Original Article

Effect of Casein Phosphopeptide-Amorphous Calcium Phosphate and Erbium:Yttrium–Aluminium–Garnet Laser on Shear Bond Strength to Demineralized Dentin

Horieh Moosavi[1,2], Atefeh Nemati-Karimooy[1,3]

[1]Department of Restorative and Cosmetic Dentistry, School of Dentistry, Mashhad University of Medical Sciences, [2]Dental Materials Research Center, Mashhad University of Medical Sciences, [3]Dental Research Center, Mashhad University of Medical Sciences, Mashhad, Iran

Abstract

Objective: The aim of this *in vitro* study was to investigate the effect of casein phosphopeptide–amorphous calcium phosphate (CPP-ACP)-containing paste (MI Paste) and erbium:yttrium–aluminum–garnet (Er:YAG) laser radiation on the shear bond strength (SBS) of an etch-and-rinse (E and R) adhesives to demineralized dentin. **Materials and Methods:** Forty-eight carries-free human dentin surfaces were prepared and demineralized using acidic solutions. Then, the samples were randomly divided into four groups ($n = 12$) receiving different surface treatments. Group A (control group): no additional treatment, Group B: 3 min application of a CPP-ACP-containing cream (MI Paste), Group C: irradiation of Er:YAG laser, and Group D: irradiation of Er:YAG laser combined with 3 min application of CPP-ACP-containing cream (MI paste). Then, a composite cylinder (Filtek™ Ultimate, 3M ESPE) bonded to the surfaces using E and R adhesives (single bond) and the SBS was measured. The SBS data were analyzed using the one-way analysis of variance test followed by Tukey *post hoc* by SPSS software. **Results:** The highest SBS to demineralized dentin was observed after the application of CPP-ACP-containing paste (MI paste) without laser radiation (17.14 ± 2.07). The second highest SBS value showed in control group (11.21 ± 1.65) in which demineralized dentin received no additional treatment. However, the application of MI paste combined with Er:YAG laser irradiation resulted in the higher SBS (8.23 ± 1.02) than laser irradiation alone (5.26 ± 1.02), even though both were lower than control group. **Conclusions:** The application of CPP-ACP-containing paste (MI paste) could increase the SBS of E and R adhesives to demineralized dentin. Furthermore, laser irradiation with and without CPP-ACP application has an adverse effect on SBS.

Keywords: Casein phosphopeptide–amorphous calcium phosphate, demineralized dentin, erbium:yttrium–aluminum–garnet laser, etch-and-rinse adhesives, shear bond strength

Introduction

In recent decades, the rapid growth of theories and materials in the field of adhesive dentistry has made cavity preparations more conservative. During tooth preparation, it is desirable that only infected dentin is removed, leaving affected remineralizable dentin. Although this affected dentin has an intact collagen matrix and no bacteria, its mineral content has reduced which resulted in lower bond strength values in comparison with normal dentin.[1-3]

Since the proper bonding of tooth-colored restorative materials to the tooth structure plays an important role in the durability and efficacy of direct composite restorations, methods that lead to increased bond strength have always been considered.

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Nowadays, laser irradiation is a new technology in the treatment of dental caries.[4] In recent years, different types of lasers were assessed either for prevention or arresting tooth caries.[4-6] Laser irradiation of dental hard tissues can cause different degrees

Address for correspondence: Dr. Atefeh Nemati-Karimooy, Department of Restorative and Cosmetic Dentistry, School of Dentistry, Mashhad University of Medical Sciences, Mashhad, Iran. Dental Research Center, Mashhad University of Medical Sciences, Mashhad, Iran, Vakil-Abad Boulevard, P. O. Box: 91735-984. E-mail: nematika@mums.ac.ir

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of chemical or morphological changes, which depends on absorption characteristics of the tissue and the type of laser.[7]

However, various studies, which have assessed the effect of laser radiation on bond strength, have shown a highly contradictory results. The type and parameters of the laser, the type of dental substrate, the applied adhesive, and even the test used to evaluate the bond strength, all are effective factors.[8-10]

Furthermore, some researchers believe that the application of calcium–phosphor compositions such as casein phosphopeptide–amorphous calcium phosphate (CPP-ACP)-containing paste on demineralized dentin may improve bond strength by mechanisms such as interaction with MDP monomers of adhesives or increasing pH.[11,12]

Nevertheless, limited studies have evaluated the effect of the simultaneous use of erbium:yttrium–aluminum–garnet (Er:YAG) laser irradiation and CPP-ACP-containing paste application on bond strength to demineralized dentin. On the other hand, fifth-generation etch-and-rinse (E and R) adhesives are still among the most common types of adhesives in the commercial market.

Therefore, our aim in this *in vitro* study was to investigate the effect of Er:YAG laser radiation and the use of CPP-ACP-containing paste (MI Paste) on the shear bond strength (SBS) of E and R adhesives to demineralized dentin. The null hypothesis of this study was that there is no significant difference in the SBS with or without different treatments.

Materials and Methods

Forty-eight caries-free human maxillary premolars, extracted for orthodontic reasons, were selected under a protocol approved by the Ethics Committee of Mashhad University of Medical Sciences (IR.mums.SD REC.1394.196). The teeth were stored in 0.5% thymol solution at 48°C and used within 1 month of extraction. All of the occlusal enamel was removed using a water-cooled low-speed diamond saw. Enamel removal was checked using a stereo microscope (Dino lite Pro, Anmo Electronics Corp, Taiwan) by ×10. The teeth were mounted in pink acrylic resin using small plastic tubes so that a flat dentin surface perpendicular to the long axis of the tooth was achieved.

The exposed dentin was polished with 400–1200 grit SiC paper for 60s under running water. To create artificial caries-affected dentin lesions, all the specimens were subjected to a pH-cycling procedure. The demineralizing solution contained 2.2 mM NaH_2PO_4, 2.2 mM $CaCl_2$, and 50 mM acetic acid, adjusted to pH 4.8, and the remineralization solution contained 0.9 mM NaH_2PO_4, 1.5 mM $CaCl_2$, and 0.15 mM KCl, adjusted to pH 7.0. For 14 days, each specimen was cycled at room temperature for 8 h in 10 ml of demineralizing solution and then for 16 h in remineralizing solution. The solutions were renewed daily.[13]

The dentin surfaces were tested under 50 g loads for 10 s with a micro-Vickers hardness tester (MH3 model, Koopaco, Iran) to check the demineralization process. Three measurements were done for each sample, and the average of them was noted as surface hardness. Then, the samples were randomly divided into four groups receiving different surface treatments. Group A (control group): the specimens of this group received no additional treatment. Group B: for the specimens of this group, a CPP-ACP-containing cream (MI Paste, GC, Japan) was applied on dentin surface for 3 min, and then, the surfaces were cleaned with a wet napkin.

Group C: in this group, the dentin surface was irradiated with Er:YAG dental laser (Pluser, LAMBDA SPA, Italy) with a spot size of 1 mm, wavelength of 2940 nm, power of 0.40 W, frequency of 10 Hz, and energy output of 40 mJ. The laser was applied using a light painting technique for 30 s with the beam directed perpendicular to the dentin surface from 5 mm distance with 0% water and 0% air.

Group D: in this group, the Er:YAG laser with the same parameters of Group C was irradiated to the surface for 30 s, while the CPP-ACP-containing cream was in place for 3 min. Then, the specimens were cleaned with a wet napkin.

Adper™ single bond 2 (3M ESPE, Dental Products USA) is a E and R adhesive, applied to the surface for 20s with light brushing motion, air thinned 5s, and light cured 10s with light-emitting diode Curing Light (JR-CL37 Foshan SCS Medical Instrument Co., Ltd, China).

Cylindrical 3 mm × 4 mm tygon tubes were placed on the surface of the specimen, filled with A2 Dentin shade of Filtek™ Ultimate dental composite (3M ESPE, Dental Products USA) incrementally and light cured with an intensity of 1200 mW/cm^2 for 40s.

All the specimens were stored in tap water at 37°C for 1 day and thermocycled for 1000 cycles between 5° and 55°C, with a dwell time of 30 s each (SANTAM Iran, SANTAM – STM zo). Shear testing was then conducted by means of a universal testing machine (Model DBBP50, BONGSHIN LOADCELL CO., LTD, Korea) with a crosshead speed of 1 mm/min. Fractured specimens were observed with a stereo microscope for determination of the failure mode, which were classified as cohesive, adhesive, or mixed.[14]

Statistical analysis

The SBS data were analyzed using SPSS software (version 16.0, SPSS Inc, Chicago, IL, USA). The normality of the data was checked using the Shapiro–Wilk test. The one-way analysis of variance (ANOVA) test followed by Tukey *post hoc* was used to compare the SBS values. Statistical significance was set in advance at the 0.05 probability level.

Results

The SBS values of 12 samples were measured in each of four groups. One-way ANOVA followed by Tukey *post hoc* test showed a significant difference in SBS between groups. The SBS data are shown in Table 1. Pairwise comparison showed

Table 1: Mean±standard deviation of shear bond strength in all groups

Group	Mean±SD
A: Control	11.21±1.65
B: MI paste	17.14±2.07
C: Laser	5.26±1.02
D: MI paste + laser	8.23±1.02

SD – Standard deviation

Table 2: Failure mode percentages after shear bond strength test for each treatment group

	Cohesive (%)	Adhesive (%)	Mixed (%)
A: Control	0 (0)	9 (75)	3 (25)
B: MI paste	0 (0)	8 (67)	4 (33)
C: Laser	0 (0)	12 (100)	0 (0)
D: MI paste + laser	0 (0)	11 (92)	1 (8)

significant differences between all four groups ($P < 0.001$). Furthermore, fracture mode distributions are shown in Table 2.

Discussion

Despite the importance of conservative dentistry in the present century, there are few studies in the field of reinforcing the demineralized dentin. Therefore, our study was conducted to evaluate the effect of Er:YAG laser radiation and the use of CPP-ACP-containing paste (MI Paste) on the SBS of an E and R adhesive to demineralized dentin. In this study, four treatment modalities were compared. The application of CPP-ACP-containing paste (MI Paste) resulted in the highest SBS, while the lowest SBS was observed in the group which the Er:YAG laser radiation on demineralized dentin was the only treatment before bonding process.

As mentioned earlier, CPP-ACP is an effective remineralizing agent. The present study showed an increase in bond strength of E and R adhesive to demineralized dentin after the application of CPP-ACP.

Previously, a study by Borges *et al*. and Kamozaki *et al*. showed that the use of a CPP-ACP compound before the application of self-etch adhesive systems could increase the SBS of some self-etch adhesives by affecting the morphology of the dentin–resin interface. These results are partly consistent with the present study.[11,15] However, some authors believe that applying CPP-ACP before the bonding procedure might be able to compromise the bond strength due to dentin tubules obliteration, preventing bond penetration throughout the collagen fibrils.[16,17] It is also stated that as MI Paste is not pure CPP-ACP, the presence of various components in the MI paste other than CPP-ACP, which is the objective of most studies, could have a negative effect on bond strength.[18]

In the present study, we applied an E and R adhesives. The point is that the bonding mechanisms of self-etch and total-etch adhesives are very different. Incorporating CPP-ACP to the demineralized dentin might compensate the mineral loss of dentin tissue. Hence, the subsequent acid etching would not result in aggressive removal of the remaining mineral content and complete collapse of collagen fibers. This can justify increasing the bond strength to the demineralized dentin following the application of CPP-ACP before etching step.

In the study of Cehreli *et al*., following the application of both E and R and self-etch adhesives, no significant change was observed on the SBS after the application of the fluoride-free CPP-ACP-containing compound, but the compound containing the fluoridated CPP-ACP as a pretreatment reduced the SBS.[19] The results of these studies were contrary to those found in the present study which might be due to the presence of fluoride in some of the compositions and application of different bonding materials and protocols.

In the present study, Er:YAG laser was irradiated from the 5 mm distance of the demineralized dentin surface in short pulse mode with the wavelength of 2940 nm, frequency of 10 Hz, power of 0.40 w, and energy of 40 mJ without air and water as a circular motion for 30 s. Since Er:YAG laser has a low penetration depth, there is no risk to the pulp and tissue around the teeth.[20] The application of such characteristics of laser radiation on dentin surface in this study decreased the SBS. Ceballos also stated that laser might decrease the bond strength because dentin ablation fuses collagen fibrils and decreases interfibrillar spaces resulting in subsequent reduction in resin diffusion into intertubular spaces and consequently less intertubular retention.[21]

It seems that reduction of bond strength in the laser group in the present study can be due to the destruction of dentin organic components and changes in surface morphology of the tooth, followed by reduction of calcium and phosphate in dentinal substrate and changes in the composition of hydroxyapatite. It is believed that after Er:YAG laser radiation, the temperature suddenly increases in the irradiated surfaces, and due to the high absorption spectrum of Er:YAG in water and hydroxyapatite, the absorbed energy in the surface layer results in degradation of the surface layer.[22]

Some studies proposed that Er:YAG laser with the ability of hard tissue ablation, if used simultaneously with fluoride compositions, could increase the penetration of ions into small spaces in the enamel and dentin. Furthermore, laser heat can create fine cracks and small spaces that facilitate fluoride penetration.[23,24]

However, in the current study, the SBS values decreased significantly after the simultaneous application of MI paste and laser. The findings of this study show that although the use of MI Paste can increase the SBS of the dentin, when applied with the Er:YAG laser simultaneously, its effect is not enough for neutralizing the destructive effects of the laser on bond strength. The results of Kamozaki *et al*. showed that the radiation of laser on softened dentin surfaces reduced bond strength following the use of self-etch adhesive, and the combination use of laser and MI Paste did not change the bond strength in comparison to not using additional treatments.[15] Although we used E and R adhesive in our study, the results were similar. The reason might be the creation of a melted surface which the acid could not penetrate completely and the etching process was impaired.

The results of the present study confirmed that in groups with higher bond strength, less adhesive and more mix or cohesive failures were happened, and in groups with lower bond strength, failures were more adhesive so that the lowest number of adhesive failures following E and R adhesive application has been observed after the use of the calcium-phosphate-containing paste (MI Paste). Similar to our results, in the study of Rezaee Soufi *et al*., the microtensile bond strength values evaluated in groups irradiated with Er:YAG laser was low, and failure mode was more adhesive.[22]

Due to the difficulty in homogenization of dental samples with natural caries, a demineralizing solution made in laboratory based on the study by Kucukyilmaz *et al*. was used in this research.[13] It should be noted that various protocols have been proposed for *in vitro* induction of caries lesions on dentin surface. Moron *et al*. stated that the artificial caries lesions of dentin vary considerably from one model to another, which, in turn, may affect the processes of remineralization.[25]

Although our study showed immediate positive effect of applying MI Paste, it should be considered that the aim of the application of MI paste is remineralizing the exposed collagen fibers which take a period of 3–4 months; longitudinal studies could create more accurate results for the bond strength to dentin.[26]

One of the limitations of this study was the use of only one adhesive. Because different adhesives may exhibit different behaviors, depending on the composition and protocol of use, the study of various adhesives in future studies can provide clear results. Furthermore, scanning electron microscope and transmission electron microscope investigations can help in finding the cause for reducing bond strength after laser radiation and enhancing bond strength after applying MI Paste. Also, clinical trials are recommended to further generalisability of the results of this experimental study.

Conclusions

Within the limitations of this study, it can be concluded that the application of CPP-ACP-containing paste (MI Paste) can increase the SBS of E and R adhesives to demineralized dentin. Furthermore, laser irradiation with and without CPP-ACP application had an adverse effect on SBS.

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Conflicts of interest

There are no conflicts of interest.

References

1. Fusayama T, Okuse K, Hosoda H. Relationship between hardness, discoloration, and microbial invasion in carious dentin. J Dent Res 1966;45:1033-46.
2. Fusayama T. Two layers of carious dentin; diagnosis and treatment. Oper Dent 1979;4:63-70.
3. Zanchi CH, Lund RG, Perrone LR, Ribeiro GA, del Pino FA, Pinto MB, *et al.* Microtensile bond strength of two-step etch-and-rinse adhesive systems on sound and artificial caries-affected dentin. Am J Dent 2010;23:152-6.
4. Mei ML, Chu CH, Low KH, Che CM, Lo EC. Caries arresting effect of silver diamine fluoride on dentine carious lesion with *S. mutans* and *L. acidophilus* dual-species cariogenic biofilm. Med Oral Patol Oral Cir Bucal 2013;18:e824-31.
5. Chin-Ying SH, Xiaoli G, Jisheng P, Wefel JS. Effects of CO2 laser on fluoride uptake in enamel. J Dent 2004;32:161-7.
6. Ana PA, Tabchoury CP, Cury JA, Zezell DM. Effect of Er, Cr:YSGG laser and professional fluoride application on enamel demineralization and on fluoride retention. Caries Res 2012;46:441-51.
7. Rohanizadeh R, LeGeros RZ, Fan D, Jean A, Daculsi G. Ultrastructural properties of laser-irradiated and heat-treated dentin. J Dent Res 1999;78:1829-35.
8. Bader C, Krejci I. Indications and limitations of Er:YAG laser applications in dentistry. Am J Dent 2006;19:178-86.
9. Koliniotou-Koumpia E, Kouros P, Zafiriadis L, Koumpia E, Dionysopoulos P, Karagiannis V. Bonding of adhesives to Er:YAG laser-treated dentin. Eur J Dent 2012;6:16-23.
10. Shahabi S, Chiniforush N, Bahramian H, Monzavi A, Baghalian A, Kharazifard MJ. The effect of erbium family laser on tensile bond strength of composite to dentin in comparison with conventional method. Lasers Med Sci 2013;28:139-42.
11. Borges BC, Souza-Junior EJ, da Costa Gde F, Pinheiro IV, Sinhoreti MA, Braz R, *et al.* Effect of dentin pre-treatment with a casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) paste on dentin bond strength in tridimensional cavities. Acta Odontol Scand 2013;71:271-7.
12. Atomura J, Inoue G, Nikaido T, Yamanaka K, Uo M, Tagami J. Influence of FCP-COMPLEX on bond strength and the adhesive-artificial caries-affected dentin interface. Dent Mater J 2018;37:775-82.
13. Kucukyilmaz E, Savas S, Akcay M, Bolukbasi B. Effect of silver diamine fluoride and ammonium hexafluorosilicate applications with and without Er:YAG laser irradiation on the microtensile bond strength in sound and caries-affected dentin. Lasers Surg Med 2016;48:62-9.
14. Versluis A, Tantbirojn D, Douglas WH. Why do shear bond tests pull out dentin? J Dent Res 1997;76:1298-307.
15. Kamozaki MB, Prakki A, Perote LC, Gutierrez NC, Pagani C. The effect of CPP-ACP and Nd:YAG laser on the bond strength of softened dentin. Braz Oral Res 2015;29. pii: S1806-83242015000100268.
16. Adebayo OA, Burrow MF, Tyas MJ. Dentine bonding after CPP-ACP paste treatment with and without conditioning. J Dent 2008;36:1013-24.
17. Adebayo OA, Burrow MF, Tyas MJ. Resin-dentine interfacial morphology following CPP-ACP treatment. J Dent 2010;38:96-105.
18. Dos Santos RA, de Lima EA, Montes MA, Braz R. Pre-treating dentin with chlorhexadine and CPP-ACP: Self-etching and universal adhesive systems. Acta Biomater Odontol Scand 2016;2:79-85.
19. Cehreli SB, Sar C, Polat-Özsoy O, Unver B, Ozsoy S. Effects of a fluoride-containing casein phosphopeptide-amorphous calcium phosphate complex on the shear bond strength of orthodontic brackets.

Eur J Orthod 2012;34:193-7.
20. Tabatabaei MH, Arami S, Khajavi F, Moradi Z. Effect of type of cavity preparation (bur, Er&58; YAG laser) and restorative materials on prevention of caries lesion. J Dent Med 2017;29:229-36.
21. Ceballo L, Toledano M, Osorio R, Tay FR, Marshall GW. Bonding to Er-YAG-laser-treated dentin. J Dent Res 2002;81:119-22.
22. Rezaee Soufi L, Kasraee S, Ghannadan K. The effect of Er:YAG and Nd:YAG lasers on microtensile bond strength of a sixth-generation two-step adhesive. J Mashhad Dent Sch 2016;40:335-44.
23. Esteves-Oliveira M, Zezell DM, Ana PA, Yekta SS, Lampert F, Eduardo CP. Dentine caries inhibition through CO(2) laser (10.6µm) irradiation and fluoride application, *in vitro*. Arch Oral Biol 2011;56:533-9.
24. Anaraki SN, Serajzadeh M, Fekrazad R. Effects of laser-assisted fluoride therapy with a CO2 laser and Er, Cr:YSGG laser on enamel demineralization. Pediatr Dent 2012;34:e92-6.
25. Moron BM, Comar LP, Wiegand A, Buchalla W, Yu H, Buzalaf MA, *et al.* Different protocols to produce artificial dentine carious lesions *in vitro* and *in situ*: Hardness and mineral content correlation. Caries Res 2013;47:162-70.
26. Tjäderhane L, Nascimento FD, Breschi L, Mazzoni A, Tersariol IL, Geraldeli S, *et al.* Optimizing dentin bond durability: Control of collagen degradation by matrix metalloproteinases and cysteine cathepsins. Dent Mater 2013;29:116-35.