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In situ assessment of the saliva effect on enamel morphology after microabrasion technique

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Abstract

Aim: This study evaluated saliva effects on enamel morphology surface after microabrasion technique.

Methods: Enamel blocks (16 mm²) obtained from bovine incisors were divided into 9 groups as follows: one control group (no treatment), four groups with microabrasion treatment using 35% phosphoric acid and pumice (H₃PO₄+Pum) and other four groups treated with 6.6% hydrochloric acid and silica (HCl+Sil). One group of each treatment was submitted to 4 frames of saliva exposure: without exposure, 1-h exposure, 24-h exposure, and 7-days exposure on *in situ* regimen. Nineteen volunteers (n=19), considered as statistical blocks, used an intraoral appliance containing the specimens, for 7 days. Enamel roughness (Ra) was tested before and after treatment, and after saliva exposure. Confocal laser scanning microscopy (CLSM) was used to evaluate qualitatively the enamel morphology. **Results:** All groups exhibited increased Ra after microabrasion. With regards to saliva exposure, the treatment with HCl+Sil presented more susceptibility to the saliva action, but no period of time was efficient in re-establishing this characteristic compared with the control group. CLSM analysis showed reduction of the micro-abrasive damages during the experimental times. **Conclusions:** Seven days of saliva exposure were not sufficient for the treated enamel to reach its normal characteristics compared with the control group.

Keywords: dental enamel; enamel microabrasion; saliva.

Introduction

The microabrasion technique is a treatment to achieve dental esthetics in cases of superficial tooth stains and enamel decalcification and defects, improving color and surface texture of the tooth^{1,2}. It is considered a safe and conservative technique, since it causes a non-significant loss of enamel, and it provides lasting and satisfactory results in a short clinical time^{1,3-4}. Currently, the microabrasive agents used are 6.6% hydrochloric acid with silica, in a commercial presentation, or pumice added to 35% phosphoric acid, with mechanical application under low-speed rotation⁵. The application of the microabrasive system is commonly followed by enamel polishing with diamond paste⁶⁻⁷ or fluoridated paste^{2,4}.

The clinical success of this technique has been documented^{1,2,5}. However, little is known about the effects of the microabrasive systems used on enamel surface, such as roughness and microhardness⁶⁻⁷ and its behavior in the oral

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environment in contact with saliva. Since microabrasion provides both erosive and abrasive challenges by an acid, and abrasive and mechanical application, it is expected that the enamel surface will undergo mineral loss due to demineralization, in which saliva and its components have a role in maintaining the integrity of the tissue⁸⁻¹⁰. Surface roughness is important for enamel, since it is related to color, smooth and brittle appearance of the tooth^{3,11}.

Compared with *in vitro* models, *in situ* and *in vivo* studies can simulate more closely the clinical conditions^{8,11} and may really reveal influences of microabrasion on enamel. The *in situ* models are more accessible than the *in vivo* studies and are considered reliable as they allow the control of some clinical variables⁸, e.g. in relation to the standardization of the enamel surface to be treated with microabrasion and exposed to the oral environment. The literature lacks *in situ* studies evaluating the treatment of microabrasion and in those cases where *in vitro* studies are available^{3,7,12-13} only few of them analyzed the influence of artificial saliva on the enamel after microabrasion⁶⁻⁷ and none of them used human saliva. The use of artificial saliva in studies about demineralization creates an optimum environment for the mineral recovery of enamel surface, since it contains only mineral ions in its composition¹⁴. On the other hand, the treatment of enamel surface with human saliva presents differences in this process, as it contains proteins such as mucin, which is deposited on the surface and may hamper the remineralization process¹⁵. So, the use of human saliva, in an *in situ* model, allows the analysis of microabraded enamel, reproducing more reliably the *in vivo* conditions.

Since there is no study evaluating the behavior of enamel

after microabrasion and relating it to human saliva exposure, the present work used an *in situ* model to investigate the effect of saliva exposure on enamel morphology after different microabrasion techniques.

Material and methods

The study design is presented in Figure 1.

Volunteers and Ethical Issues

The study protocol was approved by the local ethics committee in research (Piracicaba Dental School - Protocol No. 037/2011). Nineteen adult volunteers (20-30 years old) met the inclusion criteria exhibiting no fixed or removable orthodontic appliances, good general and oral health, no antibiotic use during 2 months prior to the study, and normal salivary flow. For standardization purposes, the volunteers were instructed to use fluoridated toothpaste (Colgate Total 12, Colgate-Palmolive, São Paulo, SP, Brazil) and manual toothbrushes (Colgate Professional Clean - Colgate-Palmolive, São Paulo, SP, Brazil) for 7 days prior to and during the course of the experiment.

Preparation of Specimens

Enamel slabs (4 x 4 x 2 mm) were obtained from bovine incisors using a precision saw and a high-concentration diamond disc. To obtain flat and standardized enamel surfaces, the blocks were leveled using water-cooled carborundum discs with increasing grit size in a circular water-cooled polishing machine. The resulting surface was then polished with felts associated with a diamond paste of 1, 1/2

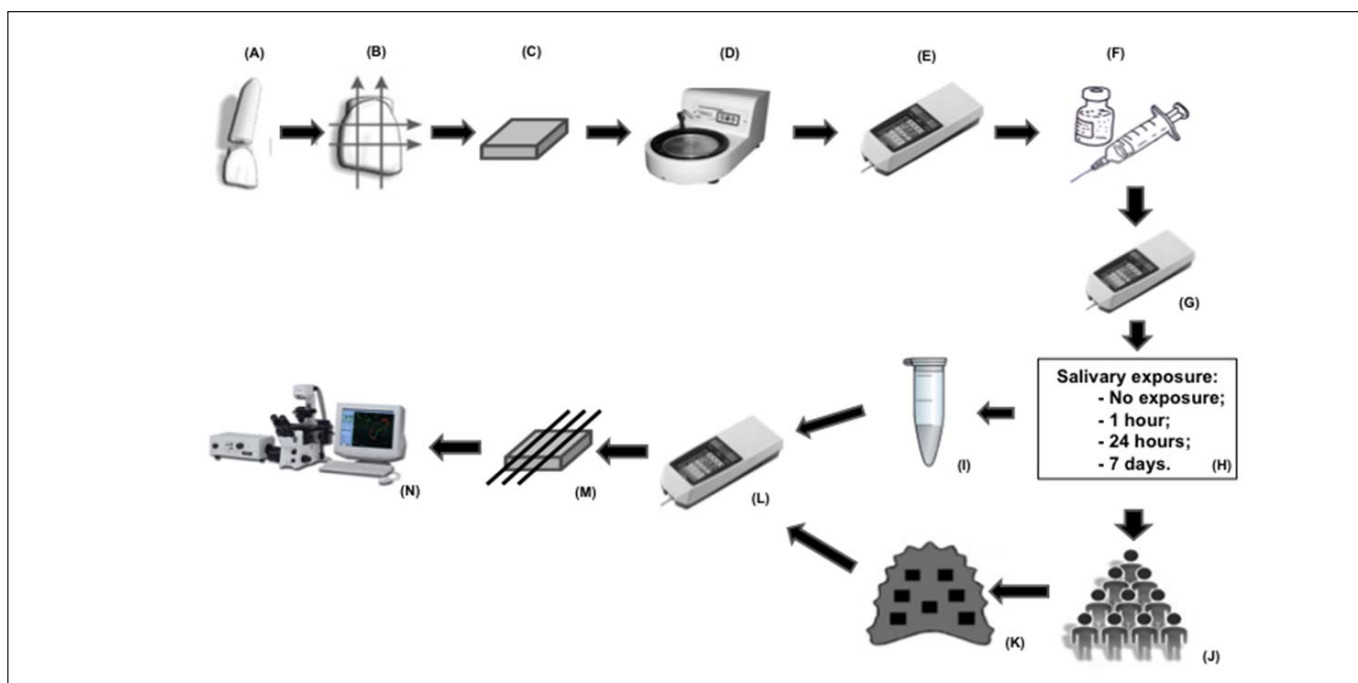


Fig. 1. Experimental design of study (a) Bovine incisor and separation of coronal portion; (b) Sectioning of crowns to obtain specimens; (c) Enamel/dentin blocks with 16 mm²; (d) Polishing of specimens; (e) Roughness analysis; (f) Enamel microabrasion; (g) Roughness analysis after treatment; (h) Division of groups according to salivary exposition; (i) Groups without salivary exposition stored in distilled water; (j-k) Groups with salivary exposition distributed for the volunteers; (l) Roughness analysis after salivary exposition; (m) Sectioning of the specimens; (n) Confocal analysis.

and 1/4µm grit sizes and greased with specific oil. Between the polishing steps and after the final polishing, all the slabs were sonicated with distilled water for 15 min. The specimens were sterilized with ethylene oxide.

Groups

Nineteen volunteers took part in the study and were considered as statistical blocks (n=19), since they used a palatal appliance containing one specimen of each group designed to receive saliva exposure. The samples were divided into nine groups (n=19) in randomized order and according to the microabrasion technique and storage time in human saliva (*in situ* phase). The experimental groups were the following: one control group, four groups treated with H₃PO₄+Pum and four groups treated with HCl+Sil. The groups with treatment were subdivided according to the *in situ* regimen: without saliva exposure, 1 h, 24 h and 7 days of saliva exposure.

Surface Treatment

The enamel microabrasion was performed by 35% phosphoric acid and pumice (H₃PO₄+Pum) or 6.6% hydrochloric acid and silica (HCl+Sil). In the first case, equal parts of 35% phosphoric acid (H₃PO₄) and pumice were measured with a metering spoon and mixed to compound the microabrasive mixture. Both products were placed on the enamel surface with a syringe until covering the specimen. The microabrasion was achieved with specific rubber cups coupled to an electric micro-motor operating at low speed (12,000 rpm). The treatment regimen was 10 applications of 10 s each.

Palatal Appliances

The intra-oral palatal appliances containing seven cavities measuring 4 x 4 x 3 mm (three at each side and one at the center of the appliance) were made for each volunteer in acrylic resin to set the slabs (Figure 1). The enamel slabs were fixed inside the cavities with wax in a manner that the enamel surface was positioned at the same level of the cavity, allowing their contact with the salivary fluid. Each volunteer used its appliance containing seven bovine dental enamel specimens, representing different experimental groups, during an experimental phase of 7 days. When the specimen of the group with 1 hour or 24 hours of salivary exposure was removed from the appliance, the cavity was full of wax. Preferably, the appliances were worn for 24 h, except during meals and periods of oral hygiene. With the appliances *in situ*, the participants were instructed not to eat, being only allowed to drink water. After meals, a period of at least 15 min elapsed prior to reinsertion of the appliance. In the evening, the appliances, but not the specimens, were cleaned with a toothbrush without toothpaste. Afterwards, the appliances were immersed for 1 min in chlorhexidine digluconate solution (Periogard; Colgate-Palmolive, São Paulo, SP, Brazil) to avoid plaque formation.

Analysis of the Enamel Roughness

The enamel roughness (Ra) was analyzed at three time frames: before and after microabrasion, and after saliva exposure using a profilometer roughness tester (Mitutoyo Surfitec 211, São Paulo, SP, Brazil). Three readings were made in three different directions on the enamel surface, and the measurements were averaged.

Confocal Laser Scanning Microscopy (CLSM)

To visualize enamel surface structures with the confocal laser scanning microscope (Leica TCS SP2-SE Microscopy, Mannheim, Germany), representative specimens of each group were sectioned to obtain slices. Each slice was polished by carbide papers and felts associated with a diamond paste of 1 µm grit size, according to the same protocol described for specimen preparation, resulting in slices about 0.3 µm thick. Subsequently, the samples were immersed into a freshly prepared 0.1 mM rhodamine B solution and left for 1 h, without further rinsing¹⁶. CSLM analysis was performed in fluorescence mode using a 40x magnification objective and oil immersion.

Statistical Analysis

After an exploratory analysis, the data were analyzed by mixed models for repeated measures (PROC MIXED), followed by Tukey-Kramer and Dunnett tests. Analysis of variance was applied since different treatments were performed in the same sample, at different moments. The significance level was set at 5%.

Results

All the experimental groups presented increased enamel roughness (Table 1) after microabrasion, with statistically significant differences in comparison with both initial analysis and control group, but without significant differences between them. After saliva exposure, only the group treated with H₃PO₄+Pum and 1-h exposure showed no decrease in enamel roughness, with statistically significant difference compared with the Ra analysis after microabrasion. In the other groups with this treatment, the groups with 24 h and 7 days of exposure to saliva showed statistical significance in reducing enamel roughness. With regard to the groups treated with HCl+Sil, all exposure periods to saliva (1 h, 24 h and 7 days) resulted in decreased enamel roughness compared with the group without saliva exposure. The groups with this treatment and saliva exposure for 24 h and 7 days to the intraoral environment showed greater reduction of roughness, with statistically significant difference compared with the group without saliva exposure and the group with 1-h saliva exposure. The effect of saliva on enamel roughness for both microabrasive systems showed that groups treated with H₃PO₄+Pum and exposed to saliva for 1 and 24 h presented the greatest enamel roughness, with statistically significant difference compared with the groups treated with HCl+Sil and the group with the same exposure time to the intraoral environment.

Table 1. Roughness analysis (Ra) means followed by pattern error of all groups.

Microabrasion	Salivary Exposition	Time		
		Before treatment	After treatment	After saliva
H ₃ PO ₄ + Pumice	No exposure	0.14 (0.03) Ca	#0.36 (0.03) Aa	#0.36 (0.02) Aa
	1 h	0.14 (0.03) Ca	#0.41 (0.05) Aa	*0.38 (0.02) Aa
	24 h	0.13 (0.02) Ca	#0.41 (0.03) Aa	*0.35 (0.04) Ba
	7 days	0.14 (0.04) Ca	#0.38 (0.03) Aa	#0.21 (0.05) Bb
HCl + Silica	No exposure	0.14 (0.03) Ca	#0.39 (0.05) Aa	#0.36 (0.03) Aa
	1 h	0.15 (0.03) Ca	#0.41 (0.05) Aa	#0.28 (0.03) Bb
	24 h	0.17 (0.04) Ca	#0.38 (0.04) Aa	#0.25 (0.04) Bbc
	7 days	0.16 (0.02) Ca	#0.38 (0.03) Aa	#0.22 (0.02) Bc
Control		0.15 (0.03)	0.17 (0.03)	0.16 (0.02)

Means followed by different letters (uppercase in horizontal and lowercase in vertical compare time within each category of microabrasion) differ ($p < 0.05$). *Differ from the group treated with HCl + Silica with the same time of salivary exposition. #Differ from the control in the same time of analysis.

The CLSM analysis showed detectable lesions depending on the group, characterized by sites of microwear on enamel surface, for both treatments. During the *in situ* regimen, there was a tendency towards reduction of this wear by filling of the micro-sites in order to recover the continuous and uniform line of the enamel surface, as found in the control. For both treatments, after 7 days of saliva exposure, almost no microwear sites could be observed and an almost unchanged line characterized the enamel surface (Figure 2).

Discussion

This study evaluated the action of saliva on the effects of microabrasion techniques, combining an acid, an abrasive agent and mechanical application^{3,12-13}, on enamel surface. The clinical protocol proposed by some authors^{1-2,4-5} advises to perform a final polishing of enamel surface after microabrasion. However, this procedure is probably against the purpose of this study, as the polishing reestablishes the enamel roughness⁶⁻⁷. As a consequence, it could be stated that if saliva has a positive effect on enamel, it would not be necessary to perform polishing after microabrasion, avoiding additional enamel wear on a demineralized surface.

The results showed changes in enamel roughness, which are considered a function of variations in both surface porosity¹⁵⁻¹⁶ and mineral loss^{15,17} resulting from the erosive-abrasive challenge of the microabrasion. In accordance with the results, both microabrasive treatments using H₃PO₄ + Pum or HCl + Sil increased the enamel roughness, with statistically significant differences compared with initial analysis and without differences between them. These results are corroborated by previous findings that reported mineral loss and increased enamel porosity after treatment with erosive¹⁷ and/or abrasive^{9-10,12,18-19} treatment and low pH¹⁴ compounds.

Enamel microabrasion may be considered an erosive-abrasive model, since the erosive and abrasive challenges cause dissolution of the hydroxyapatite crystals²⁰. Although proteins comprise a minor part of the enamel, they have the function of attaching hydroxyapatite crystals together to maintain the hierarchical structure of enamel²¹. Therefore,

the effects of microabrasion techniques on the mineral and protein components may include the mechanical behavior of enamel. Both acids showed similar alterations of enamel roughness. This may be related to the concentration of hydrochloric acid at 6.6%, different from those initially proposed for the technique, e.g., 36% and 18%^{1,4,22}, which produce a similar conditioning pattern of 35% phosphoric acid²³. With regard to the abrasive, pumice presented excessive abrasiveness and deep erosion compared with the other¹⁹. As H₃PO₄ + Pum is a microabrasive mixture customized by the clinician, the peculiarities of these components should be considered, as their sensitivity to variations may have clinical outcomes.

As the treated surface became less mineralized, it was possible to observe the saliva buffering capacity during the *in situ* regimen^{9,17}. Fluoride action may be considered even with 1 h of saliva exposure, since this component is found in oral environment at high concentration up to 10 h after the last use²⁴. In this study, the volunteers were instructed to use fluoridated toothpaste because its role should be taken into account. This analysis showed a decrease in roughness, which varied depending on the saliva exposure time *in situ* regimen and the used microabrasive system. The treatment with HCl + Sil could be considered more prone to mineral reposition of saliva than that with H₃PO₄ + Pum because, after 1 h of saliva exposure, it was possible to observe decreased roughness, with statistically significant difference compared with the analysis after microabrasion for this group. For both treatments, 24-h exposure to saliva was efficient in reducing roughness, with statistical differences compared with the analysis after microabrasion. The 7-day exposure time resulted in less roughness than the other exposure times, for both microabrasion treatments. Although the reestablishment of roughness was not observed in the total storage period in saliva applied in this study (7 days), analyzing the progressive results of increase in enamel roughness over time and comparing it with studies that used artificial saliva for 15 days, the authors hypothesized that normal enamel roughness probably would be reached even in a longer evaluation time. This result raises the necessity of performing further studies on this subject.

Comparing both treatments, the groups treated with HCl+Sil presented more evidence of superficial mineral reposition as their results after 1 h and 24 h of saliva exposure exhibited the lowest mean values of roughness when compared with H₃PO₄+Pum. The best effects of saliva with the treatment with HCl+Sil may be related to specific action of the compounds HCl and silica. The ion chloride in the buccal environment has been associated with the suppression of hydroxyapatite dissolution²⁵ beside the fact that the hydrochloric acid presents lower pH than the phosphoric acid²⁶, which favors surface rehardening and fluoride uptake^{17,20}. In relation to silica, it has been used in a bioactive material (Ca₃SiO₅) as an important agent to induce the remineralization of an acid-etched enamel²⁷, since it can provide the link between the calcium from hydroxyapatite and from saliva, leading to the formation of a new apatite layer^{18,27}. Therefore, regarding the better results achieved with this treatment, it may be assumed that this compound is formed in the enamel after microabrasion using HCl+Sil.

None of the treatments was able to re-establish the enamel roughness, as found in control group or in the initial condition, in none of the periods of saliva exposure. In previous *in vitro* findings, the enamel roughness treated with microabrasion was restored after 15 days of immersion in artificial saliva⁶⁻⁷. The differences between these studies⁶⁻⁷ and the present one are related to both time of saliva exposure and type of saliva. According to another investigation¹⁵, the deposition of proteins from saliva (e.g. mucins) on the demineralized substrate may be a factor related to the reduction of re-mineralization. Also, the absence of organic content in artificial saliva may have created an optimized system, thus enhancing the mineral reposition on the eroded surface. To the best of the authors' knowledge, this is the first study that evaluated the effect of the human saliva on the enamel after microabrasion, under *in situ* conditions. The delay in the reestablishment of the enamel roughness, which did not occur within 7 days, probably is due to the irregular deposition of ions between the enamel prisms, that happen first in the 10 and 25 μm layers, possibly due to the topographic characteristics of this region, where Retzius lines have a regular periodicity²⁸⁻²⁹.

Despite of the above information, the *in vitro* model does not reflect the dynamic conditions of the mouth, such as continuous flow and clearance of saliva, even using artificial saliva²⁹. Apart from the similar calcium, phosphate and fluoride ions in artificial saliva, human saliva has some advantages that may account for its superior protective ability, such as formation of an acquired enamel pellicle¹¹. This pellicle forms within seconds of exposure to saliva^{11,14} and it has anti-erosive potential because it acts as a diffusion barrier as well as a semi-permeable membrane^{11,23}, being important for the enamel re-mineralization. Moreover, saliva action may be enhanced by the presence of re-mineralizing agents containing fluoride^{8,24}. With regards to the roughness means obtained with the salivary exposure, it was shown that this property could be possibly restored in a short time. Therefore, it should not be stated that the recovery of mineral

content in the enamel after microabrasion is more feasible with artificial saliva, such as *in vitro* models, since they do not mimic the real circumstances of the oral environment.

The CLSM results showed that the enamel surface had remaining erosive effects from the combined action of acid, abrasive agent and mechanical pressure. The images showed an irregular surface with discontinuity points, characterized by microwear points. The recurrence of these points in the groups with only enamel microabrasion reflects the results on enamel roughness. The CLSM images (Figure 2) for the groups in early stages of saliva exposure (1 and 24 h) showed gradual reduction of these points, possibly completing the enamel surface with minerals and ions from saliva. The images also showed that this deposition occurs possibly in a random manner, which could be observed with the roughness results too. After 7 days of saliva exposure in the oral environment, the images demonstrated that the enamel surface tended to be uniform and continuous, as found in the control group, showing the re-mineralizing action of saliva.

A limitation of the present study design should be considered: in clinical situations, enamel microabrasion is performed on the buccal surface of the teeth, whereas in the current study, the enamel blocks were placed on the palate, mainly for the comfort of the volunteers and esthetic implications. Moreover, previous *in situ* studies^{8-9,11,24} chose the palatal model to simulate the condition of the human

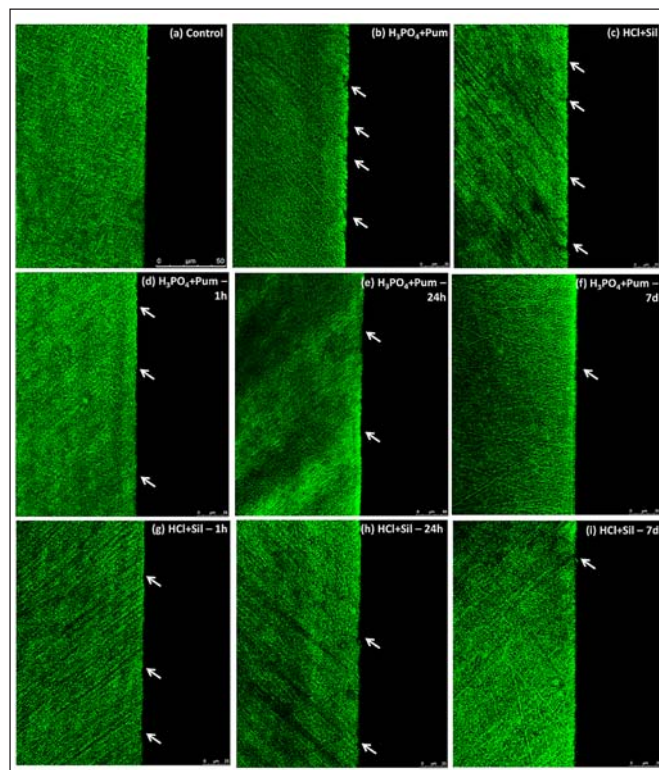


Fig. 2: Representative images of the groups obtained from confocal laser scanning microscopy (CLSM).

Arrows show surface microwear sites on enamel surface resulting from the microabrasion technique and its behavior in the oral environment. (a) Control group; (b) H₃PO₄+Pumice; (c) HCl+Sil; (d), (e) and (f) H₃PO₄+Pumice with 1 h, 24 h and 7 days of salivary exposure respectively; (g), (h) and (i) HCl+Sil with 1 h, 24 h and 7 days of salivary exposure respectively.

oral cavity and successfully proved its superiority over the *in vitro* strategy.

This is the first *in situ* study investigating the action of saliva on the enamel morphology subjected to microabrasion treatment. The results showed that the technique causes a significant alteration in enamel roughness, without differences between the tested microabrasive systems. In addition, saliva plays an important role in re-establishing the characteristics of the enamel, since a 7-day exposure to saliva reduced the roughness mean values and the alterations on enamel surface, according to the CLSM analysis.

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