Comparison Of Chlorhexidine 2% And Sodium Hypochlorite 5% As Rewetting Agents On Resin- Dentin Micro Tensile Bond Strength

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Received 29 July 2016 and Accepted 13 September 2016

Abstract

Resin- dentin interface hydrolysis is one of the greatest problems in restorative dentistry, Because of collagenolytic and proteolytic activity of dentin enzymes. The aim of this study was to compare the effect of CHX 2% and NaOCl 5% as rewetting agent on resin-dentin micro tensile bond strength. **Methods:** 45 extracted, human caries-free third molar teeth were collected. After exposing of superficial dentin, etching and rinsing of dentin were done. Specimens were divided into 3 equal groups. In group 1, after drying of dentin, CHX 2% was used as rewetting agent for 60 seconds. In group2, NaOCl 5% used as rewetting agent for 120 sec and in group 3 (control), blot dry technique with water was used for substrate. Adhesive system (SingleBond , 3M ESPE , USA) and composite build up (Filtek Z250 ,3M ESPE , USA)) were applied for all groups. After 6 month storage, μTBS measured for hourglass-shaped specimens. Specimens loaded for micro tensile bond strength until fracture occurred. **Results:** Micro tensile bond strength of group 1 significantly was higher than other groups (52.67±6.862). There was no significant difference between group 2(18.59 ± 6.081) and group 3(28.84± 6.231). (P= 0.094). **Conclusion:** CHX acts as matrix metalloproteinases inhibitor and by preventing dentin collagen degradation can preserve the bond strength after 6 months.

Keywords: dentin, micro tensile bond strength, CHX, matrix metalloproteinases inhibitor**.**

Karrabi M, Davari A, Danesh Kazemi A.. Comparison Of Chlorhexidine 2% And Sodium Hypochlorite 5% As Rewetting Agents On Resin- Dentin Micro Tensile Bond Strength. J Dent Mater Tech 2016; 5(4): 189-195.

Introduction

Nowadays patient's desire for tooth-colored restorations has increased. Despite the fact that composite properties have improved, proper resin-dentin bond is considered as a challenge. Dentin has a heterogeneous structure composed of intertubular and peritubular dentin, with different mineral content (1). Also, dentin contains endogenous matrix metalloproteinases (MMPs) (2).Host- derived matrix metalloproteinases and cysteine catepsines , activated after acid application has been claimed to degrade the collagen fibrils(3). This will lead to Resin- dentin bond failure.

Resin- dentin bond degradation may occur by (i) breakdown of the polymer phase or collagen fibrils in hybrid layer, or (ii) activation of matrix metalloproteinase (MMPs) (4, 5). During exposure of dentin to acid from caries progression or acid etching dissolution of dentinal mineral phase occurred, leads to degradation of organic matrix and collagen fibrils by host-derived collagen-degrading proteases activation and bacterial enzymes (6-8). Also, the activity of host-derived MMPs can be responsible for elution of non-polymerized monomers and the degradation of unprotected collagen within incompletely resin-infiltrated acid-etched dentin (9). Unbounded, partially demineralized human dentin substrates, exhibit collagenolytic activity. Combination of resin and/or collagen hydrolysis can degrade resin-dentin interface and weakens the physical properties of the resin–dentin bond interface that leading to reduction of bond strength (10, 11).

On the other hand, acid etching makes an amorphous gel consisted of denaturized and fragile collagen over demineralized dentin. This gel layer prevents of resin infiltration into demineralized dentin so denuded collagen fibril of hydroxyapatite (HA) and/or resin are susceptible to hydrolyses(12).

To avoid these biodegradations different strategies have been proposed, such as the demineralized collagen removal and the use of MMPs inhibitors (5, 6).

Some matrix metalloproteinase inhibitor such as Chlorhexidine can preserve bond strength by prevention or minimization of auto degradation of exposed collagen fibrils within incompletely-formed hybrid layers (10, 11).

Sodium hypochlorite (NaOCl) can act as a demineralized collagen remover .This solution is a proteolytic agent capable of removing organic material and can make a NaOCl-treated dentin that is rich in exposed hydroxyapatite crystals. This could result in a more durable bond over time because it is essentially made of mineral (5, 13).

The aim of this study is to determine the effects of different rewetting agents (CHX and NaOCl) on acid etched dentin, on micro tensile bond strength of dentin-resin interface after 6 months storage.

Our null hypothesis is that different rewetting agent applying on etched dentin, has no effect on resin-dentin micro-tensile bond strength after 6 months storage.

Methods and Material

Preparation of specimens

Forty five extracted, caries-free human third molar teeth were collected in one month. After disinfection in 0.5% chloramine solution, the teeth were stored in distilled water.

A flat surface of superficial dentin (immediately under DEJ) was exposed on the mid of occlusal surface of each tooth with water cooled diamond disk in a cutting machine (Labcut 1010; Extec Co., Enfield, CT, USA).

A diamond bur (#3195; KG Sorensen, Barueri, SP, Brazil) in a high-speed hand piece with water spray was used for surrounding enamel removal. For smear layer standardization, dentin surfaces were further polished on wet #600-grit SiC paper for 60 second.

Teeth were randomly divided into 3 equal groups (n=15). The exposed dentin surfaces were conditioned with phosphoric acid (fine etch SPIDENT, Korea) for 15 s, rinsed off (15 sec), air-dried and rewetted in first group with 2% CHX (Consepsis ultra Inc, USA) for 60 s (10), and in second group with 5% NaOCl (NaOCL,

Fischer Scientific, Hampton, NH) for 120 s (5), and in third group with water for 10 sec.

Then Adper Single Bond (3M ESPE ,USA) adhesive system applied in according to the manufacturer directions and light-cured with a LED curing unit Degolux II (Degussa AG, Gschattsbereich Dental, D-63457 hanau Wolfgang, Germany) with 800 mW/cm^2 light intensity . Z250 (3M ESPE/USA) micro filled composite resin was used for buildup of bonded area of each tooth in 4.5 mm thickness and in three layer that individually light activated for 40 s.

Preparation process has been shown in table 1.

For investigate of data validity and stability, we used a universal testing machine that has high validity and stability for measurement relation to other standard devices. Also to reduce data mistake, an experienced technician, employed for test and data registration.

Microtensile Bond Strength Test (μTBS)

After 24 h of storage in distilled water at 37ºC, according to micro-tensile technique, each tooth was longitudinally (mesio-distally) sectioned with a low speed diamond disk in a cutting machine (Labcut 1010; Extec Co.) to produce a sticks with a cross-sectional area of approximately 1 mm diameter . Each slab was

then cut into multiple 1 mm × 1 mm × 6 mm beams. After 6 Months of storage at 37°C in NaN3-artificial saliva (pH 7.1) to inhibit microbial growth, Specimens were subjected to tensile load in a universal testing machine (SD Mechatronik MTD-500/Germany) at a crosshead speed of 1 mm/min until failure occurred.

Statistical Analysis

The measured variables were entered into SPSS version 19. All data were statistically analyzed with one-way ANOVA, and the differences among the groups were assessed using the Tukey's and post-hoc tests. P-values less than 0.05 were considered statistically significant. Before analysis of data, we checked disturbance of normality by K.S (Kolmogorov- Smirnov) test. Results showed that bond strength has normal disturbance in 3 groups.(P>0.05). So we used ANOVA and Tukey's test for bond strength comparison.

Table 1. Rewetting agents, material and application mode and time in each group

Group	Rewetting agent	application mode and time
group1: CHX rewetted	CHX 2% solution	applying with micro brush for 120 sec
group2:NaOCl rewetted	NaOCl 5% solution	applying with micro brush for 60 sec
group 3: Control	Water	blot dry technique

Results

The means, standard deviations and lower and upper bond strength are presented in table 2. According ANOVA test result there was statistical significant difference between bond strength of different rewetting agents (P<0.001). According post-hoc tukey's test, there was significant difference between group 1 and 3(P<0.001) and also there was significant difference between group 2 and 3(P<0.001). But there was no significant difference between group 1 and 2. In descriptive investigation, micro tensile bond strength of CHX group (52.67 ±6.86) was higher than NaOCl group (18.59 ± 6.081) and control group (28.84± 6.231).

Lowest micro tensile bond strength was related to NaOCl group. This data has been shown in table2.

Table 2. Mean and standard deviations, lower and upper rate of Microtensile bond strength of experimental groups

Group	Means ± SD (MPa)	lower –upper μTBS(Mpa)	$P_{1,2,3}$	$P_{1,2}$	$P_{1,3}$	$P_{2,3}$
1: CHX rewetted	52.67±6.862	10.16-58.47	0.001*	0.094	0.001*	0.001*
2:NaOCl rewetted	18.59 ± 6.081	1.40-21.90				
3: Control	28.84± 6.231	12.27-35.57				

***P<0.05: considered statistically significant**

Discussion

Since the application of CHX preserved the resin-dentin bond strength, a part of hypothesis is supported. Nevertheless, the results showed that application of NaOCl has no effect on preservation of the resin-dentin bond strength.

Dentin substrate impregnating with blends of resin monomers and creation of a compact and homogenous hybrid layer are basic conditions for a stable and durable resin-dentin bond (4, 14). An etched dentin substrate with incomplete resin infiltration is susceptible to hydrolytic degradation over time. Most of this hydrolysis occurs during 6 months (15, 16). So in this study micro tensile bond strength is measured after 6 month.

During acid etching or caries progression, exposed organic matrix resulting from mineral phase dissolution, can be degraded by bacterial enzymes and host-derived collagen-degrading protease (3, 17).

Host-derived collagen-degrading proteases activity is the main factor for hybrid layer hydrolysis (18). In

oral cavity, saliva and dentin organic matrix are main sources of host-derived collagen-degrading proteases, such as the matrix metalloproteinases (MMP)(19, 20).

MMPs are thought to be responsible for degrading most of the extracellular matrix components, such as different types of collagen (14). It has been suspected that the activity of host-derived MMPs may also be involved in the degradation of naked and unprotected collagen within incompletely resin-infiltrated acid-etched dentin from factors promoting hydrolytic degradation that could explain the progressive thinning of hybrid layers and reduction in bond strength (21, 22). This factor involves residual solvent of the adhesive or the water not removed from the dentin surface (23).

In control group (group3), collagenolytic and gelatinolytic activity of partially demineralized dentin due to activation of MMPs and complementary role of cysteine cathepsins in collagen degradation (3), leads to auto-degradation of partially infiltrated collagen fibrils and reduction in bond strength.

It has been shown that, endogenous collagenolytic activity can be inhibited or reduced by the use of protease inhibitors (24).

CHX is an anti-microbial agent with MMP inhibitor activity (25). CHX inhibits dentin MMPs (MMP2, MMP8, and MMP9) and their collagenolytic activity and limit hydrolytic degeneration (26). So it improves the integrity of hybrid layer (27) and minimizes or prevents the auto degradation of exposed collagen fibril within incompletely-formed hybrid layer (11, 28).

According to Gendron,s study, two different mechanisms of action are involved in MMP inhibition: a chelating mechanism for the inhibition of MMP-2 and MMP-9; and the interaction of CHX with the essential sulfhydryl groups and/or cysteine present in MMP-active sites in the case of MMP-8 (26).

CHX binds to phosphate group in mineralized dentin crystallites or to negative carboxyl group in collagen matrix (3). so can remains bonded in demineralized and mineralized dentin. This is responsible of stable bond after CHX treatment.

In this study it was found that applying CHX as dentin rewetting agent after acid etching can preserve bond strength after 6 months. Which was similar to other studies (7, 24, 29).Moreover CHX application, can inhibit the activity of MMPs and cysteine catepsines.(30, 31) Francisconi showed that CHX application on etched dentin, prevented abrupt bond strength loss after 6 months of aging. (32) in same result, chlorhexidine presented the best physicochemical properties and preserved resin-dentin bonding stability after 12 months of water storage(1). has been shown that MMP inhibitors created uniform resin coverage and morphologic results and μTBS data suggest that CHX can inhibit MMP activity enhance dentin bonding stability with an etch- and -rinse adhesive system.(2)

Has been shown that CHX application on etched dentin, presented a less hybrid-layer degradation and a stability of resin-dentin adhesion long-term indicating that the MMP inhibition can preserve the interface integrity over time.(35)

In this study, NaOCl treatment has no effect on bond strength over 6 months.

Hybrid layer formation and resin infiltration into demineralized dentin are the most important mechanisms in modern adhesive systems. Any failure in this process, such as incomplete resin infiltration can lead to reduction in resin- dentin bond strength. (36)

After acid etching, action of surface tension forces at the air- liquid interface, may cause collapse of collagen , flattening of collagen matrix and reduction in permeability of the demineralized zone .(37)

So in this process, some of the collagen fibrils may be exposed by acid etching, but do not infiltrate by resin. Since superficial organic phase of demineralized dentin acts as barrier and prevents resin infiltration and hybrid layer formation. After that, nano leakage is expectable.(38)

NaOCl is a non-specific proteolytic agent, which can remove organic component in room temperature (5). It can remove organic phase of demineralized dentin and produces a direct bonding between resin and hydroxyapatite in dentin substrate. So hydrolytic degeneration of collagen fibril which, might decrease bonding durability is limited (39). This dentin substrate involves exposed HA crystals and has durable bond in long-term because of inorganic materials and hydrophilic properties (40). Because of de-proteinization property of NaOCl and production a hydrophilic surface , increase in wettability of dentin is expected.(41).

Gwinnet showed that collagen fibril has no effect on resin – dentin bonding and its removal by NaOCl can make a stronger bond. (42).

SEM studies have showed that NaOCl application after acid etching, causes collagen fibril removal and dentinal tubule opening and reduces inter tubular dentin. Also Naocl application exposes a network of secondary lateral channels on dentin and widens the aperture of the dentin tubules (43), which leads to enhancement of dentin permeability(5).

Also after NaOCl treatment and organic matrix removal, degradation of organic matrix can be eliminated (39).

Resin bonding to demineralized, NaOCl treated dentin will be similar to acid-etched enamel, with less mineralized substrate, more irregularity, being rougher, and with unevenly distributed porosity (40, 41).

On the other hand, incomplete removal of residual water trapped in the deepest area of NaOCl treated dentin, induces the formation of poorly polymerized polymer chains.(36) Single bond was the applied adhesive in this study, which is ethanol- water solvent based. In these systems, rate of monomer diffusion in to demineralized dentin is low and monomer cannot fill the entire porosities produced by NaOCl treating on demineralized dentin. (44, 45) So a porous interface with lower bond strength is created. Also inter tubular channel produced by NaOCl treatment, cannot be filled by large molecule of poly-alkenoic acid (46).

Regarding to positive and negative factors mentioned here, NaOCl treating has no effect on dentin bond strength. This finding was similar to Montes study (47).

Conclusion

Application of MMPs inhibitor agents for demineralization of dentin inactivates matrix-bound MMPs.

CHX acts as matrix metalloproteinases inhibitor and by preventing dentin collagen degradation can preserve the bond strength after 6 months.

Denaturized collagen removal has no significant effect on resin- dentin bond strength.

Acknowledgment

This study was supported by a grant of Re-search Vice Chancellor of Shahid Sadoughi University of Medical Sciences (Grant No: 2903).

There is no conflict of interest in this research. Funding: This research was funded by Shahid Sadoughi University of Medical Sciences, Yazd, Iran. The authors wish to thank Mr. M. Karrabi for technical assistances and statistical analysis and revising the manuscript.

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