Effect of Remineralizing Agents on Bond Strength of Resin-Composites to Primary Enamel

Faika Y Abdelmegid*/ Fouad S Salama**/ Eman I Abouobaid***/ Hassan S Halawany ****/ Mohamad K Alhadlaq*****

Aim: *The purpose of this in vitro investigation was to assess the effect of three remineralizing agents (Voco Remin Pro®, Uncle Harry's* remineralization kit, *Sunshine remineralization gel) on the shear bond strengths of two resin-composites (Tetric® N-Ceram and Filtek™ Z250 Universal Restorative) to enamel of primary molars.* ***Study design:*** *Ninety-six enamel specimens were prepared and randomly distributed to eight groups according to the control, remineralizing agents, and resin composite used. Shear bond strength was measured at a crosshead speed of 0.5 mm/min and the type of bond failure was recorded.* ***Results:*** *The highest shear bond strength (Mean±SD) in MPa was for Tetric® N-Ceram/control [21.06±1.68] while the lowest was for Filtek™ Z250/Sunshine remineralization gel [11.98±1.46]. Tukey HSD Post Hoc Tests showed significant difference between Tetric® N-Ceram/control and all other groups (p=0.0001) except Filtek™ Z250/control. In addition, there was significant difference between Filtek™ Z250/control and all other groups (p=0.0001) except Tetric® N-Ceram/control and Tetric® N-Ceram/Uncle Harry's* remineralization kit*. Mode of failure was cohesive (9.38%), adhesive (55.21%), and mixed (35.42%).* ***Conclusions:*** *The three tested remineralizing agents affect shear bond strength of the tested resin-composites to enamel of primary teeth. In general, shear bond strength values were acceptable. Mode of failure was mostly adhesive.*

Keywords: *Bond strength, Remineralizing agents, Enamel, Primary teeth*

From King Saud University, Riyadh–Kingdom of Saudi Arabia.
*Faika Y Abdelmegid, Department of Oral Medicine and Diagnostic Sciences, College of Dentistry.
**Fouad S Salama, Department of Pediatric Dentistry and Orthodontics, College of Dentistry.
***Eman I Abouobaid, Post-graduate Doctorate Student.
**** Hassan S Halawany, Department of Periodontics and Community Dentistry.
******Mohamad K Alhadlaq, Department of Pediatric Dentistry and Orthodontics, College of Dentistry.

Send all correspondence to:
Fouad Salama
Department of Pediatric Dentistry and Orthodontic
College of Dentistry, King Saud University
PO Box 60169 Riyadh 11545; Saudi Arabia
Phone: +9661-4694814
E-mail: fspdkids@gmail.com

INTRODUCTION

One of the most important concept in the field of saving tooth structure is the remineralization.[1] The new tooth remineralization technologies include compounds with or without the additional or synergistic effects of fluoride to enhance the remineralization process and improve the mechanical properties of the demineralized substrate.[2,3] Some used remineralization materials are casein phosphopeptide stabilized amorphous calcium phosphate (CPP-ACP), unstabilized amorphous calcium phosphate (ACP), a bioactive glass containing calcium sodium phosphosilicate, hydroxyapatite which contains calcium, phosphate, casein phosphopeptide-amorphous calcium phosphate fluoride (CPP-ACPF), and tricalcium phosphate fluoride (TCP-F).[4] Remin Pro® is another type of remineralizing agent, which in contrast to CPP-ACP products contains calcium, phosphate in the hydroxyapatite form.[5] In addition, fluoride and Xylitol have also been included in this product.[5] Remin Pro® contains hydroxylapatite particles much similar to calcium and phosphate ions in CPPACPF that are deposited on the bleached enamel surface and increase the micohardness of teeth.[6] Uncle Harry's remineralization kit is Remineralization Liquid which contain Alkalizing Ionic Minerals, Himalayan Pink Sea Salt, Essential Oils of Peppermint, Eucalyptus, Clove, Wintergreen, and Oregano. While Sunshine remineralization gel contains calcium pyrophosphate which has been shown to be effective in the enamel

remineralization process. Since most systems rely on phosphate and calcium compounds, their influence is mostly due to the augmentation of the natural ability of saliva to remineralize mineral loss.[4,7] A study evaluated the effect of casein phosphopeptide amorphous calcium phosphate (CPP-ACP) and CPP-ACP with fluoride (CPP-ACP-F) on the shear bond strength of orthodontic brackets bonded with two different adhesive systems concluded that the shear bond strength of the orthodontic brackets was not significantly influenced when the brackets were treated with either CPP-ACP or CPP-ACP-F and cured with light-cure bonding system, whereas decreased bond strength was seen with chemical-cure adhesive, which was within the clinically acceptable limits.[8] Another study compared shear bond strength of three self-etching adhesives to enamel bleached with carbamide peroxide, treated with casein phosphopeptide-amorphous calcium phosphate (CPP-ACP), or treated with CPP-ACP subsequent to bleaching with carbamide peroxide concluded that bleaching reduced shear bond strength to enamel, but CPP-ACP application did not affect the bond strength to intact and previously bleached enamel.[9]

To the best of our knowledge, few studies have compared the effect of remineralizing agents such as Voco Remin Pro®, Uncle Harry's remineralization kit, and Sunshine remineralization gel on shear bond strength of sound enamel of primary teeth. Thus, the purpose of this *in vitro* investigation was to evaluate the effect of three remineralizing agents (Voco Remin Pro®, Uncle Harry's remineralization kit, Sunshine remineralization gel) on the shear bond strengths of two resin-composites (Tetric® N-Ceram and Filtek™ Z250 Universal Restorative) to enamel of primary molars. The tested null hypothesis was that there is no differences between shear bond strength of resin composites to sound enamel of primary teeth after application of the three tested remineralizing agents.

MATERIALS AND METHOD

The research procedures were approved by the Ethical Committee of Human Studies at College of Dentistry Research Center. Ninety-six extracted primary molars with sound intact buccal surfaces were used in this study. All the teeth were obtained from different clinics, cleaned, and stored in 1% thymol solution. Roots were removed using low-speed carborundum disks (3M™ ESPE™, St. Paul, MN, USA) under water spray. Specimens were mounted inside a cylindrical-shaped plastic, 2.5 cm in diameter and with a height of 2.5 cm using autopolymerizing acrylic resin (Ortho-Jet, Lang Dental MFG. Co., Inc., IL, USA). The labial surfaces were slightly polished with 320-grit and 600 grit silicon carbide abrasive papers (Automata Grinding and Polishing Unit, Jeanwirtz Gmbh and Co., Charlottestrabe Dusseldorf W, Germany) with water lubrication to create a flat enamel surfaces without exposing dentin. Then, the specimens were randomly distributed into 8 groups of 12 each according to the control and remineralizing agents used [Voco Remin Pro® (VOCO America, Inc., Indian Land, SC, USA), Uncle Harry's remineralization kit (Uncle Harry's Natural Products, St Redmond, WA, USA), and Sunshine remineralization gel (Sunshine Health Products, Inc., Fort Lauderdale, Fl, USA)] as well as the resin composites used [A nanohybrid composite Tetric® N-Ceram (Ivoclar Vivadent Inc., NY, USA) and Filtek™ Z250 Universal Restorative (3M ESPE, 3M ESPE, Seefeld, Germany)]. Specimens were stored in distilled water at room temperature (27°C) for 24 hours before use. The power sample was calculated at level of significance 0.05 and estimated standard deviation = 1 with maximum difference 3 and the sample size from each group was determined to be at least 6.

Specimens were dried with a cotton roll before each application of each remineralizing agent and a thin layer of the assigned remineralizing agent was applied using cotton tip applicator. Each remineralizing agent was applied to the enamel of the assigned specimens for 5 minutes twice a day. These procedures were repeated for 24 sequential days with total time of 4 hours. For the control groups, the enamel received treatment with the remineralizing agents. The control groups and experimental groups between applications of remineralizing agents were kept in artificial saliva prepared according to Göhring *et al* ,[10] at room temperature (27°C).

All resin composite materials were applied according to the instructions of the manufacturers and light-cured with a light-emitting diode (LED) (Elipar™ S10 LED Curing Light-3M ESPE). After application of the adhesive, a standard polyvinyl chloride tube with internal diameter of 2 mm and a height of 2 mm was placed perpendicularly on the enamel surface and the resin composites were carefully inserted into the tube and cured. The specimens were stored in distilled water for 48 hours at room temperature (27°C) prior to shear bond strength testing. The shear bond strength was measured for each specimen in a universal testing machine (Inström, model no. 8500, Illinois Tool Works Inc., Norwood, MA, USA) at 0.5 mm/min crosshead speed. Bond strength was expressed in MPa. Evaluation of mode of failure and fractured surfaces were examined by two investigators using a stereomicroscope (Nikon Model C-DSD230, Nikon Co. Tokyo, Japan) at X25 magnification. Failures were classified as: Adhesive interface failure (100% of the bonded interface failed between enamel and bonding resin); cohesive failure (100% of failure in resin composite and/or enamel); or mixed failure (partial cohesive failure and partial adhesive failure).

Collected data were analyzed using software package statistical analysis (SPSS) version 21 (SPSS Inc., Chicago, IL, USA). Descriptive statistics were tabulated and one-way analysis of variance (ANOVA) was used to compare shear bond strength across different groups followed by Tukey honestly significant difference (HSD) test for multiple comparisons. A ($p<0.05$) was considered as statistically significant. Cohen's kappa statistics was calculated to quantify an agreement between the two examiners in assessing the three types of failures (adhesive, cohesive, and mixed) in each group. The Kappa value for inter-examiner reliability in assessing the three types of failures was 0.83, which indicates very good agreement between the two examiners.

RESULTS

Table 1 shows means and standard deviations of shear bond strength in MPa for all groups. One-way ANOVA showed significant difference between the groups ($p=0.0001$). The highest shear bond strength (Mean±SD) was for Tetric® N-Ceram/control [21.06±1.68] while the lowest was for Filtek™ Z250 Universal Restorative/ Sunshine remineralization gel [11.98±1.46]. Table 2. Showing comparison between all groups using Tukey HSD Post Hoc Tests. There was significant difference between Tetric® N-Ceram/Control and all other groups ($p=0.0001$) except Filtek™ Z250 Universal Restorative/Control. In addition, there was significant difference between Filtek™ Z250 Universal Restorative/Control and all other

groups (p=0.0001) except Tetric® N-Ceram/control and Tetric® N-Ceram/Uncle Harry's remineralization kit.

Stereomicroscopic assessment of the fractured surfaces and types of bond failure for each group and total frequency/percent of each type is summarized in Table 3. For bond failure, there was no statistically significant difference in the association between different groups and the three types of failure. The most common mode of failure was adhesive (55.21%) followed up with the mixed (35.42%) and the lowest was cohesive enamel failure (9.38%). No failure was recorded for cohesive composite. Table 4 shows combined types of bond failure and corresponding mean and standard error for all groups. The majority of bond failure were adhesive (53) which also showed highest mean (6.429) followed by the mixed failure (4.357). There was no cohesive failure in composite.

Table 1. Means and standard deviations of shear bond strength in MPa for different groups (n=12)

Group #	Restorative Material	Remineralizing Agent/Control	Mean	Standard Deviation	Minimum	Maximum	p value
1	Tetric® N-Ceram	Control	21.058	1.680	18.5	23.8	0.0001*
2	Tetric® N-Ceram	Voco Remin Pro®	17.350	1.639	15.1	20.3	
3	Tetric® N-Ceram	Uncle Harry's remineralization kit	17.825	1.622	15.4	20.4	
4	Tetric® N-Ceram	Sunshine remineralization gel	12.850	1.690	10.3	15.7	
5	Filtek™ Z250	Control	19.850	1.648	17.3	22.4	
6	Filtek™ Z250	Voco Remin Pro®	16.325	1.541	14.2	19.4	
7	Filtek™ Z250	Uncle Harry's remineralization kit	15.200	1.577	13.2	18.2	
8	Filtek™ Z250	Sunshine remineralization gel	11.975	1.461	9.3	14.1	

*Significant (p value <0.05)–One-way ANOVA

Table 2. Comparison between all groups using Tukey HSD Post Hoc Tests

Group #	Other Groups #	Mean Difference	Sig.
1	2	3.7083	0.0001*
	3	3.2333	0.0001*
	4	8.2083	0.0001*
	5	1.2083	0.595
	6	4.7333	0.0001*
	7	5.8583	0.0001*
	8	9.0833	0.0001*
2	3	-.4750	0.996
	4	4.5000	0.0001*
	5	-2.5000	0.006*
	6	1.0250	0.772
	7	2.1500	0.031*
	8	5.3750	0.0001*
3	4	4.9750	0.0001*
	5	-2.0250	0.053
	6	1.5000	0.314
	7	2.6250	0.003*
	8	5.8500	0.0001*
4	5	-7.0000	0.0001*
	6	-3.4750	0.0001*
	7	-2.3500	0.013*
	8	.8750	0.884
5	6	3.5250	0.0001*
	7	4.6500	0.0001*
	8	7.8750	0.0001*
6	7	1.1250	0.679
	8	4.3500	0.0001*
7	8	3.2250	0.0001*

*Significant (p value <0.05)

Table 3. Frequency of types of bond failure for each group and total frequency/percent of each type

Group #	Restorative Material	Remineralizing Agent/Control	Cohesive Enamel	Cohesive Composite	Adhesive	Mixed
1	Tetric® N-Ceram	Control	2	0	6	4
2	Tetric® N-Ceram	Voco Remin Pro®	2	0	4	6
3	Tetric® N-Ceram	Uncle Harry's remineralization kit	1	0	8	3
4	Tetric® N-Ceram	Sunshine remineralization gel	0	0	10	2
5	Filtek™ Z250	Control	2	0	5	5
6	Filtek™ Z250	Voco Remin Pro®	1	0	4	7
7	Filtek™ Z250	Uncle Harry's remineralization kit	1	0	7	4
8	Filtek™ Z250	Sunshine remineralization gel	0	0	9	3
Total Frequency			9	0	53	34
Total Percent			9.38%	0%	55.21%	35.42%

Table 4. Combined types of bond failure and corresponding mean/standard error for all groups

Failure	n	Mean	Standard Deviation
Cohesive Enamel	9	1.214	0.774
Cohesive Composite	0	0	0
Adhesive	53	6.429	3.749
Mixed	34	4.357	1.504

DISCUSSION

The null hypothesis was rejected, as there were differences between shear bond strength of resin composites to sound enamel of primary teeth after application of the three tested remineralizing agents. Considering the importance of the enamel surface layer in caries progression and bonding to restorative materials as well as the purpose of this investigation, the results showed significant reduction of the shear bond strength of primary teeth enamel after application of tested remineralizing agents except Tetric® N-Ceram/Uncle Harry's remineralization kit in comparison to the control groups.

It has been suggested that remineralizing agents have anti-erosive and anticariogenic properties.[11] When placed on the human enamel surface it can interact with hydrogen ions and form calcium hydrogen phosphate, which releases calcium and phosphate ions, which prevents the acid dissolution and protect the enamel.[11,12] The present study showed significant difference in shear bond strength between different groups. Variations in bond strength values may be related to the surface roughness and hardness of the enamel as it has been reported that the application of the whitening toothpastes to enamel increased the enamel surface roughness and decreased hardness values.[13] In this study, the application of remineralizing agents decreased shear bond strength compared to control specimens where enamel was not treated with remineralizing agents. It was reported that bleaching agents decreased bond strength due to changes in the enamel in the form of increase in surface porosity.[14,15] Moreover, some investigators reported the critical elements causing reduction of bond strength to enamel such as a decrease in microhardness and loss of calcium.[16,17] In the present study, difference in shear bond strength between different groups may also be due to composition of the remineralizing agents and resin based composite materials used. In addition, it has been proposed that changes occur in the mineral and protein components of the enamel surface layer, which could be accountable for the decrease in bond strength.[18,19] In the present study, the shear bond strengths of Remin Pro® with Tetric® N-Ceram and Filtek™ Z250 were 17.350 and 16.325 Mpa, which is the second highest, bond strength after control, which may be related to the composition of Remin Pro®. This may be supported by the study of surface roughness of sound enamel surfaces, which measured before and after bleaching, and application of MI Paste Plus, Remin Pro®, and natural saliva and showed significant reduction of surface roughness and there was no difference between MI Paste Plus and Remin Pro®.[20] Data regarding Remin Pro® is scarce since it has recently been introduced to the market. A study reported the effect of casein phosphopeptide amorphous calcium phosphate fluoride and Remin Pro® on the bleached enamel and both materials decreased the surface roughness to the same extent.[6] Remin Pro® contains HA particles much similar to calcium and phosphate ions in casein phosphopeptide amorphous calcium phosphate fluoride that are deposited on the bleached surface of enamel and increase the microhardness of teeth. Dentists should educate patients that some remineralizing agents may reduce the bond strength of resin composite to the enamel of primary teeth. However, in some cases the bond strength may still be clinically acceptable. The enamel surfaces used in this study were ground and only buccal surfaces were used. Also, the enamel in the middle third of buccal surface were used to have comparable zone from different teeth with possible similar physical and chemical characteristics. Moreover, the surface of ground enamel may slightly increase the roughness[21] and there are some influence of enamel structural on the properties of the surface such as dissimilarities in the alignment of enamel prisms and sheath.[22,23]

In the present study, stereomicroscopic assessment of the fractured surfaces showed that the most common mode of failure was adhesive (55.21%) followed up with the mixed (35.42%) and the lowest was cohesive enamel failure (9.38%). Similarly, another study reported that adhesive failures were predominant.[24] In addition, in the present study there was no significant difference in the association between different groups and the three types of failure.

This was similar to another study, which showed no significant difference in the association between different groups and the three types of failure.[25] The similarly in the results of these two aforementioned studies could be due to the similarly in methodology. The cohesive enamel failure in the present study may be due to hydrogen peroxide effect.[26,27]

In the present study, thermocycling of the specimens was not used as previous study demonstrated that thermocycling using 1800 cycles did not influence the shear bond strength of the tested materials to enamel and dentin.[28] However, it may be beneficial to test bond strength after thermocycling in future studies. In the present study, the total time of application of remineralizing agent was 4 hours. A study evaluated remineralization efficacy of stannous fluoride (SnF2), CPP-ACPF and calcium sucrose phosphate (CaSP) concluded that all remineralizing agents showed improved surface remineralization, however, complete remineralization did not occur within 7 days.[29] Also, another study showed that the average time for Casein Phosphopeptide- Amorphous Calcium Phosphate to remineralize after acid exposure is 14 days.[30] Therefore, the application of remineralizing agents for 4 hours in the present study may is considered a short-term effect of remineralizing agent on shear bond strength. As far as the authors are aware, little information is known regarding the shear bond strength of enamel of primary teeth after application of different remineralizing agents used in this study.

Remineralization concept is based on compensation of lost minerals from enamel tooth structure by improving the natural ability of saliva to remineralize enamel surfaces.[4,31] A study showed remineralization when artificial saliva was used but it was least in comparison to other groups.[32] In this study, we used artificial saliva despite the fact that we were not evaluating remineralization of demineralized enamel. Previous studies have shown that artificial saliva has no effect on the microhardness and surface roughness of enamel.[6,32,33] In addition, a high variation is seen in response to the protective agents for primary teeth, which was attributed to variation in porosity, lower content of phosphorous and calcium phosphate, and less organized microcrystals.[34,35] The results of this *in vitro* study showed that shear bond strength of the majority of the groups were higher than the results reported by various recent studies using different bonding systems, which is the accepted values for bond strength to enamel of primary teeth.[36-38]

The results of this investigation should consider the limitations of this preliminary study, including its *in vitro* setting and application of the tested remineralizing agents for only 4 hours, which may not be enough to simulate the cumulative long-term effect *in vivo*. However, the clinical condition in the mouth is not easy to mimic in the laboratory[39] and therefore, direct extrapolations to clinical conditions must be exercised with caution. However, in this *in vitro* study, standardization of experimental conditions was advantage and the results demonstrated a clear correlation between effects of remineralizating agents on shear bond strength. Another limitation of this study was the use of two resin composites only. It would be beneficial if more and different restorative materials and etch-and-rinse as well as self-etch adhesive systems is tested. Furthermore, enamel surface was flat which may do not mimic clinical situation. However, despite these limitations, the research does describe a number of positive links between *in vitro* efficacy and clinical efficacy.

CONCLUSIONS

Within the limitations of this study, it can be concluded that:

1. The three tested remineralizing agents; Voco Remin Pro®, Uncle Harry's remineralization kit, and Sunshine remineralization gel reduced shear bond strength of Tetric® N-Ceram to enamel of primary teeth.
2. Voco Remin Pro® and Uncle Harry's remineralization kit reduced shear bond strength of Filtek™ Z250 to enamel of primary teeth.
3. In general, shear bond strength values were acceptable.
4. Mode of failure was mostly adhesive.

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