

Influence of Sodium Hypochlorite on Microleakage of Three-step Total-etch Bonding Agent with Different Solvents

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Abstract

Background and Aim: some recent research have reported increased stability in mechanisms of adhesion to dentin in remove of collagen fiber of the dentin surface. The aim of this study was to evaluate the effect of sodium hypochlorite in microleakage of three step total etch dental adhesives with different solvents.

Materials and Methods: this experimental study on 80 intact bovine incisor teeth, were prepared class V cavities on buccal surface of teeth, box shap and all walls on enamel.

Then the specimens were randomly divided into eight groups (n=10).

NaOCl+etching+ SBMP (waterbase), NaOCl+etching+ All Bond₂ (Acetonebase), etching+ NaOCl+ SBMP, etching+ NaOCl+ All Bond₂, NaOCl+ SBMP, NaOCl+ All Bond₂, etching+ SBMP, etching+ All Bond₂.

Then the cavities were restored using Z₂₅₀ (3M) composite and Colt lux 75 light curing unit (350 mw/cm²). The specimens were thermo cycled for 1000 times on water baths of 5 and 55°C. After thermocycling, the specimens were immersed in a 2% aqueous solutions of basic fuchsin for 24 hours, then longitudinal section of each restoration was obtained and examined with a stereomicroscope for evaluation of micro leakage. The data were analyzed using kruskal-walis test and the mean of micro leakage of samples were compared by two- way ANOVA test.

Results: The findings showed that the higher rate of micro leakage was detected in hypo+Acid and lower rate of micro leakage was detected in Acid+hypo in different treatment method. The finding was different in hypo+Acid procedure, the higher rate of micro leakage was in SBMP. In hypo groups, the microleakage with ALL Bond 2 was higher than SBMP. In control groups, the microleakage with SBMP was higher than ALL Bond₂.

Conclusion: Application of Naocl before dentin etching for removal organic materials increase the rate of microleakage of All Bond₂, SBMP to that of control group.

Key Words: Bonding, Microleakage, Collagen fiber

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Introduction

Effective adhesion to hard dental tissue is necessary for successful clinical results of tooth colored

restorations. Good marginal adaptation prevents from recurrent caries and pulpal irritation. Dentin is less desirable for adhesion because of high or-

ganic content, variability in amount of mineral content and tubular fluid. One of the essential factors in adhesion to dentin is the presence of hybrid layer which is formed by resin impregnation into acidic demineralized dentin [1].

Absence of resin in collagen network may result in hydrolytic degradation and questionable bond durability [2]. Sodium hypochlorite is a non-specific proteolytic agent that can remove organic materials.

Sodium hypochlorite is more commonly used in root canal irrigation, blood coagulation, debridement, and disinfection of dentinal surfaces before direct and indirect restorations. Additionally, in resin restorations, sodium hypochlorite is used before etching [3]. The role of sodium hypochlorite in dentinal permeability and adhesion have been evaluated in some studies. Application of sodium hypochlorite may decrease or increase bond strength depending on the method of application or special components of dental adhesives used [2-4]. Toledano et al showed that removal of collagen by sodium hypochlorite (NaOCl) produced surface roughness and wettability [5]. Vargas et al showed that after protein removal, dentin changes into a porous structure with numerous irregularities that provides good mechanical retention and may be act as a stable interface similar to enamel. Resin polymerization into such a porous surface provides a stable substrate for composite restorations that results in adequate dentinal seal [6].

In contrast to above studies, Frankenber and Perdiago described that application of NaOCl reduces bond strength and marginal adaptation [1-2].

The aim of the present study was to evaluate the effect of sodium hypochlorite application on microleakage of a three-step total-etch dentin bonding agent with different solvents.

Materials and Methods

In this experimental study, 80 extracted bovine mandibular incisors were selected. After soft tissue debridement and disinfection, the teeth were stored and maintain in deionized water. Duration of storage was less than one month. Teeth were random-

ly divided to 8 groups each including 10 teeth. Class V cavities with the dimensions of 5*3 mm and depths of 2mm with enamel gingival walls were prepared by 0.8 high-speed fissure bur using air and water coolant.

The high-speed handpiece was changed after five cavity preparations.

Group1: In this group application of Naocl with normal etching and use of water-based scotch bond multipurpose (SBMP®) Bonding was used.

In this group cotton pellets containing 5.25% NaOCl was applied in each cavity for 150s or 2.5 min. After cavity irrigation by water spray for 5 seconds enamel and dentin were etched for 15s and 10s, respectively using %37 phosphoric acid gel and subsequently irrigated for 15s. Each cavity was dried for 2 seconds. Primer was applied in two layers. The cavity was gently dried for 5s. Adhesive was applied on primed surface in order to produce a glassy appearance. Adhesive of SBMP was applied by brush and then was thinned by spray of air. Bonding was cured by Coltolux 75 (350mv/cm²) for 10 s (according to instructions of the manufacture). Composite (Z250, 3M, ESPE, Germany) was applied in the cavity in two layers and each layer was cured for 20 seconds. The second layer was placed in the cavity after placing a mylar strip. The surface was cured for 20 seconds.

Group 2: In this group application of NaOCl, normal etching, and use of acetone-based All Bond 2 (Bisco) was done.

Similar to group 1 etching and irrigation was done. Then primer A & B was mixed and applied in 5 layers, according to the manufacturer's recommendation. The surface was air dried gently for 5 seconds. Adhesive was applied on primed surface and cured for 20 seconds according to manufacturer's recommendations. In the next step, the cavity was filled similar to previous groups.

Group 3: Similar to previous groups normal etching, use of NaOCl and application of SBMP were done and the cavity was filled with Z250 composite.

Group 4: In this group normal etching, use of NaOCl, and application of All Bonds was carried

out. In this group All Bonds was applied according to the manufacturer's recommendations. The cavity was filled with Z250 composite.

Group 5: NaOCl and SBMP were used. A cotton pellet containing sodium hypochlorite was applied on the cavity similar to other groups. After irrigation of the cavity, next steps of bonding without etching were done according to the manufacturer's recommendations. Then cavity was filled with Z250 composite.

Group 6: In this group application of NaOCl and All Bond 2 was performed. In this group All bond 2 was used without etching according to manufacturer's recommendation and cavity was filled with Z250 composite.

Group 7: In this group normal etching and use of SBMP. Etching, bonding and composite filling were carried out similar to group 1.

Group 8: Normal etching and All bond 2 was used in this group. Etching, bonding and application of composite was done similar to group 2.

In all restorations, the surface finishing was done one minute after curing with the use of finishing burs and discs.

The samples were maintained at 37 °c for one week and then thermocycled between 5 to 55 c with dowel for 1000 cycles in 30 seconds. Then the apexes of teeth were covered by red wax and all surfaces were covered by two layers of nail varnish except for a 1-mm margin surrounding the restoration in order to prevent dye penetration into restoration margins. Teeth were placed into 2% basic fuchsin solution for 24 hours in 37°C.

The teeth were completely rinsed with water and dried after incubation. Then the teeth were embedded into self cured transparent acrylic resin blocks and sectioned longitudinally in buccolingual direction with a diamond saw. The samples were observed under a stereomicroscope (Nikon Corporation, Tokyo, Japan) using a 40X magnification. Depth of penetration was measured for each sample using scaled lens. Data were analyzed using Kruskal-Wallis test in order to make comparisons between the groups as well as student t-test using

SPSS version 11.5 as the statistical tool. P values of less than 0.05 was considered significant.

Results

The results of this study showed that 5.25% sodium hypochlorite application and removing the collagen from etched or non-etched dentine resulted in statistically significant differences in amount of microleakage in different methods of preparation.

The highest rate of microleakage was observed when hypo+acid method and the lowest rate belonged to acid +hypo method.

According to result of student-t test in two independent community, in SBMP groups the mean of microleakage in hypo+acid group was significantly more than control group and in All bond2 groups the mean of microleakage in hypo group (Naocl+ All bond 2) was significantly more than control group.

The results of student-t test for independent groups showed that there was statistical significant differences in microleakage between (hypo+acid) group and control group. The mean of microleakage in SBMP group 1 (hypo+acid) was more than group 4 (control).

Pairwise comparison of microleakage showed that sodium hypochlorite application before etching resulted in a significant difference between SBMP and All bond 2. With application of sodium hypochlorite before etching the mean microleakage value was higher in comparison with application of sodium hypochlorite after etching. See table 1.

The mean of microleakage between control group and the group in which application of sodium hypochlorite without etching was carried out, there was a significant difference in all bond 2 bonding. The mean of microleakage was higher in hypo (NaOCl+All bond 2) than control group. ($p=0.001$) There were no statistically significant differences in mean of microleakage between the bonding systems. ($p>0.05$)

Kruskal walli assay was used in order to define microleakage differences based on the type of dentin treatment.

According to results of the present study there was a statistical significant difference in the mean of microleakage depend on the method of dentin treatment. So that the highest rate of microleakage was observed in the method in which sodium hypochlorite was used before etching.

There was a statistical significant difference in the mean of microleakage between group 4 (control) and group 1 (hypo+acid) of All Bond 2.

So that the microleakage in group 1 (hypo+acid) was more than group 4 (control). (Table 2)

In SBMP bonding the mean of microleakage in group 4 (control) was different from group 1 (hypo+acid) significantly, so that microleakage in group 1 (hypo+acid) was more than group 4 (con

trol). The mean difference between group 4 (control) and group 2 (hypo+acid) in SBMP bonding was not significant. (Table 3)

In all bond 2 rate of microleakage in group 4 (control) was different from group 1 (hypo+acid) significantly. So that microleakage in group 1 (hypo+acid) was more than group 4 (control).

The difference between group 4 (control) and group 2 (hypo+acid) in All Bond 2 was not significant. In SBMP rate of microleakage in group 4 (control) was not significantly different from group 3 (hypo+acid).

But in All bond 2 microleakage in group 3 (Hypo) was significantly more than group 4 (control). (Table 4)

Table 1. Comparison of micro leakage in different preparation methods

P.V	Standard deviation± difference means	Comparison two by two
0/0001	23/412±4/154	(hypo+acid)-(Acid+hypo)(under supposition of inequality variance)
0/0001	22/40±3/98	(hypo+acid)-(only hypo)(under supposition of equality variance)
0/0001	22/316±4/221	(hypo+acid)-(control)(under supposition of inequality variance)
0/735	-1/3±3/855	(Acid+hypo)-(only hypo)(under supposition of equality variance)
1/000	-1/396±4/009	(Acid+hypo)- (control)(under supposition of inequality variance)
0/982	-0/0887±3/82	(only hypo)-(control)(under supposition of inequality variance)

Table 2. Microleakage of All Bond 2 in group 4 (control) compared with All Bond 2 in group 1

95 percent confidence interval for difference means		P.V	Statistic T	Leven Assay	
Upper limit	Lower limit			P.V	Statistic T Upper limit
-11/851	۲۱۵/۴۸-	0/002	-3/344	0/649	0/210
9/290	-26/694	0/333	-0/980	0/676	0/178
20	-9/82	0/494	0/690	0/568	0333
45/69	13/81	0/001	3/783	0/02	5/897

Table 3. Mean Microleakage in SBMP bonding group 4(control) with SBMP bonding in group 1(hypo +acid)

95 percent confidence in- terval for difference means		P.V	Statistic T	Leven Assay		Standard deviation± difference means	Group 2
Upper limit	Lower limit			P.V	Statistic T Upper limit		
-9/687	-42/812	0/003	-3/211	0/643	0/219	-26/250±8/174	(A group 1) -(A group 4)
25/561	-4/894	0/178	1/374	0/731	0/120	10/333±0/7522	(A group 2) -(A group 4)

Table 3. Microleakage of All Bond 2 in group 4(control) compared with All Bond 2 in group 1(hypo+acid)

95 percent confidence interval for difference means		P.V	Statistic T	Leven Assay		Standard deviation± difference means	Group 2
Upper limit	Lower limit			P.V	Statistic T Upper limit		
-11/851	-48/215	0/002	-3/344	0/649	0/210	-30/033±8/981	(B group 1) -(B group 4)
9/290	-26/694	0/333	-0/980	0/676	0/178	-8/701±0/879	(B group 2) -(B group 4)
20	-9/82	0/494	0/690	0/568	0/333	5/08±7/36	(A group 3) -(A group 4)
45/69	13/81	0/001	3/783	0/02	5/897	92/75±7/86	(B group 3) -(B group 4)

Discussion

The aim of the present study was to evaluate the effect of sodium hypochlorite on rate of microleakage of three-step total-etch dentin bonding agents with different solvents. Bonding systems to enamel and dentin were introduced in order to inhibit penetration of bacteria or their products to tooth structure with two major objects. 1) increasing the retention of restorative materials, and 2) reducing marginal gap between tooth and restoration. Because of similarities in composition, and size as well as ease of accessibility and infection control, bovine teeth were used in this experimental study. In the present study in three-step total-etch systems, each step of etching, priming and bonding was done separately. Therefore, better wettability and penetration into demineralized dentin was possible. On the other hand, in two-step total etch systems, priming and bonding were performed together, therefore, wettability and penetration of resins into hybrid layer occurred more rapidly than three-step total-etch systems.

For this reason the rate of microleakage in two-step total-etch bonding was more than that of three-step total-etch bonding system.

The results of present study coordinates with those of Raphael pilo etal [8] in 1999.

According to the results comparing different adhesives, the least marginal seal was observed in All Bond 2, followed by SBMP. Therefore, in SBMP,

better seal and lower microleakage was observed [8]. Many studies have been done in order to reduce formation of porosities and defects through maintaining the spongy structure. In Gwinnet's study the role of quality of collagen in bond strength is debated. The authors concluded that residual substrate could not obtain a good resin bonding after deproteinization [9]. In the present study the highest amount of microleakage was related to hypo+acid method and the lowest was related to acid+hypo method. Deproteinization removes the thin and sensitive collagen layer and the etched dentinal surface remains which is rich in hydroxyapatite crystals- a surface resembling enamel. Therefore, a more stable interface is created. [12-10,6] Also, in some SEM studies the effect of deproteinization on reducing or elimination of microleakage in restoration interface is reported.

[13,14] Thus the quantitative role of collagen fibers in ideal adhesion is questionable. Gwinnett study in 1994 in agreement with the present study describes that the quality of hybrid layer is an important factor in bond strength and reduction of microleakage. Hence, the quantitative role of collagen in bond strength is questionable [15]. Even some researchers have described the possibility of interference of collagen fibrils with adhesion and reduction in bonding [10-17]. According to the present study regarding the effect of sodium hypochlorite in bonding and different methods of preparation it

was observed that the amount of microleakage in water-based SBMP bonding and acetone-based All Bond 2 was more than control group when sodium hypochlorite was applied before etching. Because sodium hypochlorite removes collagen layer and application of the acid on dentin after sodium hypochlorite results in complete or partial removal of smear layer and demineralization of subjacent dentin. It is believed that both mineral and organic materials are removed with sodium hypochlorite application before etching. In this situation no hybrid layer is left after application of hypo+acid and an increase in microleakage ensues. This is in accordance with Wilder et al and Jacobsen et al [18, 22]. Wilder et al stated that in addition to micromechanical retention, chemical bond with mineral components of dentin may be organized. For example, carboxylic acids can be attached to hydroxyapatite via ionic attachments and result in formation of calcium salts.

It is assumed that HEMA interacts with dentin chemically and physically and possibility copolymerizes and a mechanical interlock occurs. On the other hand, contrary to the present study, the possibility for chemical bonding is also stated in some bonding systems [18].

Ferrari et al in a study about SBMP reported that deproteinization could increase bond strength, but a decrease in marginal seal in enamel and dentin occurs. According to Ferrari et al increasing surface roughness caused improved bond strength but if the bonding agent did not penetrate completely into the surface, it resulted in reduction of the seal. This is in accordance with the current study. In SEM micrographs they showed that without application of sodium hypochlorite, resin tags completely penetrated in demineralized dentin and exposed collagen network producing a good tubular seal. On the other hand, after sodium hypochlorite application no adequate seal was observed [19]. Definitely this is a matter of debate for many researchers. Since Vargas et al in 1997 reported better penetration of SBMP and All bond 2 into porosities of deproteinization surfaces and formation of collateral resin tags as well as formation of more anas-

tomoses compared with etched surfaces, using TEM and SEM micrographs. However, in Vargas's study, similar to the present investigation there was no significant increase in marginal seal in SBMP, but there was a significant effect in All Bond 2. [6] In the experimental group in which only sodium hypochlorite was used, the highest microleakage value was related to All Bond 2.

In All Bond 2 microleakage in hypochlorite group (the group in which only hypochlorite was used) was more than control group. In spite of no acid application in this group, sodium hypochlorite produced a porous surface almost similar to that of etched surfaces with phosphoric acid. Wakabayashi et al, and Perdiga et al reported that wide dentinal tubules are formed with alkaline properties after preparation of the dentin surface with NaOCl for two minutes. In this way, delicate irregularities are formed on intertubular dentin resulting in obvious differences with results of phosphoric acid treatment [20,11].

In all bond 2 there was more microleakage in hypochlorite group because of lack of hybrid layer formation.

Collagen layer is not possibly removed completely with the only use of hypochlorite. This layer is possibly changed into a denaturated state which produces a gel like layer at the surface which inhibits resin penetration [19]. Definitely, the manner of sodium hypochlorite application is important.

Application of improper concentrations of sodium hypochlorite or inadequate agitation can result in formation of a gel-like surface that impedes adhesion [4]. Some researchers have reported controversial results of surface deproteinization depending on the type of bonding system [4,21,22]

Solvents such as acetone transport primer monomers to microprosities of the exposed collagen network resulting in better penetration of resin in hybrid layer and reducing microleakage. In the current study contrary to the work by Nakabayashi et al neither of the solvents did change the microleakage significantly [23]

Conclusion

Regarding the limitations and results of the present study the method of removing dentin surface collagen with 5.25% sodium hypochlorite solution can differently affect microleakage depending on the type of bonding system and method of preparing dentin surface according to the following manner:

1-Application of sodium hypochlorite before etching in SBMP bonding systems increase microleakage but such effect was not statistically significant in All Bond 2 bonding system.

2-Application of sodium hypochlorite after etching in different bonding systems (SBMP and All Bond2), did not have any signification effect on microleakage.

3-Application of sodium hypochlorite alone and without etching in All Bond 2 bonding system resulted in an increase in bond strength. Therefore it can be considered as an alternative for phosphoric acid- a point that should be more elaborated through various studies.

4-Comparing different methods, sodium hypochlorite application after etching showed the lowest microleakage value in comparison with hypochlorite application before etching, hypochlorite application alone and control.

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