Effect of Ozone Pretreatment on the Microleakage of Pit and Fissure Sealants

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Objective: *This study investigated the effect of ozone pretreatment on the microleakage and marginal integrity of pit and fissure sealants placed with or without a self-etch 6th generation adhesive.* ***Study Design:*** *Freshly-extracted, human third molars were randomly assigned into two main groups (n=48): Group A: Fissures were pretreated with ozone; Group B: Fissures were left untreated. The teeth were further randomly divided into two subgroups (n=24/each) so that half of teeth were sealed with a conventional fissure sealant (Fissurit F, Voco, Germany), while the remaining half received the same sealant bonded with a self-etch adhesive (Clearfil Protect Bond, Kuraray, Japan). Following thermal cycling (1000X), the specimens were subjected to dye penetration within 0.5% basic fuchsin for 24h. The extent of dye penetration was measured by image analysis. Kruskal Wallis and Mann-Whitney U tests were used for statistical analysis of the data (p=0.05). Two randomly-selected sections from each group were observed under SEM.* ***Results:*** *In all groups, ozone pretreatment significantly reduced the extent of microleakage (p<0.001). SEM investigation demonstrated better adaptation of the sealants in ozone-pretreated groups. Clearfil Protect Bond did not improve the marginal seal of Fissurit F (p>0.05).* ***Conclusion:*** *Ozone pretreatment favorably affected the marginal sealing ability of the tested fissure sealants.*

Keywords: *Ozone, Fissure Sealant, Primary tooth, Microleakage, Dentin bonding agent*
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INTRODUCTION

Pits and fissures constitute only 12.5% of total tooth surfaces,[1] but these sites carry more than 85% of the total caries burden in schoolchildren.[2] Thus, it has been widely accepted that pit and fissure sealants are the most effective means of reducing caries risk that arises from these sites.[3-5] The caries-preventive effect of sealants depends strongly on the physical obstruction of susceptible pits and fissures,[6] coupled with the ability of the sealant to remain intact and bonded to enamel surfaces.[1,7] Consequently, the effectiveness of sealants is mainly assessed by their ability to resist microleakage and dislodgement.[8] Clinically, microleakage may lead to marginal deterioration of the sealant material and increase the possibility of formation and/or progression of caries on occlusal surfaces.[8,9]

The application of ozone on dental hard tissues is a novel preventive and therapeutic approach, which is still being evaluated in many fields including cariology, endodontics, oral surgery and periodontology.[10] Ozone treatment has been reported to allow for remineralization of the tooth structures,[11-13] as well as for elimination of acidogenic bacteria by oxidizing the biomolecules (e.g., cysteine, methionine, etc.) featured in dental diseases.[14] Infusion of ozone into non-carious dentin can also prevent biofilm formation from Streptococcus mutans and Lactobacillus acidophilus over extended periods.[15] Finally, human studies[16-18] clearly suggest the potential efficacy of ozone in the management of incipient or minimally-cavitated lesions in pits and fissures, while provoking the least state of anxiety compared with those of traditional procedures.[16] These favorable laboratory and clinical data may logically suggest the potential use of ozone to disinfect pits and fissures prior to placement of fissure sealants.

It is generally accepted that the oxidant potential of ozone is responsible for its strong antibacterial effect.[14] However, such oxidizing potential may also modify bonding of resins to enamel[19] or interfere with the polymerization of resin-based materials including fissure sealants,[20] which in turn may jeopardize their adhesive and sealing properties.[21] In

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light of these observations, this study investigated the effect of ozone pretreatment on the microleakage and marginal integrity of fissure sealants placed with or without a self-etch adhesive. The null hypothesis tested was that, ozone pretreatment of pit and fissures may adversely affect the sealing properties and marginal integrity of the fissure sealants.

MATERIALS AND METHODS

Specimen Preparation

Freshly-extracted, unerupted human permanent third molars extracted for orthodontic treatment were collected in accordance with the research protocol reviewed and approved by the institutional review board. The teeth were cleaned of adhering tissue with a rubber cup and pumice, mounted on slow-speed, water-cooled handpiece. Therafter, they were sterilized with ethylene oxide stored in saline at 4^0C for a maximum of one month. The teeth were thawed to room temperature 24h before experimental procedures. The pits and fissures were cleaned with a low-speed water-cooled rotating brush without pumice and were examined at 20X under a stereomicroscope (Olympus, Tokyo, Japan) to discard those with any visible structural defects, cracks, or incipient lesions. Selected teeth were randomly assigned into two main groups (n=48), in which the fissures either received ozone pretreatment (Group A) or were left untreated (Group B). The ozone gas was applied to fissures with an Ozonytron device (Biozonix, Ozonytron, Vehos Inc., Ankara, Turkey) which is approved by the German Technical Inspection Association (TÜV), and the ministry of health of Turkey for use in high-level tissue disinfection and the prevention and treatment of dental caries. The Ozonytron device differs from HealOzone (KaVo, Germany) in the way ozone gas is generated, in that, the Ozonytron device produces an electromagnetic field in order to convert oxygen into ozone, which is then delivered to the target tissue with a plasma probe. For caries treatment or prevention, the manufacturer recommends using the device with a minimum concentration of 100 ppm for 30seconds.

In both Group A and Group B, the teeth were further randomly divided into two subgroups (n=24/each) so that half of teeth were sealed with a conventional fissure sealant material (Fissurit F, Voco, Germany), while the remaining half received the same sealant after application of a two-step self-etch adhesive (Clearfil Protect Bond, Kuraray, Japan) into the fissures. Light curing of all test materials was accomplished with a conventional QTH curing unit (Optilux 501, Kerr, U.S.A.) for 40 s at 480mW/cm2. In each subgroup, the curing efficiency of the QTH unit was assessed by a radiometer for standardization.

Microleakage test and image analysis

Immediately after curing, the teeth were placed in deionized water at 37°C for 24h and, thereafter, subjected to thermocycling (1000X at 5°C-55°C; dwell time=15s and transfer time=10s). Specimens were sealed with two coats of nail varnish and immersed in 0.5% basic fuchsin solution for 24 h. After thoroughly rinsing with distilled water, the samples were air-dried and embedded in autopolymerizing acrylic blocks. The specimens were then sectioned using a water-cooled, slow-speed diamond saw (Isomet, Buehler; Lake Bluff, IL, USA) in the bucco-lingual direction. Three sections (center, mesial and distal) were obtained from each specimen, and the sectioned surfaces were digitally photographed at a resolution of 1280x1024 through a stereo-microscope (Olympus, Tokyo, Japan). The images were transferred to an IBM-compatible PC as uncompressed TIFF files. The extent of dye penetration on the tooth-sealant interface was measured (in mm) using ImageJ software (V.1.42, National Institutes of Health, Bethesda, MD). The microleakage value for each section was calculated by dividing the sum of buccal and lingual dye penetration values by the sum of the lengths of buccal and lingual enamel-sealant interfaces.[22,23] The measurements were made by one calibrated operator blinded to treatment allocations. In each tooth, the mean value of three sections was recorded as the extent of microleakage for that specimen.

Statistical analysis

The microleakage values were analyzed with SPSS statistical software (version 11.5; SPSS Inc, Chicago, IL). Preliminary analysis of data using Shapiro-Wilk test revealed that the data was not distributed normally. Thus, multiple comparisons of the median values were made with Mann–Whitney-U test at $p \leq 0.05$ considered statistically significant.

Two randomly-selected sections from each subgroup were processed for ultramorphological investigation of the sealant-tooth interface with a scanning electron microscope (JSM-6400 V, JEOL, Tokyo, Japan).

RESULTS

The extent of dye penetration (in mm) for each experimental group is presented (in Table 1.) as mean, median and standard deviation. Regardless of the enamel pretreatment protocol employed, microleakage was observed in all subgroups. Figure 1 demonstrates representative images of microleakage under sealants. In both the conventional and

Table 1. The microleakage values of the test groups. The values are expressed in mm.

		Mean	Median	Standard Deviation
Group A (Ozone Pretreatment)	*Acid-Etch*	,054	,0213^a	,087
	Clearfil Protect Bond	,029	,0115^a	,037
Group B (Intact enamel)	*Acid-Etch*	,207	,169^b	,17
	Clearfil Protect Bond	,381	,318^c	,42

Median values with different superscript letters show statistically significant differences at p=0.05

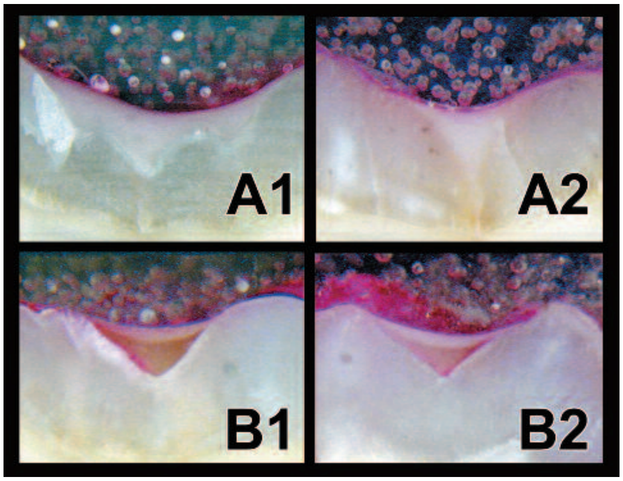

Figure 1. Representative sections of microleakage under sealants. A1=Ozone pretreatment+conventional sealant, A2=Ozone pretreatment+Bonded Sealant, B1=conventional sealant, B2=Bonded Sealant.

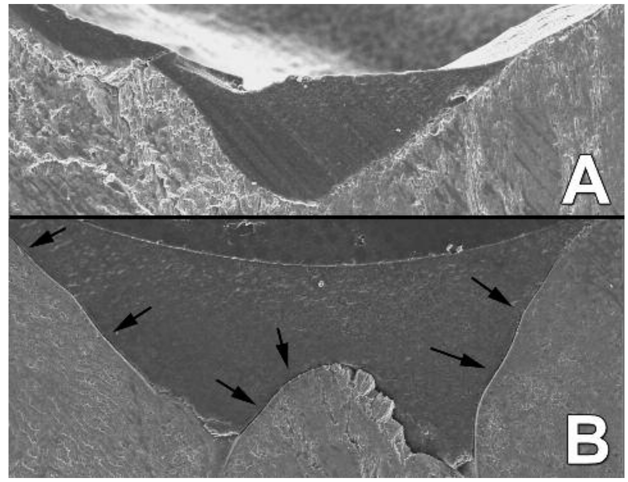

Figure 2. SEM micrographs of sealant-enamel interface with (A) and without (B) ozone pretreatment. Arrows indicate microgaps.

bonded sealant groups, the extent of microleakage in ozone-pretreated specimens were significantly lower than those of ozone-untreated (control) groups ($p<0.001$). In ozone-pretreated teeth (Group A), prior use of Clearfil Protect bond had no contributory effect on the sealing effectiveness, as sealants placed with or without the self-etch adhesive revealed similar leakage values ($p>0.05$). In the control group (Group B), teeth with conventional fissure sealants demonstrated significantly lower microleakage values than those pretreated with the Clearfil Protect Bond before placement of sealant ($p=0.009$) .

In line with their respective microleakage values, sections from the ozone-pretreated teeth demonstrated better adaptation of sealants as ultra-morphologically evidenced using SEM imaging (Figure 2).

DISCUSSION

Measurement of dye penetration on tooth sections is still the most commonly used technique to assess microleakage.[24,25] In the present study, three sections were made through each specimen to increase the reliability of measurements.[24] This technique was combined with image analysis in order to obtain quantitative results instead of a conventional subjective scoring. A relative merit of this objective approach compared with conventional subjective scoring systems was that there is no need for scoring by separate evaluators, consensus scoring in borderline cases, or statistical procedures for determining inter-examiner reliability.[23,26]

Clinical recommendations for effective placement and long-term retention of resin-based sealants typically include cleaning pits and fissures, appropriately acid-etching surfaces and maintaining a dry field uncontaminated by saliva until the sealant is placed and cured.[8,27] To reduce microleakage and sealant sensitivity to moisture contamination, the use of dentin bonding agents as an intermediate layer between the enamel and fissure sealant has been suggested.[22,28,29] The recent introduction of self-etching adhesives may provide potential improvement of the so-called "bonded sealant" technique, as these adhesive systems eliminate a separate etching-rinsing-drying procedure,[30] thereby reducing the risk of saliva contamination and the need for patient compliance.[23,29,31] Indeed, some self-etch adhesives have been shown to enhance the sealing ability or retention of fissure sealants over extended laboratory[23] and clinical test periods.[32] These reasons justify testing of a self-etch adhesive in the present study. In the absence of ozone pretreatment, the present results demonstrate that the extent of dye leakage in the bonded sealant group (self etch adhesive+fissure sealant) was significantly greater that of the conventional (acid-etch only) sealant. This finding corroborates with that of a previous study[23] which showed that under the same short-term thermocycling conditions, Clearfil SE Bond (the former version of Clearfil Protect Bond) yielded significantly greater microleakage values than that of the conventional sealant group. In both studies, this finding may be attributed to the insufficient enamel etching potential of mild self etching adhesives, characterized by the limited depth of decalcification due to total inactivation of the acid in contact with the enamel surface.[30] Together with the presence of dissolved calcium phosphates which are not removed by rinsing, this might result in a lower resistance to thermomechanical stress of the bond, and therefore in the development of marginal openings of the fissure sealing.[33,34]

In the present study, ozone fissure pretreatment led to a significant decrease in the microleakage values of both the bonded and conventional sealants, compared with their control (ozone-untreated) counterparts. Two factors may be responsible for this favorable outcome in the ozone-pretreated groups: First, the application of ozone leads to a reversible state of dehydration of the enamel,[19] Accordingly, removal of such residual moisture may significantly enhance the penetration of the hydrophobic fissure sealant material into the enamel surface and fissures. It has been shown that the dehydration caused by ozone application does not alter the microhardness and surface-free energy of enamel,[19] and does not influence bonding of resins.[19,35] Second, as a potent disinfectant, ozone dissolves the microscopic rests of organic component in the fissure, such as bacteria and their products that are not completely removed by standard fissure cleaning procedures, and produces a cleaner enamel surface and better adhesion of the material.[35] According to Dukic *et al* [35] removal of such microscopic residue from the fissure may provide a better penetration of the sealant material into the depth of the fissure. The present micromorphological findings which clearly demonstrate better adaptation and penetration of ozone-pretreated fissure sealant groups support this observation.

A primary concern of the use of ozone on dental hard tissues is the potential interaction between residual oxygen or oxide-related substances and resin-based materials, which might interfere with adhesion.[20] The results of this study showed that ozone pre treatment of fissures had no such adverse effect on the microleakage of the test materials. Based on the results obtained within the experimental conditions of this study, ozone can be applied as a prophylactic treatment prior to placement of the tested conventional and bonded fissure sealants, leading to improved sealing properties compared with their ozone-untreated counterparts. Further in vitro and clinical research is necessary to determine whether ozone treatment of fissures after cleaning and acid etching provide complete elimination or a significant reduction of remaining microorganisms.

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