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Original scientific paper

The Influence of Healozone on Microleakage and Fissure Penetration of Different Sealing Materials

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A B S T R A C T

The preventive effect of sealing materials depends on ability to penetrate into the fissures, and microleakage absence, resulting with better clinical success. The aim of present study was to investigate the influence of ozone on microleakage and penetration of nanoparticle fissure sealing resin and flowable composite, and quantitative and qualitative analysis of microleakage. Forty extracted non carious third molars were randomly divided in 4 groups. Group A: KaVo Healozone and Grandio Seal; Group B: Kavo Healozone and X-Flow; Group C: Grandio Seal; Group D: X-Flow. The teeth were thermocycled, immersed in 5% methylene dye for 24 hours, and sectioned with precision saw. The total of 149 slices were analysed with stereomicroscope for microleakage and sealant penetration. According to qualitative scores, there is a significant difference between groups C and D, group C showing better results. According to quantitative scores, there are no statistical differences between the groups. The treatment of the enamel with KaVo HealOzone after etching does not affect either microleakage or penetration proportion of flowable composite or sealing resin. There is no statistically significant difference in a degree of penetration between different groups of sealing materials. Groups of materials with flowable composite in combination with an adhesive system show a good degree of penetration into the fissure and low microleakage meaning that they can be used as a fissure sealing materials.

Key words: *ozone, pit and fissure sealants, flowable composite, dental adhesives, microleakage*

Introduction

The number of carious lesions on smooth teeth surfaces and fissures has been decreasing in number and intensity from the early 1970's until today[1]. The entire caries research today is based on prevention of occlusal caries, since it represents 80% of all carious lesions on young permanent teeth, even though occlusal surface takes up only 13% of the total tooth surface[2]. The fissure sealing is a procedure in which materials are placed into the fissures of the teeth, forming micromechanically bonded protective layer that precludes penetration of cariogenic bacteria and their contact with the source of nutrients[3]. Adequate bonding is of the most importance for retention of sealing materials and prevention of marginal microleakage; if microleakage occurs, microorganisms left at the bottom of the fissure gain access to the nutrients. This leads to bacterial growth and new start of caries process[4]. Factors, such as dental material shrinkage, contamination with salivary and debris components, and lubrication oil from dental handpieces can cause microleakage[5–7].

Recently, KaVo Healozone® (KaVo, Biberach, Germany), a device that produces ozone gas, was introduced into dentistry. The ozone is used as an antimicrobial agent, since the application of ozone during 20 seconds leads to the destruction of 99,9% of microorganisms in the carious lesion[8]. Due to the reactive potential of the ozone gas, and its high ability of oxidation, it is able to destroy bacterial membranes. Also, in this process, glycoprotein, glycolipides and other aminoacides are attacked and their enzymatic control system is inhibited and blocked. Besides its bactericidal ability, ozone can oxidise biomolecules, disabling the bacteria to survive and expand. The consequence is a destructive effect on bacterial population, that results in decreased production of acids by bacteria. Pyruvic acid, one of the strongest naturally occurring acids of bacterial origin and implicated in the progression of caries is oxidized by the ozone to acetic acid and carbon dioxide. Acetic acid is less acidic than pyruvic acid, decarboxylation reaction leading to mineral in alkaline conditions[9,10]. Other chemical agents, such as

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chlorhexidine or sodium hypoclorite, can be useful for cavity disinfection especially in combination with amalgam restorations, but when adhesive techniques and materials are used they can have inhibitory effect on bond strength[11–14]. Ozone, a highly reactive molecule capable of eliminating microorganisms and their products, can influence the sealing fissure procedure and properties of sealing material by deactivating organic components and bacteria in fissures. *In vito* studies have shown successful use of ozonated water for elimination of *S. mutans, S. salivarius*, *S. sanguis*, *S. sobrinus, A. actinomycetemcomitans, P. gingivalis, P. endodontialis* and *Candida albicans*. Also, ozonated water inhibits dental plaque accumulation *in vitro*; this suggests it can eliminate bacteria in the dental biofilm as well[15].

The aim of this study was to analyse the influence of ozone on microleakage and penetration proportion of flowable composite and nanoparticle sealing resin as a pit and fissure sealing.

Materials and Methods

Preparation of teeth

40 caries free and non cavitated third molars, extracted for orthodontic reasons, were collected. The teeth were stored in 1% solution of chloramine through 30 days. Subsequently, the teeth were rinsed in tap water, and their occlusal surface and fissure system cleaned with low-speed contraangle using bristle brush and prophylactic paste Klint (Voco, Cuxhaven, Germany), afterwards being rinsed with water from 3-in-1 syringe from the dental unit. After that, teeth were carefully dried with blown air for 3s, carefully not to overdry the teeth. They were analyzed visually using dental probe and standard illumination by 2 experienced dentists, and teeth with irregularities, such as caries or cavities were discarded and replaced with the new ones. The teeth which passed the visual examinations were sent to laser diagnostics using KaVo DIAGNOdent device (KaVo, Biberach, Germany) with device tip A. Before each measurement, the DIAGNOdent tip was cleaned with 70% ethanol and calibrated with provided ceramic standard. Only teeth with registered highest value up to 13, were selected for the study, indicating healthy enamel without caries, histologically marked with D0[16].

Sealant application

All teeth were etched for 40s with 35% phosphoric acid Vococid (Voco, Cuxaven, Germany), rinsed with water for 40s, and dried with compressed oil-free air for 20s. Materials and devices used for fissure sealing:

1. Fissure sealing material Voco Grandio Seal (Voco, Cuxaven, Germany) is a new sealing material with nano-filler particles; 70% filled by weight and volume of filler content. Material is thixotropic, meaning it is thin-flowing under pressure and movement, and stable in the absence of movement and/or pressure.
2. Flowable composite X-Flow (Dentsply, York, USA) is a smooth flowing, light curing, radioopaque composite material. It has 58% inorganic filler by weight, mostly irregular shaped particles. It can be used as a restorative material and for fissure sealing. The material is thixotropic.
3. Prime&Bond NT (Dentsply, York, USA) is a light cure self-priming dental adhesive, designed to bond composite materials to enamel and dentin, as well as to metals and ceramics. It combines primer and adhesive in a single container, which also contains di and trimethacrylate, nanofillers, amorphous silicon dioxide, photoinitiators, stabilizers, cetylamine and acetone.
4. KaVo Healozone (Kavo, Biberach, Germany) is a device which produces ozone from atmosphere oxygen with built-in ozone generator. The device has silicone cups which adapt on tooth surface producing vacuum effect, and resulting in penetration of ozone gas (2100ppm) into tooth cavities or fissures.

The teeth were randomly divided into 4 groups of 10 teeth:

Group A: Kavo Healozone (40s) + Grandio Seal (left to penetrate for 10s) + light cured 40s

Group B: KaVo Healozone (40s) + Prime&Bond NT (applied for 20s, blown to thin layer, light cured 20s) + X-Flow (left to penetrate for 10s, light cured 40s)

Group C: Grandio Seal (left to penetrate for 10s) + light cured 40s

Group D: Prime&Bond NT (applied for 20s, blown to thin layer, light cured 20s) + X-Flow (left to penetrate for 10s, light cured 40s)

After sealing and light polymerization with Elipar LED (3M ESPE, St.Paul, USA) curing unit (400mW/cm^2), the teeth were checked with dental probe for sealing material cracks, overfilling or some other irregularities, and they were immediately restored, if possible, with a sealing material. The sealed teeth with major irregularities were discarded and replaced with new ones, as described before.

Termocycling and specimen preparations

The teeth were thermocycled in distilled water for 1500 cycles between 5 °C and 55 °C with dwell time of 30s. After that, teeth apices were coated with liquid instant adhesive UHU Sekunden Alleskleber (UHU, Bühl/Baden, Germany), and above that coated with melted utility wax. The wax and teeth surfaces were covered with two layers of red nail varnish, leaving approximately 2mm of enamel around fissure and sealing material uncovered. The teeth were immersed in 5% methylene blue at 37 °C for 24 hours to allow dye penetration into possible gaps in the tooth sealant interface. Following rinsing the teeth in tap water, the wax coatings were stripped off, and again cleaned in tap water. The teeth were embedded in cold self-curing acrylic resin (Polident, Vocja Draga, Slovenia) to prevent chipping and cracks of the teeth. Using precision saw Isomet 1000 (Buehler, Illinois, USA) with low speed (250–300 r/min), each tooth was sectioned buccolingually with parallel cuts with at least 1 mm width between them, resulting in 3 to 4 slices from each tooth.

Measurements of microleakage and sealant penetration

A light stereomicroscope Olympus SZX-12 (Olympus Optical, Tokyo, Japan) at magnification of 50x was used to evaluate microleakage, fissure depth, penetration and unfilled proportion. With help of Olympus DP soft 3.1 program, with video camera attached to the stereomicroscope and linked to the computer, the photographs from tooth slices were calibrated to analyse lengths in mm.

The tooth slices were analysed with qualitative criteria according to do Rego et al.[17] and Youssef et al.[18]: 0 = no dye penetration; 1 = dye penetration restricted to occlusal third of one of the sealant walls (buccal or lingual); 2 = dye penetration restricted to occlusal third of both sealant walls; 3 = dye penetration restricted to medium third of one of the sealant walls; 4 = dye penetration restricted to medium third of both sealant walls; 5 = dye penetration restricted to pulpal third of one of the sealant walls; 6 = dye penetration restricted to pulpal third of both sealant walls; and 7 = total dye penetration along the cavity walls, including the pulpal wall (Figure 1).

The tooth slices were analysed quantitatively for fissure length, length of tooth-sealant interface, length of dye penetration, fissure depth and lengths of sealant penetration as well. In each sample, microleakage proportion was expressed as the length of dye penetration (mm) divided by the length of sealant-tooth interface (mm). The formula for microleakage proportion (M) was M=a+b/c+d. Penetration proportion was expressed as a percentage of the depth of sealant penetration(mm) divided by the total depth of sealed fissure(mm). The formula for penetration proportion (P) was P=p1/p2 (Figure 2).

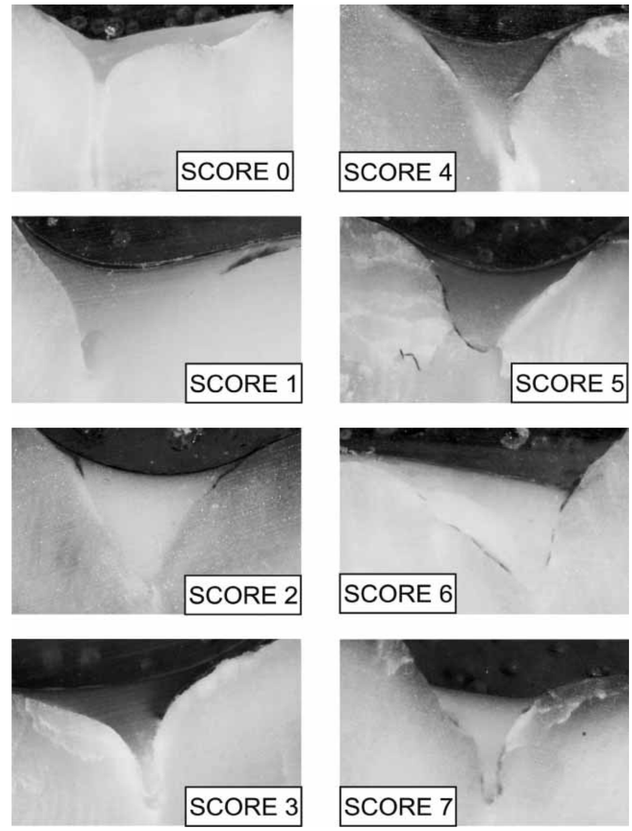

Fig. 1. Tooth slices with microleakage scores from 0 to 7.

Statistical analysis

Kolmogorov-Smirnov test ($p<0,05$) has shown that the distribution of P and M variables was not normal. Since qualitative measurement of penetration are ordinal variables, further statistical analysis of all variables was carried out using Kruskal-Wallis and Mann-Whitney nonparametric tests. For statistical analysis of quantitative measurements Kolmogorov-Smirnov test for two independent variables was used.

TABLE 1
QUALITATIVE SCORES FOR SEALING MATERIALS AND HEALOZONE TREATMENT

Group	Score/Precentage (%) of microleakage								Total
	0	1	2	3	4	5	6	7	
(A) Healozone + GrandioSeal	23 (62.16)	2 (5.4)	6 (16.21)	1 (2.7)	3 (8.1)	1 (2.7)	0 (0.0)	1 (2.7)	37
(B) Healozone + X-Flow	26 (68.42)	3 (7.89)	0 (0.0)	5 (13.15)	4 (10.52)	0 (0.0)	0 (0.0)	0 (0.0)	38
(C) Grandio Seal[a]	28 (73.68)	6 (15.78)	1 (2.63)	0 (0.0)	1 (2.63)	0 (0.0)	2 (5.26)	0 (0.0)	38
(D) X-Flow[a]	17 (47.22)	10 (27.77)	3 (8.33)	3 (8.33)	0 (0.0)	2 (5.55)	1 (2.77)	0 (0.0)	36

Equal letters ([a]) indicate statistical significance

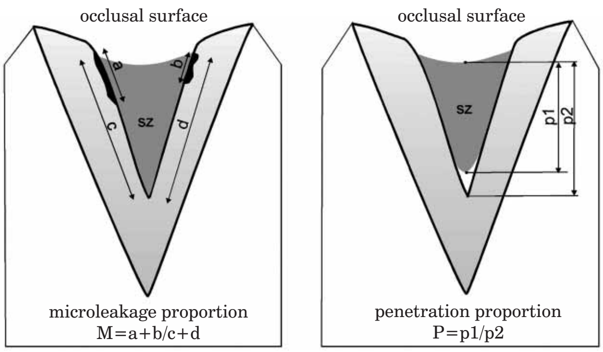

Fig. 2. Microleakage proportion and penetration proportion formula. a – microleakage length, b – microleakage length, c – fissure length, d – fissure length, sz – sealant zone, p1 – sealant penetration depth, p2 – fissure depth

Statistical evaluation was made with program solution SPSS 13 (SPSS Inc.,Chicago, USA)

Results

Total of 149 surfaces were examined by a light microscope. According to the Mann-Whitney test there is a statistically significant difference between groups C and D (p=0,025), where group C (Grandio Seal) showed better results (Table 1.). Group C showed microleakage score 0 in 73.68%, the best score among tested materials and procedures. The X-Flow material alone showed score 0 in 47.22%, the lowest result. There are no statistical differences between other groups. Scores 5, 6 and 7, were not detected in group B.

Two-sample Kolmogorov-Smirnov test showed no statistically significant difference between different groups considering quantitive measurement, i.e. coefficient of penetration and microleakage (p>0,05) (Table 2). The group B showed best result in penetration proportion, and group C showed best result in microleakage proportion.

Discussion

The success of fissure sealing procedure depends on understanding of the cariogenic process, pre-treatment of the enamel (cleaning and conditioning technique, material interaction) and type (depth and width) of the fissure involved[4,13,19–21]. These three factors can be influenced in order to improve the quality of a sealing procedure.

This research has shown that when ozone was used in enamel pre-treatment, M was lower in group B, which used flowable composite, although this was not statistically confirmed. Also, a combination of ozone and flowable composite in group B showed good P results, but there was no statistically significant difference compared to others. It is interesting that, at qualitative measurement, there were no statistically significant differences in microleakage except in groups C and D, which did not use the pre-treatment with ozone, and the group which used material Grandioseal alone was better than the group which used X-Flow material alone. It is possible that the ozone as a strong disinfectant, dissolves microscopic rests of organic component in the fissure, such as bacteria and their products that were not completely removed by standard fissure cleaning procedures, and results in cleaner enamel surface and better adhesion of the material. Also, by removing the microscopic rests from the fissure, probably a better penetration of a sealing material into the depth of the fissure is feasible, so that penetration proportion of sealing material could be high.

Ozone can be a useful disinfectant of cavities and all places that require a filling treatment, which can later result in decreased risk of residual caries and inflammation of the pulp due to the bacteria left in dental tubules, as well as of treating caries according to the principles of minimal invasive dentistry[23,24]. Removal of the microorganisms from the cariogenic tissue, and tight interface between tooth and sealing or restorative material which avoids microleakage, is crucial in caries therapy and prevention. Some studies showed that the oxygen and oxidants can influence bonding to dental materials. Interactions between residual (per) oxide-related substances and restorative materials interfere with adhesion[25,26]. This research showed that there is no statistically significant difference in microleakage on the enamel between the groups with ozone treatment and flowable composite with bonding agent, and the group without ozone treatment. The research from Schmidlin et al shows that

TABLE 2
THE RESULTS OF QUANTITATIVE MEASUREMENTS

	Group A Healozone+Grandio Seal (SD)	Group B Healozone-X-Flow (SD)	Group C Grandio Seal (SD)	Group D X-Flow (SD)
Fissure length (mm)	3.21 (0.95)	3.20 (1.12)	2.82 (1.01)	2.87 (0.91)
Dye length (mm)	0.40 (0.73)	0.30 (0.54)	0.16 (0.35)	0.39 (0.52)
60Fissure depth (mm)	1.04 (0.41)	0.83 (0.37)	0.78 (0.44)	0.91 (0.46)
Penetration(mm)	0.91 (0.31)	0.77 (0.31)	0.68 (0.34)	0.77 (0.36)
Penetration proportion-P	0.91 (0.14)	0.95 (0.10)	0.92 (0.17)	0.89 (0.15)
Microleakage proportion-M	0.13 (0.14)	0.09 (0.15)	0.07 (0.16)	0.13 (0.16)

(SD) – standard deviation

pre-treatment of the enamel with ozone showed bond strength to enamel of 30Mpa, which was similar to control group without ozone of 30.9Mpa. Also, no statistically significant difference was established between the groups with ozone and the groups without ozone for dentin specimens[14].

Do Rego et al demonstrate that the microleakage occurred in all groups of specimens, ranging from 60% to 100%, but the use of an adhesive system decreased microleakage of the fluoride resin-filled sealant[17]. Also, Moshonov et al. showed that there is no difference in microleakage between laser and acid etching groups, although the authors explain their results with usage of 1% methylene blue as a dying agent, which has large particle size in comparison to 0.2% rhodamine, 0.5% basic fuchsin or 50% silver nitrate[27].

Celeberti et al. showed no statistically significant difference in sealant microleakage, unfilled area and tag length between ozone treated and control samples, concluding that ozone can be applied as a prophylactic treatment prior to etching and sealing on prepared and intact sound enamel[28]. As the application of air and ozone showed comparable effects, it can be concluded that ozone application does not influence enamel microhardness. Furthermore, enamel dehidratation caused by ozone when applied before acid etching and rinsing does not seem to affect resin bonding to the enamel. Ozone was shown to dehydrate enamel and enhance the microhardness, which was reversible. Ozone can be applied over intact and prepared enamel prior to etching and placement of a resin material[28].

Regarding that we used ozone after etching of the fissures, which differs from the method described and recommended[28], our results also showed that there is no statistically significant difference among groups considering microleakage.

The results from Courson et al. show high rate of penetration for sealing material Consise White Sealant of 85.2% into the depth of the fissure, as well as of compomere Dycrat Seal of 92.6%[30]. Before the application of Dycrat Seal compomer, the fissures were treated with NRC self-etching primer and bonding agent Prime/Bond NT[29]. Also, Gillet et al. showed penetration depth for flowable composite Tetric Flow in conjunction with phosphoric acid and one-bottle bonding agent of 86.1%, and penetration depth for sealant resin Helioseal F of 96.9%. There was no significant difference in microleakage and penetration depth between classical bonding vs self-etching primer in Tetric Flow group[30]. Selecman et al. reported complete penetration of material into the fissure of 80% and 82% for fissure sealing resins Triage and Delton. The authors emphasise that the success of sealing depends on characteristics of a sealing material and morphology of the fissure. The morphology of the fissure considerably influences penetration of sealing material, but does not influence the microleakage and different techniques of fissure preparation as air abrasion or pumice prophylaxis do not play an important role in sealant microleakage or fissure penetrability[31]. These results are in accordance with our work, since we obtained sealant penetration rate of 94.9% for group with Healozone + Prime&Bond NT+ X-Flow, and 91.6% for Grandio seal. Shah et al. showed that there is no difference in the degree of microleakage of fissure sealants polymerised by Quartz-Tungsten halogen plasma arc curing light. The results of dye penetration for Delton Type II Opaque fissure sealing resin according to score 0, was 69.9% and 65.7% for both groups[32]. These results are in accordance with ours (62.1%, and 73.6% penetration for Grandio Seal sealant group, which is a resin sealant material).

Conclusion

Treatment of the enamel with KaVo HealOzone after etching does not affect microleakage or penetration proportion of flowable composite or sealing resin. HealOzone can be a useful device in pretreatment of the enamel before fissure sealing procedure. There is no statistically significant difference in a degree of penetration among different groups of sealing materials. Flowable composite, in combination with an adhesive system, shows good penetration proportion into fissures, and low microleakage, meaning that they can be used as a fissure sealant. The antimicrobial effect of ozone in the non cavitated fissures before sealing needs to be investigated in future researche.

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UTJECAJ HEALOZONA NA MIKROPROPUSNOST I PENETRACIJU RAZLIČITIH MATERIJALA ZA PEČAĆENJE

S A Ž E T A K

Preventivni učinak materijala za pečaćenje ovisi o njihovoj sposobnosti da penetriraju u fisuru, odsutnost mikropropusnosti, što rezultira boljim kliničkim uspjehom. Svrha ovog istraživanja bila je ispitati utjecaj ozona na mikropropusnost i penetraciju nanopunjene pečatne smole i tekućeg kompozita. 40 ekstrahiranih nekarioznih i nekavitiranih trećih molara su svrstani u 4 grupe. Grupa A: KaVo Healozone i Grandio Seal; Grupa B: KaVo Healozone i X-Flow; Grupa C: Grandio Seal; Grupa D: X-Flow. Zubi su termociklirani, uronjeni u 5% metilensko modrilo kroz period od 24 sata, te su presječeni s preciznom rezalicom. Na ukupnom broju od 149 presjeka zubi analizirana je mikropropusnost i penetracija pečatne smole uz pomoć stereomikroskopa. Prema kvalitativnom mjerenju, postoji statistički zanačajna razlika između grupa C i D, gdje je grupa C pokazala bolje rezultate. Prema kvantitativnom mjerenju, nije zabilježena statistički značajna razlika između skupina. Tretman cakline sa KaVo Healozonom nakon jetkanja ne utječe na mikropropusnost ili stupanj penetracije tekućeg kompozita ili pečatne smole. Nije utvrđena statistička razlika u stupnju penetracije između različitih skupina pečatnih materijala. Skupine materijala tekućeg kompozita u kombinaciji s adhezivnim sustavom, pokazuju dobar stupanj penetracije u fisuru i malu mikropropusnost, što znači da se mogu koristiti kao materijali za pečaćenje fisura.