Morphological Evaluation of Enamel Surface after Application of Two 'Home' Whitening Products

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Purpose: The purpose of this study was to investigate the effect on enamel surface morphology of two commercially available bleaching products (AZ Whitestrips 6% hydrogen peroxide – Procter & Gamble; Platinum TWS 10% carbamide – Colgate Oral Pharmaceuticals) and their ability to prevent enamel demineralization in the presence of cariogenic solution, with or without saliva.

Materials and Methods: Forty sound teeth were used to obtain 90 enamel fragments. Lactic acid (pH = 4.4) was used as a demineralizing cariogenic solution. The specimens were randomized into eight groups: Group A: product A + cariogenic solution; Group B: product B + cariogenic solution; Group C: cariogenic solution; Group D: (control group) stored in deionized water; Group E: product A + deionized water; Group F: product B + deionized water; Group G: product A + saliva; Group H: product B + saliva. Scanning electron microscope (SEM) analysis was performed to detect the type of lesions induced by the treatments. A score rating system was used to perform a non-parametric statistical analysis.

Results: Our study confirms that enamel alterations (i.e. removal of intraprismatic core and presence of deep porosities and pits) occur as a result of the application of a cariogenic solution (lactic acid). The enamel surface presented a honeycomb surface only in untreated samples previously stored in lactic acid solution. Conversely, both products were able to prevent enamel alterations caused by exposure to lactic acid. Saliva treatment reduced the degree of enamel lesions of both treated and untreated groups. Treatment with product A achieved better preservation of enamel integrity.

Conclusion: Whitening treatment conducted with two 'home' bleaching agents had no adverse effects on enamel surface morphology. Several morphological aspects suggest that the tested products may even prevent demineralization of the enamel surface after exposure to lactic acid.

Key words: whitening, SEM, enamel, lactic acid

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The effects produced by hydrogen and carbamide peroxides on tooth color have been known for a long time (Li, 2000). However, the technological process for transferring these discoveries into dental practice has been surprisingly slow (Haywood, 2000; Leonard, 2000). This was partially due to the fact that several decades ago (before the wide usage of fluoride products and before the advent of so-called preventive dentistry), the main problem was carious lesions. Whitening was an innovative method that allowed the patient to 'save time' by using the product at 'home', without the necessity of visiting the dentist. But even so, and in spite of the fact that tray-based systems represented a significant technological development, their assimilation in dental practice remained slow.

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Table 1 Groups of materials and combination of treatments used in the study

Group A: (N = 10) each sample treated with *Product A* and stored in cariogenic solution
Group B: (N = 10) each sample treated with *Product B* and stored in cariogenic solution
Group C: (N = 10) each sample treated with cariogenic solution
Group D: (control group) (N = 10) samples not treated and stored in deionized water
Group E: (N = 10) each sample treated with *Product A* and stored in deionized water
Group F: (N = 10) each sample treated with *Product B* and stored in deionized water
Group G: (N = 10) each sample treated with *Product A* and stored in saliva
Group H: (N = 10) each sample treated with *Product B* and stored in saliva

Some studies have investigated the effect of hydrogen and carbamide peroxide on enamel surface morphology (Basting et al, 2001; Rotstein et al, 1996). These studies considered such agents as important for their ability to modify the mechanical properties of enamel, and its surface morphology and profile (Rodrigues et al, 2001). There are only few studies that have investigated the bleaching capacity of carbamide peroxide for modifying the enamel surface. It was suggested that several repeated bleachings might affect the enamel surface. Several investigations suggested that the enamel became partially demineralized and porous after bleaching treatment and for this reason bleaching treatments must be avoided by patients affected by recurrent carious lesions. However, the difference between the types of lesions induced by bleaching on the enamel surface and the types of lesions formed on carious-affected enamel are not clear. Thus, investigations need to be performed in an attempt to evaluate whether the enamel surface may be affected by bleaching agents and if cariogenic-acid exposure may increase the risk of acid dissolution of the enamel after bleaching treatment.

In this *in vitro* study we have therefore compared two commercial bleaching products in order to investigate any ultrastructural change to the enamel surface which was examined by SEM after whitening treatment, and after acid exposure using a pump-perfusion device.

MATERIALS AND METHODS

Forty human molars free of caries, restorations and surface defects and stored in water to prevent dehydration prior to preparation, were used throughout the study. The teeth were sectioned in order to obtain 90 enamel fragments. No disinfectants were used to avoid ultrastructural modifications of dental tissues. The samples that had demineralization or irregularities covering even a small area were not included in this study. The whitening systems tested in our study were: product A, AZ Whitestrips (Procter & Gamble, Cincinnati, OH, USA); and product B, Platinum TWS (Colgate Oral Pharmaceuticals, Piscataway, NJ, USA).

Evaluation of the carioprotective effect: samples were randomized into eight groups as shown in Table 1.

Procedure for the demineralization of the samples: the pH of the demineralizing solution (lactic acid 0.1 M) was formulated at 4.4 using 32% ammonium hydroxide solution. This solution (cariogenic solution) was used to reproduce *in vitro* demineralizative lesions that occur *in vivo* in the carious process after an acid attack.

Non-stimulated saliva sample: saliva was taken consistently from a unique subject having good general and oral health (lack of systematic diseases, not on medication, healthy periodontal condition, lack of active carious lesions) with a pH of 7.0 – 7.7.

The samples were taken three hours after the oral 'home' hygiene and during this interval the subject did not take any food or beverage of any type. CRT® buffer (Ivoclar Vivadent, Schaan, Liechtenstein) was used to determine the buffer capacity of saliva by means of a test strip featuring a special indicator system. Stimulated salivation was obtained by asking the subject to chew a paraffin pellet and collect the saliva in a calibrated container over a period of 5 minutes. The buffer capacity of the saliva was determined by comparing the color of the test field with the color samples (corresponding to high, medium and low buffer capacity) after 5 minutes of reaction time. The buffer capacity of the examined saliva was medium.

The enamel fragments were divided into eight groups:

Groups A and B

Samples were respectively treated with product A and B, then rinsed for five seconds under running

Table 2 Groups A, B. These procedures were repeated daily for three consecutive days

Group	First treatment	Deionized water	Cariogenic solution	Deionized water	Second treatment	Deionized water	Cariogenic solution
A	Product A 30 min	30 min	30 min	2 hours	Product A 30 min	30 min	30 min (rinsed) 19 hours
B	Product B 1 hour		30 min	2 hours	Product B 1 hour		30 min (rinsed) 19 hours

Table 3 Group C. These procedures were repeated daily for three consecutive days

Group	Deionized water	Cariogenic solution	Deionized water	Cariogenic solution
C	1 hour	30 min	2 hours (rinsed) 1 hour	30 min (rinsed) 19 hours

Table 4 Groups E, F. These procedures were repeated daily for three consecutive days

Group	First treatment	Deionized water	Second treatment	Deionized water
E	Product A 30 min	30 min (rinsed) 2 hours	Product A 30 min	30 min (rinsed) 20 hours
F	Product B 1 hour	2 hours	Product B 1 hour	20 hours

water, gently dried and after immersion in cariogenic solution washed for five seconds under running water, and finally stored for two hours in deionized water. A second treatment was subsequently performed, followed by five seconds washing under running water and immersion in cariogenic solution. After this treatment each sample was immersed for nineteen hours in cariogenic solution (Table 2). This procedure was repeated daily for three consecutive days.

Group C

Samples were stored in deionized water, rinsed for five seconds under running water, gently dried, immersed in cariogenic solution, washed under running water for five seconds, stored for two hours in deionized water and still rinsed for five seconds. A second treatment was subsequently performed followed by five seconds washing under running water and immersion in cariogenic solution. After this treatment each sample was immersed for nineteen hours in cariogenic solution (Table 3). This procedure was repeated daily for three consecutive days.

Group D

Control group samples were stored in deionized water at 37 °C for three consecutive days.

Group E and F

Samples were respectively treated with product A and B, then rinsed for five seconds under running water, immersed in deionized water and still rinsed for five seconds. A second treatment was subsequently performed followed by five seconds washing under running water and finally the samples were immersed in deionized water for twenty hours (Table 4). This procedure was repeated daily for three consecutive days.

Group G and H

Samples were respectively treated with product A and B, then rinsed for five seconds under running water, immersed in deionized water and still rinsed for five seconds. A second treatment was subse-

Table 5 Groups G, H. These procedures were repeated daily for three consecutive days

Group	First treatment	Deionized water	Second treatment	Deionized water	Saliva
G	Product A 30 min	30 min (rinsed) 2 hours	Product A 30 min	30 min	20 hours
H	Product B 1 hour	2 hours	Product B 1 hour		20 hours

quently performed, followed by five seconds washing under running water and finally the samples were immersed in saliva for twenty hours (Table 5). This procedure was repeated daily for three consecutive days.

SEM EVALUATION

The samples were dehydrated and covered with a 20 Angstrom gold layer using a metalization process executed in vacuum. Each sample was analyzed under a Scanning Electron Microscope (Model 5400 Jeol, Japan) and evaluated independently by two observers. Each observer provided three evaluations for each sample (350X, 2000X, 5000X), for a total of six measures per sample. The arithmetic media was calculated for each group, and subsequently the respective media were compared (tables included in the manuscript as appendix).

The integrity level of the enamel surface was evaluated as per the following criteria:
Grade 0: Enamel surface remained perfectly intact with no grooves, pits and porosity.
Grade 1: Enamel surface presented a small number of irregularities.
Grade 2: Presence of surface irregularities at the superficial level, without demineralization of prismatic and/or interprismatic enamel.
Grade 3: Presence of wrinkles and light demineralization of prismatic and/or interprismatic enamel.
Grade 4: Important level of wrinkles and loss of prismatic and/or interprismatic enamel.
Grade 5: Modification and/or decomposition of morphology of prism.

Kruskal-Wallis's test for non parametric data was used.

RESULTS

SEM analysis revealed several differences among the groups tested as follows:

Control solution (Group D). The enamel surface appeared unaltered (Fig 1) and only few pit lesions were visible, probably as a consequence of *in vivo* early lesions.

Cariogenic solution (Group C). The positive control result was particularly characterized by enamel lesions at the level of prisms (Fig 2) with the loss of central core substance and exposition of some crystals (Fig 3). The interprismatic substance appeared altered and credibly modified. In some samples, dissolution penetrated deeper layers, and in others it remained more superficial; however, the final topography appeared to be overlapping in all cases.

Cariogenic solution + product A (Group A). The morphology appeared only slightly modified (Fig 4). Considerable debris was detected on the top of the enamel surface. Some resultant debris appeared to be organized as a network and/or a layer of pollution. A type of 'artificial smear layer' was often seen, with a matrix covering the surface (Fig 5). This layer of debris covered the enamel and formed a type of film that masked the surface.

Cariogenic solution + product B (Group B). The observed morphology was similar to the previous group, but presented widespread and compacted debris between the demineralized areas (Fig 6). This resultant debris appeared to be characteristic of this solution and filled the grooves and pits created by cariogenic solution.

Deionized water + product A (Group E). The enamel morphology appeared intact. There was much debris located in a non-uniform way on the enamel surface (Fig 7). This debris was able to partially cover the enamel surface.

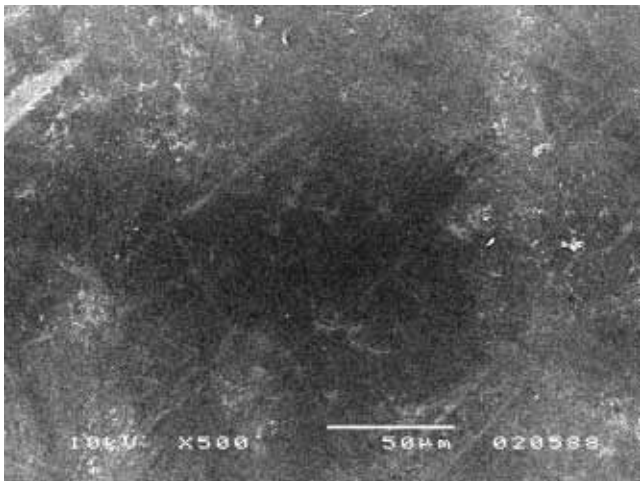

Fig 1 Control solution (group D): the enamel surface appeared unaltered and only few pit lesions were visible, probably as a consequence of *in vivo* early lesions. (x500)

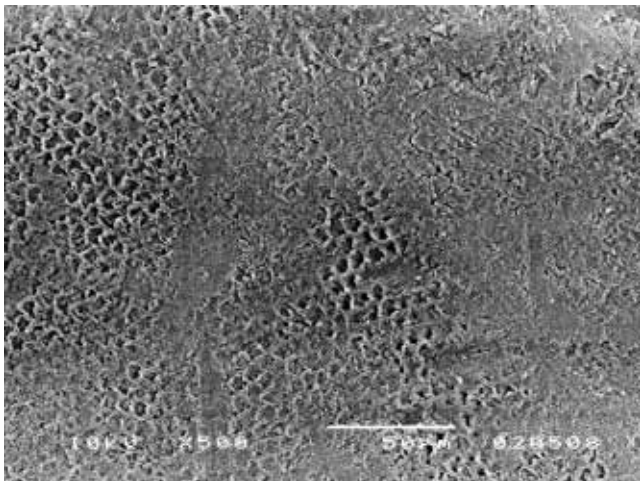

Fig 2 Cariogenic solution (Group C): the positive control result was particularly characterized by enamel lesions at the level of prisms with the loss of central core substance and exposition of some crystals. (x500)

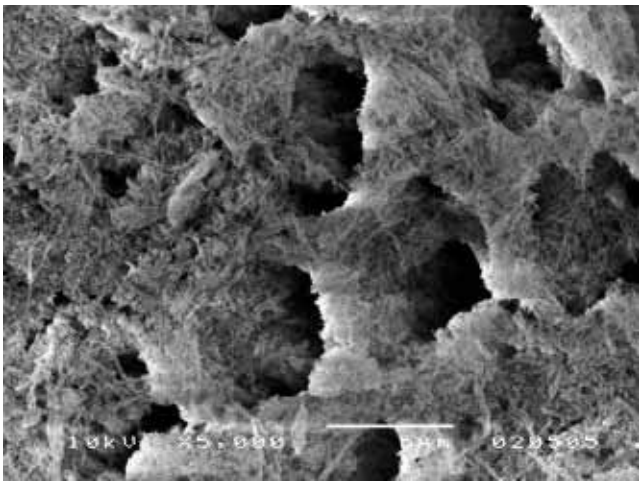

Fig 3 Cariogenic solution (Group C): the interprismatic substance appeared altered and credibly modified. (x5000)

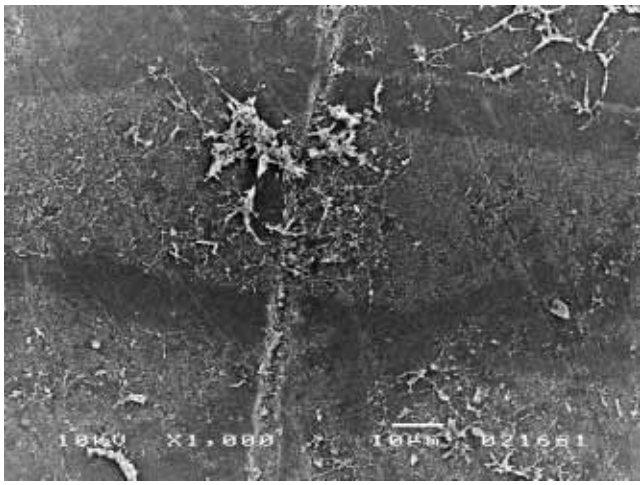

Fig 4 Group A: the morphology appears only lightly altered (Fig 4). Considerable debris was detected on the top of the enamel surface. Some resultant debris appeared to be organized as a network and/or a layer of pollution. (x1000)

Deionized water + product B (Group F). The enamel appeared whole and only a few crateriform lesions were visible, probably as a consequence of *in vivo* (early) micro carious lesions (Fig 8). The surface appeared to be comprehensively sprinkled with fine debris.

Saliva + product A (Group G). The enamel morphology did not reveal superficial alterations as a result of the received treatment. It was evident in this case also that a type of biofilm or artificial smear layer masked the enamel surface (Fig 9).

Saliva + product B (Group H). The morphology of these samples did not show superficial alterations and was characterized by superficial debris layered on the enamel surface. Only a few samples were partially and lightly spoilt (Fig 10).

The number of samples and the media of ranks for each treatment are shown in Table 6. The mean scores are shown in Table 7. Kruskall-Wallis's test (175.0; $p < .01$) produced highly significant results. Data in Fig 11 are shown as Box-and-Whiskers-Plots.

The results obtained from this study showed that there was no significantly statistical difference between groups A, D, E, F, G, H, while samples of group B revealed greater demineralization com-

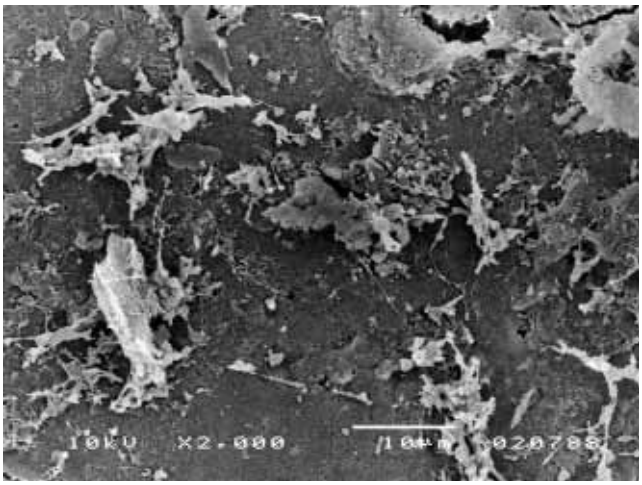

Fig 5 Group A: a type of 'artificial smear layer' was often seen, with a matrix covering the surface. This layer of debris covered the enamel and formed a type of biofilm that masked the surface. (x2000)

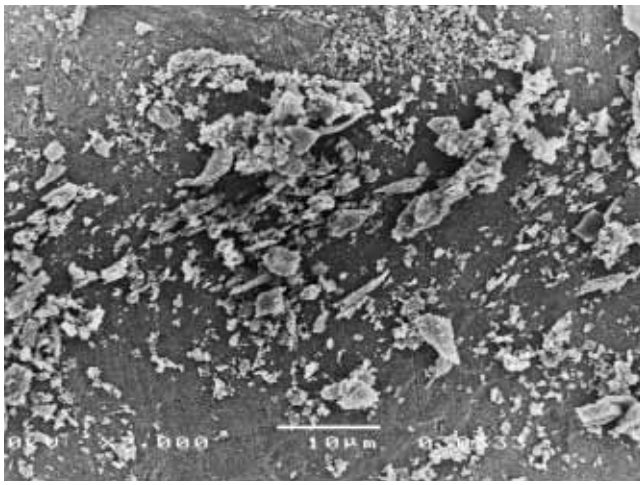

Fig 6 Group B: the observed morphology is similar to the previous group, but presented widespread and compacted debris between the demineralized areas. (x2000)

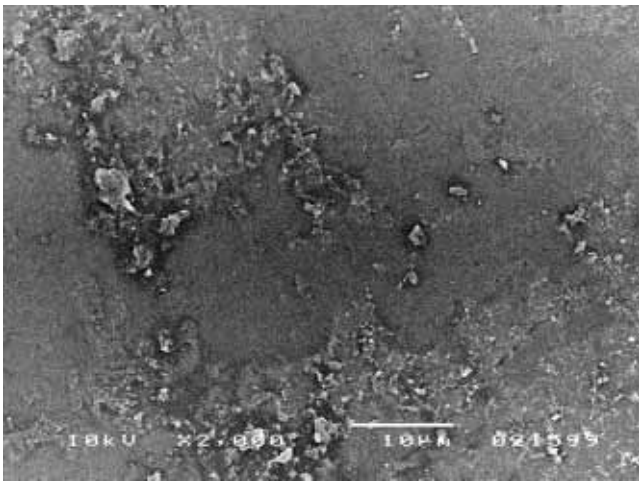

Fig 7 Group E: enamel morphology appears intact. There was much debris located in a non-uniform way on the enamel surface. (x2000)

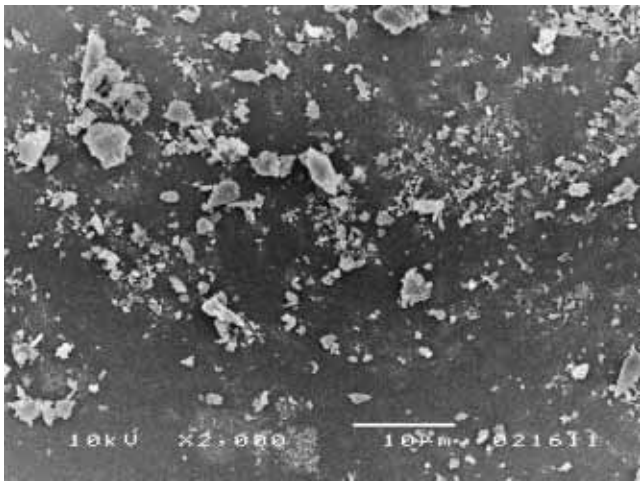

Fig 8 Group F: enamel appears whole. The surface appears to be comprehensively sprinkled with fine debris. (x2000)

pared to the previous groups. Group C, treated with lactic acid, showed a superficial alteration of enamel that was significantly higher than other groups.

DISCUSSION

It has been clearly demonstrated in the literature (Maupomè et al, 1999; Amaechi et al, 1999; Frank, 1990) that cariogenic solution substantially modifies enamel morphology, and that lactic acid typically causes the loss of the interprismatic substance. Studies on early carious lesions (Hubbard, 1982) indicated that the acid attack initially involves spots of minor mineral density on the enamel surface located in conjunction with the growth lines or Retzius strias, in which a minor number of apatitic crystals is present. The acid attack further affects the interprismatic areas and the periphery of enamel prisms, being characterized by a greater organic content. However, it is only in the final phases that demineralization begins to affect the central part of the prisms (core). As to *in vitro* demineralization, obtained by exposure of enamel to organic or inorganic diluted acids, the acid attack produces a dissolution pattern, observable under SEM,

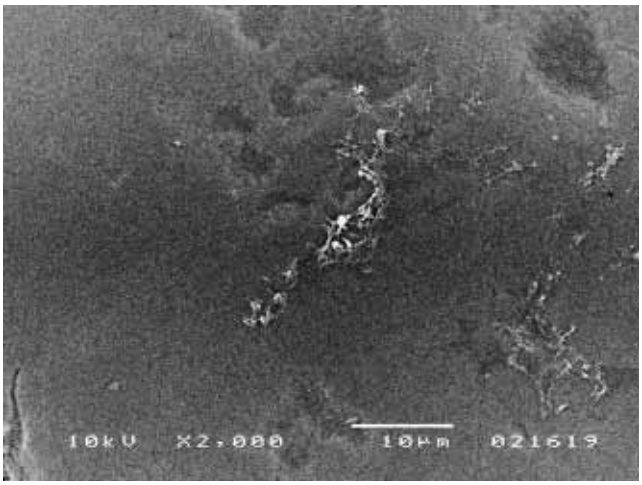

Fig 9 Group G: enamel morphology did not reveal superficial alterations as a result of the received treatment. Also it is evident in this case that a type of biofilm or artificial smear layer masked the enamel surface. (x2000)

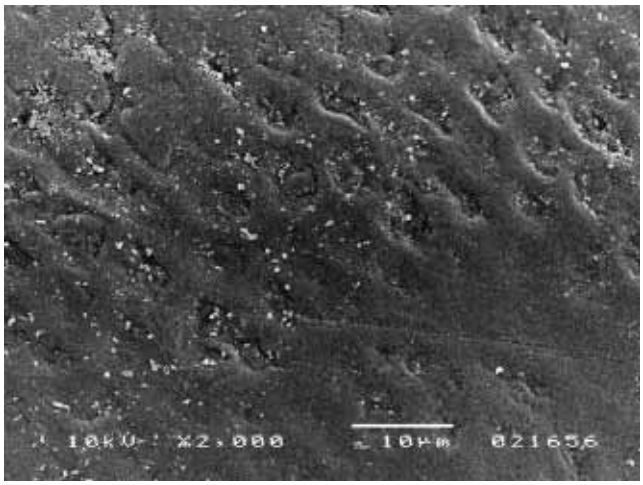

Fig 10 Group H: the morphology of these samples did not show superficial alterations and was characterized by superficial debris layered on the enamel surface. (x2000)

Table 6 Kruskal-Wallis Test for score by group

Group	Sample Size	Average Rank
A	60	148.244
B	60	203.905
C	60	312.818
D	60	115.366
E	60	128.095
F	60	124.833
G	60	154.190
H	60	151.929

Table 7 Mean scores for the groups A–H

GROUP	MEAN
A	0.41
B	1.08
C	3.64
D	0.08
E	0.18
F	0.14
G	0.36
H	0.36

which marks the main involvement of the prismatic cores, with a final morphology similar to that of a beehive and thus called 'honeycomb'.

Actually this is true only for enamel subjected to prolonged exposures of dilute acids: brief contacts or exposure to very concentrated acid solutions lead to a prevalent demineralization of the prism's periphery. Therefore, in the initial phases, even *in vitro* demineralization begins to affect areas (e.g. interprismatic enamel and periphery of prisms) characterized by a greater porosity, and for this reason these become more pervious to acid attack. The prismatic cores are only later affected by dissolution following extended acid exposure.

In the final phase, and after long exposure, the enamel surface is characterized by a bee's nest morphology, where the prism core appears to be dissolved more rapidly, since the peripheral areas seem more prominent due to the orientation of hydroxy-apatite crystals. Actually, this ought to be the result of a reprecipitation process of dental mineral at the junctions of prisms, caused by the immobility of the solutions used in *in vitro* experiments that allows a precipitation of the products in the first phases of dissolution (Tyler, 1976). Even our study confirms such action. However, our research proved that product A reduced and attenuated the effects of cariogenic solution, thereby reducing the loss of interprismatic substance.

That effect confirms some preliminary clinical indications that suggest a certain degree of enamel protection afforded by some whitenings. The mech-

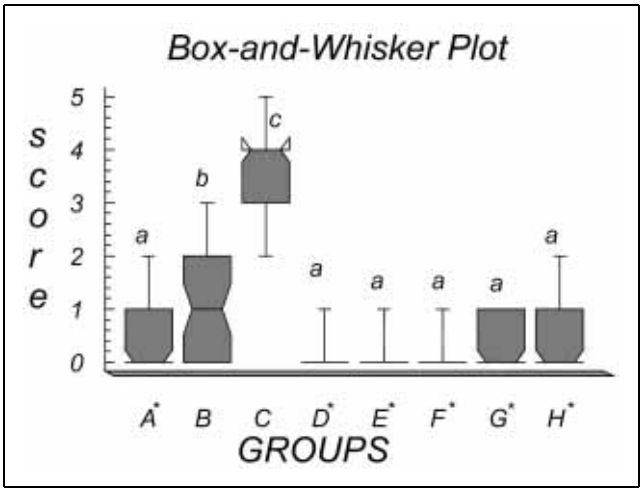

Fig 11 The Kruskal-Wallis test tests the null hypothesis that the medians of score are the same within each of the 8 levels of treatment. * = Same statistical group.

anisms which allow the enamel to be less damaged should probably be sought in the formation of a type of 'artificial film' which strongly reduces the flow and times of contact between lactic acid and enamel. It is worth remembering that carbamide peroxide, in the presence of water, becomes divided into hydrogen peroxide and urea. Subsequently, the disproportionation of hydrogen peroxide into oxygen and water takes place, representing the active, while urea is hydrolyzed in carbon dioxide and ammonium. Some whitening systems, such as product A, also include Carbopol® , a polyacrylic acid neutralized by triethanolamine with the aim of increasing the formulation viscosity (thereby increasing adhesion to the tooth surface) and inhibiting the action of salivary catalase, which antagonizes the whitening effect of hydrogen peroxide (McGuckin et al, 1992). Thus it is possible that such a formulation could be at the root of such an 'artificial film', which, as we have previously assumed, is able to reduce the contact of lactic acid with dental enamel, both quantitatively and in the terms of time.

A recent study conducted by Turkün et al (2002) that investigated the effects of two 10% carbamide peroxide whitenings, showed that the tested products caused slight alterations of enamel morphology that were immediately noticeable after application of whitening. Notwithstanding the above, the grade of such alterations depended on the whitening product and on the time of application. However, within a period of approximately three months, we can see an almost complete reversibility of the above-mentioned alterations.

In a morphological study by Lopes et al (2002) which investigated enamel morphology after the application of two 'home' whitening systems and separated solutions of 10% carbamide peroxide (3.6% hydrogen peroxide and 6.4% urea), the authors concluded that they had not noticed alterations of enamel in the sample treated with commercially available products containing 10% carbamide peroxide, while non-uniform modifications were observed. This further confirms that different formulations of whitening systems lead to different interactions with dental enamel, comes from a recent work by Auschill et al (2002) in which the effect of three whitening systems on the enamel surface (two 'home' and one in-office) were investigated. In this study the authors did not notice alterations to the enamel surface in any analyzed sample.

Thus, in the present study we hypothesize that the application of product A induces the formation of a type of adhesive artificial film that protects the enamel surface, thereby reducing the acid attack and the intra and interprismatic demineralization. Such a film is probably very thin, and sufficiently stable and impermeable to prevent the acid solution (and therefore demineralization) from acting on the enamel surface. Therefore, it can be considered a type of barrier (or a buffer) that is capable of preventing acid contact with the enamel surface. Our SEM analyses clearly showed the presence of such a film on the surface of the enamel.

Similarly, carbamide peroxide in conjunction with other compounds can produce a relevant buffer effect, thereby maintaining local pH above 4.5–4.8, which is the level that is considered critical and of sufficient value to induce serious demineralization in exactly the same way as observed in the samples treated with lactic acid.

The present study suggests, therefore, that there may a clinical benefit from the use of some whitening solutions – both to prevent the risk of demineralization and subsequently the risk of white spots. The *in vivo* clinical studies for the evaluation of such results are currently under investigation.

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