Original Article

The Effects of Disinfection with Er, Cr:YSGG Laser, Application of CPP–ACP and Sodium Hypochlorite on Shear Bond Strength

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Abstract

Background: The effects of commonly used antimicrobial and anticariogenic agents on the adhesion of pit and fissure sealants were investigated in this study. **Aims:** The aim of this study is to evaluate the effects of erbium, chromium: yttrium-scandium-gallium-garnet (Er, Cr:YSGG) laser disinfection, casein phosphopeptides–amorphous calcium phosphate (CPP–ACP) containing paste and sodium hypochlorite application before the placement of a resin-based pit and fissure sealant on the shear bond strength of primary tooth enamel. **Materials and Methods:** The shear bond strength test evaluated the bond strength of sealants on the buccal enamel surfaces of primary molar teeth. The study groups were pit and fissure sealant without any preapplication, pre application of disinfection with Er, Cr:YSGG laser, disinfection with Er, Cr:YSGG laser and CPP–ACP containing paste, sodium hypochlorite, sodium hypochlorite and CPP–ACP containing paste and CPP–ACP containing paste. The pit and fissure sealants were placed using 4 mm diameter, 2 mm height cylindrical plastic tubes. Shear force was applied to each sample. The surfaces of the broken samples were detected under stereomicroscope and were grouped as adhesive, cohesive, and mixed. The results of the study were evaluated using the SPSS 16.0 package program for statistical analysis **Results:** The groups where sodium hypochlorite and sodium hypochlorite with CPP-ACP were applied showed the lowest bond strength ($p < 0.05$). It was observed that most of the failures in these groups were adhesive-type failures. No significant difference was observed between the shear bond strengths of the other groups ($p < 0.05$). **Conclusions:** Er, Cr: YSGG laser and CPP-ACP containing paste are alternative methods for pre-application of fissure sealants.

Keywords: *Adhesives, casein phosphopeptide–amorphous calcium phosphate, Er, Cr: YSGG laser, pit and fissure sealants, primary tooth, shear strength, sodium hypochlorite*

Introduction

Tooth decay is a preventable infectious bacterial disease and preventive efforts against tooth decay have gained great importance in the dental profession. The term "pit and fissure sealant" which is in the scope of preventive therapy, is used to describe a chemically active material that is either chemically cured or visible light cured, enters the occlusal pits and fissures of caries susceptible teeth. More precisely the invasion of caries-producing bacteria can be prevented. This layer also prevents existing dental caries-producing bacteria from accessing their own food resources.[1,2]

Researchers have determined that the caries-inhibiting effect of milk and dairy products is due to the calcium and phosphate ions in the milk as demonstrated *in vitro*,

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animal and *in situ* caries models studies.[3-5] Casein phosphopeptide (CPP) is produced from digestion of casein in milk by trypsin enzyme and has an important capacity for stabilizing and transferring of calcium and phosphate. CPP can stabilize the calcium phosphate in the form of CPP–amorphous calcium phosphate (ACP) complex.[6-8] CPP–ACP is currently found in topical jellies, sugar-free gums, and peppermint tablets. There are extensive clinical and laboratory findings showing that CPP–ACP is both a remineralizing and an anticariogenic agent.[5,9]

As previously mentioned, bacteriostatic methods can be used before pit and fissure application. The term "laser," which is stimulated emission of radiation by increasing the light intensity, comes from the merging of the initials of the words "Light Amplification by Stimulated Emission of Radiation."[10] Laser technology is used in pediatric dentistry for primary diagnosis, preventive dentistry, restorative dentistry, endodontics, and soft tissue applications.[11,12] One of the fields of use of laser technology in preventive dentistry is its use as an alternative to antibacterial agents for cavity disinfection.[13-16] Erbium, chromium: yttrium-scandium-gallium-garnet (Er, Cr: YSGG), a laser commonly used in dentistry, emits energy at a wavelength of 2,790 nm.[17,18] The Er, Cr: YSGG laser system has been shown to effectively and cleanly cut enamel, dentin, cementum, and bone when used under air-water cooling.[18,19] Also the bacteriocidal activity of Er, Cr: YSGG laser has been documented.[20,21]

Hitherto sodium hypochlorite (NaOCl) has been the most commonly used antimicrobial agent. NaOCl is a broad-spectrum antimicrobial agent that can be effective against bacteria, bacteriophages, viruses, spores, and yeasts. A concentration of 5% NaOCl has been shown to be effective on streptococcus mutans.[22,23]

In this study, the effects of commonly used antimicrobial and anticariogenic agents on the adhesion of pit and fissure sealants were investigated. The bond strength of different materials to dental tissues is one of the most important criteria for the decision of the suitability of a dental material for clinical use. Bond strength measurements performed under laboratory conditions provide important information about the adhesion between the materials and the tooth structure.[24,25] The aim of this study is to evaluate the effects of different types of disinfection methods: Er, Cr: YSGG laser disinfection, CPP–ACP containing paste, and sodium hypochlorite application before the placement of a resin-based pit and fissure sealant, on the shear bond strength to primary tooth enamel.

Subjects and Methods

This *in vitro* study was approved by the Ethical Committee of the xxx University on the July 5th, 2013. For the bond strength test, 102 human mandibular second primary molars, free of caries and other macroscopic defects and extracted for various reasons, were collected and used within 6 months. Teeth were stored in distilled water that was renewed every week. The teeth, intended for the bond strength test, were divided into six experimental groups.

For the placement of the teeth and application of the shear test acrylic blocks were prepared. The dimensions of the surface of the universal test device (Testometric Ax, M500-25kN, Rochdale, England) were measured and silicone molds were prepared accordingly. Roots of the primary molar teeth were separated from their crowns with diamond burs. The buccal enamel surfaces of the teeth were placed in the cold acrylic. The surfaces on these molds were prepared in such a way that they would be exposed parallel to the plane of the ground surface and be at the center of the mold. The upper surfaces of the specimens were polished with fine grained #600-grit SiC paper in order to get a flattened surface. Then the primary molar samples, which were buried in the acrylic block, were divided into six groups of 17 samples each. G Power 3.1.9.2 package program was used for sample calculation. As a result of sample calculation and power analysis, it was decided that there should be at least 17 samples in each group.

Group 1: Er, Cr: YSGG laser + CPP–ACP + fissure sealant

Group 2: Er, Cr: YSGG laser + fissure sealant

Group 3: NaOCl + fissure sealant

Group 4: NaOCl + CPP–ACP + fissure sealant

Group 5: CPP-ACP + fissure sealant

Group 6: fissure sealant (control group)

Group 1: The sample surfaces were irradiated with an Er, Cr: YSGG laser (WaterlaseiPlus, Biolase, San Clemente California, CA, USA) emitting photons at a wave length of 2,780 nm and a pulse duration of 140 µs. Laser energy was delivered through a fiberoptic cable to a sapphire tip terminal of 600 µ min diameter and 6-mm long, positioned perpendicular to the buccal surfaces. A power of 0.75 W (%15 water and %15 air) with 20 Hz was used at a 1–2 mm focal distance. The laser was applied to sample surfaces five times for 10s application with 5s intervals. GC-Tooth Mousse (GC, Tokyo, Japan) with CPP–ACP was applied with a cotton pellet on the laser applied enamel surfaces. After waiting for 3 min, it was washed with water for 10 s and air‑dried for 10 s.

Group 2: The sample surfaces were irradiated with Er, Cr: YSGG laser emitting photons at a wave length of 2,780 nm and a pulse duration of 140 µs. Laser energy was delivered through a fiberoptic cable to a sapphire tip terminal 600 µm in diameter and 6 mm long, positioned perpendicular to the buccal surfaces. A power of 0.75 W (%15 water and %15 air) with 20 Hz was used at a 1–2 mm focal distance. The laser was applied to sample surfaces for five times for 10 s application with 5 s intervals.

Group 3: 2.5% NaOCl was applied on to the sample surfaces with a cotton pellet and left for 20 s and then dried for 10 s.

Group 4: 2.5% NaOCl was applied on to the sample surfaces with a cotton pellet then left for 20 s and dried for 10 s. After that GC‑Tooth Mousse with CPP‑ACP was applied with a cotton pellet to the enamel surfaces followed by the laser exposure. After waiting for 3 min, it was washed with water for 10 s and air‑dried for 10 s.

Group 5: GC‑Tooth Mousse with CPP–ACP was applied with a cotton pellet to the laser exposed enamel surfaces. After waiting for 3 min, the area was washed with water for 10 s and air‑dried for 10 s.

Group 6: This group was identified as the control group. No preparation was made before the application of the fissure sealant.

37.5% ortho‑phosphoric acid (Kerr Gel Etchant, Kerr, Washington DC, USA) was used to etch the surface of the tooth surfaces after the preparation process. The enamel surfaces were etched for 30 s, washed for 15 s, and air‑dried for 10 s. After the acid etching process, resin‑based fissure sealant ClinPro Sealant (3M ESPE) was applied with plastic tubes prepared at 2 mm height and 4 mm diameter. In line with the manufacturer's recommendations the sealant was polymerized with an LED beam device (Woodpecker‑Built In C) for 20 s. After waiting at room temperature for half an hour, the plastic tubes were removed from the resin blocks by cutting with a lancet.

Following the preparation, the samples were kept in distilled water at room temperature for 24 h until they were connected to the universal testing machine. The specimens were embedded in acrylic blocks and fixed to the universal testing machine (Testometric Ax, M500‑25 kN, Rochdale, England) for a shear test. The loader which was attached to the moving part of the device until the fissure sealant fractured (S = 1 mm/min, break sensitivity = 5, and cell = 2,500 kgf). The force at break was recorded as Newton (N), then the bond strength was calculated in megapascals (MPa). Shear bond strength (MPa) = N/πr2.

After the shear bond strength test, the micromorphology of the fractured surfaces was examined using a stereomicroscope. The failure modes were also determined as adhesive, cohesive or mixed.

The results of the study were evaluated using the SPSS 16.0 package program for statistical analysis. G Power 3.1.9.2 package program was used for sample calculation. The sample size was calculated at 95% power and 5% significance level. It was decided that each group should have 17 samples. Shear bond strength values of the groups were summarized as mean, median and standard deviations. Mann–Whitney *U* test was used to compare the quantitative measurements without normal distribution between two groups. Kruskal–Wallis test was used to compare the quantitative measurements without normal distribution between more than two groups. Statistical significance level was taken as 0.05 in all tests.

Results

Shear bond strength values of all groups are shown in Table 1. According to Kruskal–Wallis test results, all groups were statistically different from each other ($p < 0.001$).

The comparison of the shear bond strength values of the NaOCl and NaOCl+ CPP–ACP groups with the other groups is shown in Table 2. The difference between "NaOCl" group and "NaOCl+ CPP–ACP" group was not statistically significant ($p = 0.459$). A statistically significant difference was found when the "NaOCl" and the "NaOCl + CPP–ACP" groups were compared with "laser + CPP–ACP," "laser," "CPP–ACP" and "control" groups ($p < 0{,}001$). The shear bond strength values of these groups were found to be significantly lower than the other groups.

The distributions of the failure modes observed according to the samples are shown in Table 3. In the

Table 1: The mean shear bond strength values (MPa) obtained from the groups and the standard deviation values

Group	Number	Mean±SD (MPa)	Median	*P*
Laser + CPP–ACP	17	18.23±5.9	18.03	<0.001
Laser	17	18.29±7.6	16.86	
NaOCl	17	6.42±2.5	5.57	
NaOCl+CPP–ACP	17	6,23±3.2	4.76	
CPP–ACP	17	15.61±7.9	13.05	
Control	17	16.09±6.8	14.52	

**P*<0.05 is statistically significant; Kruskal–Wallis test

Table 2: Comparison of the shear bond strength values of NaOCl group with other groups

Group NaOCl	Mean (MPa)	Other Groups	Mean (Mpa)	P*
NaOCl	6.42±2.5	Lazer + CPP–ACP	18.29±7.6	<0.001
		Lazer	18.23±5.9	<0.001
		NaOCl + CPP–ACP	6.23±3.2	0.459
		CPP–ACP	15.6±7.9	<0.001
		Control	16.09±6.8	<0.001
NaOCl + CPP–ACP	6.23±3.2	Lazer+ CPP–ACP	18.29±7.6	<0.001
		Lazer	18.23±5.9	<0.001
		NaOCl	6.42±2.5	0.459
		CPP–ACP	15.6±7.9	<0.001

*$P<0.05$ is statistically significant; Mann-Whitney *U* test

Table 3: The distribution of fracture types according to groups

Groups	Adhesive *n* (%)	Cohesive *n* (%)	Mixed *n* (%)	Total *n* (%)
Laser + CPP–ACP	5 (29.4)	7 (41.1)	5 (29.4)	17 (100)
Laser	4 (23.5)	9 (52.9)	4 (23.5)	17 (100)
NaOCl	10 (58.8)	2 (11.7)	5 (29.4)	17 (100)
NaOCl + CPP–ACP	11 (64.4)	2 (11.7)	4 (23.5)	17 (100)
CPP–ACP	4 (23.5)	7 (41.1)	6 (35.2)	17 (100)
Control	6 (35.2)	7 (41.1)	4 (23.5)	17 (100)

groups of "laser," "laser + CPP–ACP," "CPP–ACP" and control groups cohesive and mixed fracture types are the majority. In the "NaOCl" and "NaOCl + CPP–ACP" groups, the adhesive fracture type was the majority.

Discussion

While tooth decay is an avoidable infectious bacterial disease, pit and fissure sealants are one of the most effective interventions of preventive dentistry.[1,2] Also there are extensive clinical and laboratory findings showing that CPP-ACP is both a remineralizing agent and an anticariogenic agent. Furthermore, laser technology is used in pediatric dentistry for primary diagnosis, preventive dentistry, restorative dentistry, endodontics and soft tissue applications.[9,11,12,26] One of the areas of laser technology in preventive dentistry is its usage as an alternative to other cavity antibacterial disinfection agents.[13,16] The antimicrobial agent that is most widely used until now has been NaOCl.[23] The aim of this study was to evaluate the effects of Er, Cr: YSGG laser disinfection, CPP-ACP containing paste and sodium hypochlorite application, before the placement of ClinPro pit and fissure sealant, on the shear bond strength to primary tooth enamel.

According to the results of our study, there was no statistically significant difference between shear bond strength values of the control group and the laser irradiated group for disinfection before Clinpro Sealant was placed.

The recent studies by Arslan *et al*.[13] and Shafiei *et al*.[15] evaluated the effect of Er, Cr; YSGG laser on the shear bond strength among different bonding systems. The studies showed that cavity disinfection did not affect the shear bond strength, similar to our study results.

Çelik *et al*.[22] showed that the application of cavity disinfection with Er, Cr: YSGG laser increased the bond strength of both self-etch and etch-and-rinse adhesive systems. The difference in the results of the two studies may be due to the differences of permanent dentine and the test methods.

Oznurhan *et al*.[16] reported that the KTP laser cavity disinfection improved the bond strength (MI,MI paste: a casein phosphopeptide-amorphous calcium phosphate paste). The inconsistency with the results of this study may depend on different laser types used and also that the bonding tests were undertaken on tooth dentine. In our study, we tested the shear bond strength on primary tooth enamel. A further study by Öznurhan *et al*.[27] reported that KTP laser application increased the bond strength. In this study, the increase in bonding may be due to the type of adhesive system that was used. Other differences in our study, such as the usage of a different laser system, the use of primary tooth enamel and the choice of the shear bond test method, may have resulted in lower bonding values compared to other studies.

Borges *et al*.[28] evaluated the effects of MI Paste, a CPP–ACP-containing paste, and different adhesive system applications on the bond strength of the Fluroshield fissure sealant. CPP–ACP was found to increase the bond strength of the fissure sealant. Barreto *et al*.[29] investigated the effects of CPP–ACP on silorane and methacrylate-based restorative systems and on the dental bond strength to permanent dentin, and reported that CPP–ACP application did not affect the bond strength. In this study one of the CPP–ACP groups showed no statistically significant difference between the bonding strength values of both test groups and the control group.

In the study conducted by Kamozaki *et al*.[30] the effects of CPP-ACP and Nd: YAG laser applications on the microtensile bond force of the softened dentin tissue were investigated and CPP–ACP-containing paste did not affect the bond strength of softened dentin tissue. In our study, NaOCl solution at 2.5% concentration was used in two groups. The NaOCL group and NaOCL with CCP–ACP group. While there was no statistically significant difference between shear bond strength values of these two groups, it was found that bonding strength was lower in both groups compared to the control group.

Cha and Shin[31] evaluated the effects of NaOCl solution at 6% concentration on the bond strength of a self‑etch adhesive system to dentine. After the solution was applied in one group, the surface of the tooth was washed, and the other group was not washed. While there were no changes in bond strength in the washed group, the non‑washed group showed lower bond strength values similar to our study.

Harleen *et al*.[32] investigated the effect of 5.25% NaOCl solution on the bond strength to permanent tooth enamel before acid etching, NaOCl was left on the tooth surface for 60 s and washed. In our study, we didn't wash the tooth surface after NaOCl application. As a result of the study, it was reported that NaOCl does not affect bonding. The different results from our study may have been due to the usage of permanent teeth, the washing of NaOCl and the application of the adhesive system.

In the study conducted by Elkassas *et al*.[33] the effects of 5.25% NaOCl on the micro‑shear bond strengths of two different dentin adhesives were investigated. It has been reported that while NaOCl increases the self‑etch adhesive strength, it reduces the bond strength of the total‑etch adhesive as in this current study. Arslan *et al*.[13] reported that 2.5% NaOCl application for 20 s did not affect the bond strength of a silorane‑based composite. The variation may be the result of differences such as NaOCl washing and self‑etch adhesive use.

In this study, the effects of CPP‑ACP, Er, Cr: YSGG laser and NaOCl on the bond strength of a fissure sealant material applied to the primary teeth were evaluated in‑vitro. When all the obtained data were evaluated, it was concluded that Er, Cr: YSGG laser and CPP‑ACP‑containing paste could be used safely before applying a fissure sealant, but NaOCl solution caused failure in the fissure sealant material. We suggested that Er, Cr: YSGG laser is an alternative method for disinfection of primary tooth enamel and CPP–ACP containing pastes can be used before fissure sealant. The results of the *in vitro* tests alone are not to be seen as a result of the evaluation of the material, but may be considered as preliminary information about the clinical performance of the material. The relationship between in‑vitro tests and clinical performance is inevitable, and in‑vitro testing can be planned to continue with further clinical trials.

Conclusion

The results suggested that Er, Cr: YSGG laser is an alternative method for disinfection without damaging the adhesive properties of fissure sealants. CPP–ACP containing pastes can be used on primary tooth tissues without resulting in any decrease of adhesion of fissure sealant materials.

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Conflicts of interest

There are no conflicts of interest.

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