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Effect of Saliva Contamination and Decontamination on Bovine Enamel Bond Strength of Four Self-etching Adhesives

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Clinical Relevance

Salivary contamination before and after priming could significantly reduce the enamel bond strength of self-etching adhesives. Proper isolation should be performed before and during application of the adhesives and placement of the resin composite. Thorough water-spraying could significantly improve the μTBS of saliva-contaminated enamel.

SUMMARY

Objective: This study evaluated the effect of saliva contamination on the bovine enamel microtensile bond strengths (μTBS) of four self-etching adhesives. Materials and Methods: The labial enamel surfaces of extracted non-carious bovine incisors were serially wet ground. The enamel surfaces were not contaminated (Group A), contaminated with saliva before/after priming (Groups B/C) or they were water-sprayed after salivary contamination occurred before/after priming (Groups D/E). Four self-etching adhesives and the corresponding resin composites from the same manufacturer (Clearfil SE Bond + Clearfil AP-X, Kuraray Co; Xeno III + Ceram X, Densply; Frog + Ice, SDI; FL Bond II + Beautifil II, Shofu Inc) were applied onto the enamel surfaces. The μTBS tests were performed with a micro tester (BISCO, Inc). The enamel surface was analyzed with AFM (Atomic Force Microscopy) before/after salivary contamination occurred or after the saliva-contaminated enamel was water-sprayed. The data were analyzed using one-way ANOVA, factorial design ANOVA and post hoc Tukey's HSD multiple comparisons.

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Results: Salivary contamination significantly reduced the μTBS of all the adhesives in the current study ($p<0.001$). Thorough water-spraying could significantly restore the μTBS of saliva-contaminated enamel to some degree ($p<0.05$) or fully restore it for Clearfil SE Bond, but it could not remove some proteins adsorbed on the enamel surface. Conclusion: Hydrophilic self-etching adhesives are negatively influenced by salivary contamination. Thorough water-spraying could significantly improve the μTBS of the saliva-contaminated enamel. Proper isolation should be performed before and during application of the adhesives and during placement of the resin composite.

INTRODUCTION

Since the concept of adhesive dentistry was introduced by Buonocore,[1] dental adhesives have been widely used in orthodontics, preventive dentistry and restorative dentistry. Achieving good moisture control is a prerequisite for permanent stable bonding of conventional hydrophobic resin bonding agents.[2] Saliva contamination is one of the most common problems encountered during bracket bonding, placement of fissure sealants on partially erupted mandibular molars or the restoration of para- or subgingival cervical lesions with resin composites.

In contrast to conventional hydrophobic bonding agents, self-etching adhesives contain not only hydrophobic, but also hydrophilic monomers. Two-step self-etch adhesive systems contain hydrophilic monomers in a self-etching primer and hydrophobic monomers in a bonding resin. One-step, one-bottle self-etching adhesives are "all-in-one" mixtures of hydrophilic and hydrophobic monomers. Both the hydrophilic and hydrophobic monomers are mixed just before the application of one-step, two-bottle self-etching adhesives. The self-etching adhesives simplify the application procedure from multiple steps (etching, rinsing, priming and bonding) into two steps (a combination of etching and priming, and bonding) or one step.

The majority of previously published reports have focused on the influence of saliva on orthodontic adhesives[3-18] or fissure sealants,[19-23] and only a few reports have dealt with the effect of salivary contamination on resin restorations.[24] Controversial data have been reported regarding the effect of saliva contamination on the enamel bond strength of adhesives,[3-27] because it depends not only on the individual adhesive used, but also on the adhesive in combination with the primer.[10,14]

For a traditional hydrophobic orthodontic luting agent (Transbond XT, 3M Unitek, Monrovia, CA, USA), all previous studies revealed that saliva contamination at any point in time (before or after application of the primer) deteriorates the enamel bond strength.[3,7,9,12-16,18] However, the application of a protective liquid polish (BisCover, BISCO, Inc, Schaumburg, IL, USA) or curing the primer on the enamel surface prior to saliva contamination could prevent a reduction of the enamel bond strength.[14,25] Interestingly, reapplication of the primer after contamination with artificial saliva could restore bond strength to its original level.[3]

For hydrophilic orthodontic adhesives, such as Transbond MIP (a moisture-insensitive primer, 3M Unitek), Transbond SEP (Transbond Plus self-etching primer, 3M Unitek) or Assure hydrophilic primer (Reliance, Itasca, IL, USA), many studies demonstrate significantly lower bond strength when the enamel was contaminated with saliva before priming,[3,8] after priming[3,8-9] or both before and after priming.[8-9,11] Furthermore, re-priming after contamination of the primer-treated surface with saliva could not completely restore the bond strength of Transbond MIP (3M Unitek)[3,8] or Assure (Reliance).[3] However, some contrary findings reveal that saliva contamination before priming has no significantly adverse effect on human enamel bond strength obtained with Transbond MIP (3M Unitek)[7,11,13] or Assure (Reliance).[12]

In the case of Assure[3-4,16,25] or Transbond MIP,[3,8,25] the bond strength is negatively influenced by saliva contamination but is clinically acceptable for bracket bonding.[5]

With regard to Transbond SEP (3M Unitek), some studies demonstrated no reduction in enamel bond strength for saliva contamination both before[8-9,16] and after priming.[6-8,16] However, contrary findings from other studies indicated significantly lower enamel bond strength in the case of salivary contamination after priming or both before and after priming.[9] However, Transbond SEP or iBond (a self-etching adhesive, Heraeus Kulzer, LLC, South Bend, IN, USA) were less influenced by water and saliva contamination than MIP and Transbond XT.[9,18] More interestingly, Turk and others[17] demonstrated that the bond strength of Transbond SEP was not significantly reduced by saliva contamination before priming for a short storage time in water (5, 15 or 30 minutes) and was significantly reduced after 24 hours of water storage, but it was low and clinically acceptable for bracket bonding.

Furthermore, prior to placement of precoated brackets (APC II, 3M Unitek), an application of Angle I, a self-etching primer (3M ESPE), can maintain adequate shear bond strength for APC II bracket bonding if saliva contamination occurs either before or after prim-

ing.[26] However, saliva contamination that occurs both before and after priming significantly deteriorates shear bond strength.[26] For Adper Prompt L-Pop, saliva contamination has a detrimental effect on enamel bond strength if saliva contamination occurs before priming or both before and after priming, but not after priming.[27]

Extensive research has been performed relating to the influence of salivary contamination on the performance of orthodontic resin bonding agents. However, to date, the effect of saliva contamination on the enamel bond strength of resin composite restorations using self-etching adhesives has been scarcely reported.[24]

Thus, the aim of the current study was to investigate the effect of saliva contamination on the enamel bond strength of different self-etching adhesives. The null hypothesis tested was that saliva contamination at different time points during the treatment procedure has no significant effect on the enamel bond strength of self-etching adhesives.

METHODS AND MATERIALS

Forty non-carious bovine incisors stored in 0.1% thymol solution at 4°C were used within three months of extraction. The labial enamel surfaces of the teeth were ground serially with 300-, 600- and 1200-grit SiC paper (MoPao 260E, Shangdong, China) under running water.

Bonding Procedure Without Contamination (Control, Group A)

Four self-etching adhesives (Clearfil SE Bond, Kuraray Co, Tokyo, Japan; Xeno III, Dentsply, Konstanz, Germany; Frog, Southern Dental Industries [SDI], Bayswater, Victoria, Australia; FL-Bond II, Shofu Inc, Tokyo, Japan) were applied onto the enamel surfaces strictly according to the manufacturers' instructions. Subsequently, the respective resin composites from the same manufacturer (Clearfil AP-X, Kuraray Co; Ceram X, Dentsply; Ice, SDI; Beautifil II, Shofu Inc) were placed on the adhesive-pretreated enamel surfaces in two increments, each 2 mm thick, and light-cured for 40 seconds, respectively.

All light curing was performed using a MACO curing light with a power output of 800 mW/cm^2 (MACO, SLC-VIIIB, Hangzhou, China). The application procedures and components of the adhesives and resin composites are summarized in Table 1.

Bonding Procedures After Saliva Contamination

Contamination Procedure 1 (Group B)

Fresh whole saliva was provided by a healthy female principal investigator (Jiang Q) who was instructed to restrain from eating and drinking one to two hours before saliva collection. The bovine enamel surfaces were microbrushed with fresh whole saliva, left undisturbed for five seconds and gently air blown for three-to-five seconds. Subsequently, the adhesive and resin composite application took place as in Group A.

Contamination Procedure 2 (Group C)

The adhesives were applied to the bovine enamel surfaces as in Group A but not light-cured; they were contaminated with fresh whole saliva applied with a microbrush, left undisturbed for five seconds, gently air blown for three-to-five seconds and light-cured for 20 seconds. Placement of the resin composites was the same as in Group A.

Bonding After Decontamination Procedures

Decontamination Procedure 1 (Group D)

After the bovine enamel surface had been contaminated with fresh saliva as in Group B, the surface was thoroughly water-sprayed for 30 seconds and gently air blown for three-to-five seconds. The subsequent treatments (adhesive application and resin composite placement) were the same as Group A.

Decontamination Procedure 2 (Group E)

After saliva contamination had been performed on the uncured primed enamel surface as in Group C, the enamel surface was thoroughly water-sprayed for 30 seconds and gently air blown for three-to-five seconds. Subsequently, the enamel surfaces were retreated with the adhesives. Light curing of the reapplied adhesive layer and placement of the resin composite were performed as in Group A.

Microtensile Bond Strength (μTBS) Test

After all the specimens had been stored in tap water at room temperature for 24 hours, they were perpendicularly sectioned through the resin-enamel interfaces using a slow-speed diamond saw (Isomet 1000, Buehler, Lake Bluff, IL, USA) under continuous water cooling. The specimens were prepared into beams about 1 x 1 x 8 mm. The microtensile bond strength test was performed with a Micro Tensile Tester (BISCO, Inc) at a tensile speed of 1 mm/minute. After the bond strength test, the exact dimension of each fracture surface was measured with a micrometer device. The μTBS was calculated in MPa.

Atomic Force Microscopy (AFM)

After the labial enamel surface of a bovine incisor had been polished as mentioned above, a two-sided parallel enamel disk was prepared with a thickness of about 1 mm. The enamel surface was observed with an atomic force microscope (NanoScope IVa AFM, Veeco/DI, Plainview, NY, USA) using the tapping mode. Subsequently, the enamel surface was exposed to whole fresh saliva applied with a microbrush for five seconds, gently air blown for three-to-five seconds and again observed with AFM. After AFM analysis, the enamel surface was thoroughly water-sprayed for 30

Table 1: *Self-etching Adhesives and Resin Composites Used in This Study*

Products (manufacturers, batch #)		Ingredients	Application Procedure of Adhesives and Resin Composites	Codes
Clearfil SE Bond (Kuraray Co, Tokyo, Japan)	Primer (00047A)	MDP, HEMA, water, initiator	Apply the primer for 20 seconds, gently air blow, apply the bond, gently air blow, light-cure for 20 seconds.	SE
	Bond (00111A)	MDP, HEMA, dimethacrylates, initiator, microfiller		
Clearfil AP-X	AP-X (00631)	barium glass, silanated colloidal silica (filler content 85.0 wt%), Bis-GMA, TEGDMA	Place two increments, each 2 mm thick each, light-cure each increment for 40 seconds.	
Xeno III (Densply, Konstanz, Germany)	liquid A (0612000217)	HEMA, ethanol, water, aerosil, stabilizers (BHT)	Apply a mixture of liquid A and B (1:1) for 20 seconds, gently air blow, light-cure for for 20 seconds.	XIII
	liquid B (0612000217)	Pyro-EMA, PEM-F, UDMA, CQ BHT, ethyl-4-dimethylaminobenzoate (co-intiator)		
Ceram X	Ceram X (0506001472)	76 wt% 2.3 nm organically modified ceramic nano-particles,10 nm nanofillers, ~1 μm conventional glass fillers, a methacrylate modified polysiloxane, a dimethacrylate resin	Place two increments, each 2 mm thick, light-cure each increment for 40 seconds.	
Frog (SDI, Southern Dental Industries, Victoria, Australia)	Primer (080158)	phosphoric acid ester monomer, HEMA, water, dimethacrylate monomer, photoinitiators, stabilizer	Apply the primer for 20 seconds, gently air blow, apply the bond, gently air blow, light-cure for 20 seconds.	FR
	Bond (080262)	phosphoric acid ester monomer, HEMA, dimethacrylate monomer, silicon dioxide filler, photoinitiators, stabilizer		
Ice	Ice (071177N)	60~82 wt% 0.6 μm silica filler, UDMA	Place two 2-mm thick increments, light cure each increment for 40 seconds.	
FL-BOND II (Shofu Inc, Kyoto, Tokyo, Japan)	Primer (040707)	Water, ethanol, carboxylic acid monomer, phosphoric acid monomer, Initiator	Apply the primer for 20 seconds, gently air blow, apply the bond, light-cure for 20 seconds.	FB
	Bond (040710)	S-PRG filler based on fluoroboroaluminosilicate glass, UDMA, TEGDMA, 2-HEMA, Initiator		
Beautifil II	Beautifil II (080726)	S-PRG filler based on fluoroboroaluminosilicate glass, UDMA, TEGDMA, 2-HEMA, Initiator	Place two increments, each 2 mm thick, light-cure each increment for 40 seconds.	

Abbreviations:
MDP: methacryloyloxydecyl dihydrogenphosphate; TEGDMA: triethylene glycol dimethacrylate; HEMA: 2-hydroxyethyl methacrylate;
Pyro-EMA: tetramethacryloyloxyethyl pyrophosphate; FEM-F, pentamethacryloyloxyethylcyclohexaphosphazene monofluoride;
UDMA: urethane dimethacrylate or 1,6-di (methacryloyloxyethylcarbamoyl)-3,30,5-trimethylhexane;
CQ: camphorquinone or camphoroquinone or 1,7,7-trimethylbicyclo-[2,2,1]-hepta-2,3-dione (photo-initiator);
BHT: butylhydroxytoluene or butylated hydroxytoluene or 2,6-di-(tert-butyl)-4-methylphenol (inhibitor); S-PRG: surface pre-reacted glass ionomer.

seconds, gently air blown for three-to-five seconds and observed with AFM for a third time.

Statistics

Statistical analysis was carried out using the SPSS software package (SPSS Software, version 16.0, SPSS Inc, Chicago, IL, USA). Comparison of the μTBS of the four adhesives was performed using one-way analysis of variance (ANOVA). Factorial design ANOVA followed by post hoc Tukey's HSD multiple comparisons were used to statistically analyze the μTBS in the subgroups (with and without salivary contamination).

RESULTS

Mean Microtensile Bond Strength (μTBS)

The different adhesives and the different enamel surface treatments significantly influenced the bovine enamel microtensile bond strengths ($p<0.001$). All μTBS data are summarized in Table 2. Multiple comparisons of the μTBS of all subgroups in each adhesive group are shown in Table 3.

Comparison of the four adhesives in the control group (A) indicated that XIII revealed significantly higher μTBS than FB ($p<0.01$); however, no significant difference in μTBS was found among the adhesives SE, XIII

and FR ($p>0.05$). Saliva contamination significantly reduced the μTBS of all the adhesives involved in this study ($p<0.001$, Tables 2 and 3). However, decontamination procedures (water-spraying) could not restore the μTBS to its original level ($p<0.05$), except for SE. The μTBS obtained on the enamel surface contaminated with saliva before adhesive application (Group B) could be significantly improved by thorough water-spraying before placement of the adhesive (Group D) ($p<0.05$). Interestingly, the μTBS measured after contamination of the adhesive-treated enamel surface with saliva was significantly improved by thorough water-spraying before reapplication of the adhesive (Group E) using the materials XIII and FR ($p<0.05$), but not in the case of the SE and FB adhesives SE and FB ($p>0.05$).

AFM

The surface morphology of the highly polished bovine enamel disk revealed individual enamel crystallites and showed some scratch lines (Figures 1a and 1b). The enamel disks contaminated with fresh saliva revealed many small dots or clusters of proteins deposited on the enamel surface, and the enamel crystallites were partially covered by the adsorbed protein layer (Figures 1c and 1d). The saliva-contaminated enamel surface sprayed with water revealed some residual protein dots with the enamel crystallites being clearly detected (Figures 1e and 1f).

DISCUSSION

All materials used in the current study performed well without saliva contamination, suggesting that all the materials are suitable for clinical application. The material XIII revealed a significantly higher bond strength than FB ($p<0.05$); however, there are not significant differences in μTBS among SE, FR and FB ($p>0.05$). In previous reports, the bond strength to ground enamel ranged from 23.0 to 57.2 MPa for SE,[28-31] from 19.5 to 38.4 MPa for XIII[28-29] and was above 24 MPa for FB.[32] Discrepancies regarding the previously reported bond strength values might result from the different surface treatments, different modes of measurement and different operators.[33-34]

Regarding the effect of saliva contamination on the enamel bond strength of self-etching adhesives, in previous studies, self-etching adhesives were usually used prior to bracket bonding[6-10,16-18,26-27] or placement of the fissure sealants[21-23] and they were rarely applied with salivary contamination for resin restorations.[24]

In the current study, short-term contamination with saliva either before or after priming had a detrimental effect on the enamel μTBS of self-etching adhesives ($p<0.001$). The null hypothesis was totally rejected. This finding is consistent with previous reports.[24] The current μTBS findings clearly indicate that even short-term salivary contamination of the enamel surface strongly interferes with the obtainable bond strength. Saliva composition includes inorganic compounds, enzymatic molecules and organic macromolecular, proteinaceous compounds.[35] Thus, even a short-term application of saliva to the enamel surface and subsequent air drying will result in protein adsorption and the formation of the initial (basal) pellicle layer.[36] Salivary macromolecules (glycoproteins) adsorbed on the saliva-contaminated enamel surface will follow diffusion of the self-etching primer into the enamel surface (hybrid layer). Thereby, glycoproteins may

Table 2: *Mean Microtensile Bond Strengths for All Experimental Groups (Means ± SD; MPa, n=12 in Each Subgroup, ANOVA)*

Groups		Dental Materials			
		SE	XIII	FR	FB
Surface Treatment	A	25.68 ± 4.35[a,b]	28.46 ± 4.55[a]	25.42 ± 4.18[a,b]	22.43 ± 2.38[b]
	B	17.57 ± 3.12	18.24 ± 3.77	11.47 ± 2.82	16.27 ± 1.99
	C	19.93 ± 2.66	16.00 ± 3.73	11.74 ± 2.62	17.13 ± 2.48
	D	21.90 ± 4.06	22.66 ± 3.25	15.72 ± 1.68	19.83 ± 1.53
	E	23.11 ± 1.94	22.51 ± 2.35	15.23 ± 2.12	19.39 ± 1.98

Note: A: *Non-contamination.* ***B:*** *Salivary contamination before priming.* ***C:*** *Salivary contamination after priming.* ***D:*** *Water-rinsing after the enamel contaminated with saliva.* ***E:*** *Water-rinsing after the primed enamel contaminated with saliva. Within Group A, the different superscript letters indicate significantly different μTBS between the adhesives (p<0.01), while the same superscript letter indicates insignificant difference (p>0.05).*

Table 3: *Multiple Comparisons of the Mean μTBS for All Subgroups in Each Adhesive Group (ANOVA Followed by Post Hoc Tukey's HSD Test)*

Groups		Dental Materials															
		SE				XIII				FR				FB			
Surface Treatment		E	D	C	B	E	D	C	B	E	D	C	B	E	D	C	B
	A	ns	ns	<.01	<.001	<.01	<.01	<.001	<.001	<.001	<.001	<.001	<.001	<.01	<.05	<.001	<.001
	B	<.01	<.05	ns		<.05	<.05	ns	.	<.05	<.01	ns		<.01	<.01	ns	
	C	ns	ns			<.001	<.001			<.05	<.01			ns	<.05		
	D	ns				ns				ns				ns			

Note: *A, B, C and D is the same as above-mentioned. ns: not significant. The significance level is 0.05.*

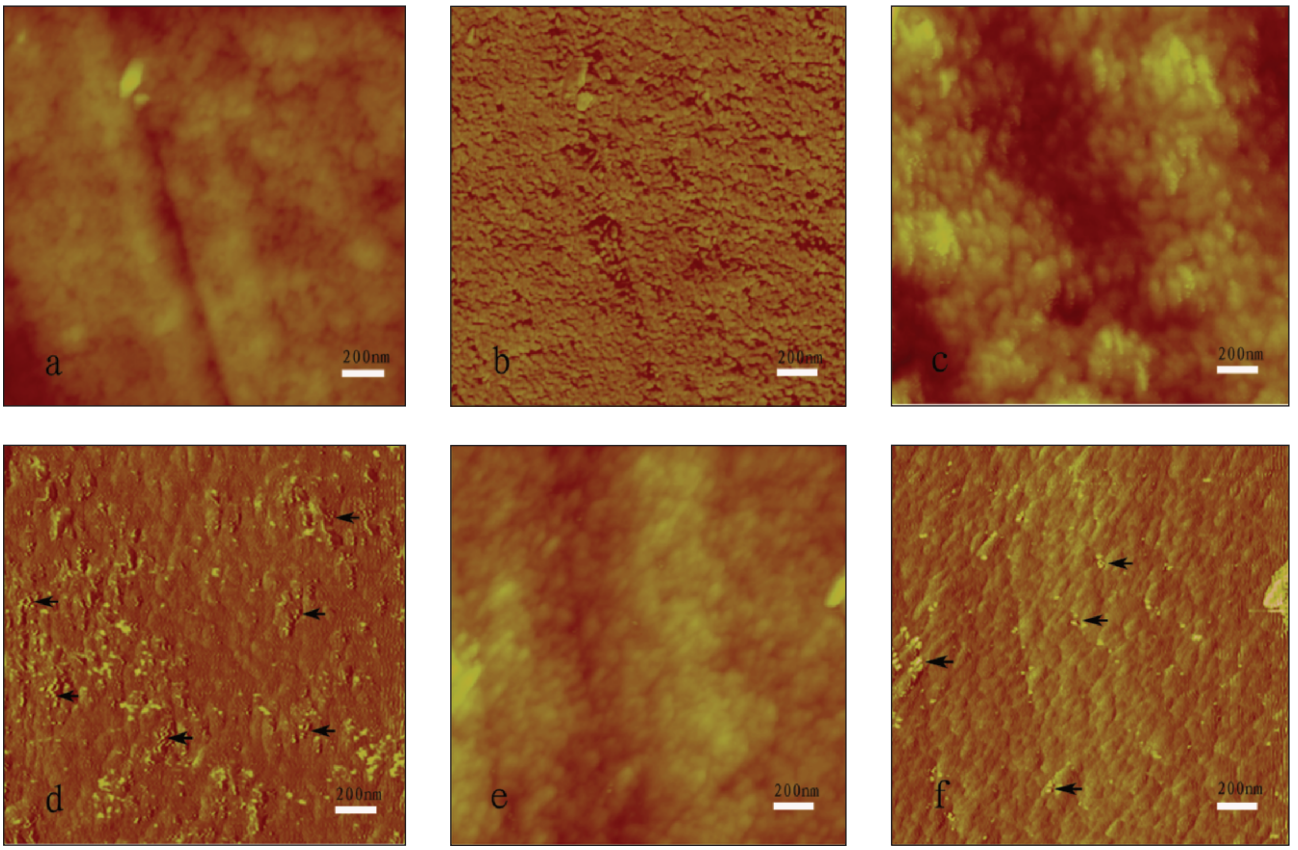

Figure 1: *AFM analysis of bovine enamel surfaces (Figures 1a,c and e: topographic images; Figures 1b,d and f: phase images). The polished enamel surface without salivary contamination reveals the individual hydroxyapatite crystallites (Figures 1a and 1b). After contamination with saliva (Figures 1c and 1d), the individual enamel crystallites are partially masked by the adsorbed proteins, and small dots or clusters of adsorbed proteins are deposited on the surface (phase image, Figure 1d). After the saliva-contaminated enamel surface has been sprayed with water, the individual enamel crystallites become clearly visible again (Figure 1e), and some residual protein dots are detected on the surface (phase image, Figure 1f). (bars = 20 nm).*

compete with hydrophilic monomers during the hybridization process,[37] prevent polymerization of the primer[24,38-39] and reduce bond strength.[9,17,24,26,40] As a consequence, the nano-interaction zone[41] and the inter-crystallite nano-interaction/-retention layer[42] will not be well created due to saliva protein interference.

Interestingly, simple water-spraying of the saliva-contaminated enamel surfaces could completely restore the bond strength of SE and greatly improve the bond strength of XIII and FB. Salivary proteins on the enamel surfaces could not be removed by simple water-rinsing. This was confirmed by the current AFM findings and is consistent with Silverstone's findings.[43] Silverstone observed that salivary exposure times greater than one second will lead to an adherent organic coating that is not removed by conventional washing methods.[43] Self-etching adhesives penetrate through the basal pellicle into the enamel substrate during the hybridization process[44] and, to some degree, the tiny protein (pellicle) layer adsorbed onto the enamel surfaces might still interfere with the bond strength of the adhesive. Most interestingly, simple water-rinsing of the saliva-contaminated primed enamel surfaces, air-drying and subsequent reapplication of the adhesives could restore the μTBS values, at least for SE and, to some extent, for XIII and FB. All the adhesives used in the current study possess a functional monomer, such as phosphoric acid esters (PAEs) and some hydrophilic monomers (see Table 1). Therefore, some hydrophilic monomers, saliva proteins and soluble calcium salts of PAEs after the reaction of PAEs with hydroxyapatite[45] on primed enamel surfaces could be washed out by water rinsing. Some insoluble calcium salts of PAEs will remain chemisorbed on the enamel surfaces,[45-47] thus providing an easy-to-wet surface for subsequently applied bonding and also providing the potential for chemical bonding. SE contains methacryloyloxydecyl dihydrogen-phosphate (MDP), and the other manufacturers do not declare which types of PAEs are in the adhesives. Among the self-etching adhesives involved in the current study, saliva contamination could dramatically reduce the μTBS of the adhesive Frog. The current findings indicate that SE is more saliva-tolerant than

the other adhesives tested[6] and, among the adhesives used in the current study, Frog is the most sensitive to saliva contamination. Chemical bonding efficacy is expected to contribute to the adhesive potential to tooth hard tissues,[46] suggesting that the chemical bonding efficacy of MDP is better than that of the other PAEs, according to the current μTBS data.

Giomer (Beautifil II) is a newly developed type of aesthetic dental restorative material that uses surface pre-reacted glass ionomer (S-PRG) technology to form a stable glass ionomer phase in the material.[48] Unlike compomers, the reaction of polyalkenoic acid with fluoroaluminosilicate glass particles takes place before inclusion in the silica-filled urethane resin.[32] Giomer requires an application of FL-Bond II (a self-etching adhesive containing S-PRG nanofillers) prior to placement. Giomer (FL-Bond II/Beautifil II) in the current study performed similarly to the bond strength after one-hour storage in the previous report; however, it performed lower than the bond strength after seven-day storage.[32] For the first time, the current findings indicate that saliva contamination had an adverse effect on the bond strength of Giomer

CONCLUSIONS

1. Hydrophilic self-etching adhesives are negatively influenced by saliva contamination. Proper isolation should be performed before and during application of the adhesives and placement of the resin composite.
2. Salivary protein on the enamel surface could not be removed by water-rinsing and possibly interfered with the obtainable bond strength. The chemical bonding efficacy of some functional monomers, such as MDP, is promising when dealing with saliva contamination.

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