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Original Article

Effects of ozone application on fissure sealants prepared different surface etching modalities: An in vitro study

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Abstract
Aim: The aim of this study was to compare the microleakage levels of different surface treatment of fissure sealants.
Material and Methods: A total of 60 standardized non-erupted third molar teeth were divided into 5 groups (n=12) according to the pre-treatment method; Group 1 (G1): Clearfil Universal Bond Quick (self-etching) + ozone +fissure sealant (FS), Group 2 (G2): Clearfil Universal Bond Quick (self-etching) +FS, Group 3(G3): Clearfil Universal Bond Quick (etch-and-rinse) +ozone +FS, Group 4 (G4): Clearfil Universal Bond Quick (etch-and-rinse) +FS, Group 5 (G5): phosphoric acid etching +FS (control). Following surface pre-treatments, fissures of all teeth were sealed. Then, all specimens were subjected to thermal cycles (5000 times of 5 degrees C/55 degrees C for 1 minute each). All surfaces of the teeth were covered with nail polish twice up to a distance of 1mm to fissure sealant. All teeth were immersed in a 0.5% basic fuchsine dye for 24 hours. To examine microleakage, teeth separated into 2 halves bucco-lingually with saw. Microleakage scores were evaluated under stereomicroscope with a magnification of x40. The scores were statistically analyzed with Kruskal-Wallis and Mann Whitney U tests.
Results: G2, G3 and G4 showed statistically significant differences compared to control group, except G1. Although G2 showed highest microleakage level, G4 sowed lowest microleakage level G5 Ozone led to decreased microleakage in images in groups 3, 5 and 6.
Conclusions: The fissure sealing ability of with the universal adhesive in etch-and-rinse or self-etch modes was similar to that of conventional acid etching. Ozone pretreatment favorably affected the marginal sealing ability of the tested fissure sealants.

Keywords: Ozone; Fissure Sealant; Primary tooth; Microleakage; Universal bond.

INTRODUCTION

Preventive dentistry is an important part of dentistry and it has not only dental hygiene procedure but also some treatment modalities. In pediatric dentistry, fissure sealant (FS) is a widely used treatment option in the field of preventive dentistry (1). The term fissure sealant defines a micromechanically bonded material which penetrates into the occlusal fissures and pits of caries-susceptible teeth and by this way creating a protective layer that keeps caries producing bacteria away from teeth surface especially pits and fissures. These areas also has a great structure for growing bacteria (2). These sealants are existing as opaque, clear or tinted. Although the tinted and Opaque FSs are more accurate evaluated by the dentist at recall, no product of the above has demonstrated a superior retention rate (3).

The most important feature of effective restorations is the marginal sealing and adhesion capacity of sealants which is important for their ability to resist microleakage and dislodgement (4). Microleakage between cavity and restoration cause the penetration of various ions, microorganism, and liquids and in turn leads to postoperative sensitivity, recurrent caries, discoloration and pulpal pathology (5,6). Thus, preventing marginal leakage is so vital for not only the sort-term success but also long-term success of fissure sealants and also microleakage is strongly related with surface roughness and connection between fissure sealant and enamel. The widely used and well-known method for roughening enamel surfaces is applying phosphoric acid without adhesives (7). However, a number of studies stated that fissure sealants can also be applied following adhesive systems (8,9). Generally, adhesives used in dentistry are

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categorized as total-etch (two or three-step adhesives) or self-etch (one- or two-bottle adhesives). Recently a new generation of one-bottle adhesives called "universal", "multi-mode" or "multipurpose" adhesives was developed. They are simpler, low technique-sensitive, and decrease the patient chair time. These are desired advantages in pediatric dentistry (10).

Presence of microorganism under the fissure sealant cause caries, because of that application of fissure sealant on disinfected area is so important. Some disinfection method was showed, one of them is ozone application. Lately, ozone has been employed for both remineralization of initial pit and fissure caries (non-invasive treatment) and surface pre-treatment prior to fissure sealants (11,12). Some studies suggest that adjunct use of ozone is a useful prophylactic anti-microbial treatment before the etching and application of fissure sealants, that has no negative interactions with the physical properties of enamel and adhesive restorations (13,14). Artificial aging is a significant part/factor in composite repair as it imitates environmental effects. The frequently methods to imitate aging and stress interfacial bonds are thermocycling and water storage (15).

The purpose of this in vitro study was to compare the effect of microleakage of fissure sealant prepared different etching and different surface treatment modalities. The null hypotheses tested in present study were that 1) etching type and (b) different surface treatment modalities would not affect the microleakage level after FS treatment.

MATERIAL and METHODS

This in vitro study was conducted with the approval of the Ethics Committee of Gaziantep University 2017/382.

Tooth preparation

Sixty human non-erupted third molars were included for the study. Soft tissue residues on selected teeth were removed, the pit and fissures were cleaned with water cooled hand piece and brush. Then, the teeth were examined at 20x under a stereomicroscope (Leica M165C, Leica Mycrosystem Ltd., Heerbrug, Germany) to exclude defects or cracks. Teeth were stored at room temperature in 0.1% tymol solution until the experimental period and the storage solution was changed once a week.

Groups and used material

The teeth were randomly divided into 5 groups (n=12) by used random number generator (www.random.org), according to the occlusal surfaces pre-treatment method before fissure sealant application as follows:

•G1 - Clearfil Universal Bond Quick (self-etching)+ozone application+fissure sealant

•G2- Clearfil Universal Bond Quick (self-etching)+fissure sealant

•G3- Clearfil Universal Bond Quick (etch-and-rinse)+ozone application+fissure sealant

•G4- Clearfil Universal Bond Quick (etch-andrinse)+fissure sealant

•G5- Phosphoric acid etching (control group) +fissure sealant

All materials were applied according to the manufacturer's instructions showed at Table 1.

Table 1. Materials used in present study

Materials	Chemical composition	Manufacturer
Clearfil Universal Bond Quick	Bis-GMA, HEMA, MDP, ethanol, water, initiators, silica, hydrophilic amide monomer, silane coupling agent	Kuraray Noritake Dental, Tokyo, JAPAN.
Ozone	Ozone gaseous	OZONYTRON X; Mymed, Töging, GERMANY.
Fissure Sealant	Bis-GMA, TEGDMA, pyrogenic silicilic acid	Voco Gmbh, Cuxhaven, GERMANY.

*Abbreviations: Bis-GMA, bisphenol A glycidyl methacrylate; HEMA, 2-hydroxyethyl methacrylate; MDP, 10-methacryloyloxydecyl dihydrogen phosphate; TEGDMA, triethilene glycol dymethacrylate

Application modalities

Application procedures mentioned above were performed as follows;

In ozone application including groups, before adhesive application, the pits and fissures were pretreated with a CA probe tip attached to ozone generator (OZONYTRON X; Mymed, Töging, Germany) at a concentration of 100 ppm for 30 seconds in compliance with the manufacturer's instructions.

In acid pretreated groups; the occlusal surfaces were etched with 37% phosphoric acid gel for the 30 seconds, rinsed with water for 15 seconds, and dried for 5 seconds with air syringe.

In adhesives group with self-etching mode; adhesive applied to dried tooth surface and then medium air pressure applied to surface for 5 s. Lastly, adhesive photocured for 10 s.

In adhesives group with etch and rinse mode; prior to the self-etch mode steps, pits and fissures were etched with 37% phosphoric acid gel for the 10 seconds, rinsed with water for 10 seconds, and dried for 5 seconds with air syringe.

Following the pretreatment procedures of pit and fissures, sealants were applied regarding with the manufacturer's instructions. Fifteen seconds per each application was waited for the penetration of sealants and they were then light-cured for 20 seconds.

After sealant application, the samples were stored in distilled water for 1 day at 37ºC. The specimens were then aged in a thermocycling bath (5000X at 5°C-55°C; dwell time=15s and transfer time=10s) for providing thermal stress. Then the root apices were sealed with wax, all teeth surfaces except 1 mm around the margins of the fissure sealant agents were covered with double layer nail varnish and immersed in 0.5% basic fuchsine solution (Wako Pure Chemical Industry; Osaka, Japan) for 24 h for microleakage. Next the specimens were rinsed with water and sectioned bucco-lingual direction with Isomet (Bisco

Micro Tensile Tester, Bisco Inc., Schaumburg, ABD) under water irrigation. One intercalibrated examiner (Z.U.G), who was blinded to surface modification modalities, scored the microleakage stage using a stereomicroscope with magnification of 40X (Leicamicrosystems stereo microscope, Stereo and microscope systems; Heerburg, Switzerland).

Microleakage per section side was scored with a 3 point rating scale.

Score 0: indicated no microleakage visible,

Score 1: revealed microleakage up to half of the fissure,

Score 2: microleakage more than half of the fissure but not all fissure.

Score 3: Microleakage observed in all of cavity

Statistical analysis

Before the test procedure "Power Analysis" test was applied in order to determine the sample size of study groups. All data from the microleakage assessment analyzed using a software (SPSS v22 for MAC, USA). All data distribution was checked by Kolmogorov-Simirnov and Shapiro-Wilk tests and intra/inter group comparison was made by Kruskal-Wallis test and Dunet C post hoc test.

RESULTS

Microleakage scores of all groups were represented in Table 2. The least microleakage scores were observed in G3 while the most ones were belong to G2. Although G4 and G1 represented more leakage scores compared G3, the differences were not statistically significant ($p > 0.05$). The microleakage scores of G2 were statistically higher ($p < 0.05$) compared to all other groups. Microleakage scores of group 5 were not statistically different from group 1 ($p > 0.05$).

Table 2. Comparison of microleakage scores distribution (0 to 3)

Groups	Scores				Median (Min-Max)	
	0	1	2	3		
Group1	6	9	5	0	1	1 (0-2)[ab]
Group 2	0	0	13	7	2	2 (2-3)[c]
Group 3	14	6	0	0	0	0 (0-1)[a]
Group 4	11	4	5	0	0	0(0-2)[a]
Group 5	3	8	9	0	1	1(0-2)[b]

[*]Kruskal-Wallis non-parametric test was used to compare the groups for statistically significant differences at 5%significance level. Same superscript capital letters in rows indicate no difference N=20

DISCUSSION

Fissure sealant is one of the preventive dentistry applications avoiding the occurrence and progression of caries (16). In the present study, the microleakage scores following different fissure sealant surface pre-treatments were evaluated. Dye penetration is still the most commonly used technique to evaluate the microleakage throughout the margins of restoration (17). This technique can also be utilized with image analysis software instead of a conventional visual scoring in order to both obtain quantitative results and avoid subjective scoring. This study compared the microleakage scores of the FS–tooth interface in permanent teeth after the application of UA in etch-and-rinse and self-etch modes with or without ozone.

The development of equipment employing gaseous ozone in the dental practice has provided new therapeutic possibilities in dental surface pre-treatment (14). The oxidant potential of ozone provides its antibacterial efficiency (18). However, oxidizing reaction can also interfere with monomer polymerization and affect adhesion negatively (19). In the study of Celiberti et al. (13), the effect of ozone surface pre-treatment on sealant tag length, microleakage and unfilled area ratio were evaluated. Ozone application did not influence the degree of microleakage. It was stated that the dehydration following ozone application does not alter the microhardness and surface-free energy of enamel. Thus, adhesion of fissure sealant agents is not adversely affected. In another study, it was shown that ozone pre-treatment enhanced the adhesion of fissure sealant agents (20). Likewise, in our study, ozone application positively influenced the microleakage scores irrespective of the adhesive system used.

Furthermore, residual debris present in pits and fissures is another factor affecting the adhesion of fissure sealant agent. According to the study of Dukic et al. (21) removal of this residual content of fissures may provide additional penetration of the sealant materials. The findings of the present study may be correlated with the cleansing effect of ozone-pretreatment into pits and fissures. It is obvious that ozone pre-treatment of fissures had no adverse effect in terms of microleakage. Indeed, it can be considered that ozone pre-treatment acts as a prophylactic treatment before the application of both conventional and bonded fissure sealants. Ozone pre-treatment improved the sealing of fissure sealant materials regardless of the type of agent. According to the results of the present study we assume that the decreased microleakage and increased sealing provided by ozone pre-treatment can also prevent secondary caries. However, further clinical researches are required to better clarify this issue. Placement of fissure sealants are technically sensitive treatment modalities (22). Conventional fissure sealants are applied following acid etching. However, in order to reduce microleakage and to provide moisture control, the use of dentin bonding agents as an intermediate layer between the enamel and fissure sealant has been suggested (23). American Dental Association and American Academy of Pediatric Dentistry suggest the application of adhesives prior to sealant application to gain better retention (24).

For pediatric patients, the self-etch adhesives have the ease of use, requires less technical sensitivity and acid etching is not needed (25). However, etch- and-rinse adhesive systems provide better penetration to enamel surface compared to self-etch adhesive systems. This may result in a better bond strength (26). To overcome the weakness of previous single-step self-etch adhesives, universal adhesives were introduced since 2011 into daily use (27). These systems combine the use of the simplest option for both one-step self-etch (SE) and two-steps etch-and-rinse (ER) (28). They are able to adhere different types of hard tissues (i.e., sound, carious, sclerotic dentin, as well as enamel) (29). Furthermore, they can be used to bond to different surfaces, such as glass ceramics, zirconia, and alloys (30). Universal-bonding agents are composed of nano-fillers, which provide stress absorption, increased bond strength (31). Combined use of them with acid etching has been suggested by many authors to achieve optimal bonding (32,33). In the study of Unverdi et al., it was shown that fissure sealants applied following acid-etching and ER or SE adhesive procedures lead to better retention compared to conventional sealant over a period of 24 months (34). Our study concluded that application of sealant along with universal bonding agents enhanced sealant agents' retention and when etch and rinse mode was applied, bond strength was superior compared to self-etch mode.

Yoshida et al. reported that applying universal adhesives for a period of time (nearly 15 seconds) may enhance the quality of chemical bonding regardless of the etching mode (35). On the other hand, within a few years, many manufacturers have produced different kinds of universal adhesive agents which can be used during a reduced application time and do not require waiting time before light curing of the adhesive (36). A novel product-G-Premio Bond (GC, Tokyo, Japan) is claimed to represent increased bond strength within a shortened application time. Current data about the effects of the application-time of universal adhesives on bonding efficacy is limited. Although reduced application time is considered as appealing for clinicians, this might negatively affect bonding because the quality of bonding of self-etch adhesives is directly proportional with the chemical reaction between functional monomers and hydroxyapatite which can be optimized by prolonged application (29,37). However the results of a previous study stated that certain universal adhesives can be used with reduced application time (9). Sai et al. (8) reported that using universal adhesives requiring no waiting time with self-etch mode is acceptable but if the substrate is uncut enamel surface (as in fissure sealant application), choosing etch and rinse mode is more appropriate. This finding is in accordance with the results of the present study.

CONCLUSION

Within the limitations of this study, ozone application before fissure sealant treatment can positively affect the microleakage. And tested different adhesives showed similar microleakge score.

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