Morphological changes in the surface of enamel of primary teeth after application of ozone gas

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Abstract: The aim of the study was to confirm if ozone gas provides morphological changes that promote increased adhesion forces of polymers to primary teeth enamel. The acquisition of enamel surface images using an AFM and an optical profiler made it possible to estimate the roughness indicators R_a, R_z and R_{max}. It was assumed that the application of ozone gas provides morphological changes in the enamel of primary teeth based on increased surface roughness. It was also noticed that ozonated enamel is more prone to the action of H^+ ions, which provides even more development of an adhesive surface.

Keywords: ozone gas, polymers, enamel teeth, surface roughness.

Zmiany morfologiczne powierzchni szkliwa zęba poddanej działaniu ozonu

Streszczenie: Zbadano wpływ działania ozonu na zmiany morfologiczne powierzchni szkliwa zęba, sprzyjające zwiększeniu adhezji polimerów do tej tkanki. Obrazy uzyskane za pomocą AFM i profilometru optycznego pozwoliły na określenie wskaźników chropowatości R_a, R_z i R_{maks}. Stwierdzono, że zastosowanie ozonu powoduje zwiększenie chropowatości powierzchni szkliwa zębów mlecznych. Zaobserwowano, że szkliwo ozonowane jest bardziej podatne na działanie jonów H^+ niż szkliwo nieozonowane, co umożliwia dodatkowe rozwinięcie powierzchni i lepszą adhezję polimerów do tkanki zęba.

Słowa kluczowe: ozon, polimery, szkliwo zęba, chropowatość powierzchni.

The fundamental factors that influence the strength and quality of adhesion include extensiveness, morphology and the chemical composition of two neighboring surfaces, the method of tissue preparation, type and qualities of bonding materials, micro-chemical and/or chemical connections and the presence of inter-molecular interactions. The physical and chemical features of the enamel surface, such as smoothness and low value of surface energy, practically rule out the possibility of a direct connection with polymer. Only with the Acid Etch Technique, which consists of a selective dissolution of enamel using acid in order to develop the enamel surface and achieve better conditions for micromechanical retention it is possible to overcome these natural barriers [1, 2]. The basis of the Acid Etch Technique are monomers from bonding agents that are able to infiltrate the enamel surface and subsequently result in radical polymerization initiated by special polymerization lights *e.g.*, diode, halogen, plasma arc or argon laser, that is then changed into a solid body. After hardening (polymerization) of the polymer, a mechanical connection between tissue and material is achieved.

Aprismatic enamel dissolves in a different way than prismatic enamel because it is highly mineralised and contains many inorganic substances. During surface dissolution of this enamel type, irregular, very scarce and non-retention etching patterns are formed. Hence, achieving optimum strength of polymers' adhesion to aprismatic enamel is problematic [1, 3].

In order to improve the micro-retention properties of primary tooth enamel, Garcia-Godoy and Gwinnett [4] recommended the mechanical preparation of tissue before etching. This claim suggests that a similar effect can also be obtained in areas of aprismatic permanent teeth (*e.g.*, near the cervical zone of the tooth), as well as in mechanically unprepared prismatic enamel.

Lasers can also be used for the preparation of the tooth surface prior to adhesive procedures. The possible effects of application of a laser include removal of the smear layer and a series of specific processes that comprise melting and recrystallization. Therefore, ablation of enamel with the use of Er:YAG and CR:YSGG lasers can be applied in order to develop its surface [5].

Polymers can be connected to enamel using self-etch bonding agents that do not require initial acid etching. They contain acid monomers that dissolve the peripheral and central part of enamel prisms, penetrate in the area between these parts and infiltrate them [6]. After they penetrate deep into the tissue, they dissolve the inorganic part causing the formation of an adhesive connection

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characterized by micro-mechanical retention [7—9]. Unfortunately, the force of adhesion to enamel created in this way is often lower than in the case where initial acid etching of surface is required [3, 6]. In order to increase the force of adhesion of self-etching bonding agents, 10–15 seconds of pre-etching with 36 % H_3PO_4 is recommended. The improvement can also be achieved by extending the bonding material's application time (even up to 120 seconds) [6, 9].

When analyzing the properties of materials used in dentistry, attention was paid to changes occurring on the enamel surface after the application of a strong oxidant. Erosion of the surface and increased roughness after the application of hydrogen peroxide were observed using SEM imaging [10]. In some experiments, it was also possible to observe the elimination of the prismatic layer and the denudation of enamel prisms [11]. It was also stated that ozoning of deciduous teeth before etching their enamel with 36 % H_3PO_4 and applying bonding agent containing acidic monomers increases the bonding force of polymers to enamel tissue. In the case of a polymer bonding agent requiring tissue preparation with acid, the adhesion force had an increased average value of 5.05 ± 0.47 MPa to 5.41 ± 0.52 MPa, and respectively for polymer bonding agent containing acidic monomer from 3.13 ± 0.48 MPa to 3.79 ± 0.54 MPa [12]. The results of this study allow us to propose a hypothesis that the use of ozone gas provides beneficial changes in the aprismatic enamel surface. In the available literature, there are no studies regarding the influence of ozone on the development of primary teeth enamel adhesive surface.

The aim of the study was to confirm if ozone gas provides morphological changes that promote increasing adhesion forces of polymers to primary teeth enamel and to determine a level of these changes on the basis of three roughness indicators measurements. The study contains imaging of untouched primary teeth enamel and this surface after application of H_3PO_4 and a self-etching bonding agent with and without previous ozone gas application.

EXPERIMENTAL PART

Materials

— Self-etch bonding agent Xeno III (Dentsply) — composition: tetramethacryloxyethyl pyrophosphate (Pyro-EMA), pentamethacryloxyethyl cyclophosphazen fluoride (PEM-F), urethane dimethacrylate resin (UDMA), hydroxyethylmethacrylate (HEMA), ethanol, SiO_2.

— 36 % H_3PO_4 (De Trey Conditioner 36, Dentsply).

Sample preparation

Seventy human primary teeth were used in the research. After extraction, the teeth were rinsed under running water, cleaned mechanically using a toothbrush and toothpaste, and stored in a physiological saline solution at a temperature of approximately 4 °C. 140 enamel samples (5 mm × 5 mm) were obtained from the vestibular area.

In the first experiment, 120 samples were used. Samples were divided into 6 groups of 20 samples each: intact enamel (group 1), enamel exposed to ozone gas (group 2), enamel treated with 36 % H_3PO_4 (group 3), enamel exposed to ozone gas and treated with 36 % H_3PO_4 (group 4), enamel treated with Xeno III (group 5), and enamel exposed to ozone gas and treated with Xeno III (group 6). The ozonation consisted in covering the sample with a silicone cap (∅ 3 mm) and exposing it to ozone gas for 60 seconds using a HealOzone ozone gas generator (KaVo Dental GmbH, Biberach, Germany). Samples in groups 3 and 4 were treated with 36 % H_3PO_4 for 20 seconds. The surfaces of enamel samples from groups 5 and 6 were covered with a material Xeno III bonding agent. The material was applied to the enamel surface for at least 20 seconds and subsequently removed by compressed air streaming.

Methods of testing

Samples were examined in an AFM Multimode Nanoscope (Veeco Instruments Inc., Plainview, NY, USA) [13]. Values of three roughness indicators were estimated as a result of digital processing for each of the acquired images: R_a (average roughness), R_z (10-point height) and R_{max} (maximum height) according to ISO 4287:1997. Precise definitions of these parameters are disclosed in Table 1.

T a b l e 1. Definitions of roughness indicators

Roughness indicator	Definition
R_a	Arithmetical mean deviation The average deviation of all points roughness profile from a mean line over the evaluation length
R_z	The average absolute value of the five highest peaks and the five lowest valleys over the evaluation length
R_{max}	Maximum peak-to-peak-valley height The absolute value between the highest and lowest peaks

In the second experiment, the 20 remaining samples were divided into two groups of 10 samples each: in one of them, the enamel samples were intact, and in the other they were exposed to ozone gas in the same way as in the first experiment. Although an optical profiler was used (Optical profiler Wyko NT 9300, Veeco Instruments Inc., Plainview, NY, USA), flattening of the samples by removal of the aprismatic surface layer was not necessary since images of small size (500 μm × 500 μm) were ac-

quired. Values of the parameter R_a were estimated as a result of digital processing for each of the acquired images.

The calculation of mean values and standard deviations, as well as the t-Student and Cochran-Cox tests, were used for the analysis of the obtained results. Shapiro-Wilk tests were performed for the obtained values of roughness indicators in all groups in order to check whether their distributions were normal.

RESULTS AND DISCUSSION

Selected images of the enamel surface acquired using AFM for each group are presented in Figs. 1—6.

The results of the Shapiro-Wilk test led to the conclusion that almost all of the results of the first experiment are normally distributed: for each group and parameter, a p value in the range of [0.07; 0.97] was obtained except for R_z in group 3 ($p = 0.002$). The mean values and standard deviations of the parameters R_a, R_z and R_{max} obtained for each of the six groups in the first experiment are presented in Table 2.

The value of the roughness indicator R_a was more than 3.5 times greater in the group of ozonated enamel samples than in the group of intact enamel samples.

The results of another Shapiro-Wilk test led to the conclusion that the results of the second experiment for both groups ($p = 0.64$ for control group, $p = 0.47$ for ozonated group) were also normally distributed. The mean values

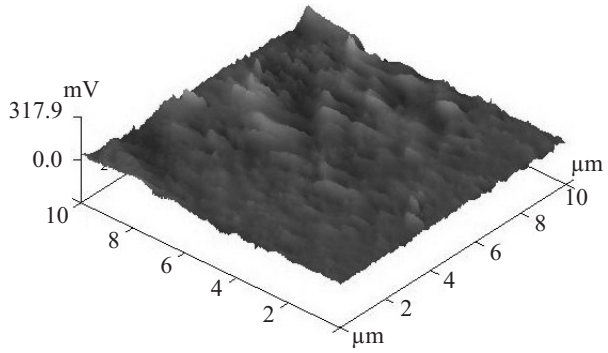

Fig. 1. Selected AFM images of intact enamel

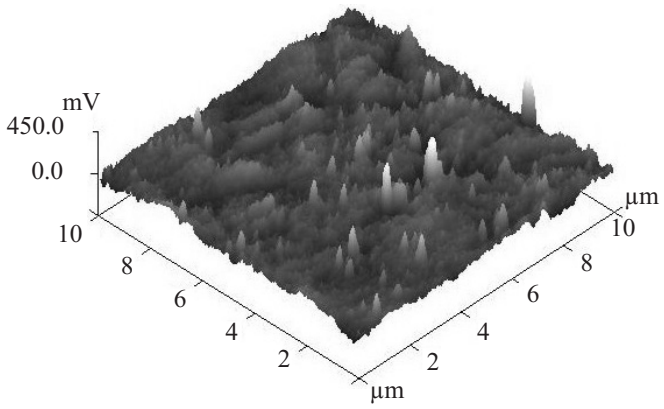

Fig. 2. Selected AFM images of enamel exposed to ozone gas

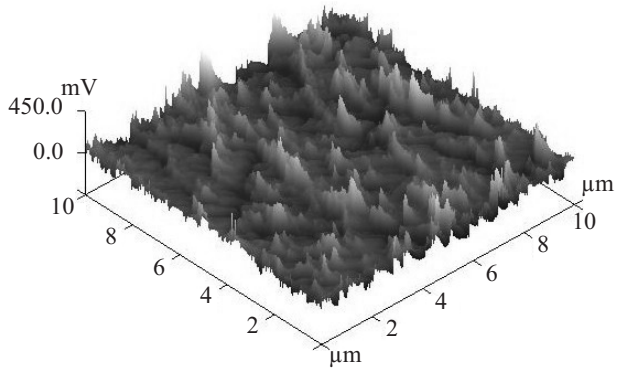

Fig. 3. Selected AFM images of enamel treated with 36 % H_3PO_4

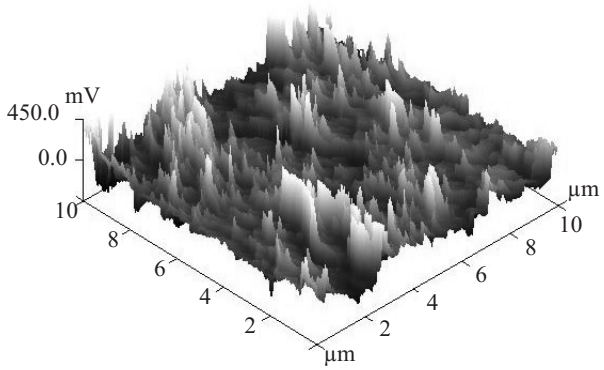

Fig. 4. Selected AFM images of enamel exposed to ozone gas and treated with 36 % H_3PO_4

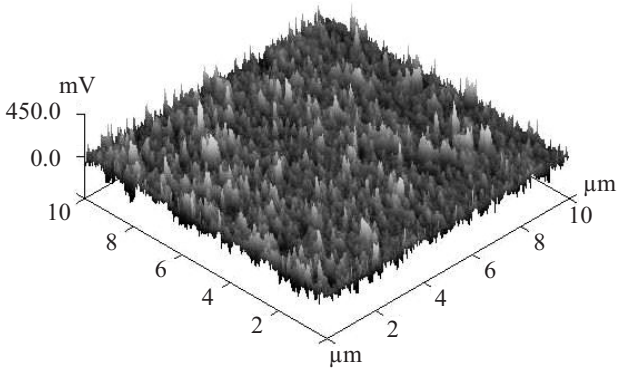

Fig. 5. Selected AFM images of enamel treated with Xeno III

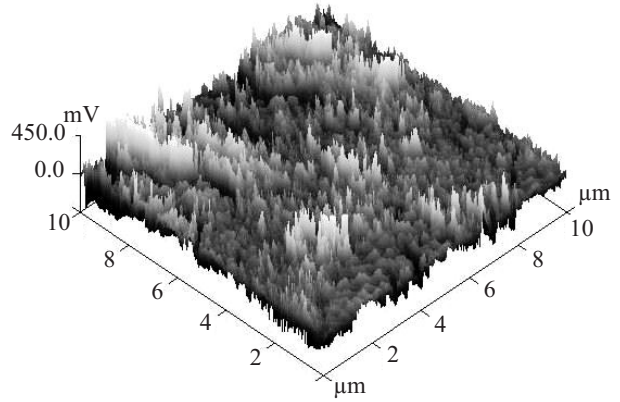

Fig. 6. Selected AFM images of enamel exposed to ozone gas and treated with Xeno III

of the parameter R_a obtained for both groups in the second experiment are presented in Table 3.

T a b l e 2. Mean values and standard deviations of the parameters R_a, R_z and R_{max} in different groups, estimated by processing AFM images

Group	Mean			Standard deviation		
	R_a	R_z	R_{max}	R_a	R_z	R_{max}
1	18.05	92.85	202.6	5.90	30.36	61.09
2	32.90	130.3	322.6	10.32	35.00	74.93
3	74.86	572.9	614.5	12.19	177.7	137.3
4	94.89	651.4	788.4	19.54	228.8	168.2
5	38.49	144.45	372.6	8.08	29.9	56.38
6	39.68	243.75	378.65	8.25	74.02	79.9

T a b l e 3. Mean values, standard deviations and variability coefficients of the parameter R_a for control and ozonated groups, estimated by processing optical profiler images

Group	Mean	Standard deviation	Variability coefficient, %
Control	2.62	0.58	22.2
Ozonated	9.97	0.76	7.6

In the images acquired using the AFM for group 1 (intact enamel), a smooth surface with a few small convexities and depressions can be mainly observed. In group 2 (enamel subject to ozone) more convexities and depressions, almost uniformly distributed across the whole surface, as well as a few fissures can be observed. In group 3 (enamel subject to acid), a very rich structure of enamel can be observed, including cavities with numerous fissures and faults, and enamel prisms. In group 4 (enamel subject to ozone and acid), the most diversified structures can be observed, including numerous deep craters and clearly visible prisms lying in parallel. Images acquired using the optical profiler had properties similar to those acquired using the AFM. In group 5 (enamel treated with Xeno III), a close to regular distribution of grooves over the whole surface of the samples was observed. These measurements confirm previous reports (a high resolution SEM study) indicating a relatively regular (without creation of craters) action of acidic monomer on enamel [8]. In group 6 (enamel exposed to ozone gas and treated with Xeno III), a very irregular structure of enamel was observed.

Until now, no studies have been conducted with the aim of evaluating the influence of ozone on the level of primary teeth enamel surface development. Only Celiberti *et al*. have made an attempt to evaluate, under laboratory conditions, the influence of ozone on unprocessed enamel of permanent teeth [14]. They observed that the exposure of enamel tissue of prismatic structure to ozone did not affect its physical properties, nor did it influence the effect of phosphorous acid etching. They also noticed that 20 seconds application of ozone could have a reversible effect of dehydration manifested by increased micro-hardness. In the described study, the time of exposure of enamel to ozone was longer by 60 s, a condition which probably caused different results.

In the AFM experiment, the exposure to ozone gas (without applying H^+) caused an increase of the mean value of the parameter R_a (*cf.* results for groups 1 and 2). Similar effects were observed for mean values of the parameters R_z and R_{max}. In the optical profiler experiment, the mean value of the parameter R_a was also significantly higher for the ozonated group than for the control group.

The surface of samples subjected to 36 % H_3PO_4 in the AFM experiment (groups 3 and 4) were significantly more developed compared to the surface of samples not subjected to acid application (groups 1 and 2). Average values of all roughness indicators were higher for samples that were exposed to ozone gas (group 4) than for those that were not (group 3). The images of enamel in group 3 are difficult to qualify as classic type I or II etch patterns [1] because the structural elements of tissue have been visualized on a different scale.

In the case of self-etch bonding agent (groups 5 and 6), the mean values of all roughness indicators were greater in comparison to group 2 and much lower than samples subjected to acid application. The analysis of the received images found no statistically significant ($p > 0.05$) differences between roughness indicators R_a and R_{max} for groups 5 and 6. However the value of indicator R_z was found to be almost two times greater in group 6 than in group 5. This looks to be an interesting finding because a previous study [12] sets a higher growth of the adhesive force in the case of self-etching bonding agent after previous ozone gas application to enamel in comparison to bonding agent requiring initial acid etching and experimental ozone gas application. It can be supposed that, in developing the enamel surface, the most important point is the existence of high absolute differences between the highest top and lowest grooves of the surface roughness profile.

In the context of the described experiments, it was possible to observe an increase of surface roughness in primary teeth after their exposure to ozone gas. Changes in the structure of the enamel surface in primary teeth, as well as in their microhardness and mineral content, caused by exposure to ozone, have also been observed in other studies. However, the time of exposure was longer — 120 s [15].

The obtained results may also indicate a substantial loss of the mineral part of the enamel under the influence of H^+ and support the conclusion that ozonating aprismatic enamel before the application of acid or acid monomers improves the micro-mechanical properties of enamel in terms of roughness. Thus, such a procedure of sur-

face preparation before adhesive bonding creates better conditions for complete penetration of a bonding agent.

Dukić *et al*. demonstrated that the use of a HealOzone device after enamel etching did not have any impact on micro-penetration and sealing properties of low filler content composites [16]. However, as opposed to the study described in this paper, the sequence of ozone gas application and etching was reversed, which could have had an influence on the results and conclusions.

Exposure of enamel to ozone gas before etching with phosphorous acid may also be useful for adhesive mounting of fixed orthodontic devices' retention elements [17]. This mainly concerns buccal surfaces of primary molar teeth where the largest areas of aprismatic enamel can be found.

The results of measurements with the use of AFM and an optical profiler described in this paper confirm a previous hypothesis of the occurrence of morphologic changes in the ozonated enamel surface that are beneficial for polymer adhesion [15, 18].

CONCLUSIONS

In the conditions of this study, it was assumed that applying ozone gas provides morphologic changes in the enamel of primary teeth based on increasing its surface roughness. It was also noticed that, in comparison to enamel without ozone application, ozonated enamel is more prone to the action of H^+ ions, which provides even more development of an adhesive surface. These findings confirm the hypothesis that an application of ozone gas creates the conditions beneficial for micro-mechanical bonding of polymers to aprismatic enamel.

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