Effect Of Long Term Water Storage on Microleakage of New Self –Etch Adhesives

[a]Maryam Moezizadeh, [b]Zahra Aghamohammadi
[a]Associate Professor Department of Operative Dentistry School of Dentistry Shahid Beheshti University of Medical Sciences, Dental college Tehran - Iran
[b]Department of Dental Material School of Dentistry Shahid Beheshti University of Medical Sciences, Dental college Tehran – Iran

Abstract

Background and Aims : The present study evaluated the microleakage of three self-etch bonding systems (Clearfil SE Bond, Clearfil Protect Bond and Clearfil Tri-S Bond) at occlusal and cervical margins of Class V composite restorations with the effect of water storage at different time intervals.
Methods: Standard Cl. V cavities were prepared on buccal and lingual surfaces of teeth and were randomly assigned to 9 groups of 7. They were treated as follow: Groups 1 , 4 and 7 : A two - step self-etch adhesive ,Clearfil SE Bond (SE), Groups 2 , 5 and 8 : A two - step self-etch adhesive, Clearfil Protect Bond (PB) and Groups 3 , 6 and 9 : An one - step self-etch adhesive Clearfil Tri S Bond(TS) . Clearfil AP-X Composite resin was used to restore the teeth. After thermocycling and storage time of 24 hours, 6 and 12 months, specimens were immersed into 0.5% basic fuschin dye solution for 24 hours, sectioned longitudinally and dye penetration was evaluated on occlusal and cervical margins using Stereomicroscope.
Results:
After 24 hours , for Clearfil SE Bond amount of microleakage at occlusal surface was higher than gingival surface which was statistically significant ($P<0.05$).
For Clearfil Protect Bond bonding agent showed no statistically significant differences between microleakage of occlusal and gingival margins. ($P=0.51$)
For Clearfi Tri-s Bond was not statistically significant differences in amount of microleakage between occlusal and gingival surface ($p=0.16$) .
For 6 months , for SE bonding agent none of the samples showed any leakage neither at occlusal nor at gingival margins. For PB bonding agent, showed no statistically significant differences between occlusal and gingival margins. ($p=0.06$). For TS group, no leakage was seen at occlusal and gingival margins.
After one year water storage for SE bond , there was not statistically significant differences between the microleakage of occlusal and gingival margin . ($P = 0.14$)
Results for PB bond group showed that there was not statistically significant differences between the microleakage of occlusal and gingival margin . ($P = 0.35$)
For TS group, there was statistically significant differences between the microleakage of occlusal and gingival margin . ($P < 0.008$)
Conclusion: Microleakage was greater at occlusal margin than cervical one.
Occlusal leakage for the specimens of TS Bond demonstrated the most values.
KEYWORDS: Dentin Bonding Agents; microleakage; Class V Restoration

Introduction

Advances in denting bonding technology has attributed to rapid progression of adhesive dentistry in the past few years. [1,2] In order to have a successful and long durable

restoration, an efficient bond and adhesion between restoration and tooth structure is essential so that an optimal seal can be achieved.

In the absence of complete polymerization of resin , polymerization shrinkage occurs, result in gap formation, particularly at dentin interface and making way for microleakage which facilitate ingress of bacteria, their toxins, fluids, molecules or ions between the cavity walls and restorations. [2,3]

Adhesive systems have been simplified and improved in order to provide better long term performance. In this regard, self-etch adhesives were introduced as an alternative to etch and rinse adhesives, because of their many advantages like reduced technique sensitivity and application time. [4]

The current classification of self-etch adhesives relies on the number of the steps constituting the system. In one system, etchant and primer both are there in one bottle (self-etch primer) and dentist should apply bonding agent in two steps, and in the other system, etchant, primer and adhesive all present in one bottle (self-etch adhesive) and only one step is required for it's application which is easier and more useful for uncooperative patients like children or very old patients who cannot sit on operating chair for long time. [4-6]

In these adhesive systems a continuous layer is formed between the composite and dentin surface, as simultaneous demineralization of dentin and resin monomer impregnation occurs together. [4-7]

In order to have a successful treatment for adhesive restoration, demineralizing dentin substrate to produce proper resin impregnation is very important, so that sufficient mechanical strength of cured resin adhesive can be achieved. [8,9]

Even though current composite resin/ adhesive systems bond to human dentin well, but due to polymerization shrinkage, new adhesive systems are not capable of totally prohibiting gap formation between the prepared tooth and restorative material. [1-3,10,11]

Oral microorganisms from saliva could enter the gaps between restorations and cavity walls. Invaded bacteria along the tooth - restoration interface may cause secondary caries and damage to the pulp. [10]

When we use self-etch adhesives, in which smear layer is not washed away, residual bacteria can be anticipated. Therefore adhesive systems with antibacterial activity , is very useful in the destruction of bacteria which could have harmful effects and damage the pulp. [12]

This would lead to a better prognosis associated with minimal restoration treatments of dental caries to provide resin-based materials with antibacterial activity. A new monomer, methacryloxylodecyl pridinium bromide (MDPB) has been developed .MDPB is a compound of an antibacterial agent quarternary ammonium with a methacryloyl group and exhibits strong antibacterial activity against oral streptococci. The incorporation of MDPB has been reported to be effective in providing dentin bonding systems with antibacterial activity before and after curing. [10-15]

Long term durability of bonding systems is very important and sine most of bonding agents loose their integrity and degrade over time, the aim of present study was to evaluate microleakage of three self-etch adhesives at occlusal and cervical margins of Cl.V composite restorations after 24 hours , 6 months and one year of water storage.

Materials and Methods

Sixty- three freshly extracted human premolars were debrided and stored in a 1% thymol solution at room temperature. Within about 15 days of extraction, Cl. V cavity preparations were cut in to the buccal and lingual surfaces of each tooth with straight carbide fissure burs at high speed. Preparations were centered on the cementoenamel Junction with dimensions of 2 mm depth, 2mm height and 3 mm width. Occlusal margin of preparations was kept at enamel and cervical margin was at cementum. Teeth were randomly divided into 9 groups of 7 each. In groups 1 , 4 and 7, a two- step self- etch adhesive Clearfil SE Bond (SE) ((Kuraray Medical Inc., Tokyo, Japan)) was used.

At first content of first bottle (primer) was applied on the cavity surface for 20 seconds, air blasted, then bonding agent (second bottle) was applied, air blasted and light cured using QTH light cure (Arialux, Iran) for 10 seconds.

In groups 2 , 5 and 8, two- step self- etch adhesive, Clearfil Protect Bond (PB) (Kuraray Medical Inc., Tokyo, Japan) was used. First bottle (primer) was applied on the surface of cavity for 20 second, air blasted, then second bottle bonding was applied, air blasted and light cured for 10 seconds.

In groups 3 , 6 and 9, one step self- etches adhesive Clearfil Tri s Bond (TS) (Kuraray Medical Inc., Tokyo, Japan) was used. The bonding agent was applied on cavity surfaces for 20 seconds, air blasted and then light cured for 10 seconds.

The name, composition and manufacturer of materials are given in Table 1.

After application of bonding agents, all cavities were restored with composite resin AP-X (Kuraray Medical Inc., Tokyo, Japan) using incremental layer technique, each layer was light cured for 30 seconds at 600 mw/cm^2 using Quartz- tungsten halogen light. The restorations were finished using carbide burs and polished using a polishing disk.

All teeth were stored in distilled water at 37^{o}C, after storage, the restored teeth were thermo cycled for 500 times at temperatures of 5 ± 55^{oC}. The dwell time was 10 seconds in each water bath, with transfer time of 5 seconds between each bath.

The samples were then blotted dry with a paper towel and roots were sealed with flowable composite. A nail varnish was applied to all surfaces of the teeth except for 1mm adjacent to the restoration margins. The teeth were embedded in acrylic resin blocks.

Specimens of groups one to three were kept in distilled water for 24 hours and groups 4, 5 and 6 were kept for 6 months , and groups 7,8, and 9 for one year in an incubator (Pooya Electronic Co, PI – 455 G) at 37 OC.

After storage, all specimens were immersed in 0.5% basic fuschin dye solution for 24 hours. The teeth were rinsed in running water blotted dry, then sectioned longitudinally from the facial to lingual surface with a water- cooled diamond wheel saw (Isomet, Buehler, lake Bluff, IL, USA)

Two longitudinal sections were performed. Dye penetration at the occlusal and gingival margin was examined using a stereomicroscope (Olympus-Japan) at 40 times magnification and scored according to the following criteria:

0= no dye penetration

1= dye penetration up to 1/3 of distance between margin and axial wall

2= Dye penetration up to 2/3 of distance between margin and axial wall

3- Dye penetration to and along the axial wall.

For statistical analysis Mann- Whitney U test and Kruskal- Wallis test was used.

Results :

The results of 24 hours storage showed that :

For Clearfil SE Bond Mann- Whitney U test showed that amount of microleakage at occlusal surface was higher than gingival surface which was statistically significant ($P<0.05$).

For Clearfil Protect Bond bonding agent, Mann-whiteny U test showed no statistically significant differences between microleakage of occlusal and gingival margins. ($P=0.51$) (Figure -1).

For Clearfil Tri-s Bond, there was not statistically significant differences in amount of microleakage between occlusal and gingival surface ($p=0.16$)

Results of 6-month water storage of samples showed that for SE bonding agent none of the samples showed any leakage neither at occlusal nor at gingival margins.

For PB bonding agent, Mann- Whitney U test showed no statistically significant differences between occlusal and gingival margins. ($p=0.06$).

For TS group, after 6months, no leakage was seen at occlusal and gingival margins. Mann-Whitney U test showed that for SE bond at occlusal surface there was a statistically significant differences between 2 times of evaluation (24 hours and 6 months) ($P <0.009$) (Figure – 2)

For gingival margin Kruskal- Wallis test showed no statistically significant differences for TS group, so, storage time had no effect on microleakage of gingival surface. The same was observed for SE bond also. But for PB group, Mann-Whitney U test showed statistically significant differences at gingival margin between 24 hours and 6 months ($P<0.03$) .

After one year water storage , results for SE bond : Mann – Whitney U- test showed that there was not statistically significant differences between the microleakage of occlusal and gingival margin . ($P = 0.14$)

Results for PB bond group showed that, there was no leakage at gingival margin . Mann – Whitney U- test showed that there was not statistically significant differences between the microleakage of occlusal and gingival margin . ($P = 0.35$)

For TS group, Mann – Whitney U- test showed that there was statistically significant differences between the microleakage of occlusal and gingival margin . ($P < 0.008$) (Figure -3)

Kruskal -Wallis test showed that there was statistically significant differences between three groups at occlusal margins after 6 months and one year water storage ($P=0.00$ and $P< 0.005$ respectively).

Mann – Whitney U test showed that there was statistically significant differences between TS and PB groups after 6 months ($P<0.05$) and also between PB and SE groups . ($P < 0.05$)

But there was not statistically significant differences between TS and SE groups. ($P=1$)

After one year water storage there was statistically significant differences between TS and PB groups.($P<0.004$) , but there was not statistically significant differences between TS and SE groups ($p=0.31$)

Discussion

Self-etch adhesives are widely used for many years, mainly due to their ease of use, time saving, low technique sensitivity, and good performance in clinical trials. [16-19]

Nevertheless, characterization of in-vivo-aged adhesive dentin interfaces revealed signs of bond degradation. [20,21] Besides micromechanical interlocking through hybridization, the potential benefit of additional chemical interaction between the functional monomer and residual hydroxyapatite has regained attention. [20,23]

One of the components of mild two self-etch adhesives is specific functional monomers which has been shown to interact chemically with hydroxyapatite that remains available within the submicron hybrid layer. The specific molecular nature of the functional monomer and the subsequent dissolution rate of its calcium salts have been shown to determine actual chemical bonding efficacy and stability. [23,24]

In the present study three self-etch adhesives, Clearfil SE (SE), Clearfil protect (PB) and Clearfil Tri S (TS) were used which all of them were from the same factory (Kurary, Japan). And amount of microleakage at occlusal and cervical margins of Cl. V cavities was evaluated at 24 hours , 6 months and one year water storage.

Clearfil SE bond is a mild self-etch adhesive which is supplied as 2 bottles adhesive.

The primer in this bonding contains l0-methacryloxyclecyl dihydrogen phosphate (10MDP) which improves bonding to tooth structure. 10 MDP not only interact most intensively with hydroxyapatite, but also has the most hydrolytically stable bond with calcium. [20,26]

The results of present study showed that after 24 hours water storage, amount of microleakage at occlusal surface was more than cervical margin for SE bond. But no microleakage was observed after 6 months and one year water storage. Inspite of having more leakage at occlusal surface of samples compare to cervical ones, but there was not statistically significant differences between microleakage of occlusal and cervical margins.

pH of SE bond is 1.9, this could be the reason for higher amount of microleakage at occlusal margin than cervical, since this bonding agent is a week or mild self- etch adhesive and is not able to completely etch enamel and create an excellent bond, but, the functional monomer (10MDP) in this bonding can have chemical bond to dentin and even improve the bond over time. [19,26]

So, this chemical bonding to calcium can compensate the degradation of micromechanical bond that occur over time because of hydrolytic activity, which is one of the advantages of this system.

Many researches have shown very good results and good water stability of Clearfil SE bond and this bonding among the self-etch adhesives is one of the most reliable ones. [3,27]

Since dentin demineralization is less pronounced , very thin hybrid layer (1 micrometer) is formed

and smear plug occludes the orifice of the dentinal tubules, which is partially infiltrated by resin, a reduced resin tag formation occurs with this systems. [31,32] Despite the limited thickness of hybrid layer, SE bond has been reported to result in very high dentin bond strength comparable to or even higher than the ones obtained with total-etch adhesives. [8,33]

In studies conducted by Peumans M et al. in 2007, and Van Meerbeek B. in 2005, they concluded that after 2 and 5 years of application of this bonding agents, patients had no sensitivity and no problems. In addition to show good performance in clinical trial, it has also shown good results in in-vitro studies. [3,19]

In self-etch bonding systems that smear layer is not totally removed, it is possible that microorganisms in smear layer remain, so, use of adhesives which have antibacterial property will be very useful, in addition to destroy the bacteria, they can improve the bonding efficacy and also success of restoration. [34,35]

Protect Bond (PB) is a two- step self-etch adhesive to which fluoride is added. The primer of this bonding agent contains MDPB, it also contains MDP and its pH is 2. Antibacterial property of self-etch adhesives depends on different factors, but, acidity of self-etch primer is an important factor in their antibacterial effect. [35]

Imazato S. and his colleagues reported that unpolymerized MDPB shows strong antibacterial property. Adding acidic monomer to primers can reduce pH of them low enough to reduce the bacterial activity. Results of study conducted by Ozer F et al. concluded that bacteriocidal effect of MDPB monomer in primer is more effective than other self – etch adhesives. [11-15,34,35]

PB primer contains adhesion- promoting monomer (MDP), and antibacterial effect of PB is because of its primer and the adhesive part (second bottle) of it has shown no antibacterial effect on Agar Well Technique. [35]

The results of present study showed that amount of microleakage at occlusal margin was more than cervical, but there wasn't statistically significant difference between them.

The microleakage at 24 hours was more than 6 months and it has reduced over time. The reason could be that monomer in PB are changed because of their antibacterial effect which these changes and other changes in formulation can have unfavorable effect on short term bonding capacity.

MDPB has no acidic group which probably affect the short term interaction of bonding with tooth surface but with passing the time and completion of chemical reaction between MDP and enamel, this effect will be compensated, in the present study improving the seal and decreasing the microleakage of this bonding could be due to continuous chemical reaction in composite after 2h hours. [36]

In addition to that, since this bonding agent is hydrophilic in nature , absorbs water and swells and as a result swelling could improve sealing and reduce microleakage. [25,36]

Some of the studies have shown that mineral loss from enamel and dentin during water storage could result in decrease in mechanical properties of tooth. This can also cause decrease in strength of material, so, releasing fluoride by adhesives can reduce loss of minerals from enamel and dentin. [37]

In order to reduce the number of steps used in bonding process and saving time, many new self-etch adhesives have been marketed. In this system, etchant, primer and bonding agent all are present in one bottle (self-etch adhesive), and only one solution is applied on the tooth surface and light cured, and immediately after that composite resin is applied.

Since this type of bonding agent uses only one step, technique sensitivity associated is reduced , as a result better and more efficient bond can be achieved , of course, not to mention that it is very time consuming and is very useful for uncooperative patients like children and old patients who can not sit on dental chair for long time. [6] Clearfil Tri S (TS) is an one- step self-etch adhesive that showed greater leakage at occlusal margin than cervical after 24 hours water storage. After 6 months, the leakage was lesser at occlusal margin but, at cervical margin time had no effect on amount of microleakage.

The reason for higher microleakage at occlusal surface could be due to single-step application of this bonding which means water and other solvents like alcohol and

acetone all present in single bonding solution, which is expected to be removed by air spray before curing completed, but, for whatever reason, if solvent remains, and does not evaporate completely, could have negative effect on polymerization reaction and as a result hydrolysis of components in bonding agent on long term could decrease the bonding efficacy. [38]

Another reason for greater microleakage at 24 hours could be due to higher concentration of this bonding and also complicated chemical bonding. Two phenomenon have been described to play role in microleakage ; 1- Presence of water in resin adhesive 2- Diffusion of water from dentinal tubules to adhesive. So there is a direct relation between microleakage and amount of water at resin-dentin interface after curing. It has been reported that single-step self-etch adhesives have more ionic and hydrophilic resin monomers and it is possible that water remains at tooth-resin interface after curing and can reduce seal of restoration. [39]

Results of present study have showed good success rate of two step self- etch adhesive after one year. But with one step self-etch adhesive, it was not very successful.

Any way it is not yet clear completely that which factor has main effect on dentin bonding agents.

So, Clearfil Tri S bond, inspite of being easier and faster to work, has not shown good long term performance which needs more clinical and laboratory studies.

Conclusion

None of the latest generations of adhesive resin bonding systems can create gap-free margins in Cl.V composite restorations. The amount of microleakage at occlusal margin was greater than cervical margin. All three bonding agents had more microleakage at 24 hours than 6 months, which for SE and TS at occlusal margin and for PB at cervical margin was statistically significant and there was statistically significant differences between microleakage of TS and PB after one year water storage.

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Table 1: Name, composition and manufacturer of materials used

Materials	Composition	Manufacturer
Clearfil SE Bond	Primer: 10-Methacryloyloxydecyl dihydrogen phosphate (MDP) 2-Hydroxyethyl methacrylate (HEMA) Hydrophilic dimethacrylate dl-Camphorquinone N,N-Diethanol-p-toluidine Water Bond: 10-Methacryloyloxydecyl dihydrogen phosphate (MDP) Bis-phenol A diglycidylmethacrylate (Bis-GMA) 2-Hydroxyethyl methacrylate (HEMA) Hydrophobic dimethacrylate dl-Camphorquinone N,N-Diethanol-p-toluidine Silanated colloidal silica	Kuraray, Medical Inc., Tokyo, Japan
Clearfil Protect Bond	Primer: 10-Methacryloyloxydecyl dihydrogen phosphate (MDP) 10-Methacryloyloxydodecylpyridinium bromide (MDPB) 2-Hydroxyethyl methacrylate (HEMA) Hydrophilic dimethacrylate Water Bond (Fluoride-bonding agent): 10-Methacryloyloxydecyl dihydrogen phosphate (MDP) Bis-phenol A diglycidylmethacrylate (Bis-GMA) 2-Hydroxyethyl methacrylate (HEMA) Hydrophobic dimethacrylate N,N-Diethanol-p-toluidine Silanated colloidal silica Surface treated sodium fluoride	Kuraray, Medical Inc., Tokyo, Japan
Clearfil S^3 Bond	Bond: 10-Methacryloyloxydecyl dihydrogen phosphate (MDP) Bis-phenol A diglycidylmethacrylate (Bis-GMA) 2-Hydroxyethyl methacrylate (HEMA) Hydrophobic dimethacrylate dl-Camphorquinone Ethyl alcohol Water Silanated colloidal silica	Kuraray, Medical Inc., Tokyo, Japan

Figure 1-Comparison of microleakage of occlusal and gingival surfaces after 24 hours

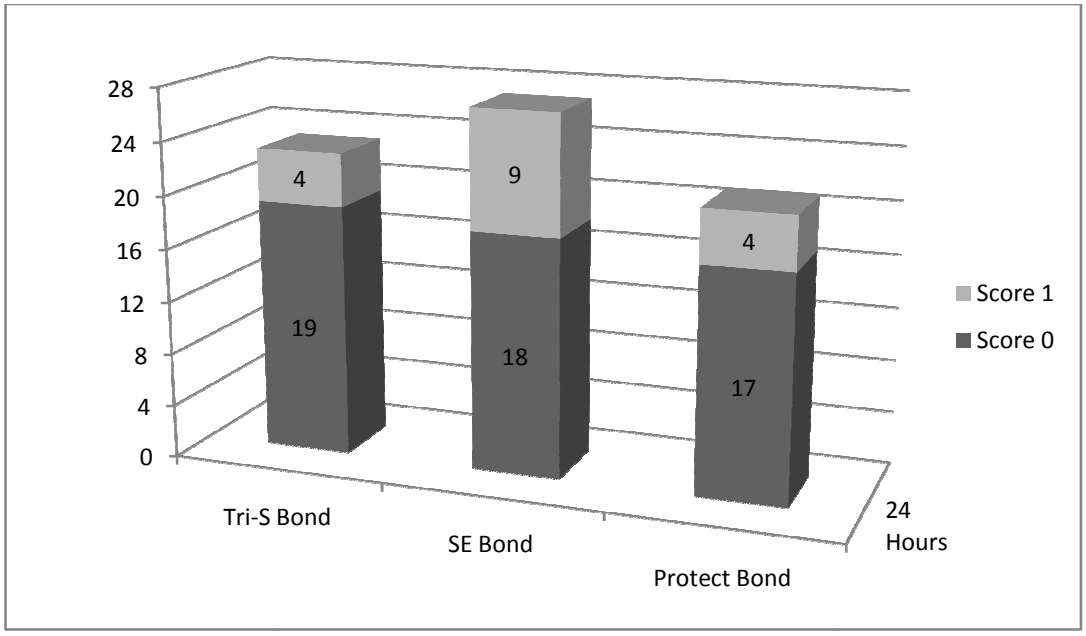

Figure 2 -Comparison of microleakage of occlusal and gingival surfaces after 6 months

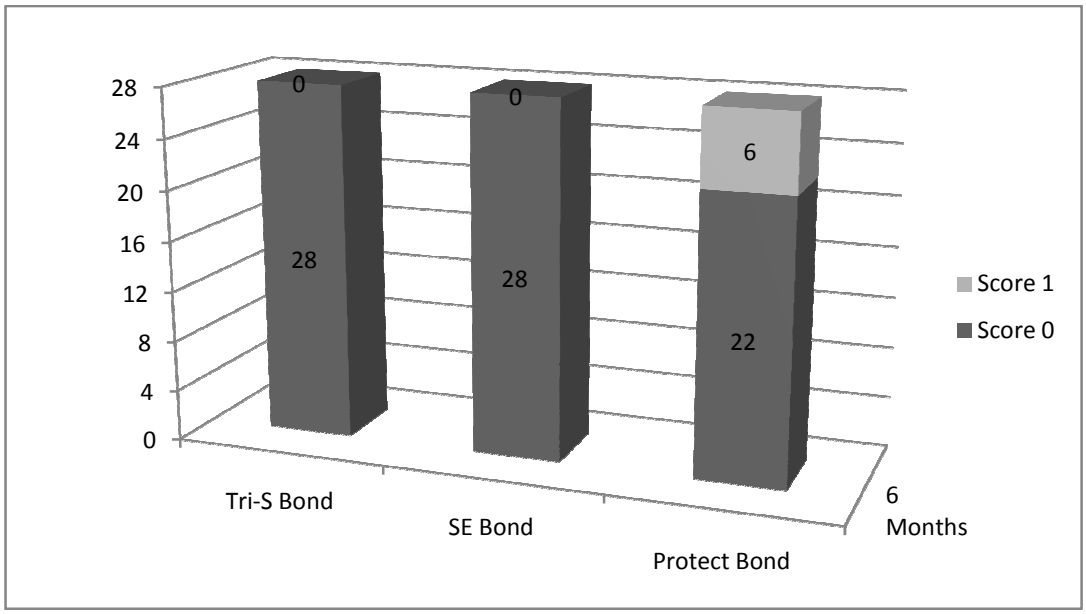

Figure 3-Comparison of microleakage of occlusal and gingival surfaces after 1 year