Original Article

The Effect of Calcium Phosphate-containing Desensitizing Agent on the Microtensile Bond Strength of Multimode Adhesive Agent

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Abstract

Objective: The aim of this study was to investigate the effect of calcium phosphate containing desensitizing pretreatments on the microtensile bond strength (MTBS) and microleakage of the multimode adhesive agent to dentin. **Materials and Methods:** In this study, twelve noncarious, freshly extracted human third molar teeth for MTBS and 20 premolar teeth for the microleakage test were used. The teeth were restored using Clearfil Universal Bond + Clearfil APX and Teeth mate Desensitizer (TMD). For MTBS test, Group 1: Self-etch, Group 2: Etch and rinse (G1 and 2, nondesensitizer treatment served as a control), Group 3: TMD/self-etch, Group 4: Acid-etch/TMD/etch and rinse. For microleakage test, Class V adhesive cavities (3 mm × 2 mm × 2 mm) were prepared and restored as mentioned before. The restored teeth were subjected to thermal cycling. The MTBS test was performed in all procedures. The MTBS data were submitted to a one-way ANOVA and *post hoc* Tukey test ($P < 0.05$). One tooth in each group was prepared for scanning electron micrograph examination. Marginal microleakage was measured based on the penetration of a 0.5% basic fuchsin dye. Dye penetration was then scored. The data were submitted to the Kruskal–Wallis and Wilcoxon signed ranks tests ($P < 0.05$). **Results:** Control groups exhibited a higher mean MTBS value than TMD groups, and there were statistical differences between the groups. TMD groups also demonstrated significantly less microleakage than control groups ($P < 0.05$). **Conclusions:** This study proves that the application of TMD with a multimode adhesive bonding system produced significantly lower MTBS and microleakage.

Keywords: *Calcium phosphate, desensitizer, microtensile bond strength, multimode dentin bonding agent*

Date of Acceptance:
31-Jan-2016

Introduction

Dentin hypersensitivity is characterized by short and sharp, pain arising from exposed dentin in response to stimuli (thermal, tactile, osmotic, evaporative, and chemical)[1] which may occur as a result of wear, caries, noncarious cervical lesions, or after dental procedures such as cavity preparation or reduction of vital abutment teeth.[2-4]

Dentin hypersensitivity can be treated with invasive (gingival surgery, pulpectomy, application of resins, and laser) and noninvasive (topical agents and dentifrices that contain a desensitizing ingredient) procedures. Noninvasive treatment options are considered to be the simplest, most cost-effective, and most efficacious first line of treatment for most patients.[5] Topical agents containing fluoride, oxalate, potassium nitrate and calcium phosphate occlude dentinal tubules and decrease the permeability of dentin.[6-11]

Calcium-containing desensitizing pastes should be used before bonding to occlude dentinal tubules, thereby managing immediate sensitivity and preventing

Access this article online

Quick Response Code:

Website: www.njcponline.com

DOI: 10.4103/1119-3077.187322

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How to cite this article: Siso SH, Dönmez N, Kahya DS, Uslu YS. The effect of calcium phosphate-containing desensitizing agent on the microtensile bond strength of multimode adhesive agent. Niger J Clin Pract 2017;20:964-70.

post-operative sensitivity during and after adhesive restoration.[12,13] Currently, calcium phosphate containing desensitizers have evoked considerable interest due to their biocompatible property, their outstanding characteristic in dentinal tubule occlusion and favorable reduction in dentin permeability in the oral environment.[14,15] Teethmate Desensitizer (TMD; Kuraray Noritake Dental Inc., Tokyo, Japan) is a recently developed calcium-phosphate containing material; tetracalcium phosphate (TTCP; Ca4[PO4]2O) and dicalcium phosphate anhydrous (DCPA; CaHPO4), whose combination could spontaneously transform to hydroxyapatite (HA; Ca10[PO4]6[OH] 2).[16]

Adhesive composite resin restorations may be performed after dentin hypersensitivity treatment procedures. However, the effect of desensitizers on the bond strength of adhesive restorations is controversial. Pashley *et al*.[17] reported that dentin surfaces were less favorable bonding substrates after using desensitizing agents.

Yang *et al*.[18] proposed that calcium-containing pastes, when applied after etching, could provide a new potential strategy to achieve effective tubule occlusion without affecting bonding effectiveness during etch and rinse adhesive restoration in clinical practice.

Recently, some manufacturers have released more versatile adhesive systems that give the dentist the opportunity to decide which adhesive strategy to use: Etch and rinse or self-etch.

This new family of dental adhesives is known as "universal" or "multi-mode" and represents the latest generation of adhesives on the market.[19,20] They are designed under the "all-in-one" concept of the already existing one-step self-etch adhesives but also incorporate the versatility of being adaptable to the clinical situation.[21]

The question still remains whether clinicians should consider using these new adhesives with prior calcium phosphate-containing desensitizing agent.

Therefore, this study has focused on the compatibility of calcium phosphate-containing desensitizing pastes when used with multimode adhesive systems. The purpose of this study was to evaluate the effect of calcium phosphate-containing desensitizing agent on the microtensile bond strength (MTBS) and microleakage of a multimode adhesive resin.

Materials and Methods

Ethical aspects

All teeth were collected after the donor's informed consents were obtained according to a protocol approved by the Ethics Committee of the Bezmialem Vakif University, Turkey (March 25, 2015, 4384).

Teeth and surface preparation

Microtensile bond strength test

Twelve noncarious, freshly extracted human third molar teeth, were used in this study. Soft and infected tissues were cleaned from the extracted teeth. After this, they were stored in 1% chloramine T at 4°C for 1 week to prevent bacterial growth. The teeth were sectioned parallel to the occlusal surface to expose the mid-coronal dentin by using a high-speed diamond bur (G & Z Instrumente Gmbh, Lustenau, Austria). The exposed dentin surfaces were ground using 600-grit silicon carbide paper under running water for 60 s to create a standard smear layer formation.

Microleakage test

Twenty caries-free human premolar teeth were used in this study. Two Class V cavities were prepared in each tooth, one in the buccal surface and the other in the lingual surface, with occlusal margins at the enamel and cervical margins at the cementum/dentin level. Dimensions of the cavities were 3 mm wide, 2 mm high, and 2 mm deep, prepared with a 1.6 mm/1 pieces diamond bur (G & Z Instrumente Gmbh, Lustenau, Austria) in a water-cooled high-speed handpiece. No bevels were placed. The cavities were restored according to manufacturer recommendations (as detailed below in Group 1,2,3,4) and polished with aluminum oxide polishing disks (Sof-Lex, 3M ESPE Dental Products, St. Paul, MN, USA). All specimens were then stored in distilled water at room temperature (24°C) for 24 h.

Experimental design

The prepared teeth were randomly divided into four groups (n = 3, for MTBS test, n = 10, each group for microleakage test) as follows.

Group 1

To the self-etch procedure, Clearfil Universal Bond (CUB) (Kuraray, Japan) was applied to dentin surface and rubbed for 10 s, then dried by blowing mild air for 5 s, and light cured (Demi Ultra, LED Ultracapacitor, Kerr, USA) for 5 s according to manufacturer instructions.

Group 2

To the etch and rinse procedure, specimens were etched with 37.5% phosphoric acid (Kerr Etchant, Kerr Corporotion, California, USA) for 15 s and rinsed thoroughly with water, gently air dried for 5 s, and then according to the self-etch procedure.

Group 3

The flattened dentin surfaces were initially pretreated with calcium phosphate containing desensitizing paste (TMD; Kuraray Noritake Dental Inc., Tokyo, Japan), respectively, according to the manufacturers' instructions and subsequently rinsed and dried, then CUB was performed as Group 1.

Group 4

Initially specimens were etched with 37.5% phosphoric acid for 15 s then treated with TMD paste as Group 3 then CUB was performed as Group 2.

The teeth were restored with composite resin (Clearfil AP-X, Kuraray, Japan). For MTBS test; 2-mm-high composite resin core buildups were created with the help of a matrix (Super Mat Adapt Supercap Matrix, Kerr, Switzerland). Incremental technique was used for this purpose, and each increment (2 mm) was cured for 20 s using an LED light curing unit (Demi Ultra, LED Ultracapacitor, Kerr, USA). The application protocols suggested by each manufacturer are listed in Table 1.

The specimens were subjected to 1000 thermal cycles between 5°C and 55°C. The dwelling time in the water was 30 s, and the transfer time was 10 s (SD Mechatronic Thermocycler, Germany).

Microtensile bond strength test

After treatment, all teeth were sectioned with a slow-speed saw (Isomet, Buehler Ltd., Lake Bluff, IL, USA) under water cooling into multiple 0.9 mm × 0.9 mm beams, with the "nontrimming" version (13) of the MTBS test. The obtained composite-resin-dentin sticks (n = 23) were performed in tension using a universal testing machine (SD Mechatronic MTD 500, Germany) at a crosshead speed of 0.5 mm/min until failure. The cross-sectional area at the site of failure was measured to the nearest 0.01 mm with a digital caliper (Model CD-6BS; Mitutoyo, Tokyo, Japan), from which the MTBS was calculated and expressed in MPa. Data were submitted to one-way ANOVA and *post hoc* Tukey tests. The significance level was set at = 0.05. One tooth in each group was prepared for scanning electron micrograph (SEM) examination.

Failure mode analysis

After MTBS test, the debonded dentin specimens were evaluated using a stereomicroscope (Nikon SMZ 800) at ×30 and classified as follows: A: Adhesive failure between dentin and resin; CD: Cohesive failure in dentin; CC: Cohesive failure in composite; and M: Mixed failure involving a maximum of 50% each of the adhesive and cohesive resin composite failures. In addition, the representative failures of each subgroup were observed under SEM.

Microleakage test

After thermocycling, coronary and radicular surfaces of the teeth, except the restorations and 1 mm around their margins, were isolated with two layers of nail varnish. The apexes of the teeth were sealed with composite resin to avoid penetration of the tracer toward the pulp. The specimens were then immersed in a solution of 0.5% basic fuchsin dye for 24 h to produce a visible stain while in the incubator (37°C). After this procedure, any surface adhered dye was carefully rinsed away with tap water. Dye penetration around the specimens was used to determine the presence of a gap around the restoration. Then, each tooth was sectioned longitudinally in a bucco-lingual plan through the center of the restoration with a water cooled, slow speed diamond blade (Mecatome T180, Presi, France) to obtain two sections of each tooth. The marginal sealing ability as indicated by the depth of dye penetration around the enamel or dentin margins was evaluated under a stereomicroscope (Nikon SMZ 800) at ×30. The following scoring scale was used to assess the extent of dye penetration at the tooth-restoration interface; Score 0: Without evidence of infiltration in the tooth/restoration interface; Score 1: Infiltration of the tracer up to one-third of the walls of the restoration; Score 2: Infiltration of the tracer in more than one-third of the walls of the restoration, without reaching the axio-cervical or axio-occlusal angles; Score 3: Infiltration of the tracer reaching the axio-cervical or axio-occlusal angles and going toward the pulp.

Statistical analysis of the results was obtained by the Kruskall–Wallis test (comparing the adhesives and the restorative materials) and the Wilcoxon signed ranks test (comparing the occlusal and cervical margins).

Results

Microtensile bond strength results

Mean MTBS values were calculated from all experimental groups and are shown in Table 2. The control groups (Groups 1 and 2) exhibited a higher mean MTBS value than TMD Groups (Groups 3 and 4), and there were statistically significant differences ($P < 0.05$). Moreover, there were significant differences among the control groups ($P < 0.05$). Highest MTBS value was seen in Group 2 (27.74 ± 7.84).

The distribution of failure modes, as observed by stereo microscopy, is shown in Table 3. Most failures were recorded in all groups as an adhesive failure. Moreover, the minimal mixed failure was seen in Group 3.

Table 1: Materials, compositions, and application procedures in this study

Materials (manufacturer)	Classification	Components	Application procedure
Clearfil Universal Bond (Kuraray, Noritake Dental Inc., Tokyo, Japan)	Adhesive resin	10 MDP, Bis-GMA, HEMA, hydrophilic aliphatic dimethacrylate, colloidal silica-silane coupling agent, di-camphorquinone, ethanol, water	Self-etch Apply bond and rub 10 s, dry by blowing mild air 5 s, light cure (Demi ultra, [Kerr] at 1100 mW/cm^2) for 5 s Etch and rinse Apply a phosphoric acid to the entire cavity (enamel and dentin) 10 s, then rinse and dry and then according to self-etch procedure
Clearfil AP-X (Kuraray, Noritake Dental Inc, Tokyo, Japan)	Hybrid composite	Bis-GMA, TEGDMA, silanated barium glass filler, silanated silica filler, silanated colloidal silica, di-camphorquinone inorganic filler %71, particle size 0.02–17 µm	Incremental placement and light curing each increment with dental visible light curing with Demi Ultra (Kerr), for 20 s (1100 mW/cm^2)
Kerr Etchant (Kerr Corporation, California, USA)	Etchant gel	37.5% phosphoric acid	Apply for 15 s, water rinse for 15 s, then dry with clean, oil-free air without desiccating the dentin
Teethmate desensitizer (Kuraray Noritake Dental Inc., Tokyo, Japan)	Calcium phosphate-containing desensitizing paste	Powder: TTCP DCPA Liquid: Water, preservative	Mix liquid and powder for more than 15 s Apply the slurry by rubbing for more than 30 s Rinse the excess slurry with water spray or by having the patient rinse

TTCP=Tetracalcium phosphate; DCPA=Dicalcium phosphate anhydrous; Bis-GMA=Bisphenol A-glycidyl methacrylatem; TEGDMA=Triethylene glycol dimethacrylate; MDP=10-methacryloxydecyl dihydrogen phosphate; HEMA=2-hydroxy ethyl methacrylate

Table 2: Means and standard deviations of microtensile bond strengths for each group

Groups	MPa ± SD
Group 1 (self-etch)	21.8 ± 6.53[a]
Group 2 (etch and rinse)	27.74 ± 7.84[b]
Group 3 (self-etch with TM)	16.17 ± 4.96[c]
Group 4 (etch and rinse with TM)	11.78 ± 5.3[c]

Mean values exhibiting different letters were significantly different. SD=Standard deviation; TM=Teethmate

Table 3: Failure mode distribution of fracture specimens after microtensile bond strength test

Groups	Failure type			
	A (%)	CC (%)	DC	M (%)
Group 1 (self-etch)	12 (52.17)	2 (8.69)	-	9 (39.13)
Group 2 (etch and rinse)	13 (56.52)	-	-	10 (43.47)
Group 3 (self-etch with TM)	18 (78.26)	3 (13.04)	-	2 (8.69)
Group 4 (etch and rinse with TM)	13 (56.52)	-	-	10 (43.47)

TM=Teethmate; CC=Cohesive in composite; DC=Cohesive in dentin; A=Adhesive; M=Mixed

Microleakage results

The microleakage scores are given in Table 4. According to the Kruskal–Wallis test, when the groups were compared in terms of microleakage scores, there were significant differences between control groups and TMD groups in occlusal and gingival leakage

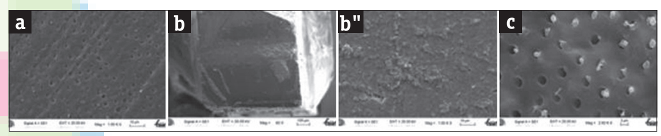

Figure 1: Control groups scanning electron micrograph figures. (a-c) scanning electron micrograph of different control group specimen's, but (b") higher magnification of same specimen (b)

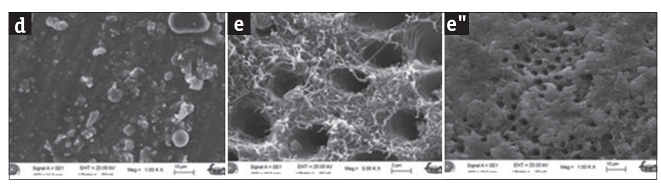

Figure 2: Teethmate groups scanning electron micrograph figure. (d and e) scanning electron micrograph of different specimen's , but (e"); higher magnification of same specimen (e)

scores ($P < 0.05$). In TMD groups, less microleakage was seen than in the control groups.

Scanning electron micrograph examination of dentin surface

Figures 1a, b, b", c and 2d, e, e" show the microstructure alterations of the dentin surfaces after treatment with or without TMD. In the control groups, both adhesive failure and mixed failure in the self-etch procedure were seen. The etch and rinse procedure shows an adhesive failure too. Dentinal tubules occluded by resin tags demonstrate that the failure was at the top of the hybrid layer. Following application of

Table 4: Microleakage scores and Kruskal–Wallis test results of the groups

Groups	Occlusal score				Gingival score				Occlusal median	Gingival median
	0	1	2	3	0	1	2	3		
Group 1 (self-etch)	2	4	3	11	2	4	3	11	3.00	3.00
Group 2 (etch and rinse)	-	-	2	18	-	-	-	20	3.00	3.00
Group 3 (self-etch with TM)	2	8	2	8	1	10	2	7	1.50	1.00
Group 4 (etch and rinse with TM)	7	6	1	6	7	5	-	8	1.00	1.00

TM=Teethmate

TMD, the dentinal tubules were occluded to different extents [Figure 2d, e, e"].

Discussion

In the present study, newly developed calcium phosphate-containing desensitizing paste (TMD) was applied to seal dentinal tubules before bonding with multimode adhesives (self-etch and etch and rinse). The results of the MTBS test showed that the application of TMD significantly affects MTBS during multimode adhesive bonding agent, especially when the multimode bonding agent is applied to the etch and rinse procedure, as the lowest MTBS value was found in this procedure. TMD was applied on the etched dentin surface, as dentinal tubules were mostly occluded with particles and a protective layer was formed [Figure 2e"].

The effectiveness of TMD in forming a layer on dentin regardless of pretreatment and maintaining tubule occlusion should be attributed to its chemical composition. TMD consists of TTCP and DCPA as the major starting components. The mixing of these two components provides a thick paste which can penetrate into the dentinal tubules [Figure 2e] by scrubbing on dry dentin surface. This occluding effect resulted in the immediate dentinal permeability reduction and hence, clinical hypersensitivity reduction could be expected. Previous studies on the combination of TTCP and DCPA demonstrated that this compound in an aqueous environment could transform to HA as the final product.[16,22] The mechanism of transformation was described as the dissolution of calcium and phosphate ions from TTCP and DCPA powder, which then precipitated as HA on the surface of the particles in the mixture[22] contributing to the setting and solidification process. This expected formation of HA gives a superior advantage over other desensitizers such as oxalate-containing mixtures, as the calcium-phosphate rich layer of TMD could act as a substrate for crystal growth by conducting calcium and phosphate ions from the surrounding supersaturated solution.[23]

In previous studies, different types of desensitizers have been used[24] such as monopotassium oxalate, sodium fluoride, strontium chloride + calcium carbonate, and gluma desensitizer.[25] These desensitizers when used with an adhesive system have given conflicting reports.

Arısu *et al.*[26] reported that desensitizing treatment procedures (except Clearfil SE Bond Nd: YAG laser) reduced the bond strength of a Clearfil SE Bond (two-step self-etch adhesive) to dentin. They used three topical agents (Vivasens [potassium fluoride], BisBlock [Oxalate], fluoride gel) for desensitizing. In contrast with other oxalate desensitizers, BisBlock's patented technique for the total-etch procedure occurs before oxalate and adhesive placement.[27]

This technique provides a durable effect because calcium is removed from the surface and oxalate crystals form deep within the dentinal tubules.[28]

Yiu *et al.*[29] reported that oxalate desensitizers did not negatively affect the bond strength of adhesives, such as Single Bond (3M ESPE) or One-Step (Bisco Inc.). However, Pashley *et al.*[17] reported a reduced bond strength because of crystal precipitation on the dentin surface. Pashley *et al.*[17] and Tay *et al.*[27] reported that when oxalates were used on acid-etched cavities that contained enamel margins, the enamel surfaces became covered with calcium oxalate crystals. In this study, oxalates are not used which contain calcium phosphate as Teethmate's (TM) has, even though MTBS results of our study are consistent with the study of Pashley *et al.*

The manufacturer claims that it has no film thickness and can be used easily under restorations. When TMD is applied on a decalcified (by acid etching and rinsing) dentin surface, it might create HA crystals, providing a localized source for occluding open dentinal tubules. Thanatvarakorn *et al.*[30] reported that the calcium-phosphate rich layer of TMD had interacted closely with the dentin surface. Dong *et al.*[31] showed that the calcium phosphate-based bioactive materials can effectively form a chemical bond with dentin tissue. Endo *et al.*[32] reported that the application of TMD within the tubules was effective on the inhibition of dentin demineralization. The obliteration of dentinal tubules by repeated application of TMD prevents demineralization, and the occluded dentinal tubules reduce dentinal fluid movement with consequent clinical improvement of dentin hypersensitivity. The current study supports the use of TMD to prevent postoperative sensitivity before applying the multimode adhesive to decrease microleakage of Class V composite restorations. Findings of the current study, however, contradict the manufacturer reports and show that the etch and rinse approach of a multimode (self-etch and etch and rinse) adhesive's (Clearfil Universal Bond) did not bond ideally to dentin surfaces. The MTBS value has shown the lowest bond degree of the etch and rinse group, therefore TMD is not recommended on dentin surfaces before the placement of direct restorations.

Microleakage has been a focus in detecting the performance of any restorative material used in tooth restoration.[33] The amount of microleakage is administrated by marginal adaptation of the restorative material to the tooth and is affected by polymerization shrinkage and the coefficient of thermal expansion among the tooth structure and the restorative material.[34]

Consequently, when temperature changes happen in the oral cavity, the tooth and the restoration extend and shrink at dissimilar rates, generating a gap at the restoration-tooth interface where microleakage can occur.

In vitro multimode adhesive resin bonding and microleakage performance of TMD are being described for the first time in this study. In the existing literature, there is only one study of TMD and it evaluated dentin permeability reduction and its integration with dentin surface before and after immersion in artificial saliva.[30] Therefore, the results of the present study were compared with the results reported by the manufacturer.

Our study showed a reduction of dentin's permeability in the microleakage method so that TMD may be applied to the dentin self-etch procedure of a multimode adhesive. Further studies are required to determine the clinical success of the newly introduced material.

Conclusion

Within the limitation of this study, calcium phosphate-containing desensitizing agent used with an multimode adhesive system provided to etch and rinse procedure lowest bond strength and a better marginal seal than the control group. To prevent postoperative sensitivity, the clinicians can reliably use self-etch procedure of multimode adhesive resin with a TMD under cavities.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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