Effect of a re-wetting agent on bond strength of an adhesive to primary and permanent teeth dentin after different etching techniques

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Abstract

Objective: This study investigated the effect of a re-wetting agent on the microtensile bond strengths (μTBS) of primary and permanent dentin after acid or laser etching.

Materials and Methods: Twelve permanent and 12 primary molar teeth were ground to expose an occlusal dentin surface. Each group teeth were randomly divided into groups; I–II: 37% phosphoric acid etching with/without re-wetting agent, III–IV: Erbium: Yttrium aluminium garnet laser etching with/without re-wetting agent. An etch-and-rinse adhesive was used, and vertical sticks were obtained for the microtensile test.

Results: μTBS of permanent teeth was higher than that of primary teeth ($P < 0.05$). Re-wetting agent groups were similar with control groups in both etching groups in the permanent teeth ($P > 0.05$). Re-wetting agent group was similar with the control group in acid etch group ($P > 0.05$) and lower than the control group in laser etch group in primary teeth ($P < 0.05$).

Conclusion: Acid etching in permanent teeth; laser etching in primary teeth was found more successful. The use of re-wetting agent did not provide an advance on bond strength of the adhesive in both primary and permanent teeth after acid-etch or laser-etch.

Key words: Acid-etch, dentin bond strength, laser-etch, permanent teeth, primary teeth, re-wetting agent

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Introduction

Bonding of adhesive resin to enamel is simple, but bonding to dentin is more mixed and difficult because of dentin's structural properties and composition.[1,2] While the number of tubules in deep dentin is approximately 45,000/mm^2, the surface dentin is 25,000/mm^2.[3] Dentin permeability and dentin moistness depend on the diameter of the dentinal canals, the number of remaining dentin thickness, dentin fluid concentration, and the presence of the smear layer.[4] Water content and permeability of the dentin are not the same all over due to differences in the number and diameter of dentin tubules. Reduced intertubuler dentin area complicates adhesion in deep dentin compared to superficial dentin.[5]

In the total etch technique, the smear layer is removed, the dentinal tubules open and the intertubular and peritubular dentin decalcify when phosphoric acid-etching is applied. Recently, the use of erbium: Yttrium aluminum garnet (Er: YAG) laser has been introduced as an alternative to phosphoric acid in etching methods.[6] Laser removes the smear layer and opens the tubules. Hence, a

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microscopically rough dentin surface with a micro-retentive pattern occurred.[7] The laser etches the surface, sterilizes dentin surface, and saves time for both patients and clinicians.[8] Furthermore, acid etching has disadvantage that the unpleasant taste in the mouth and this may lead to undesired behavior, particularly in pediatric patients.

When the dentin air-dried after the etchant is rinsed, collagen fibers collapse and shrink. This shrinkage reduces penetration of resins.[9] In wet bonding systems, water provides support for the collagen fibrils to remain in the appropriate position.[3] Thus, the connection is increased.

After the etching procedure, enamel must be dried without drying dentin to regulate the ideal amount of moisture before application of adhesive system but it is clinically very difficult. In such situations, dentin should moisturize before application of the adhesive. Until now, various re-wetting agents have been tested.

The aim of this study was to investigate the effect of a re-wetting agent on the microtensile bond strengths (μTBS) of an adhesive on sound primary and permanent dentin after acid or laser etching. The hypotheses to be investigated in this study were:

- There are differences in the bonding values of re-wetting agent group and control group
- There are no differences in the bonding values of acid etched and laser etched dentin
- There are no differences in the bonding values of primary and permanent teeth groups.

Materials and Methods

Sampling

Twenty-four noncarious recently extracted permanent and primary teeth; each 12 were used in this study. All teeth were stored at 4°C in physiologic saline after extraction. The debrises on the teeth were cleaned. Teeth were ground to expose a flat occlusal dentin surface using diamond burs. All the dentine surfaces were grinded with 600-grit silicon carbide paper under running water to obtain homogeneous surface.

Experimental groups

Both the primary and permanent teeth were randomly divided into two groups according to the etching procedures.

Acid etching groups

Conventional 37% phosphoric acid (Pulpdent, Etch Royale, USA) was applied for 15 s on a dry dentin surface, was rinsed thoroughly with air-water spray from an air syringe for 10 s. The etched dentin surface was gently dried for 5 s with an air syringe.

Laser etching groups

The Er: YAG laser (Fidelis Plus III; Fotona, Ljubljana, Slovenia) was used for laser etching. The noncontact hand piece (R02) was used at 1.2 W with a pulse duration of 180 μs a (medium short-pulse mode). It operates at a wavelength of 2.94 μm; the repetition rate was 10 Hz. The area of the beam was 1.8 $cm^2 \times 10^{-2}$ cm^2. Laser etching was performed with water and air cooling of the laser unit. The beam was aligned perpendicular to the dentin at a distance of 8 mm and was moved in a sweeping motion by hand over the area for 15 s. The irradiated specimen was dried in an oil-free air source for 15 s.

Then each group was divided into two subgroups: Re-wetting agent and control groups.

The re-wetting agent (Aqua-Prep F) is used according to manufacturer's instructions after the acid etched, air dried for 2–4 s. A dry, but not desiccated surface was obtained before applying Aqua-Prep. Dentin appeared dull, and etched enamel appeared frosted. Then, two drops of Aqua-Prep dispensed into a mixing well. With a brush, applied to the dentin and enamel surfaces. Aqua-Prep allowed to soak for 20 s. Gently air dried to remove excess Aqua-Prep. The resulting surface had a shiny appearance.

An etch-and-rinse (Adper Single Bond 2) adhesive was applied to all specimens and the teeth were restored with composite resin (Filtek Z250) which was built up incrementally with 1.5 mm layers to a height of 4–5 mm and polymerized with a curing light (T LED, Elca Technologies, Imola, Italy) for 20 s. Specimens were then stored in water at 37°C for 24 h. Table 1 shows the adhesive systems and re-wetting agent used in the present study.

Microtensile bond strengths test

After immersion, the restored teeth were vertically sectioned both mesial-distally and buccal-lingually along their long axis with a slow speed diamond saw (Isomet 1000, Buehler Ltd., Lake Bluff, IL, USA) in order to obtain five 1 mm^2 stick-shaped specimens from each

Table 1: Materials used in this study

Adhesives/ re-wetting agent	Manufacturer	Composition
Adper single bond 2 Two-step, etch-and-rinse	3M Dental Products, MN, USA	2-HEMA, water, ethanol, Bis-GMA*, dimethacrylates, amines, methacrylate-functional copolymer of polyacrylic and polyitaconic acids
Filtek Z250 Microhybrid	3M ESPE (St. Paul, MN, USA)	TEGDMAc, Bis-GMAa, Bis-EMAd, UDMAb microhybrid Zirconia/silica, particle size 0.01-3.5 μm, 82 wt%, 60 vol%
Aqua-Prep F	Bisco Inc., USA	18% HEMA, 2% NaF, water

*Bis-GMA=Bis-phenol-A-diglycidyl rnethacrylate; HEMA=2-hydroxyethyl methacrylate; TEGDMAc=Triethylene glycol dimethacrylate tooth (n = 20 specimens). Thicknesses of specimens were measured using digital calipers (Mitutoyo, Tokyo, Japan). These were fixed with cyanoacrylate glue (Zapit; DAVA, Corona, CA, USA) to two surfaces on a linear actuator-driven, offset micro-tensile testing device (BISCO; Schaumburg, IL, USA).

Failure mode

After the µTBS test, the test surfaces of the dentine and resin were examined with an optical microscope (Leica Microscopy Systems, Germany) under ×40. The dentin surfaces of debonded specimens were examined to determine the failure mode. The failure modes were classified as follows: Adhesive failure if 100% of the bonded interface failed between the dentine and the bonding resin; cohesive failure if 100% of the failure was in the resin composite; or mixed failure if the failures were partially adhesive and partially cohesive.

Evaluation using scanning electron microscopy

The aim of scanning electron microscopy (SEM) (JEOL JSM-6390 LV, JEOL Ltd., Tokyo, Japan) analysis was to observe the micromorphology of the two primary and two permanent sound dentine after the use of two different etching procedures. Surface preparation procedures were carried out the same as for the µTBS testing, and then the tooth substrates were fixed in 2.5% glutaraldehyde in 0.1 M phosphate-buffered solution for 24 h at room temperature. Specimens were dehydrated with increasing ethanol concentrations and submitted to chemical drying in hexamethyldisilazane. After drying at room temperature (24°C), the specimens were gold sputter-coated and caries-affected dentine surfaces were observed by SEM. The entire surface was scanned, and the most representative areas were photographed at ×2000 magnifications.

Statistical analysis

The results of the Levene statistics ($P < 0.05$) and the Shapiro–Wilk statistics in all groups ($P < 0.05$) not demonstrated variance homogeneity. The µTBS data were statistically compared by two-way analysis of variance (ANOVA), Kruskal–Wallis and Mann–Whitney test with Bonferroni correction. The Chi-square test was used to compare the incidence of the different fracture modes among the etching techniques. The data were analyzed with SPSS Version 13 for Windows statistical program software. The level of significance was 5% ($P < 0.05$).

Results

Microtensile bond strengths

According to the ANOVA results, there were no significant differences between re-wetting agent groups and control groups (P = 0.54). There were significant differences between etching procedures (P = 0.001) as in primary and permanent teeth (P = 0.007) [Table 2]. Mean µTBS and standard deviations for the experimental groups are shown in Table 3.

Microtensile bond strengths of the permanent teeth were higher than primary teeth only in the acid etch-control group ($P < 0.05$), the other groups were similar ($P > 0.05$).

In the primary teeth groups, µTBS of the control group were higher than laser etch-re-wetting agent group ($P < 0.05$). Furthermore, µTBS of laser etch-control group were higher than acid etch-control group ($P < 0.05$). In the present study, though insignificant, the µTBS of acid etch-re-wetting agent were higher than the control group. The highest µTBS was observed in laser etch-control group. There were no significant differences among the other groups ($P > 0.05$).

In the permanent teeth groups, there were no significant differences between re-wetting agent and control group in both laser and acid etch group ($P > 0.05$). The highest µTBS was shown in acid etch-control group. µTBS of acid etch-re-wetting agent group were statistically higher than laser etch-re-wetting agent group ($P < 0.05$). Furthermore, µTBS of acid etch-control group were significantly higher than laser etch-control group ($P < 0.05$).

Table 2: Two-way ANOVA

Source	Type III Sum of squares	Df	Mean square	*F*	Significant
Teeth	314.384	1	314.384	7.467	0.007
Etching procedures	483.303	1	483.303	11.479	0.001
Re-wetting agent	159.201	1	159.201	3.781	0.054
Teeth × etching procedures	738.311	1	738.311	17.536	0.000
Teeth × re-wetting agent	14.340	1	14.340	0.341	0.560
Etching procedures × re-wetting agent	255.581	1	255.581	6.070	0.015
Teeth × etching procedures × re-wetting agent	345.156	1	345.156	8.198	0.005
Error	6399.580	152	42.103		
Total	613,00.635	160			
Corrected total	8709.857	159			

ANOVA=Analysis of variance

Table 3: The µTBS values (MPa) (mean±SD)

	Permanent teeth	Primary teeth
Acid etch (control)	24.92±7.93^{aA}	14.28±5.22^{bB}
Acid etch-re-wetting agent	21.92±5.34^{aA}	18.35±7.94^{abA}
Laser etch (control)	16.74±6.04^{bA}	20.57±9.02^{aA}
Laser etch-re-wetting agent	14.55±4.52^{bA}	13.71±4.11^{bA}

Same lowercase letters indicate an insignificant difference within the same column, same uppercase letters denote an insignificant difference within the same row (P>0.05). SD=Standard deviation; µTBS=Microtensile bond strengths

Failure mode

Specimen failure modes were evaluated. Table 4 shows the distribution of failure mode (adhesive, cohesive, and mixed) for the teeth types and etching procedures. According to statistical analysis (Chi-square-test), there were significant differences in failure modes among the groups in the permanent teeth ($P = 0.012$). Furthermore, in the primary teeth, there were significant differences in failure modes among the groups ($P = 0.000$). For all the permanent teeth, adhesive and mixed type failures are observed more than cohesive failure type. While the mixed type of failure was dominant in the laser etch-re-wetting group, adhesive type of failure was dominant in the acid etch-control group. For all the primary teeth, mixed type failure was observed more than the others. The mixed type of failure was dominant in the laser etch-re-wetting group.

Scanning electron microscopy

Representative SEM images of the primary and permanent dentin after acid or laser etching were shown in Figures 1-4.

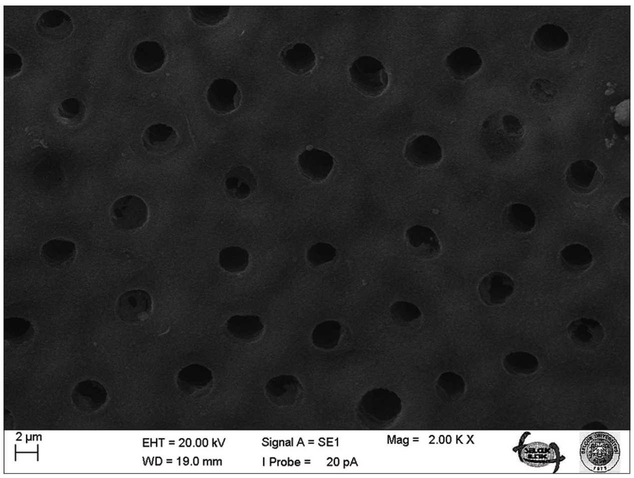

Figure 1: Scanning electron microscopy of the primary dentin surface after acid etching with 37% phosphoric acid at x2000

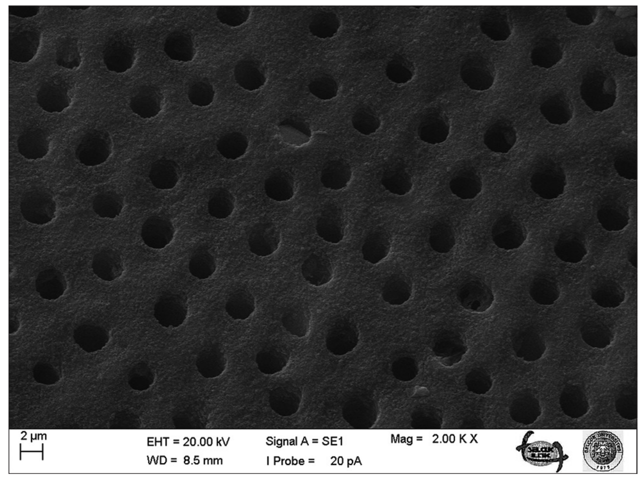

Figure 2: Scanning electron microscopy of the permanent dentin surface after acid etching with 37% phosphoric acid at x2000

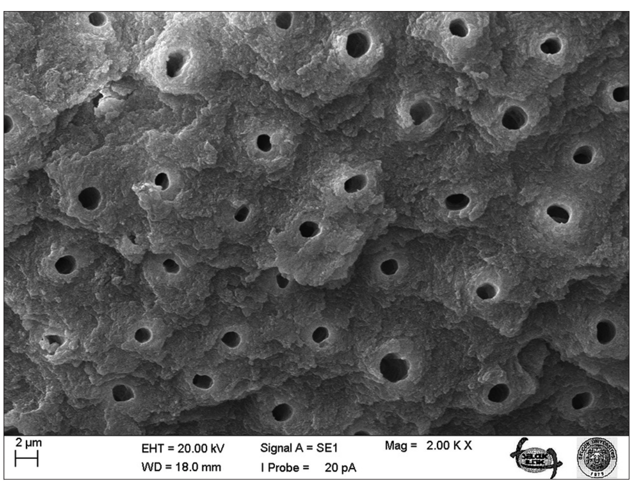

Figure 3: Scanning electron microscopy of the primary dentin surface after erbium:Yttrium aluminum garnet laser etching at x2000

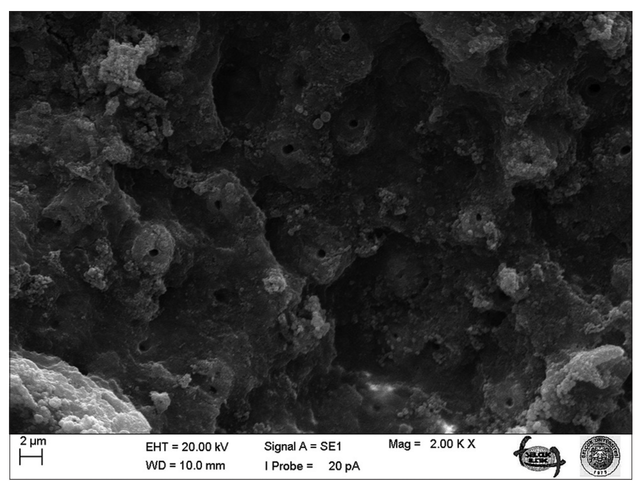

Figure 4: Scanning electron microscopy of the permanent dentin surface after erbium:Yttrium aluminum garnet laser etching at x2000

Table 4: Distribution of failure modes for the teeth types and etching procedures

	Permanent teeth			Total	Primary teeth			Total
	Adhesive	Cohesive	Mixed		Adhesive	Cohesive	Mixed	
Acid etch-control	14	0	6	20	6	5	9	20
Acid etch-re-wetting agent	10	1	9	20	12	1	7	20
Laser etch-control	8	5	7	20	6	11	3	20
Laser etch-re-wetting agent	5	2	13	20	1	4	15	20

The primary and permanent dentin surface after acid etching showed open orifices of the dentinal tubules with smooth dentin surface without smear layer [Figures 1 and 2].

The primary dentin surface after Er: YAG laser etching (1.2 W, 180 μs, 10 Hz) showed a scaly, irregular, and rugged appearance. Open dentinal tubules were clearly visible with more prominent peritubular dentin than intertubular dentin due to the preferential removal of intertubular dentin [Figure 3].

The permanent dentin surface after Er: YAG laser etching showed much more irregular surfaces with deep and shallow areas and some opened dentin tubules. Furthermore, few crack areas were observed, and some dentinal tubules were still partially occluded with smear layer [Figure 4].

Discussion

This study investigated the effect of a re-wetting agent on the μTBS of sound primary and permanent dentin after acid or laser etching. The results of this study did not support the hypothesis that: (1) There are differences in the μTBS of re-wetting agent and control group, (2) there are no differences in the μTBS of acid etched and laser etched dentin, (3) there are no differences in the μTBS of primary and permanent teeth. In fact, there were significant differences among teeth types and etching procedures. But there were no significant differences among re-wetting and control groups.

After acid etching of enamel, enamel must be dried to determine the adequacy of etching. Excessive drying of dentin after acid conditioning causes collapse and shrinkage of collagen fibers. That means penetration of the hydrophilic resin into the fiber is reduced. Thus, μTBS of resin is lower. Therefore, it is important to rehydrate the dentin prior to bonding after air-drying. Re-wetting of dried dentin expands the demineralized collagen network and increases the permeability of the substrate to subsequent diffusion of the hydrophilic resin monomers.[10] Solvents of primers like ethanol carry the monomer of primer into spaces between fibers via replacing remaining water on the dentin surface by monomers. If excess water was incompletely replaced by monomers, this is called over-wet phenomenon.[11] An inadequate μTBS may occur due to phase separation of the hydrophobic and hydrophilic components resulting in blister and globule formation in resin-dentin interface.[11] If the degree of dentin moisture is necessary, drying the dentin and using a re-wetting agent before the application of the adhesive resin might provide optimum moisture. Therefore, a re-wetting agent was used in the present study.

Phosphoric acid etching to provide better bonding to dentin is considered as the gold standard. Recently, laser etching is an alternative to acid etching for enamel and dentin because it has some advantages such as its antibacterial effect and more stable surface produced by laser etching and less acid-soluble compounds in dentin, consequently reduced susceptibility to secondary cavities.[12] During laser irradiation the water present in the tissue vaporizes, leading to micro explosion and then removal of the organic and inorganic portion of dentin.[13] It was thought that a re-wetting agent following laser etching should be used due to vaporized water after irradiation to promote bonding. In the present study laser etching was also examined besides acid etching.

Dentin adhesion depends on both the adhesive system and the dentin substrate.[14] However, some differences exist between the dentin of permanent and primary teeth as well as the number of dentinal tubules, the degree of mineralization, and inorganic component.[15,16] Most of the studies about re-wetting agents were carried out on permanent teeth. In the present study, both permanent and primary teeth were used.

In some studies, thermocycling and the water storage protocols of specimens have been performed to mimic the natural aging process of dental restoration so far to evaluate possible effects of aging conditions. However, Akin *et al.* reported that dentin had not been affected by the aging methods used to simulate the degradation of the adhesive interface.[17] Bagheri *et al.* also reported slightly increase after 8 weeks immersion in distilled water.[18] In the present study, aging procedure was not performed.

In wet bonding technique that has been shown to enhance μTBS, organic solvents, like acetone or ethanol is added in the composition of most adhesives to provide the application of adhesives on moist dentin. The solvent promote the infiltration of resin tags through the nanospaces of the collagens.[9,19] Single bond that was used in the present study is an ethanol- and water-based adhesive system. It was reported that resin infiltration into the full width of the demineralized dentin was observed when the collagen matrix was kept moist prior to bonding.[20] Al Qahtani *et al.* stated that re-wetting of the etched and dried dentin with HurriSeal desensitizer resulted in high shear bond strengths for all bonding agents.[21] The presence of 35% hydroxyethyl methacrylate (HEMA), benzalkonium chloride and 0.5% sodium fluoride and water may provide this result. The HEMA component that is a wetting agent can reduce the surface tension and reopen the spaces between fibers re-establishing the level of μTBS.[22] It was reported that ethanol-and water-based Single Bond Adhesive group that was applied to moist dentin showed the highest μTBS, and there was no significant difference between moist dentin and Aqua-Prep applied dried dentin groups. In all adhesive groups, dry dentin showed the lowest μTBS.[23] In the present study, there are no differences in the μTBS of re-wetting agent and control groups. While insignificant differences were observed between re-wetting agent and control groups in both acid and laser etched groups of permanent teeth and in acid etched group of the primary teeth, re-wetting agent group was observed as statistically lower than control group in laser etched group of the primary teeth. Soares *et al*. reported that there was no significant difference between shear bond strength values of the bond systems applied alone and applied with re-wetting agent.[24] Van Meerbeek *et al*. reported that when the water-based adhesives were dry-bonded, no ultrastructural evidence of the collapsed demineralized collagen, incompletely or not at all infiltrated by resin, could be detected.[25] The results of the present study are consisted with those of Soares *et al*. and Van Meerbeek *et al*. It is important that the lack of studies focusing on the use of combination of Er: YAG laser etching with re-wetting agent on primary teeth make comparisons difficult with the findings of the conducted researches.

In the current study, μTBS of acid etch groups of permanent teeth were significantly higher than laser etch groups of permanent teeth. In the SEM images of permanent teeth, acid-etched dentin showed more frequent opened tubules than laser etched dentin, and acid-etched dentin showed larger orifices of tubules [Figure 2]. Much more irregular surfaces and few cracks on lased permanent dentin might affect bonding adversely. Lased dentin surfaces also have microfragment-like structures which might have an adverse effect on the adhesion.[26] It was reported that the μTBS of acid-etching were higher than laser etching in permanent teeth.[27,28] Similar results were reported by Ramos *et al*.,[29] Ceballo *et al*.,[30] Chimello *et al*.[31] consistent with the present study. However, in the primary teeth, μTBS of laser etch-control group were significantly higher than acid etch-control group in the present study. SEM images revealed that the laser etched permanent dentin surface showed more irregular flaky surface than the laser etched primary dentin surface, and primary dentin tubules were more clearly observed. Furthermore, smear layer that observed on laser etched permanent dentin surface may affect the μTBS [Figures 3 and 4]. Monghini *et al*. reported that Er: YAG laser irradiation of primary teeth dentin, prior to the adhesive protocol, adversely affected bond strength.[32]

Primary teeth dentine is more reactive to acidic conditioners than the permanent teeth dentine because of the lower concentration of calcium and phosphate in peritubular and intertubular dentin.[33] Manufacturers' instructions for adhesives don't different from permanent teeth when used for bonding to primary teeth.[34] In the current study, conventional 37% phosphoric acid was applied for 15 s, laser parameters were also same in both primary and permanent teeth. According the teeth type, there were no significant differences in laser etch group but, permanent teeth were statistically higher than primary teeth only in the acid etch-control group. Kornblit *et al*. observed irradiated dentine of the deciduous teeth is identical to those found in permanent teeth, when using an Er: YAG laser.[16] However, a study of total-etch adhesives has revealed similar μTBS to both types of dentin.[35]

The limitation of this *in vitro* study is that a SEM observation of re-wetting applied dentin was not examined due to dehydration step of SEM procedures. The interpretation of the results is also limited because there is no study in the literature related to the effect of laser etch and re-wetting agent to bonding of adhesives to primary dentin. In addition, because of *in vitro* study design, it could not be possible to provide intertubuler dentin liquid as *in vivo* situations. Therefore, moisture degree after re-wetting agent should be evaluated *in vivo* in further studies.

Conclusions

Within the limitations of this study, we can draw the following conclusions: The use of re-wetting agent did not increase μTBS in both acid etch and laser etch groups. Acid etching in permanent teeth; laser etching in primary teeth was found more successful.

The present study gives ideas to clinicians about etching procedures and re-wetting agents in both dentitions. Further studies are needed about clinical usage of re-wetting agents. Investigations should be done using adhesives with different contents to understand and promote bonding in the primary teeth.

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