive systems combinations as follows : (A) Adper Single Bond 2 (etch and rinse)/ Filtek Z 350 XT (nanofilled), (B) Adper Single Bond 2/ Filtek Z 250 (microhybrid) (C) Adper SE plus (two step self etch) /Filtek Z 350 XT (D) Adper SE plus /Filek Z 250 . (E)Adper Easy One (one step self etch) /Filtek Z 350 XT (F) Adper Easy One /Filtek Z 250. After the restorative procedure, the restorations were submitted to demineralization-cariogenic solution (lactic acid, pH 4.5, 0.1M) for four weeks. 2 teeth in each group were immersed in de-ionized water as control group. The margins of restorations and perimarginal enamel were examined by scanning electron microscope (SEM). The width of demineralized zone around restorations were measured at cervical, occlusal and approximal margin.*
Results: *There was statistically significant difference ($p<0.05$) between groups E and F with rest of groups of restorative materials concerning the approximate width of demineralized perimarginal enamel zones.*
Conclusion*: Demineralization of perimarginal enamel zone caused by cariogenic solution could be seen around all composite restorations .*
KEYWORDS: *Perimarginal enamel, Composite resins, Demineralization, Scanning electron microscopy.*

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I. INTRODUCTION

The clinical use of resin composites has expanded considerably over the past few years due to increased esthetic demands by patients, new improved formulations and simplification of bonding procedures.It has been seen that the bonding agents and composites do influence the marginal integrity of tooth restoration interface [1]. Further more invitro studies have demonstrated that enamel adjacent to the margin of the restoration (defined as the perimarginal enamel zone) was more severely affected by artificial cariogenic solution in comparison to enamel surface away from the restoration [2]. Further the cutting action of diamond burs can lead to enamel micro-cracks and polymerization contraction stress created on adjacent enamel surface which increases its acidic cariogenic dissolution [3] and [4]. This leads to formation of enamel zone of multiple deteriorations which may be more susceptible to action of demineralizing solution. Comparison of the width of demineralization zone among recently developed bonding agents and composites is important for the long term success of restoration and it will help in selection of adequate bonding system and appropriate resin composite for long term clinical success of resin composite restorations. The various methodologies for invitro evaluation of perimarginal enamel demineralisation include micro hardness of perimarginal enamel, polarized microscopy and SEM examination of tooth restoration margin. The insitu studies involves microbiological evaluation of plaque adjacent to tooth restoration margin.

Extensive review of literature shows paucity of studies on width of perimarginal enamel demineralization among newly developed nano-filled composites and self etching bonding agents. Thus the purpose of present invitro study is to evaluate the perimarginal enamel of cavities filled with two type of resin composites, Filtek Z 250 (microhybrid) and Filtek Z 350 XT (nanofilled) bonded with three different types of bonding agents Adper Single bond 2, Adper SE plus and Adper Easy one (an etch and rinse, a two step self-etch and one step self-etch) under high cariogenic challenge by subjecting to demineralizing solution of lactic acid at a pH of 4.5 by SEM examination.

II. MATERIALS AND METHODS

Seventy-two human third molar teeth were used. Following extraction the teeth were stored for two days at room temperature in 3% sodium hypochlorite to remove organic debris. Subsequently they were scaled with ultrasonics, washed with distilled water for the removal of any calculus or soft tissue debris and then immersed in 10% formalin solution until use. In all 72 teeth roots were cut at cervical region with diamond discs. Class V cavities were prepared on both buccal and lingual surfaces of teeth with medium grit diamond burs with high speed hand piece under air water spray. All cavities were prepared above the cemento-enamel junction so that all margins are in enamel and all enamel margins were finished with fine diamond points and enamel hatchet. The cavity dimensions were standardized and made 4x2x1.5mm.Vernier caliper was used to measure the cavity dimensions. The cavity margins were not bevelled. All cavities were examined under a stereomicroscope to investigate marginal integrity after bur prepration. According to type of bonding agent and restorative resin teeth were randomly divided into six groups of 12 teeth each, with two teeth as control in each group as given in the table below:

Group	Type of bonding agent and composite Resin used	Total no. of teeth in test sample subgroup	Total no. of teeth in control subgroup
A.	Adper Single bond 2 and Filtek Z 350 XT	10	2
B.	Adper Single bond 2 and Filtek Z 250	10	2
C.	Adper SE plus and Filtek Z 350 XT	10	2
D.	Adper SE plus and Filtek Z 250	10	2
E.	Adper easy One and Filtek Z 350 XT	10	2
F.	Adper easy One and Filtek Z 250	10	2

Cavity surfaces were etched with 35% phosphoric acid gel (Scotchbond Etching Gel, 3M ESPE) for 15 seconds, rinsed for ten seconds, and gently dried with a cotton pellet to remove excess water keeping tooth surface moist. Two consecutive coats of Adper Single Bond 2 were then applied to the etched surfaces, left for 15 seconds and gently thinned with air and light-cured for 10 seconds. Cavities were than filled in two increments with Filtek Z350 XT (12 teeth) and Filtek Z250 (12 teeth). Each increment was followed by 20 seconds of curing time. The two-step self-etch sixth generation adhesive, Adper SE plus was applied by first treating the cavity with Liquid A so that a continuous red-colored layer appeared on the surface. Liquid B was then applied and scrubbed into the surface of the bonding area for 20 seconds. The red color disappeared quickly, indicating that the etching components had been activated. After the treated Cavity surface was air dried thoroughly for 10 seconds to evaporate water, a second coat of Liquid B was applied and lightly air-thinned, then light-cured for 10 seconds. This was followed by restoration with two resin materials Filtek Z3250 XT (12 teeth) and Filtek Z250 (12 teeth). Seventh generation all in one single step, Adper Easy One adhesive was applied on the cavity surface for 20 Seconds, dried for 5 Seconds-and light cured for 10 Seconds. After the application of bonding agent the cavities were similarly restored by two types of resin composites as in previous bonding systems. Each restoration along with perimarginal surface was immediately finished with fine diamond points and graded soflex discs (3M ESPE), stored in water at room temperature for 20 min, and gently washed with deionized water. Out of 12 teeth in all six groups, only10 teeth in each group (i.e 10 with 20 cavities) were immersed in lactic acid solution for demineralization (pH 4.5, 0.1 M) in 40-ml tubes for 28 days. The solution was changed after every 24 hrs. 2 teeth (Control Group) with 4 cavities were kept in deionized water (pH6.7) for 28 days in 20ml tubes.

Preparation of samples for SEM analysis:

i. The samples after 28 days of immersion in lactic acid were placed in deionized water for 24 hrs.
ii. The samples were then treated with ascending concentrations of alcohol for dehydration up to absolute alcohol. The samples were then air dried.
iii. Before gold sputtering, the samples were kept in vacuum evaporator for absolute dehydration.
iv. The samples were gold sputtered and examined in SEM.

Criteria For SEM analysis of Perimarginal enamel zone demineralization

According to SEM analysis, demineralized perimarginal enamel zone presents as several signs of demineralization / deteriorations such as:

i. Porosities caused by loss of tissue between and within enamel prisms
ii. Typical honeycomb configurations and alteration of rods
iii. Enamel micro cracks/ fractures/ interfacial gaps representing signs of perimarginal enamel deterioration

Measuring demineralized zone width

The width of the demineralized zone was measured with scales on the microscope screen. It was performed on four sides of the restoration (mesial, distal, occlusal and gingival) and widest zone width among all was recorded as width of demineralization. The width of perimarginal enamel demineralisation in Group A [Image 1] and Group F [Image2] are135µm and 250µm respectively.

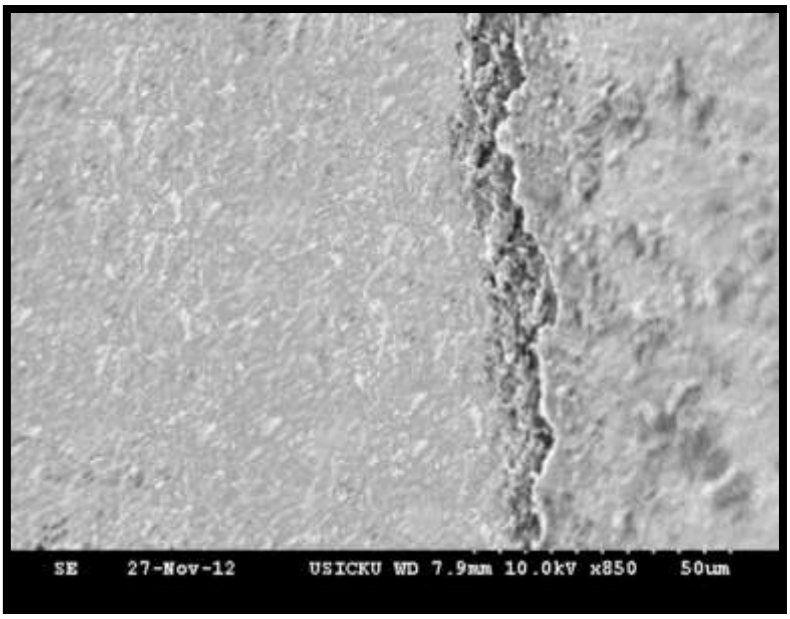

Image 1

SEM photomicrograph of demineralized perimarginal zone in cavities restored with Single bond 2 and Filtek Z 350 XT (Group A) treated with lactic acid for 4 weeks. The width of perimarginal enamel demineralisation is 135µm.

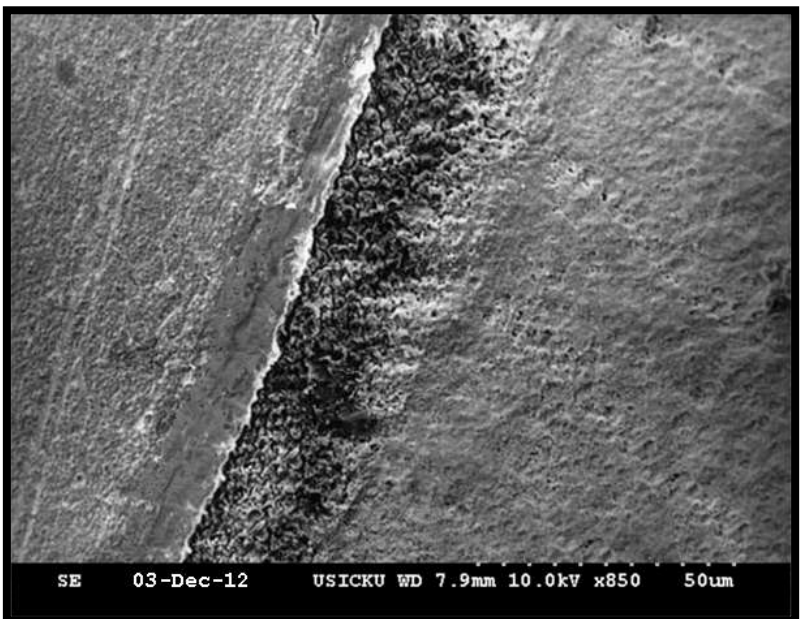

Image 2

SEM photomicrograph of demineralized perimarginal zone in cavities restored with Adper easy One and Filtek Z 250 (Group F) treated with lactic acid for 4 weeks. The width of perimarginal enamel demineralisation is 250µm.

III. STATISTICAL ANALYSIS

The following statistical parameters were presented: Standard deviations (SD), Minimum (Min) and Maximum (Max). Comparison of average width of demineralized areas was performed by one way analysis of variance (One-way ANOVA) followed by Post-hoc Dunnett test. The data analysis was performed using SPS 10.0 statistical program.

IV. RESULTS

SEM examination showed demineralization only in test sample sub groups while no demineralization was seen in control sample subgroups. The average widths for groups A,B,C,D,E and F were 135.00±66.58987µm, 138.7500 ± 33.90680 µm, 152.500±91.73044 µm, 166.2500±52.73756 µm, 197.5000 ± 60.09860 µm and 202.5000 ±104.47236µm respectively [Table 1].

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
A	20	135.00	66.58987	14.88995	103.8350	166.1650	50.00	350.00
B	20	138.750	33.90680	7.58179	122.8811	154.6189	75.00	200.00
C	20	152.500	91.73044	20.51155	109.5688	195.4312	50.00	375.00
D	20	166.250	52.73756	11.79248	141.5681	190.9319	75.00	300.00
E	20	197.500	60.09860	13.43846	169.3730	225.6270	75.00	300.00
F	20	202.500	104.47236	23.36073	153.6054	251.3946	50.00	400.00
Total	120	165.4167	75.53613	6.89547	151.7629	179.0704	50.00	400.00

Table 1

The mean width of demineralization along with Std. Deviation of samples of different groups.

The comparison of mean width of demineralization of group A with groups B, C and D showed statistically no significance. However on comparison with group E and F, statistically significant results were noticed [Table2].

(I) GROUPS	(J) GROUPS	Mean Difference (I-J)	Std. Error	p-value	95% Confidence Interval	
					Lower Bound	Upper Bound
A	B	-3.75000	22.84218	.870	-49.0002	41.5002
	C	-17.5000	22.84218	.445	-62.7502	27.7502
	D	-31.25000	22.84218	.174	-76.5002	14.0002
	E	-62.5000	22.84218	.007	-107.7502	-17.2498
	F	-67.5000	22.84218	.004	-112.7502	-22.2498

Table 2

The intergroup comparison of mean width of demineralization of group A with rest of the groups with p value scores

The comparison of mean width of demineralization of group E with groups A, B, C and D showed statistically significant results. However the comparison with group F was statistically not significant.[Table3]

(I) GROUPS	(J) GROUPS	Mean Difference (I-J)	Std. Error	p-value	95% Confidence Interval	
					Lower Bound	Upper Bound
E	A	62.5000	22.84218	.007	17.2498	107.7502
	B	58.75000	22.84218	.011	13.4998	104.0002
	C	45.000	22.84218	.043	-.2502	90.2502
	D	31.2500	22.84218	.05	-14.0002	76.5002
	F	-5.000	22.84218	.827	-50.2502	40.2502

Table 3
The intergroup comparison of mean width of demineralization of group E with rest of the groups with p value scores

V. DISCUSSION

The use of composites as a direct restorative material has been universalized. The introduction of different varieties of composites for almost all clinical situations has lead to large number of composite restorations being placed by dental professionals.Tooth-restoration interface governs the durability and success of dental restorative procedure. Enamel margin immediately adjacent to restoration is sensitive to influences of various restorative procedures and presents a weak link between tooth and restoration. It is susceptible to development of secondary caries due to possible presence of marginal gaps, porosities, microfractures and imperfect adaptation of restorative materials [5],[6] and [7]. Consequently, the diagnosis of secondary caries is the main reason given by dentists for replacement of all types of restorations in permanent and primary teeth accounting for more than half of all operative work done [8] and [9].

The use of etch and rinse adhesive technique is gold standard in adhesive dentistry [10].For etch and rinse adhesive system, typically at least two steps are required: selective dissolution of hydroxyapatite crystals by etching followed by rinsing and then in situ adhesive resin polymerization. Reports of postoperative sensitivity with etch and rinse [11], its technique sensitivity and multisteps has given rise to other user friendly self etching adhesive techniques with lesser clinical steps which have popularized. The bonding mechanism of Self etching primers are based upon simultaneous etching and priming of the smear layer covered enamel and dentin using an acidic primer, followed by application of the adhesive resin. The two step self etch adhesives are characterized by separate chemical formulations for priming and bonding, utilizing a non rinsing self etching hydrophilic primer that is followed by the application of a comparatively more hydrophobic bonding agent [12]. One step self etch adhesives incorporate all the steps of etching, priming and bonding in single application. Self etch adhesives rely on their ability to infiltrate through smear layer and partially dissolve hydroxyapatite to generate a hybrid layer with minerals incorporated. Because prepration is not rinsed it is user friendly owing to less steps and less technique sensitive as dentine moisture control is not required [12]. Because the water is an essential component of these systems to enable ionization of acidic monomers for demineralization of dental hard tissues, the technique sensitivity associated with variation in state of hydration of demineralized collagen matrix is eliminated. Moreover the excess residual water during polymerization may be one of the reasons for poor bond strength [10], they also behave as semipermeable membranes and allow water movement from dentin to composite adhesive interface [13].This makes self etch bonding inferior to etch and rinse.

A study by Prati C [14], has demonstrated that enamel close to the margin of composite restoration was more severely affected by artificial cariogenic solution than enamel elsewhere on the same restored tooth. This area known as perimarginal enamel presents with altered morphology which is more easily affected by cariogenic / demineralizing environment and thus shows a higher susceptibility towards secondary caries than rest of the tooth in a similar setting.

Perimarginal enamel demineralization has been extensively studied by different methodologies to evaluate effect of various restorative techniques, materials and other procedures on this sensitive area. These methodologies include determination of perimarginal enamel microhardness (Claudia Silami et al [15] (2005), (C F Pinto et al [16] (2010), the examination of perimarginal enamel by SEM (Prati C et al [14] (2003), the examination of perimarginal enamel by microradiography and confocal microscopy after artificial demineriladtion and in situ microbiological composition of dental biofilm in perimarginal enamel area. In this study class V cavities were prepared on both buccal and lingual surfaces of teeth with all margins in enamel, the margins were finished with fine diamond finishing burs to remove marginal enamel overhangs and later verified by stereomicroscope. The cavities were restored with test materials according to manufacturer's recommendations and subjected to high cariogenic challenge of lactic acid at a ph of 4.5 for a period of 28 days. This lead to the formation of demineralization changes on restored teeth. The tooth restoration margin was than studied under scanning electron microscope for induced alterations produced by demineralizing solution. The study demonstrates that in vitro demineralization procedure used in this study was responsible for the early

erosion of enamel margin since no changes were produced in samples stored in deionized water so enamel changes were related to acid exposure. Further the SEM study clearly demonstrated that the enamel changes were most clearly visible close to the margin of restoration and the enamel distant from the margin was either free of changes or relatively with few minor changes.

In the present study, Group A- Adper single bond 2 (etch and rinse adhesive system) with Filtek Z350(nanofilled) composite produced minimum demineralization zone widths among all adhesive systems. This was most probably due to high bond strengths of Adper Single bond 2 an etch and rinse adhesive system to enamel which is approximately 20 to 30 Mpa [12].Such a high bonding strength holds the composite to the walls of cavity and counter acts the forces and stresses of polymerization shrinkage, and also results in good adaptation of material to the cavity with minimum or no gap formation at enamel restoration margin which may be the route of penetration of lactic acid to cause demineralization. These results are in accordance with previous studies by Stefan Dacic [17] and Clauda Silami [16]et al.

. Adper SE plus produced slightly wider demineralization zone with respect to group A and B (etch and rinse adhesive systems, Adper single bond 2) but the result were not statistically significant p value > 0.05. This is largely due to enamel etching pattern of Adper SE plus, a strong two step self etch adhesive, comparable to phosphoric acid in Group A and group B. Since the pH of Adper SE plus is less than 1 it may cause appreciable enamel etching resembling etch and rinse [12] and microporosities resulting in deeper penetration of resin tags and there by higher micromechanical bond strengths. More over in Adper SE plus the components are strategically distributed in bottles. Liquid A contains water, HEMA and a pink dye which is first applied into cavity and water meets the monomer in second step upon placement of Liquid B on to cavity. This prevents hydrolytic degradation of the components and improves shelf life of the material. A continuous brushing procedure is advised to force the contact of all components and help in evaporation of excess water for better polymerization and durable bonding. The colour change indicator confirms the adhesive coverage and its activation and makes the procedure precise. More over simultaneous demineralization and infiltration of bonding agent makes the demineralization and bonding agent infiltration mismatch less possible [10]. Though it forms shallow resin tags than phosphoric acid etched enamel but they are uniform and consistent and studies have consistently demonstrated that length of resin tags as in etch and rinse contribute little to bond strengths [12]. All this improves interface bonding and resistance to erosion by demineralizing solution. This is in accordance to a study by C F Pinto [16] who found no difference in microhardness value of enamel around cavities bonded with Clearfil SE bond (Kurary Medical Inc) a two step self etch adhesive and Adper Single bond (3M ESPE) a etch and rinse adhesive.

In this in-vitro study group E (Adper easy One and Filtek Z 350 XT) and F (Adper easy One and Filtek Z 250),demonstrated appreciably wider zones of demineralization. The results showed significant differences with etch and rinse system (group A and B). These results may be due to high pH of 2.3 of Adper Easy One monomer. The high pH may have resulted in insufficient etching of enamel and therefore lower bonding strengths resulting in more marginal discrepancies and increased deterioration when subjected to cariogenic challenge.

Due to increasing types of bonding agents with ever simplifying user friendly techniques in the market, it is important to access their performance with clinically oriented studies. The results obtained in this study suggest that etch and rinse adhesive system with newly developed nanofilled resin composite can be a better choice over single step adhesive system for long term clinical success of restoration in patients with high caries index and poor oral hygiene. It also suggests that a two step self etch adhesive should be preferred over single step adhesive when a less technique sensitive procedure has to be undertaken. But clinical significance of present study should be cautiously interpreted due to in-vitro conditions of high cariogenic challenge and absence of thermal and mechanical conditioning of samples. Hence, more clinically oriented studies need to be performed for further evaluation of results.

VI. CONCLUSION

Etch and rinse adhesive system (Adper Single bond 2) produced least amount of width of demineralization followed by two step self etch adhesive system (Adper SE plus). One step self etch adhesive (Adper Easy one) produced significantly wider zones of perimarginal enamel demineralization than other two systems. Nanofilled composite performed better than microhybrid with all the adhesive systems.

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