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Microtensile bond strength to phosphoric acid-etched dentin treated with NaF, KF and CaF_2

Pinar Altinci[a,b], Murat Mutluay[b,c], Leo Tjäderhane[d,e], Arzu Tezvergil-Mutluay[b,f]

a Finnish Doctoral Program in Oral Sciences, Turku, Finland.

b Department of Restorative Dentistry and Cariology, Institute of Dentistry, University of Turku, Turku, Finland.

c Institute of Dentistry, University of Eastern Finland, Kuopio, Finland.

d Department of Oral and Maxillofacial Diseases, University of Helsinki, and Helsinki University Hospital, Helsinki, Finland.

e Research Unit of Oral Health Sciences and Medical Research Center Oulu (MRC Oulu), Oulu University Hospital and University of Oulu, Oulu, Finland.

f Turku University Hospital, TYKS, University of Turku, Turku, Finland.

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Correspondance to:

Pinar Altinci, DDS

Department of Restorative Dentistry and Cariology,

Institute of Dentistry, University of Turku

Lemminkaisenkatu 2, 20520, Turku, Finland

E-mail: paltinci@hotmail.com

Tel. +358 2 3338340

Fax +358 2 3338390

ABSTRACT

Purpose: Fluoride compounds have been reported to play a protective role in the host-derived enzymatic degradation of demineralized dentin matrices. The objective of this study was to evaluate the effect of fluoride pretreatment on the immediate and long-term microtensile bond strength (μTBS) of resin-dentin interfaces**.**

Materials and methods: Mid-coronal dentin surfaces of ninety-nine teeth were etched with 32%wt phosphoric acid and randomly assigned to pretreatment with NaF, KF or CaF_2 solutions (6, 24 and 179 mM F content) for 30 s before the application of bonding agent (Scothbond Multi-Purpose, 3M ESPE). No fluoride pretreatment and a commercially available bonding agent with KF (Excite F, Ivoclar Vivadent) were used as controls. After composite built-up, the specimens were sectioned into μTBS test beams, stored in artificial saliva at 37 °C for 24 h, 6 or 12 months and tested. Fracture types were evaluated under scanning electron microscope. The data were analyzed with ANOVA and Scheffe post-hoc tests (α=0.05). Pearson Chi-Square test was used to compare the distribution of failure types.

Results: Fluoride compound, fluoride concentration and storage time showed significant effect on μTBS ($p < 0.05$). NaF, KF and Excite F groups showed higher μTBS than the control ($p < 0.05$). After 24h, μTBS ranged between 35.26(±10.25) MPa in control to 54.65(±14.60) MPa in NaF 24 mM group ($p < 0.05$), and the bond strength of 6 and 24 mM F groups were also found higher than the control ($p < 0.05$). After 12 months, all F groups showed stable bond strength ($p > 0.05$), except CaF_2 179 mM ($p < 0.05$). According to Chi-square test, fracture types were also significantly influenced from the test factors ($p < 0.05$).

Conclusions: NaF and KF treatment after acid-etching step of adhesive bonding procedures do not adversely influence the initial or long-term bond strength to dentin, and can improve the maintenance of bond strength durability.

Keywords: Adhesion, Dentin, Durability, Surface treatment

1. Introduction

Dentin tissue consists of type I collagen fibrils, non-collagenous proteins, hydroxyapatite minerals, free and collagen-bound water molecules and collagenolytic enzymes, primarily matrix metalloproteinases (MMP) -2, -8 and -9 as well as cysteine cathepsin K [1-3]. Bonding to dentin is achieved with the infiltration of resin monomers into the exposed collagen matrix after demineralization by acid-etching or acidic adhesive primers. After the dissolution of hydroxyapatite minerals during adhesive bonding procedures, endogenous dentin enzymes become activated and slowly break down the collagen fibrils unless the fibrils are remineralized or fully impregnated by resin monomers [4-6]. However, in studies, partially or totally demineralized zones have been detected beneath the hybrid layer, increasing nanoleakage while decreasing the mechanical strength of the adhesive interfaces under loading [7,8]. Thereby, the degradation of hybrid layers occurs by the hydrolytic degradation of the resins, and the enzymatic degradation of the organic components, limiting the clinical longevity of adhesively bonded restorations [7,9,10]. Strengthening the collagen fibrils either by remineralization or cross-linking strategies and the inhibition of proteolytic enzymes using enzyme inhibitors have been the focus of recent studies [11-14].

Fluoride (F) is an antimicrobial material inhibiting several bacterial enzymes [15,16], and shows anti-cariogenic property by enhancing remineralization and preventing demineralization of dental hard tissues via the conversion of hydroxyapatite to less soluble calcium fluoride or fluorapatite minerals [17-19]. Dentin bonding systems and composite resin restoratives with varying levels of F content have been developed to prevent secondary caries formation, and enhance clinical success of adhesive restorations [20-23]. It has been also shown that salivary MMPs and dentin matrix-bound CCs could be inhibited by NaF and KF, which may be additionally beneficial for adhesive interface stability [24-26].

Incomplete polymerization of adhesive resins can be one of the factors increasing the water sorption and solubility of the resin components and permeability of hybrid layer, leading to hydrolytic degradation at the adhesive interfaces [27,28]. On the other hand, this permeability at the adhesive interface allows the release of fluoride ions from fluoridated adhesive resins while reducing the mechanical strength of adhesive interface depending on the solubilized and released fluoride ion quantity [29]. Therefore, the fluoride treatment of acid-etched dentin surface can be an alternative method for fluoride delivery to the adhesive interfaces to improve the degradation resistance of hybrid layers [30]. In this respect, the short- and long-

term bond strength to fluoride-treated dentin surface should be investigated in order to examine the degradation events at the resin-dentin interface [7,10,21].

The aim of this study was to evaluate the short- and long-term microtensile bond strength (μTBS) of an adhesive resin applied onto phosphoric acid etched-dentin surface pretreated with different concentrations of various fluoride compounds. The tested null hypothesis was that F treatment with NaF, KF and CaF_2 after the phosphoric acid-etching step of dentin bonding procedures does not improve the short- or long-term durability of dentin-resin adhesive interface.

2. Materials and methods

The schematic diagram of study protocol is depicted in Figure 1. A total of ninety-nine, freshly extracted, caries-free, third molars were used in this study according to the approval of Ethical Committee, Faculty of Medicine, University of Oulu (#19/2006). The teeth were firstly disinfected and stored in 0.02% NaN_3 solution at 4 °C for a maximum of one month until use. Then, the mid-coronal dentin was exposed by the removal of occlusal enamel with a low-speed diamond saw (Isomet saw, Buehler Ltd., Lake Bluff, IL, USA) under water-cooling. After wet-grinding with 600-grit SiC paper for 60 s to create standardized smear layer, the teeth were randomly divided into 11 different groups according to the pretreatment used (n=9/group) The groups were: no F treatment (control) and treatment with NaF, KF or CaF_2 solutions with the F contents of 6, 24 and 179 mM. F in each solution and commercially available bonding agent containing ≤2.5% (approximately 195 mM) KF (positive control) (Excite F, soft touch single dose, Ivoclar Vivadent). The fluoride solutions were prepared by mixing the respective amount of F compound with distilled water and the pH was adjusted to 7 before application, considering the pH-dependent proteolytic activity of dentin matrix-bound MMPs and CCs.

Bonding agent, restorative material, manufacturers, and their application protocols are shown in Table 1. A three-step etch-and-rinse adhesive system (Adper Scothbond Multi-Purpose, 3M ESPE) was used for the bonding procedures. Following etching with 32%wt phosphoric acid gel (Scothbond Universal Etchant, 3M ESPE), dentin surface was rinsed with water spray for 15 s, and blot-dried with cotton pellets. The F solution was applied with a

microbrush for 30 s, and the surface was blot-dried. Bonding agent was applied and light-cured (Elipar S10, 3M ESPE AG, Seefeld, Germany; 1186 Mw/cm_2 measured with CheckMARC, Blue Light Analytics, Halifax, Canada) according to the manufacturer's instruction (Table 1). Composite resin (Filtek Supreme XTE, 3M ESPE) build-up of 4 mm thickness was incrementally layered (2 mm layer thickness) and light-cured for 20 s per layer.

After restorative procedures, the specimens were stored in distilled water at 37 ^{o}C for 24 h, and were sectioned into μTBS test beams (0.9 mm x 0.9 mm). The beams originated from the same specimen were randomly divided into three groups according to the storage condition and assigned to be tested after 24 h (immediate), 6 months or 12 months of storage in artificial saliva (5 mM HEPES, 2.5 mM $CaCl_2.H_2O$, 0.02 mM $ZnCl_2$ and 0.3 mM NaN_3) [30] at 37 ^{o}C. The storage solution was not changed but its pH was monitored monthly. After storage, microtensile test was performed using a microtensile tester (Bisco Microtensile Tester; Bisco, Schaumburg, IL, USA) at a crosshead speed of 0.5 mm/min. The microtensile bond strength (MPa) was obtained by dividing the load at failure by the bonding surface area of the beam (mm^2). The fractured beams were removed and the fractured interfaces were sputter coated with gold (SC7620 Sputter Coater, Quorum, East Sussex, UK) and examined using scanning electron microscopy (SEM, Phenom-World, Eindhoven, The Netherlands) at 10 kV to identify the fracture types as 'cohesive in composite', 'cohesive in dentin', 'adhesive failure at the resin-dentin interface' and 'mixed'; a mixture of adhesive and cohesive failure within the resin-dentin interface [31].

The microtensile bond strength of all beams from the same tooth was averaged for statistical purposes and tooth was used as a statistical unit. Pre-test failures during specimen cutting and after aging procedures were included as 0 MPa in the calculation of mean and included in the statistical analysis. The microtensile bond strength data were analyzed using modified Levine test for homogeneity of variances. Since the equality of variance assumptions of the data were not violated, data were subjected to three-factor ANOVA to determine the main significant effects of pretreatment, concentration and storage time.; further, for interactions, two-way ANOVA and Sheffe's post hoc tests were performed. The data of fracture type were evaluated using Pearson Chi-Square test to compare the distribution of failure types. For all statistical tests, IBM SPSS v.23 (NY, USA) software was used at $p = 0.05$.

3. Results

The mean (±SD) μTBS of the control, the groups of NaF, KF, CaF_2 and Excite F are shown in Figure 2. The interactions between each test factor as 'F compound', 'F concentration' and 'storage time' were significant for μTBS ($p < 0.05$). After 24 h, among all F groups only NaF 24 mM showed significantly higher bond strength than the control ($p < 0.05$). In comparison to 24 h, the 6-month bond strength of 6 mM NaF and CaF_2 groups showed a significant increase ($p < 0.05$), but only 6 mM NaF maintained its bond strength for 12 months. However, compared to the control, none of F groups demonstrated a significant difference in μTBS after 6 months ($p > 0.05$). On the other hand, the μTBS differences between 24 h and 12-month as well as 6-month and 12-month were statistically significant ($p < 0.05$).

The comparison of F compounds showed no significant difference between NaF and KF ($p > 0.05$), whereas CaF_2 was significantly different than both NaF and KF after 24 h and 12-month testing ($p < 0.05$). When the F concentrations were compared after 12-month, significant differences were detected between 6 and 24 mM as well as 24 and 179 mM F.

The fracture types for the control, the groups of NaF, KF, CaF_2 and Excite F are displayed in Figure 3. According to Chi-square test, there were significant interactions between the fracture types and the each test variable ($p < 0.05$). At the 24 h testing, mostly mixed type fractures occurred in all test groups, except CaF_2 179 mM group showing 65% cohesive in composite type fractures. After 6 months, the control specimens primarily showed mixed type fractures, whereas in almost all fluoride groups, including Excite F, there were markedly less interfacial fractures especially in 6 mM groups. After 12 months, KF 24 mM and 179 mM groups showed the least (approximately 30%) mixed type fracture, and all failures in the 179 mM CaF_2 group were the mixed type involving pretesting failures ($p < 0.05$).

The representative SEM images of the fracture interfaces taken after the 12-month tests are shown in Figure 4. Under SEM, open dentin tubules and poorly resin infiltrated areas were observed in all groups, and it was particularly remarkable for the CaF_2 179 ppM specimens after 12 months.

4. Discussion

Fluoride containing etch-and-rinse and self-etch adhesives have been previously investigated to assess the anti-cariogenic effects of fluoride [21,22,32]. The expected outcomes were the sealing of the microgaps around restoration margins with the acid-resistant minerals, and the remineralization of the exposed but not resin covered collagen fibrils in hybrid layer. However, it was shown that the bond strength of self-etch adhesives and resin-based cements decreases when applied on sound dentin surface treated with fluoride containing dentin desensitizing agents [33-36]. This was explained by the prevention of resin infiltration into dentin due to the precipitated acid-resistant minerals. In the present study, NaF, KF and CaF_2 in the F concentrations of 6, 24 and 179 mM were treated to the acid-etched dentin surface before adhesive resin application in order to enhance the hybrid layer stability. These F concentrations were selected based on the fact that NaF and KF with F level $\geq$ 24 mM could inhibit the dentin matrix-bound CCs [28]. Considering cysteine cathepsins may also involve in MMP activation in demineralized dentin matrices [37], it was thought that fluoride, either via direct inhibition of CCs or indirect inhibition of MMPs, could be beneficial in the maintenance of adhesive interface durability. After 24 h, in addition to the slight μTBS increases detected in the most F groups, in particular NaF 24 mM gave significantly higher bond strength than the control group. After 12 months, there were no significant differences between the μTBS of F groups and the control, with the exception of the pretesting fractures seen in CaF_2 179 mM group. On the other hand, multivariate ANOVA further indicated that there was significant interaction between the μTBS to dentin and the treatment with NaF and KF, furthermore the effective F concentrations were 6 and 24 mM. Hence, the tested null hypothesis is partly rejected. This study showed that depending on the used compound and concentration, fluoride can improve the bond strength to acid-etched dentin, and may also enhance the long-term adhesive interface stability.

In control group, the bond strength to dentin did not show significant decrease, which was in line with the other studies reporting insignificant changes in the μTBS to dentin after 6 months [38] and 12 months [39]. Carvalho et al. [40] recommended the inclusion of minerals into the incubation solutions to ensure the activation of the endogenous dentin enzymes during μTBS testing. In the present study, the specimens were stored in artificial saliva containing zinc and calcium which are the required ions for the proteolytic action of MMPs [31]. Yet, in our study, the degradation of the hybrid layer could not be observed as a

significant reduction in the μTBS of the control as expected. On the contrary, there were significant increases in the bond strength values, which were also statistically significant for the 6 mM NaF and CaF_2 groups after 6 months. Due to the water sorption and the hydrolysis of resin monomers, the decrease in the elastic modulus of the hybrid layer and the nanoleakage increase were also highly significant with this adhesive resin when the specimens were tested after 12 months [8]. Hence, it can be thought that the increase in the μTBS over time might be related with the increasing elasticity of adhesive interface as a result of water sorption.

In the present study, the F level of 6 mM has been found effective in the improvement of bond durability, although this concentration was not adequate for the CC inhibition [28]. On acid-etched dentin, F ions do not only react with the dissolved calcium and phosphate ions on the superficial surface, but also with the free ions within the deep demineralized dentin [41]. Hence, owing to the new formed minerals, the mineral support of collagen fibrils vulnerable to enzymatic degradation can be improved, which can further explain the role of fluoride against to the enzymatic degradation. In accordance, it was also reported that the treatment with NaF saturated solution for 1 min could improve the immediate bond strength of self-etch adhesives to demineralized dentin [30]. Hence, it can be suggested that F in low levels may mainly provide mineral support for the defective hybrid layers whereas high F levels may interfere with the resin infiltration in dentin.

In this study, all fluoride groups, except CaF_2 179 mM, gave stable μTBS with a slight reduction during 12-month time, which can be regarded as the maintenance of hybrid layer integrity. The fracture type analysis demonstrated fracture type alterations from ‘mixed’ to ‘cohesive in composite resin’ or ‘cohesive in dentin’ fractures after 12 months, which could also be an indication of a durable bond. Together with the reduced cohesive strength of composite resin due to water sorption during storage [42], the fractures occurred within the composite resin build-up with nearly the same tensile forces, which were previously sufficient to fracture the specimens from adhesive interface. Therefore, considering both μTBS and fracture type analysis findings together, it can be asserted that the treatment of phosphoric acid-etched dentin surface with 6 or 24 mM of NaF and KF containing solutions can improve the adhesive interface durability. Similar to the KF groups, Excite F also showed a stable bond strength. On the contrary, CaF_2, a difluoride compound that has lower solubility

in water compared to monofluorides, caused mainly pretesting fractures due to the inadequate resin infiltration into dentin as confirmed by the SEM images.

In this study, Excite F gave similar immediate μTBS to dentin with its predecessor Excite [43,44]. A recent study examined the adhesive resin beams prepared by Excite F and Excite F infiltrated demineralized dentin beams in terms of the apparent modulus of elasticity and mass changes after storage in water for 24 h, 1, 3 and 6 months [45]. The authors reported that, regarding these parameters Excite F was not affected by the storage conditions and gave stable results. In addition, the present study showed that, considering the favorable results obtained with KF-treatment groups, the fluoride content of Excite F can also be beneficial for the maintenance of bond strength by recovering the mineral support of exposed, but not resin covered collagen fibrils. However, it should also be noted that the degradation of adhesive interface might be observed with extending the storage time up to 4 years [46].

Kato et al. [47] showed that degradation of dentin organic matrix by bacterial collagenase could be inhibited using 1.23 % NaF gel. It was also stated that, considering the activity differences of proteolytic enzymes, this anti-enzymatic effect could be significantly different on dentin matrix bound enzymes. Our previous study showed that dentin matrix-bound cysteine cathepsins, but not MMPs, may be inhibited by high F concentrations [28,29]. Regarding the favorable results obtained with NaF and KF compounds in the current study, F might also be effective in either silencing the enzymes via remineralization in low concentration, or with indirect inhibition due to the inhibited cysteine cathepsin activities. Action pathway of F ions can be further clarified with the examination of the enzymatic activities within and beneath the hybrid layer in further studies. Considering the complexity of the degradation process, μTBS testing after a longer storage time together with further nanomechanical analysis would give more information regarding the effects of potentially beneficial F compounds NaF and KF as pretreatment agents during dentin bonding, or when F compounds are incorporated into the adhesive resin composition.

5. Conclusions

Considering the limitations of this study, it can be concluded that NaF and KF treatment of acid-etched dentin surface do not negatively influence the initial or long-term bond strength.

According to the detected interactions between the bond strength to etched dentin and F treatment using NaF and KF with 6 and 24 mM F concentrations, fluoride containing pretreatment agents may be a potential method of improving the durability of adhesive interfaces.

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Figure Legends

Figure 1. The bar graphs of μTBS mean (±SD) μTBS of the control, NaF, KF and CaF_2 groups after 24 h (Fig. 1A), 6-month (Fig. 1B) and 12-month (Fig. 1C) storage in artificial saliva. The same uppercase letters indicate no significant difference between the groups at the same fluoride group ($p > 0.05$). The same lowercase letters indicate no significant difference between the groups at the same time-period ($p > 0.05$). After 24 h, the μTBS of NaF 24 mM was significantly higher than the control whereas there was no significant difference among the groups after 6 months. CaF_2 6mM gave lower bond strength to dentin compared to control after 12 months while CaF_2 179 mM caused mostly protesting failures.

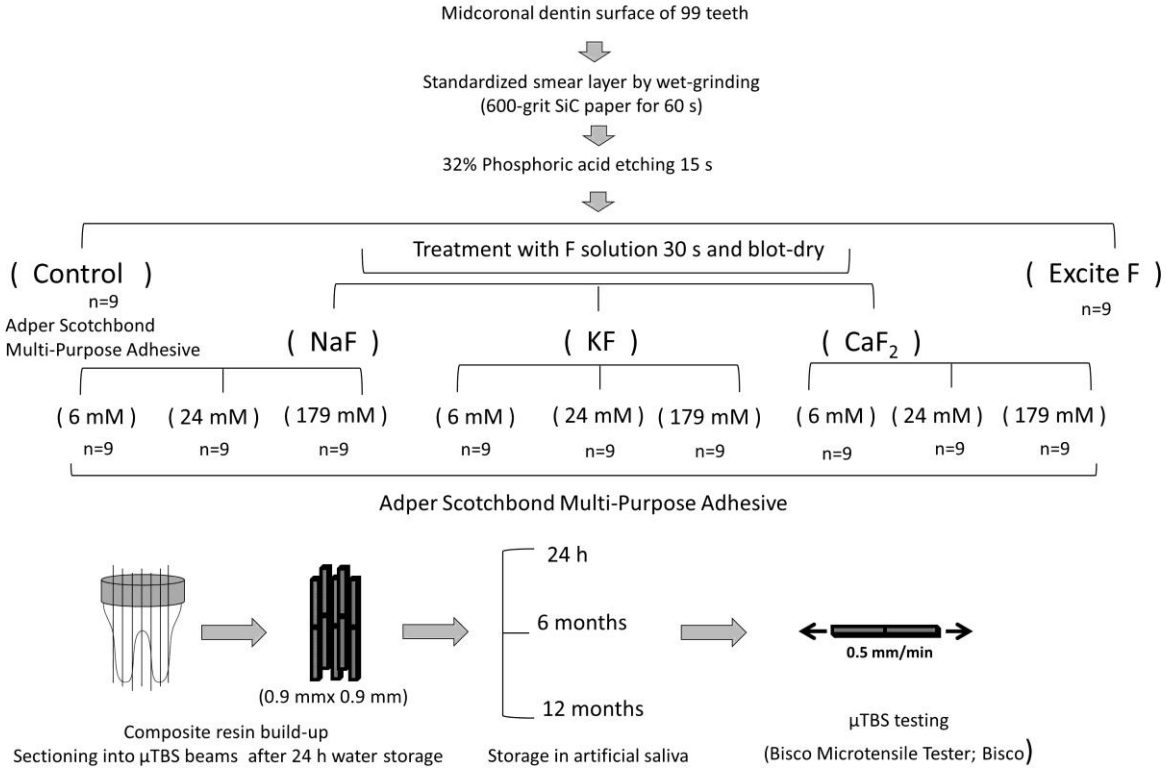
Figure 2. Fracture type analysis.

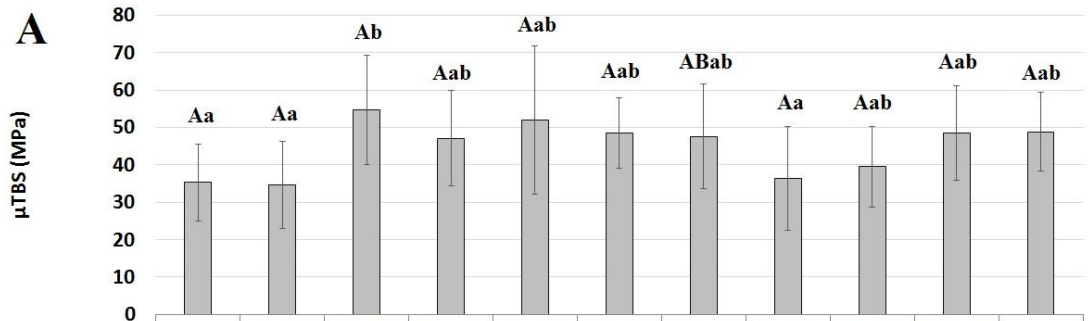
Figure 3. Representative SEM images from the fracture analysis of the specimens tested after 12 months of storage in artificial saliva. For all test groups, 'Adhesive interface' fractures mostly occurred at the adhesive resin-composite interface with partial involvement of exposed dentin tubules, except CaF_2 179 mM group in which dentin tubules were not mostly infiltrated by the adhesive resin.

Table 1. The materials used in the study

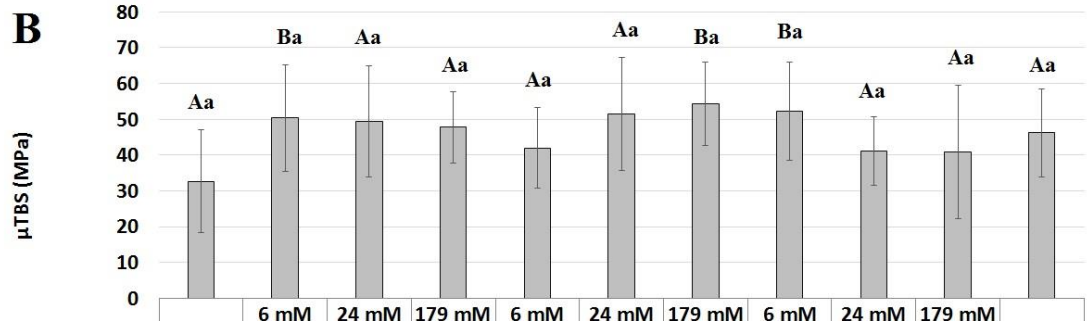
Material	Chemical Composition	Application Protocol	Manufacturer	Lot no
Scotchbond Universal Etchant	Phosphoric acid 32% by weight	Etch dentin surface for 15 s; rinse with water for 15 s; remove excess water by air syringe or blotting.	3M ESPE AG, Neuss, Germany	515105
Adper Scotchbond Multi-Purpose	HEMA, PAMA, GPDM, polyalkenoic acid, water	Apply the primer; gently dry for 5 s.	3M ESPE AG, Seefeld,	N325842

Primer			Germany	
Adper Scotchbond Multi-Purpose Adhesive	Bis-GMA, HEMA, dimethacrylates, polyalkenoic acid, initiators	Apply; light-cure for 10 s.	3M ESPE AG, Seefeld, Germany	N329945
Excite F	HEMA, dimethacrylate, phosphonic acid acrylate, silicone dioxide, initiators, stabilizers and KF (≤2.5%, approximately 195 mM KF), ethanol	Apply and agitate the surface for at least 10 s; disperse to a thin layer with a weak stream of air; light-cure for 10 s.	Ivoclar Vivadent Schaan, Liechtenstein	R54345
Filtek Supreme XT (A2 Shade)	Bis-GMA, UDMA, TEGDMA, and bis-EMA(6), PEGDMA resins and the combination of nano-size silica, zirconia and zirconia/silica filler particles.	Place in 2 mm increments; light-cure for 20 s.	3M ESPE AG, Seefeld, Germany	N474941
Abbreviations: Bis-EMA: Bisphenol-A-polyetheylene glycol dimethacrylate; BisGMA: Bisphenol-A-diglycidyl dimethacrylate; GPDM: glycero-phosphate dimethacrylate; HEMA: 2-hydroxyethyl methacrylate; UDMA: Urethandimethacrylate; KF: potassium fluoride; PEGDMA: Poly(ethylene glycol) dimethacrylate; PAMA; phtalic acid monomethacrylate; TEGDMA: Triethyleneglycol dimethacrylate.				

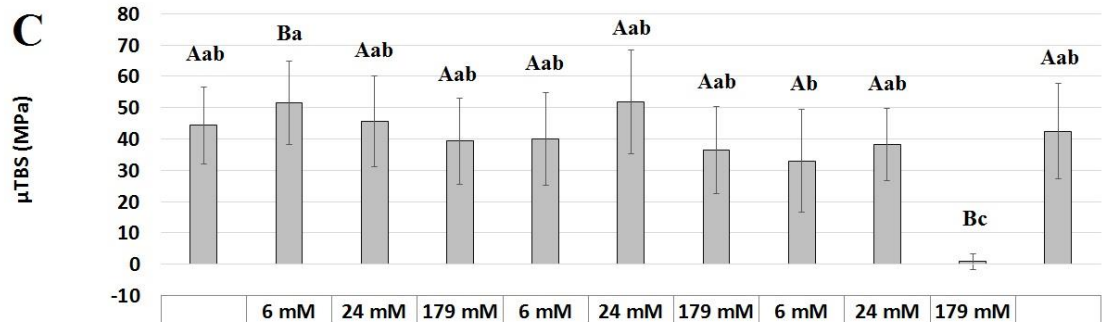

Midcoronal dentin surface of 99 teeth
Standardized smear layer by wet-grinding
(600-grit SiC paper for 60 s)
32% Phosphoric acid etching 15 s
Treatment with F solution 30 s and blot-dry
(Control)
n=9
Adper Scotchbond
Multi-Purpose Adhesive
(Excite F)
n=9
(NaF)
(KF)
(CaF2)
(6 mM)
(24 mM)
(179 mM)
n=9
Adper Scotchbond Multi-Purpose Adhesive
24 h
6 months
12 months
0.5 mm/min
(0.9 mmx 0.9 mm)
Composite resin build-up
Sectioning into µTBS beams after 24 h water storage
Storage in artificial saliva
µTBS testing
(Bisco Microtensile Tester; Bisco)



	Control	6 mM	24 mM	179 mM	6 mM	24 mM	179 mM	6 mM	24 mM	179 mM	Excite F
		NaF			KF			CaF2			
24 h mean µTBS	35,27	34,70	54,66	47,11	51,89	48,56	47,58	36,34	39,49	48,47	48,84



	Control	6 mM	24 mM	179 mM	6 mM	24 mM	179 mM	6 mM	24 mM	179 mM	Excite F
		NaF			KF			CaF2			
6-month mean µTBS	32,67	50,31	49,28	47,73	41,95	51,53	54,29	52,25	41,10	40,85	46,22



	Control	6 mM	24 mM	179 mM	6 mM	24 mM	179 mM	6 mM	24 mM	179 mM	Excite F
		NaF			KF			CaF2			
12-month mean µTBS	44,31	51,63	45,56	39,31	40,01	51,85	36,48	33,06	38,24	0,88	42,40