Clinical and Experimental Health Sciences

Effect of Different Mouthwashing Regimens on Adhesion of a Universal Adhesive:A Microshear Bond Strength and Scaning Electron Microscopy Evaluation

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ABSTRACT

Objective: To evaluate the effect of probiotic or chlorhexidine-based mouthwashes and coconut oil pulling therapy on microshear bond strength of a universal adhesive, used with two application modes.

Method: Ninety-six enamel specimens were prepared using bovine incisors and the surfaces were grounded. Then the specimens were randomly divided into 4groups and each group were subjected to a mouth washing regimen with one of three agents-chlorhexidine mouthwash, probiotic-based mouthwash, coconut oil pulling – or stored in artificial saliva(control) for 7 days(n=24). After the procedure, all groups were divided into 2 subgroups, and a universal adhesive was applied with etch-and-rinse or self-etch mode(n=12). Composite micro-cylinders were bonded to the enamel surfaces and micro-shear-bond strength was measured after 24hours water storage. Failure modes were determined using a stereomicroscope and SEM analysis was also performed. The data were analyzed using Mann-Whitney-U and Kruskal-Wallis tests.

Results: No significant differences were observed between the different mouthwash groups, regardless of application modes($p > .05$). There were no significant differences in microshear-bond strength, within the same mouthwash groups, between self-etch or etch-and-rinse modes, except for oil pulling group. Etch-and-rinse group showed higher bond strength than self-etch group in specimens subjected to oil pulling($p < .05$).

Conclusion: Etch-and-rinse mode might be preferable on patients who practice oil pulling.

Keywords: oil pulling, universal adhesive, shear-bond strength, mouthwash

1. INTRODUCTION

Recently, with the increasing consciousness about oral health, people started to use various products to enhance their oral hygiene routines. Among these products, mouthwashes are commonly used to reduce plaque accumulation and to combat caries. There are numerous types of mouthwashes containing different active agents in the dental market. Chlorhexidine gluconate (CHX) is considered as one of the most effective agents in plaque control (1). Despite their indisputable antibacterial and antiplaque efficacy, CHX containing mouthwashes, have some serious drawbacks, such as staining of teeth and restorative materials, which limit their use for a certain time (2). Another effective anti-plaque agent used in mouthwashes are probiotics, described as living bacteria that have various benefits to general health, if used in adequate amount (3). In recent years, their effect on oral health is gained importance and a number of studies reported different effects from reduction of mutans streptococci(4) to reduction of halitosis (5).

Along with the commercial mouth rinsing products, some people incline traditional medicine and home-made remedies. Oil pulling, is an Ayurvedic therapy, which is performed by whisking a tablespoon of oil in the mouth for about 20 minutes, with an empty stomach (6). But since the long application time can be tiring, 5 to 10 minutes application is considered adequate. The oils used in this practice are edible oils that are commonly available in the household, which makes this therapy achievable.

As the use of different mouth rinsing techniques become widespread, the knowledge of their interaction with restorative materials grows in importance. Universal or multimode adhesives are a newer type of adhesive, which can be used in etch-and-rinse, self-etch or the combination of two – selective etch – strategies. Because of requiring relatively less technical sensitivity and allowing flexible use, Universal adhesives' popularity have been increasing since the day they have first launched. While their practicality

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provides a large user base, there are some points to be emphasized regarding universal adhesives. When these materials used with self-etch strategy on enamel, their bond strength were shown to be lower due to their lower aggressiveness causing incapability to fully demineralize enamel (7). However, in several studies, it was reported that when etch-and-rinse strategy was used, enamel bond strength values were significantly increased (8-10). On the other hand, there are also clinical studies that reported there were no significant differences in terms of retention between application strategies on enamel (10, 11). As controversial findings on bond strength of universal adhesives utilized with different strategies are included in the literature, there are no clear data on the effect of different mouth washing agents on bond strength of universal adhesives.

Therefore, the purpose of this in-vitro study is to evaluate the effect of CHX or probiotic based mouthwashes and oil pulling therapy on microshear bond strength of a universal adhesive used with two application modes.

The null hypotheses tested were;

1. There would be no difference in microshear bond strength between the specimens exposed to different mouthwashes, regardless of adhesive application mode.
2. There would be no difference between the microshear bond strength of different adhesive application modes, regardless of the mouthwash used.

2 .METHODS

Materials used in this study were presented in Table 1. Ninety-six bovine enamel specimens were prepared from freshly extracted bovine incisors collected from slaughterhouse as a product of regular cattle slaughtering for human consumption. The bovine teeth were stored in 0.1% thymol solution for one month and cleaned with pumice using a rubber cap.

After the teeth were cleaned, enamel blocks (4mm×4mm×4mm) were prepared by cutting from the middle third of the buccal surfaces with a low speed diamond saw (Isomet, Buehler, Lake Bluff, IL, USA) under water cooling. The enamel blocks were then, embedded in acrylic resin (Meliodent, Bayer Dental, Berkshire, UK) blocks. The enamel surfaces were ground flat using 600 grit SiC paper. After that, the specimens were randomly divided into 4 groups (n=24).

Group I: The specimens were stored in artificial saliva during the whole test period to act as control group.

Group II: The specimens were immersed in a CHX based mouthwash (Kloroben, Drogsan, Ankara, Turkey) for 1 min, twice a day for one-week period. Between the immersion periods, the specimens were stored in artificial saliva and the saliva was refreshed every day.

Group III: The specimens were immersed in a probiotic based mouthwash, using the same procedure with Group II. For the preparation of probiotic based mouthwash; a probiotic sachet (Quadbiotic, MCG Pharma, Ankara, Turkey) was dissolved in 100 ml distilled water.

Group IV: The specimens were immersed in coconut oil (The Life Co., Istanbul, Turkey) for 5 min two times a day for one week and stored in artificial saliva in intervals.

At the end of the immersion periods, all four groups were subdivided into two groups according to the adhesive procedure accomplished;

Sub-Group ER: A universal adhesive (Tetric-N-Bond Universal, Ivoclar Vivadent) was applied using etch-and-rinse mode. A 37% orthophosphoric acid (Panora, Imicryl, Turkey) was applied for 30 s on enamel surface, rinsed off for 15 s and air dried. The adhesive was applied with scrubbing motion for 20 s and dispersed with air until an immobile film layer was observed. Then the adhesive was polymerized using a light-curing device (Henry Schein, HS-LED Light 1200, NY, USA) for 20 s.

Sub-Group SE: The same universal adhesive was applied using self-etch mode. For this procedure, the adhesive was applied with scrubbing motion for 20 s, dispersed with air and light-cured for 20 s.

After the adhesive was applied, resin composite (Tetric-N-Ceram, Ivoclar Vivadent, Zurich, Switzerland) micro-cylinders with 0.7 mm diameter were bonded to enamel specimens using plastic tubes. After removing the tubes with a scalpel, the microshear bond strength testing was performed. The shear load was applied by a thin metal wire, placed at the adhesive interface with a crosshead speed of 0.5 mm/s until the failure occurred using a test machine (LRX, Lloyd Instruments, Chicago, USA). The microshear bond strength values were expressed in MPa after measuring the cross-sectional area at the site of fracture with digital calipers. After testing, modes of failure were examined using a stereomicroscope under 30x magnification and categorized as adhesive failure, cohesive failure and mixed failure.

2.1. Scanning Electron Microscopic Evaluation

One representative specimen from each group was prepared for SEM evaluation. Each sample was cut in half perpendicular to the bonded interface, exposing the adhesive interface at the center of the tooth surface. The exposed adhesive interfaces were then polished with 1000 grit silicon carbid paper and then with diamond paste, under water cooling. For decontamination, the samples were soaked in 10% neutral buffered formalin solution for 8 hours. The specimens were fixed on metal stubs and then gold sputtered (one cycle of 120 s) in a vacuum chamber, using a sputtering device (MED 010, Balzers Union, Balzers, Liechtenstein). The surfaces were examined by scanning electron microscopy (Tescan GAIA 3) to evaluate the enamel-resin interface.

2.2. Statistical Analysis

Statistical analysis was performed with SPSS 21.0, Inc., (SPSS, Inc., Chicago, IL, USA) for Windows. All data sets were subjected to normality testing using the Kolmogorov–Smirnov test. Since the data were failed the normality test, the means microshear bond strength values of the groups were compared by Kruskal Wallis test. Multiple comparisons were done using Mann Whitney U test. The degree of significance was defined as p= .05.

Table 1. *Materials used in the study.*

Materials	Type	Manufacturer	Composition
Kloroben	Mouthwash	Drogsan, Ankara, Turkey	Clorhexidine gluconate, benzydamine hydrochloride
Quadbiotic	Mouthwash	MCG Pharma, Ankara, Turkey	Saccharomyces Boulardii, Lactobacillus rhamnosus, Lactobacillus acidophilus, Bifidobacterium longum
Coconut Oil	Mouthwash	The Life Co., İstanbul, Turkey	Coconut oil
Tetric-N-Bond Universal	Resin composite	Ivoclar Vivadent, Zurich, Switzerland	Ethanol, phosphonic acid acrylate, Bis-GMA, HEMA, UDMA, diphenyl (2,4,6 – trimethylbenzoyl) phosphine oxide
Tetric-N-ceram	Adhesive	Ivoclar Vivadent, Zurich, Switzerland	UDMA, Bis-GMA, Ethoxylated Bis-EMA, TEGDMA, Barium Glass, Yitterbium Triflouride, Silicon dioxide, Additives, Stabilizers, Catalysts
Phosphoric acid	Dental conditioning gel 37%.	Dentsply, Brazil	Phosphoric acid, colloidal silica, Surfactant, and pigment.

Abbreviations: Bis-GMA: bisphenol A-glycidyl methacrylate, HEMA: Hydroxyethyl Methacrylate, UDMA: Urethane Dimethacrylate, TEGDMA: Tetraethyleneglycol Dimethacrylate

Table 2. *Descriptive statistics for µSBS of self-etch and etch-and-rinse modes within each mouth washing group*

Groups	Application modes	Median	Minimum	Maximum	Z	*p*
Group I Control	Self-etch	9.77	4.76	17.19	-1.937	*.053*
	Etch-and-rinse	14.75	6.75	29.57		
Group II Chlorhexidine mouthwash	Self-etch	11.39	4.37	29.33	-0.624	*.533*
	Etch-and-rinse	16.90	8.23	24.34		
Group III Probiotic-based mouthwash	Self-etch	13.58	2.16	32.51	-0.0624	*.533*
	Etch-and-rinse	15.66	3.90	27.59		
Group IV Coconut oil pulling	Self-etch	7.83	2.35	18.66	-2.528	*.011**
	Etch-and-rinse	15.83	4.17	47.46		

** indicates significant difference (p<.05).*

3. RESULTS

No significant differences were observed between the different mouthwash groups, regardless of application modes (p= .375 for SE groups, p= .935 for ER groups). Also, no significant differences in microshear bond strength values were found, within the same mouthwash groups, between self-etch or etch-and-rinse modes, except for oil pulling group (p= .533 for CHX, p= .533 for probiotic, p= .053 for control) Etch-and-rinse group showed higher bond strength than self-etch group in specimens subjected to oil pulling. (p= .011) Although not significant, all other mouthwash groups showed higher microshear bond strength values, when the adhesive was applied in etch and rinse mode. (Table 2)

All mouth rinse groups showed mostly cohesive failures when the adhesive system was used in etch-and rinse mode. In self-etch mode, oil pulling group showed mostly adhesive failures (Figure 1).

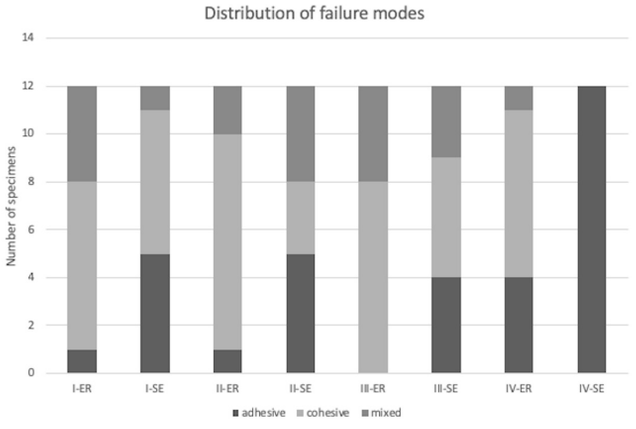

Figure 1. *Represents distribution of failure modes across all groups.*

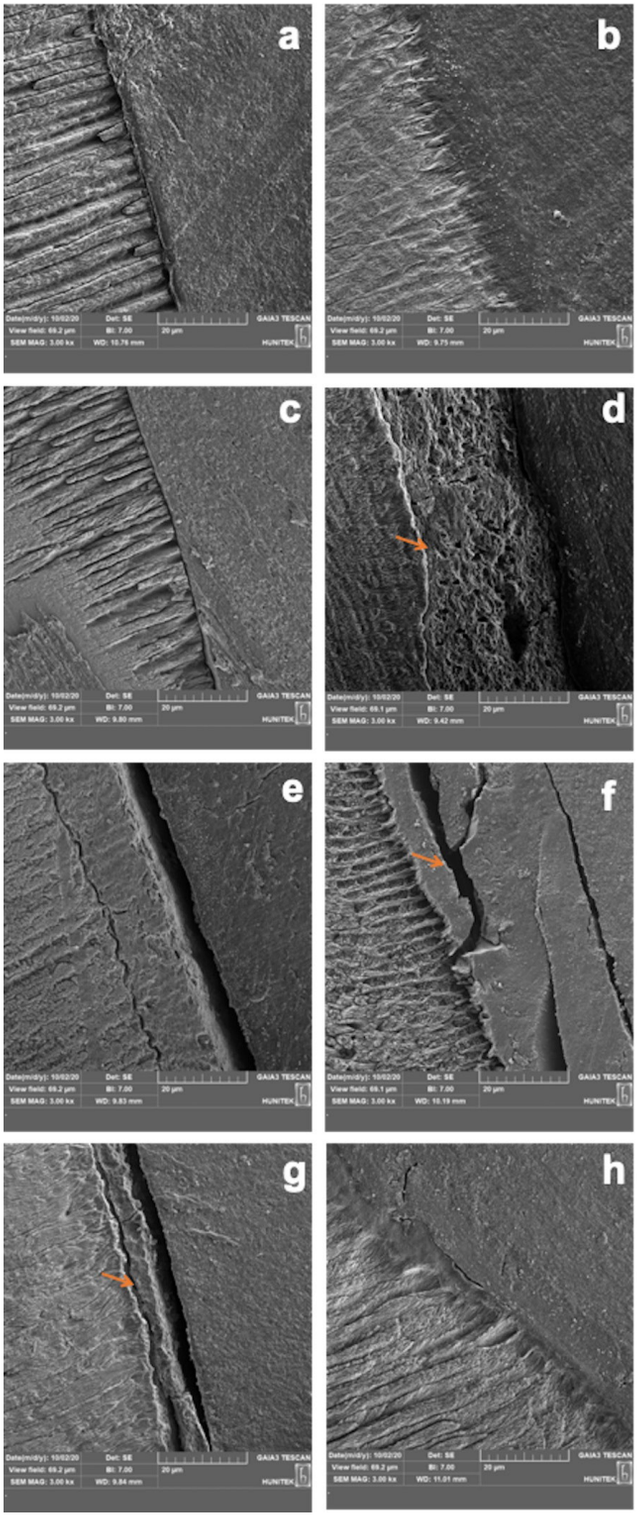

Figure 2. *SEM photos of representative samples from all groups. a: I-SE, b: I-ER, c:II-SE, d: II-ER, e:III-SE, f: III-ER, g: IV-SE, h: IV-ER. In all ER groups (b,d,f,h) it was observed that an intact adhesive interface and deep micro retention was occurred. In SE groups (a,c,e,g), however , the resin tags were shorter, and partial spacing were observed. In group II-ER, a very thick adhesive layer, possibly caused by adhesive ponding in sectioned area(arrow) was observed. In group III-ER, cracks in adhesive, possibly caused by the preparation of SEM process, can be observed. (arrow) In group IV-SE, a layer between adhesive and tooth tissue and also distinct spacing was observed.(arrow)*

Representative SEM images of adhesive-enamel interfaces are shown in Figure 2. In general, all etch-and rinse groups showed intact anchoring between the adhesive and enamel and deep penetration into demineralized enamel tissue forming well-defined, long resin tags; whereas in self-etch groups resin tags were shorter and partial disintegrated areas between adhesive and tooth tissue were present. Only in oil pulling group, distinct spacing between tooth tissue and adhesive was observed in self-etch mode where, remnants of coconut oil was also present between adhesive and tooth tissue. On the other hand, etch and rinse group revealed an even and reliable adhesive interface without any sign of residual coconut oil.

4. DISCUSSION

Individuals with good oral hygiene habits, frequently prefer minimally invasive methods and esthetic adhesive restorations when a restorative treatment is needed. Therefore, the knowledge of the effect of mouthwashes on the success of adhesive restorations can guide the clinicians through material choice. Thus, to fill a gap in the literature, the effect of current rinsing methods on the bonding efficacy of Tetric-N-Bond – one of the most preferred universal adhesives of the day – was evaluated in this study.

Shear bond strength test is a distinguishing tool to assess the bonding efficacy in the tooth restoration interface. Recently the microshear bond strength test, which defines as a shear bond strength test applied with a bonded cross-sectional area of 1mm2 or less, gained popularity. It is believed that the smaller bonded area leads to a more uniform stress distribution providing more precise measurements (12).

The present study compared the effect of probiotic/CHX mouthwashes and oil pulling on microshear bond strength of a universal adhesive used with two different application methods. According to the current results; there were no differences between the tested mouthwashes regardless of application modes. Therefore, the first null hypothesis had to be accepted.

CHX is a cationic bisbiguanide, which is commonly used to maintain oral hygiene and considered as the gold standard amongst the antiplaque agents (13). Sinha et al.(14) reported that the use of CHX as a dentin pretreatment agent increases the shear bond strength of resin composite to dentin. However in another in-vitro study, the use of CHX as dentin disinfectant was found to decrease the shear bond strength to dentin (15). As far as the authors' knowledge, there are a limited number of studies regarding the shear bond strength of adhesive materials to chlorhexidine-exposed enamel. In the present study, the use of chlorhexidine-based mouthwashes had no adverse effects on microshear bond strength of the tested universal adhesive with either of the two application modes. Frey et al.(16) evaluated the effect of CHX application on different concentrations to enamel prior to bracket bonding. They reported that the use of CHX based mouthwash didn't influence the shear bond strength,

which is in accordance with the results of the present study. Similarly, in two different in-vitro studies Bishara et al(17,18), reported that; the shear bond strength to enamel was not affected by application of CHX.

Recently, the use of probiotics for providing a balanced oral flora has become the main topic. The probiotics can compete with the harmful bacteria for adhering hard and soft tissues inside the oral cavity, thus and so may prevent the periodontal diseases and caries (19). There are several studies regarding the probiotics' effects in oral cavity (4, 13, 20-22). They have been shown to reduce, plaque accumulation (1) and streptococcus mutans levels in saliva (21). However, based on the authors' knowledge, there is no published data regarding the interaction of probiotic based mouthwashes exposed dental tissues with adhesive agents. In the present study, the use of probiotic mouthwash didn't affect the microshear bond strength of the universal adhesive to enamel regardless of different application modes, compared to control group. Previously, probiotic mouthwash was found to be more effective than CHX in reducing gingival inflammation (23, 24). Based on the present study's findings, it may be suggested to prefer probiotic mouthwashes over CHX, since probiotic mouthwashes don't have the certain drawbacks of CHX such as discoloration or alteration in taste. However, the reason why the probiotics not interfering with the bond strength in the present study, might be related to the type of adhesive agent used and its composition. Tetric-N-Bond Universal is a mild-etching adhesive with a pH level of 2.5-3.0. The matrix of Tetric-N-Bond Universal is a combination of hydrophobic, hydrophilic and intermediate natured monomers, which allows this material to successfully interact with both hydrophilic tooth, and hydrophobic resin restorative substrates (25). Different types of adhesives with different compositions might present results inconsistent with the present study. Thus, the study must be repeated with various types of adhesives to understand the nature of the interaction of probiotics and adhesives.

Oil pulling therapy has become popular among many people, that try to avoid the use of chemicals. As the healthy lifestyle becomes the new trend, the idea of synthetic therapeutics is toxic for the body, leads more and more people to use natural remedies. Therefore, the knowledge of the interaction between the oils used in this therapy and teeth tissues gain importance. In the present study, coconut oil pulling application didn't affect the microshear bond strength of the universal adhesive comparing to other groups. However, within the same group, application with self-etch mode revealed lower microshear bond strength values than etch-and-rinse mode. In the present study, no difference was observed between the application modes, regardless of the mouthwash applied, except for oil pulling group. Therefore, the second null hypothesis had to be partially rejected. This finding might be explained with the fact that the lauric acid exist in coconut oil, can react with the saliva components to form a soap like substance that reduces plaque and bacterial adhesion (26, 27). In parallel with that, in the present study, a similar layer between adhesive and tooth tissue was observed in SEM photos (Figure 2). The film layer formed on tooth tissue might have adversely affected the bond strength of universal adhesive used with self-etch mode, while the acid etching step in etch-and-rinse mode might have eliminated the film layer. Therefore, it might be suggested to use etch-and-rinse mode in patients who frequently performs oil pulling while performing adhesive restorations.

In control group, no significant differences were observed between etch-and-rinse and self-etch mode. Normally, the etch-and-rinse method was known to be the gold standard when working on enamel tissue. In previous in-vitro studies, etch-and-rinse method have shown higher shear bond strength values compared to self-etch method when applied on enamel tissue (28, 29). There might be several reasons that cause this situation. In the present study bovine teeth were used whereas human teeth were used in the mentioned studies. Although Bovine teeth has the most similar Ca:P rate to human enamel, it has some morphological differences in its crystalline structure which may have caused the contradiction with other previous studies (30). Also, the difference between the compositions or the pH levels of the adhesive used in the current study and the previously mentioned studies may have created the conflict. On the other hand, some clinical studies reported that; there were no significant differences in terms of retention rate between etch-and-rinse and self-etch modes of universal adhesive, supporting the present study's findings (11, 31). However, in these studies, universal adhesive was applied on non-carious cervical lesions that contains both enamel and dentin tissue. Since self-etch mode is the preferred bonding method in dentin tissue because of presenting higher bond strength values compared to etch-and-rinse method, it may have balanced the weak bonding to enamel tissue. Also, further follow-up periods may result in significant differences between retention rates. In any case, because of insufficient data on universal adhesives, it is hard to obtain an exact interpretation.

The findings of this in-vitro study provide a preliminary overview about the effects of some most frequently preferred rinsing methods on the adhesive performance of a popular universal adhesive system. Having some certain limitations due to the nature of all in-vitro studies, these results have to be interpreted carefully and it is recommended to perform further studies with larger groups of specimens to achieve more reliable and comprehensive data. Furthermore, further in-vitro and in-vivo studies must be performed with different mouthwashes and adhesive systems.

5. CONCLUSION

Based on the limitations of this study;

1. The mouth washing habits with different agents, didn't interfere with either etch-and rinse or self-etch application modes of the current universal adhesive.
2. Etch-and-rinse adhesive strategy might be more reliable on patients who performs oil pulling with coconut oil.

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