Original article

Effect of mouth rinses on tooth enamel surface

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Abstract: This study aims to evaluate the effect of mouth rinses on the color, microhardness, and surface roughness of tooth enamel. Sixty dental blocks were collected from human third molars and divided in five groups ($n = 12$): the control group (CG) without immersion in mouth rinse, Listerine Zero, Colgate Plax Fresh Mint, Listerine Whitening, and Colgate Luminous White. The groups were subjected to initial color analysis; the microhardness and roughness of the enamel surfaces were evaluated. Next, the samples were subjected to immersion in mouth rinses or brushing with conventional fluoride toothpaste (CG) according to the manufacturer's instructions; after a 12-week treatment, the color, microhardness, and roughness were once again assessed and compared with the initial analysis. Data were tabulated and analyzed through a two-way analysis of variance (ANOVA) (color and roughness) followed by Tukey's test. Microhardness was analyzed through the Kruskal-Wallis test followed by Dunn's test; the level of significance was 5%. All groups immersed in mouth rinses had a higher level of microhardness loss than CG; additionally, all groups showed changes in the enamel surface. Enamel surface loss was observed using a roughness test, and the mouth rinses promoted a higher level of color changes than CG. Given the results, it can be concluded that the mouth rinses led to significant changes in tooth enamel.

Keywords: change color, enamel surface, microhardness, mouth rinses

Introduction

Over-the-counter (OTC) refers to products that are sold freely to consumers in supermarkets, drugstores, convenience stores, and on the Internet. This type of product does not require a prescription or professional application [1-5] because it is self-applicable and can be found in various presentations, such as whitening dentifrices, mouth rinses, chewing gum, and paint-on films. [1,2,6]. They are used as oral hygiene auxiliaries, for halitosis prevention, and sometimes as bleaching agents because some of these products contain low concentrations of a bleaching agent in their formulations [1,4,5].

Mouth rinses are also OTC products and are essentially composed of water, antimicrobials, a coloring agent, and salts [5]; sometimes alcohol is used as solubilizer and preservative [7,8], and hydrogen peroxide or a bleaching agent [2,5,6,8] is used in concentrations of approximately 3% to 6% [1]. It is known that some mouth rinses showed efficacy when used as bleaching agents as described by Torres et al. [4] and Lima et al. [2]; however, the daily contact of such products for a long period of time without professional supervision can promote changes in the surface of the tooth enamel. The implication of continuous or prolonged use of mouth rinse is still controversial; studies suggest that continuous use can cause changes in the surface of the tooth enamel [2,5,9,10], while there are authors [8] who did not find significant alterations in human tooth enamel subjected to mouth rinse by simulating a daily application period with cycles of immersion times and intervals different than those indicated by the manufacturers and that correspond to daily clinical practice.

Furthermore, patients have been increasingly concerned about more effective oral hygiene habits; the use of chemical control agents (i.e., mouthwash) has been adopted to complement tooth brushing and flossing. An investigation has shown that when enamel is exposed to an inorganic aqueous solution that is unsaturated in relation to hydroxyapatite and fluorapatite, the enamel surface is altered in that a microlesion forms that is microscopically similar to the erosion that is naturally develops in the oral cavity [11]. Thus, the indiscriminate use of mouthwash, even for different purposes, may cause morphological changes on the surface of the enamel [2,9-13] and result in unexpected and unwanted consequences to the dental structure.

Therefore, due to the indiscriminate use of these products, it has become timely to evaluate the effects of different types of mouth rinses available in the market, including the different compositions and forms of use, the change in the enamel's color due to the addition of hydrogen peroxide. Moreover, to evaluate the impact on the enamel surface of the tooth subjected to immersion, simulating the protocol of daily clinical use indicated by the manufacturers regarding brushing, immersion time, interval, and total period of treatment. The hypothesis tested states that there will be no change in the microhardness, roughness, or color of the enamel after the daily use of mouth rinse for 12 uninterrupted weeks, simulating clinical application, as indicated by manufacturers.

Materials and Methods**Power analysis**

The sample size was determined according to microhardness data collected from the study by Berger et al. [14]. A power analysis indicated that at least nine teeth per group would have an 80% chance of obtaining significance at the 0.05 level.

Sample selection and preparation

The present study was submitted and approved by University of North Parana ethics committee (protocol #1.810.114). Sixty human third molars without cavities or enamel defects extracted for therapeutic reasons were selected. The teeth were cleaned and stored in 0.5% chloramine T for seven days immediately after extraction and then stored in distilled water at 4°C until the experiment was initiated.

Enamel blocks were collected from the vestibular and lingual surface of each tooth (7 mm wide $\times$ 4 mm deep $\times$ 7 mm high). The blocks were fixed on acrylic discs using wax. The enamel surfaces were abraded with 600, 1,200, and 2,000 grit silicon carbide paper and polished with abrasive paper and 1 μ m and 0.25 μ m diamond paste in an electric polisher (AROPOL-2V, Arotec S/A Ind. e Comércio, Cotia, Brazil). The specimens were immersed in an ultrasonic bath with deionized water for 10 min (Ultrasonic Cleaner 1400, Odontobras, Ribeirão Preto, Brazil) to remove all the waste. The prepared specimens were examined in a stereomicroscope (Bel Microimage Analyser, Bel Photonics, Monza, Italy) to confirm the absence of cracks or other surface defects. Next, they were stored in moisture to avoid dehydration.

The superficial microhardness in all of the samples was determined using the Shimadzu micro hardness tester (HVM-G 21S, Shimadzu Corp., Kyoto, Japan) equipped with a Knoop-type indenter at a static charge of 25 grams applied every 10 s according to Araújo et al. [11]. Three indentations (100 μ m from one another) were made in the center of the fragments of each sample. The first indentation in the initial mark was made in the upper

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Table 1 Group, manufacturer and lot, composition and pH of the mouth rinses used in the study

Group	Trade name, manufacturer, and lot	Composition	pH measured
CG	-----	distilled water	----
LZ	Listerine Zero, Johnson & Johnson do Brasil Indústria e Comércio de Produtos para Saúde Ltda, São Paulo, Brazil / 1287B02	water, sorbitol, propylene glycol, thymol, menthol, methyl salicylate, eucalyptol, flavor (<i>d</i> -limonene), sodium lauryl sulfate, poloxamer 407, sodium saccharin, sucralose, benzoic acid, sodium benzoate, CI 42053	3.63
CPF	Colgate Plax Fresh Mint, Colgate-Palmolive Ind. Ltda. São Paulo, Brazil / 7045BR121C	water, glycerin, propylene glycol, sorbitol, poloxamer 407, flavor, cetylpyridinium chloride, sodium fluoride, potassium sorbate, sodium saccharin, citric acid, CI 42053, CI 15985.	5.05
LW	Listerine Whitening, Johnson & Johnson do Brasil Indústria e Comércio de Produtos para Saúde Ltda. / 0285C	water, alcohol (8%), hydrogen peroxide, sodium phosphate, poloxamer 407, sodium lauryl sulfate, sodium citrate, mint flavor, menthol, eucalyptol, sodium saccharin, sucralose	5.38
CLW	Colgate Luminous White, Colgate-Palmolive Ind. Ltda. / 6179BR122C	water, glycerin, propylene glycol, sorbitol, tetrapotassium pyrophosphate, polysorbate 20, tetrasodium pyrophosphate, zinc citrate, pvm/ma copolymer, flavor, benzyl alcohol, sodium fluoride, sodium saccharin, CI 42051	6.20

left corner; 1,500 μm on the horizontal plane and 1,500 μm on the vertical plane, and the mean values were obtained by the indentations represented the sample.

The mean overall hardness of all blocks was calculated, and those presenting values lower or higher than 10% were excluded from the study. Finally, the dental enamel blocks were selected and randomly divided into five experimental groups ($n = 12$). Two statistical tests were performed sequentially: the Kolmogorov–Smirnov test for normality and the one-way ANOVA test with a significance level of 5% to verify whether the selected samples were statistically equal at baseline ($P > 0.05/P = 0.775$). Each sample was measured at two points in time: when the study began and after the mouth rinse application.

Table 1 shows the experimental group, manufacturer, batch, composition, and pH of each mouth rinse used in the study. The microhardness (Knoop), color, and surface roughness (Ra) of each enamel sample were assessed and set according to the baseline values prior to the bleaching treatment.

Color evaluation

The samples were dried in absorbent paper before color evaluation. A trained examiner performed the measurements of each sample under standardized environmental conditions with the aid of an acrylic guide at 3 mm aperture positioned in the center of the sample in order to enable tip-positioning of the digital spectrophotometer (VITA Easyshade Advance, Zahnfabrik, Bad Sackingen, Germany). The spectrophotometer was calibrated using a calibration plate according to the manufacturer's recommendations. The following coordinates were recorded for each sample: L^* , a^* , and b^* . Value L^* represented the degree of lightness; it could vary from black (0) to white (100). Values a^* and b^* represented the red ($+a^*$), green ($-a^*$), yellow ($+b^*$), and blue ($-b^*$) degree in the samples [15–17]. Each sample was measured at two points in time: when the study began and after the mouth rinse application.

Surface roughness evaluation

The Ra surface roughness (mean surface roughness) of the samples was found using a digital profilometer (SJ-410, Mitutoyo, Tokyo, Japan). Three measurements were made in three different directions (vertical, horizontal, and transversal) at different points in time at a length of 0.25 mm [18] and speed of 0.01 mm/sec in each sample. The mean of the three measurements at each time point was calculated and used in the statistical analysis. Each sample was measured at two points in time: when the study began and after the mouth rinse application.

Mouth rinse application

Samples were immersed in mouth rinses according to the manufacturer's instructions:

Group CG (control group): Brush with fluoride toothpaste for 2 min. Do this two times per day (morning and evening).

Group LZ (Listerine Zero): Brush with conventional fluoride toothpaste for 2 min, then immerse in the LZ mouth rinse solution for 30 s. Do this two times per day (morning and evening).

Group CPF (Colgate Plax Whitening): Immerse in CPF mouth rinse solution for 30 s, then brush with conventional fluoride toothpaste for 2 min. Do this two times per day (morning and evening).

Group LW (Listerine Whitening): Immerse in the LW mouth rinse solu-

tion for 60 s, then brush with conventional fluoride toothpaste for 2 min. Do this two times per day (morning and evening).

Group CLW (Colgate Luminous Whitening): Brush with conventional fluoride toothpaste for 2 min, then immerse in the CLW mouth rinse solution for 60 s followed by re-brushing. Do this two times per day (morning and evening).

All treatments were performed in constant agitation in a shaker at 37°C, during all treatment time with the mouth rinses. Furthermore, samples were stored in distilled water at 37°C during the immersion intervals then replaced on a daily basis [19]; this occurred in all groups. Color reading, microhardness, and surface roughness of each enamel sample was once more determined 24 h after the last immersion.

Data analysis

The final microhardness values were compared to the initial values, and the percentage of surface microhardness loss (%ML) was calculated, as follows:

$$100 - [\text{microhardness after treatments} \times 100 / \text{initial microhardness}]$$

The results of the final and initial roughness were compared, and the loss of the surface smoothness of the enamel was obtained in micrometers (μm). The delta E (ΔE) total difference, or the distance between the two colors initially and after the treatments, was calculated according to the following formula: $\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$ where the applied values were those of the coordinates L^* , a^* , and b^* obtained at initial and final points in time.

Statistical analysis

The statistical analysis was performed using Minitab 18 (Minitab, State College, PA, USA). First, the homoscedasticity and normality of all the data were assessed using Bartlett's test and the Kolmogorov–Smirnov test, respectively. The microhardness data were not present homoscedasticity ($P = 0.0013$) and normal distribution ($P = 0.004$), therefore were subjected to the Kruskal–Wallis test ($P < 0.05$), which identified a difference among the groups. Next, Dunn's test ($P < 0.05$) was applied. The values of color and roughness were normal ($P = 0.052$ and $P = 0.058$, respectively) and had a homoscedasticity of ($P = 0.151$ and $P = 0.64$, respectively), and thus were subjected to ANOVA, which showed a significant difference among the groups and justified the application of Tukey's test ($P < 0.05$) at a 5% significance level.

Results

Microhardness changes

Microhardness data were used to calculate power of statistical test. The power analysis demonstrated a power of 0.99 when the highest difference among the groups was adopted, a Knoop microhardness (KHN) of 47.85, and a standard deviation of 16.73. Table 2 describes the mean values (standard deviation) of the percentage of surface microhardness loss (%ML) according to the experimental groups and were calculated based on the comparison between the initial and final values of surface microhardness.

It was observed that the control group, which was not exposed to the mouth rinses, had a lower %ML and was statistically lower; it was different from the groups subjected to the mouth rinses. The LZ group presented the highest %ML. All the groups had microhardness changes and were different from the CG; this result indicates that contact with mouth rinses

Table 2 Average values (standard deviation) of % Microhardness loss (%ML), surface roughness (Ra) and color change (ΔE), according to the experimental groups ($n = 12$)

Groups	% ML	μm^*	ΔE
CG	9.45 (1.54) D	-0.130 (0.025) E	3.30 (0.87) C
LZ	57.30 (4.55) A	-0.534 (0.027) A	8.74 (2.93) B
CPF	39.24 (5.08) C	-0.264 (0.036) C	7.95 (3.63) B
LW	46.94 (5.45) B	-0.385 (0.035) B	12.89 (3.85) A
CLW	42.13 (5.53) BC	-0.166 (0.026) D	8.35 (3.78) B

Means followed by uppercase letters (columns) are statistically different according to Dunn's test (%ML) and Tukey's test (Ra and ΔE), ($P < 0.05$). *minus sign (-) indicates surface enamel loss

caused changes in the microhardness of human tooth enamel. The LW and CLW groups were statistically equal.

Surface roughness changes

The mean values of surface roughness (Ra) according to the experimental groups are described in Table 2. All groups presented enamel surface loss and were different from each other. However, the CG group had the lowest loss, and the LZ group had a higher enamel surface loss compared to the other groups followed by the LW > CPF > CLW > CG.

Color change evaluation

Table 2 describes the mean (standard deviation) ΔE values from the samples according to the experimental groups. The mean color coordinate (L^* , a^* , and b^*) in each group was statistically equal to that of the baseline (T_0). It was observed that the CG group presented the lowest variation in color and was different than the other groups. The LW group showed the highest variation in color and was different than the control group and the groups subjected to the mouth rinse immersion. The CLW, LZ, and CPF groups presented variation in color statistically similar to one another and were different from the LW and CG groups.

Discussion

The current in vitro study investigated changes in color, microhardness, and surface roughness of human tooth enamel subjected to daily applications of different mouth rinses. After 12 weeks of daily applications, the results indicated that the frequent utilization of these products can result in significant changes to the surface of the enamel. Thus, the hypothesis, which stated that there will be no change in the microhardness, roughness, or color of the enamel after the daily use of mouth rinse for 12 uninterrupted weeks as indicated by manufacturers, was rejected.

A decrease in the microhardness values and an increase in the tooth enamel surface roughness in the samples that received mouth rinses applications was observed. The group that was subjected to LZ immersion presented the highest %ML and the highest surface enamel loss, although all groups showed microhardness changes and superficial enamel loss. This result is similar to those of previously performed in vitro studies [5,9-13]. It found significant changes to tooth enamel surfaces subjected to mouth rinse and, in addition, products containing alcohol may induce changes to oral tissues [3].

The pH value critical to tooth enamel is 5.5 [5]; the LZ mouth rinse has 3.63, which is lower than that supported to the dental enamel and lowest of the pH among the mouth rinse tested in the present study. There is a probability that LZ's low pH value can promote enamel surface demineralization, and, consequently, the microhardness decreases and the surface roughness increases; such results corroborate studies by Lima et al. [2] and Fernandes et al. [5], who stated that continuous tooth exposure to acid products can result in numerous complications. Leonard et al. [9], Araújo et al. [11], and Potgieter et al. [12] have also found significant changes in microhardness and tooth enamel surface roughness and suggested that users of these products should be informed of the risks of their prolonged use. These substances should be used with caution [4].

However, in an in vitro study, Pelino et al. [8], contrary to the findings of the present study, found no changes in tooth enamel structure after the use of different mouth rinses simulating the application for 90 and 180 days, using daily cycles 30 or 60 min, respectively; however, when simulating a long period of daily application with different cycles of immersion times and intervals, the study did not comply with the protocol of daily clinical

use regarding brushing, immersion time, interval, and total period of treatment as indicated by the manufacturers and adhered to in the present study. This may produce different results than what occurs clinically since brushing one's teeth with fluoride toothpaste prior to or after immersion in the mouth rinses, as indicated by the manufacturers and performed in the present study, may result in changes in the enamel surface not detected in Pelino et al.'s study [8].

The ΔE values demonstrate that if there is a color change between the two time periods, some authors [7] only consider the color change visually detectable when the ΔE is greater than 3.7, the threshold value of ΔE that was adopted in this study. By observing the samples' mean values of ΔE , it was verified that the LW group showed the highest color variation and differed from the other groups subjected to immersion in mouth rinses as well as from the control group, which did not present a clinically significant change ($\Delta E < 3.7$) [7,20]; but the groups treated with other mouth rinses also presented significant color variation similar to one another and different than the LW and CG groups. This result corroborates previous studies [2,8] and the results obtained by Torres et al. [4], which demonstrated color changes promoted in tooth enamel by Listerine Whitening and Colgate Plax Whitening similar to conventional bleaching with 10% carbamide peroxide gel. This effect can be explained by time of use, although these agents have low hydrogen peroxide concentration and were used for a long period of time (12 weeks). However, such results differ from findings [21] in which mouth rinses did not alter tooth surface color after 14 and 30 days; such divergence of results can be justified by the different durability of the daily application period since in Nahsan et al. [21] the maximum period was 30 days, whereas in the present study the period was 90 days. Demarco et al. [1], through a literature review, stated that mouth rinses have a low level of bleaching agents and, consequently, promote a non-significant bleaching effect. However, the presence of hydrogen peroxide in the LW composition may justify a greater color variation detected in the samples of its group.

Given the results, it is possible to infer that all the rinses presented significant changes in tooth enamel color, although some do not promote a bleaching effect as Listerine Whitening and Colgate Luminous White do. The color change and tooth enamel demineralization caused by such products after a long period of exposure are possibly related to pH values below the critical value for enamel and/or erosion caused by brushing immediately after contact with the acids in the mouth rinses. As reported by Lima et al. [2], it is believed that the color change produced by the mouthwash was probably caused by enamel surface demineralization resulting from the low pH. This observation is of the utmost importance in order to alert the population to the drawbacks of prolonged use of such substances, which include adverse effects on tooth enamel.

For this study, different brands and compositions of commercially available mouth rinses were selected. These mouth rinses can be freely purchased and self-applied by patients without the knowledge or supervision of a professional. During the entire period of application, among the intervals of immersion in the mouth rinses, the samples were kept in distilled water because storing them in artificial saliva could have resulted in a protective salivary film [22], which could have influenced the microhardness and surface roughness values.

The clinical application of the products was simulated for 12 weeks following the manufacturers' instructions; however the results should be interpreted with caution since this was a laboratory study that evaluated the action of mouth rinses outside the oral environment, which is a complex environment to reproduce in a laboratory. In the oral cavity, the presence of saliva may positively impact the enamel surface remineralization [21] and influence the action of the mouth rinses in a clinical environment; therefore, more *in situ* and clinical studies are necessary. Thus, based on the results of this study, one can conclude that mouth rinses cause significant changes in the microhardness, roughness, and color of tooth enamel. However, further clinical research is required to test these products in an oral cavity; thereby the effects of diet and oral habits on the bleaching result could be observed.

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Conflict of interest

All the authors deny any conflict of interest.

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