ORIGINAL RESEARCH

Effect of Post-bleaching Surface Microroughness with Whiteness HP Blue vs Whiteness HP Maxx on Different Locations of Bovine Enamel

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Abstract

Aim: To compare the effect after bleaching with 35% hydrogen peroxide with and without calcium gluconate on surface microroughness in an *in vitro* study on the bovine enamel.

Materials and methods: The study had an *in vitro* experimental design. The following groups were formed: group I: MV (mesiovestibular) enamel, group II: V (vestibular) enamel, and group III: DV (dystovestibular) enamel, undergoing treatment with Whiteness HP Blue (H_2O_2 at 35% with calcium gluconate), Whiteness HP Maxx: H_2O_2 at 35% (without calcium gluconate), and physiological serum. A Surftest SJ-210 digital roughness meter (Mitutoyo) was used to evaluate microroughness.

Results: The highest mean microroughness was found in group II (V) with 0.23 ± 0.13 µm and 0.17 ± 0.02 µm for Whiteness HP Blue and Whiteness HP Maxx, respectively. The *post hoc* analysis of surface microroughness subjected to H_2O_2 with and without calcium gluconate showed that there were only statistically significant differences between Whiteness HP Blue and the control ($p = 0.032$).

Conclusion: There were no significant differences in surface microroughness on comparing the Whiteness HP Maxx group with the control group and the Whiteness HP Blue group.

Clinical significance: The clinical importance of this study was that it allowed us to know the direct impact that bleaching agents with and without calcium have on dental structures.

Keywords: Hydrogen peroxide, *In vitro* study, Roughness, Teeth whitening.

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Introduction

Dental esthetics are important in terms of physical beauty, and therefore dental whitening plays a very relevant part of the field of dentistry. Compared to other treatments that require wearing dental structures, dental whitening is a fairly conservative treatment that involves the use of different substances that act on the teeth in order to brighten them. The procedure used depends on the etiology and intensity of the color alteration, making it important for the dentist to determine the cause of the color alteration during the esthetic evaluation in order to establish the most adequate method to restore esthetic balance using techniques with the minimum loss of dental structure and at the same time fulfilling the function of thinning.[1–3]

The most widely used dental office brightening agents are hydrogen peroxide at variable concentrations, ranging from 3 to 38%, and carbamide peroxide at concentrations of 10–37%. For whitening at home, it is recommended to use hydrogen peroxide at 3–9% and carbamide peroxide at 10–22%.[4–7] Hydrogen peroxide is a transparent liquid capable of penetrating enamel and dentin due to its molecular weight. It is able to oxidize a wide range of organic and inorganic compounds, produce discoloration, and subsequent thinning of the substrate. Among its most notable adverse effects is that it can produce burns on contact with soft tissues and generate postoperative sensitivity. It has been demonstrated that hydrogen peroxide can affect the dental structure. On the other hand, several studies have described that reinforcement with remineralizers such as calcium gluconate and calcium phosphate in lightening agents can decrease the effect of demineralization and postoperative sensitivity.[7–10]

Currently, a variety of teeth-whitening agents are available in the market. These are based on hydrogen peroxide with and without calcium gluconate, which can be included or separately. Before performing a whitening treatment, it is essential to determine and evaluate the effects of the lightening agents on

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the tooth enamel. With this information, the operator can choose the most adequate and innocuous treatment, since in addition to obtaining the lightening effect, it is necessary to guarantee the preservation and integrity of dental hard tissues and ensure that the teeth are not worn down by other pathologies such as bruxism.[3] For example, the Whiteness HP Maxx is a whitener that is created based on 35% hydrogen peroxide for dental use that has as its main benefit the heat blocker that will reduce future sensitivity caused by halogen light, while Whiteness HP Blue is applied with an alkaline and stable pH producing less tooth sensitivity.

Thus, the aim of the present study was to compare surface microroughness on different locations of the bovine enamel following bleaching with Whiteness HP Blue vs Whiteness HP Maxx in an *in vitro* study.

Materials and Methods

This study was carried out in Laboratory No. 4 of the Faculty of Mechanical Engineering of the Universidad Nacional de Ingeniería (UNI) and in the Faculty of Dentistry of the Universidad Nacional Federico Villarreal (UNFV).

Study Design and Sample

A quantitative approach was used because microroughness variables were measured numerically. The methodological design was experimental, prospective, and longitudinal. The dependent variable was surface microroughness of the adamantine tissue and the independent variable was hydrogen peroxide-based bleaching agents with and without calcium gluconate. The sample was made up of bovine incisor teeth. The sample size of 45 blocks of the bovine enamel was calculated using data from a previously carried out pilot study and with a formula for comparing means with an alpha of 0.05 and a beta of 0.80 with the Stata 15 statistical software.

Experimental Groups

Whiteness HP Maxx is a 35% hydrogen peroxide-based lightener, while Whiteness HP Blue has as main ingredients 20% or 35% hydrogen peroxide, neutralizing agents, glycol, inert blue pigment (HP Blue 20%) or inert violet pigment (HP Blue 35%), calcium gluconate, and deionized water. Both agents are produced by DENTSCARE LTDA—Brazil with Registration at ANVISA No. 80172310044.

The following groups were formed to evaluate surface microroughness:

Group I: MV (mesiovestibular) enamel treated with Whiteness HP Blue (H_2O_2 at 35% with calcium gluconate), Whiteness HP Maxx (H_2O_2 at 35% without calcium gluconate), and physiological serum ($n = 15$ specimens).

Group II: Enamel V (vestibular) treated with Whiteness HP Blue (H_2O_2 at 35% with calcium gluconate), Whiteness HP Maxx (H_2O_2 at 35% without calcium gluconate), and physiological serum ($n = 15$ specimens).

Group III: DV (dystovestibular) enamel treated with Whiteness HP Blue (H_2O_2 at 35% with calcium gluconate), Whiteness HP Maxx (H_2O_2 at 35% without calcium gluconate), and physiological serum ($n = 15$ specimens).

Group IV: Control: Physiological serum

Inclusion Criteria

- Healthy bovine incisors free of carious lesions
- Bovine incisors without any wear
- Bovine incisors between 3 years and 4 years old

Exclusion Criteria

- Discolored bovine incisors
- Bovine incisors with fissures
- Bovine incisors with alterations in the enamel structure

Preparation of the Specimens

The samples included lower bovine incisor teeth from Frigorífico La Colonial S.A.C. Ate Vitarte, Peru) certified with Voucher No. 003864 by a specialist veterinary doctor. A total of 45 teeth were collected, all of which had been extracted immediately after the slaughter of the bovines and stored in physiological serum contained in sterile amber bottles. The teeth were then cleaned with a sterile gauze, leaving all the pieces free of impurities. The teeth were stored in artificial saliva (Salival Laboratorios Unidos SA Lima-Peru) until use (Fig. 1A).

Whitening Process

The central incisors of the bovines were used because it is very difficult to find patients who have endodontic extraction of the incisors. Therefore, to guarantee the viability and feasibility of the study, bovine teeth were used since they share similar characteristics to human teeth, especially in their morphology. Following extraction, the teeth were subjected to the whitening process with Whiteness HP Blue and Whiteness HP Maxx, respectively. The teeth were dried and separated. Then the phases were mixed at a ratio of 3 to 1 drop according to the manufacturer's instructions. According to the manufacturer's instructions, the whitening products were mixed in different phases at a ratio of 3 to 1 drop. Thereafter, using a mixing plate, phase 1 (peroxide) was mixed with phase 2 (thickener). Then, with the help of a microbrush, the surfaces of the bovine teeth were completely covered with the whitening product of each respective group. The gel layer was applied with a thickness between 0.5 and 1 mm. Finally, a photopolymerizer was applied for 20 seconds on each tooth at a distance of approximately 10 mm from the tooth surface (Fig. 1B).

Microroughness Assessment

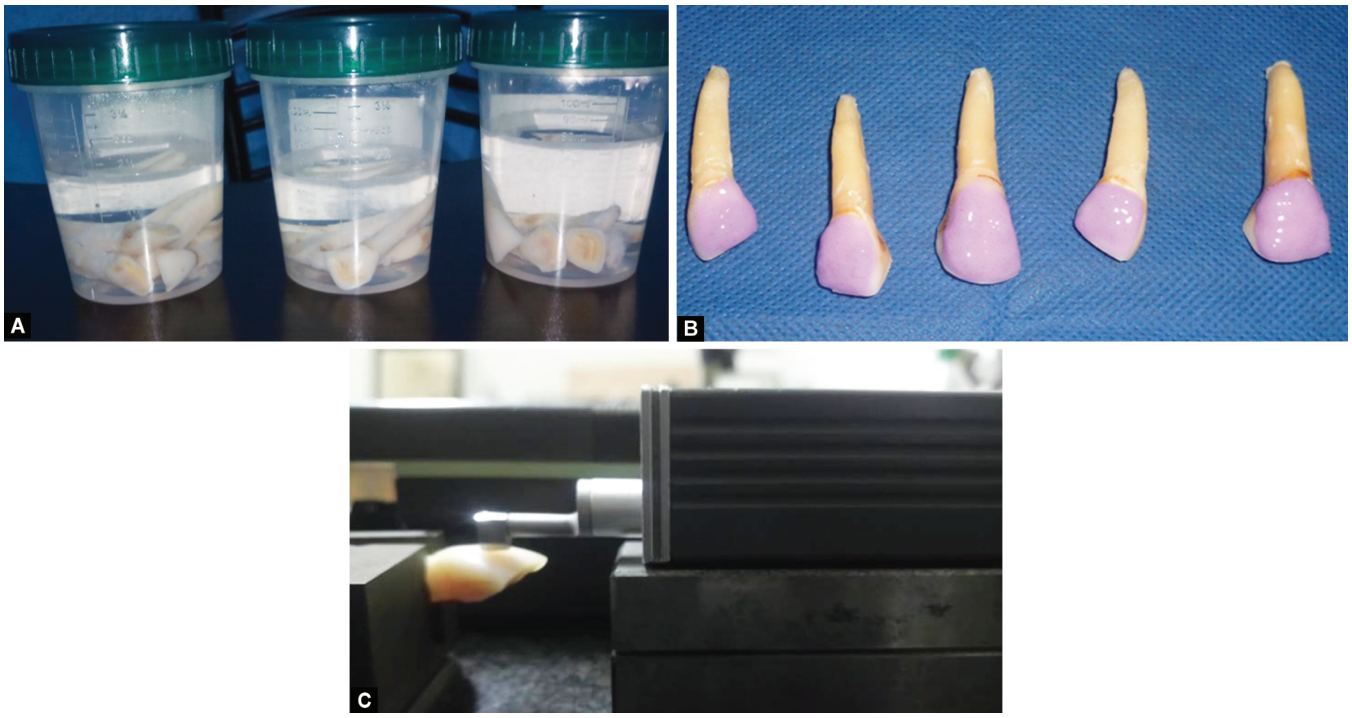
For the evaluation of microroughness, a Surftest SJ-210 178 series digital roughness tester—Mitutoyo portable surface roughness meter (Borsigstraße 8-10 D-41469 Neuss-Germany)—was used. This device has a LED display, a diamond point stylus, and a reference standard for roughness measurement. Three measurements of each sample were made at 0.25 mm/second with an approximation of 0.01 µm. The results were recorded in the representative cards. All the measurements of surface microroughness were carried out at a temperature of 21°C with a relative humidity of 70% recorded in the Lb4-1392-2018 voucher (Fig. 1C).

Statistical Analysis

Descriptive analysis of the variable surface microroughness was performed and expressed as mean and standard deviation. The Shapiro Wilk test was used to test normality, and the Levene test was used for the analysis of homogeneity of variances. The ANOVA test was used to perform statistical inference. Finally, the Tukey test was used to perform the post-hoc analysis, establishing a significance level of $p < 0.05$.

Results

The group treated with Whiteness HP Blue (H_2O_2 at 35% with calcium gluconate) showed the highest mean surface microroughness with 0.19 ± 0.05 µm, while the enamel specimens treated with Whiteness



Figs 1A to C: (A) Specimens preserved in artificial saliva; (B) Bovine teeth subjected to the thinning agent; (C) Measurement of the superficial microroughness of the tooth

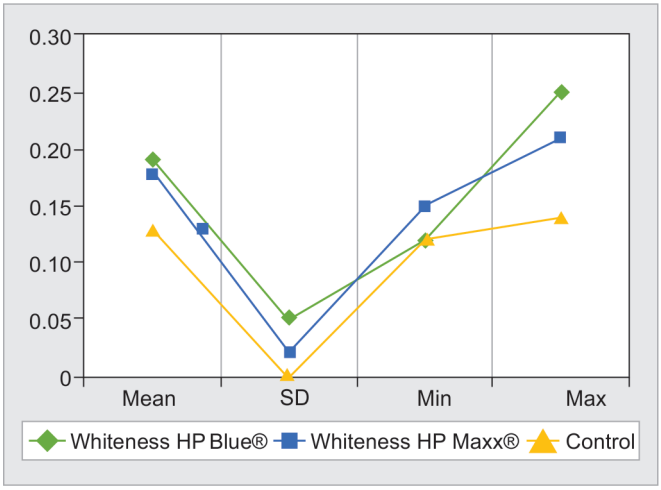


Fig. 2: Analysis of surface microroughness of the bovine enamel subjected to hydrogen peroxide with and without calcium gluconate

HP Maxx (H_2O_2 at 35% without calcium gluconate) showed a mean of 0.18 ± 0.02 µm and the control (physiological serum) presented a microroughness of 0.13 ± 0.08 µm (Fig. 2). The inferential analysis showed statistically significant differences in microroughness among the three groups evaluated ($p = 0.001$) (Table 1).

On the other hand, when comparing the surface microroughness of MV, V, and DV bovine enamel treated with 35% hydrogen peroxide with and without calcium gluconate, the V group showed the highest mean microroughness with 0.23 ± 0.13 µm and 0.17 ± 0.02 µm for Whiteness HP Blue and Whiteness HP Maxx, respectively (Fig. 3). Comparison using the ANOVA test only showed statistical differences in microroughness among the three experimental groups and the V and DV enamel areas ($p = 0.001$; both) (Table 2).

Table 1: Comparison of surface microroughness of bovine enamel subjected to hydrogen peroxide with and without calcium gluconate

Group	*Mean*	*SD*	*Min*	*Max*	*p*
Whiteness HP Blue	0.19	0.05	0.12	0.25	
Whiteness HP Maxx	0.18	0.02	0.15	0.21	0.001
Control	0.13	0.08	0.12	0.14	

All specimens were measured in µm.
Whiteness HP Blue: H_2O_2 at 35% with calcium gluconate
Whiteness HP Maxx: H_2O_2 at 35% without calcium gluconate
Control: Physiological serum
*ANOVA test
Significance level $p < 0.05$

Finally, the *post hoc* analysis of the surface microroughness submitted to the hydrogen peroxide in both groups only showed statistically significant differences between Whiteness HP Blue and controls ($p = 0.032$) (Table 3).

Discussion

Enamel, which is also called the adamantine tissue, is an acellular tissue of ectodermal origin. It has a shiny smooth surface due to its high translucency, with dentin being easily perceived forming a yellowish-white coloration. The surface of the enamel is mainly of 0.1–0.2 mm and is chemically formed by an organic matrix (1.8%), inorganic matrix (95%), and water (3.2%). Taking this into account, apart from the wear and alteration of enamel surfaces, they are also vulnerable and prone to bacterial attack. The degree of calcification is determined by the permeability of the enamel with saliva. Two types of chromatic alterations can alter the color of the tooth: extrinsic and intrinsic. These chromatic alterations can negatively affect the appearance of a smile, damaging a tooth or set of teeth. Extrinsic disorders are the most common alterations,

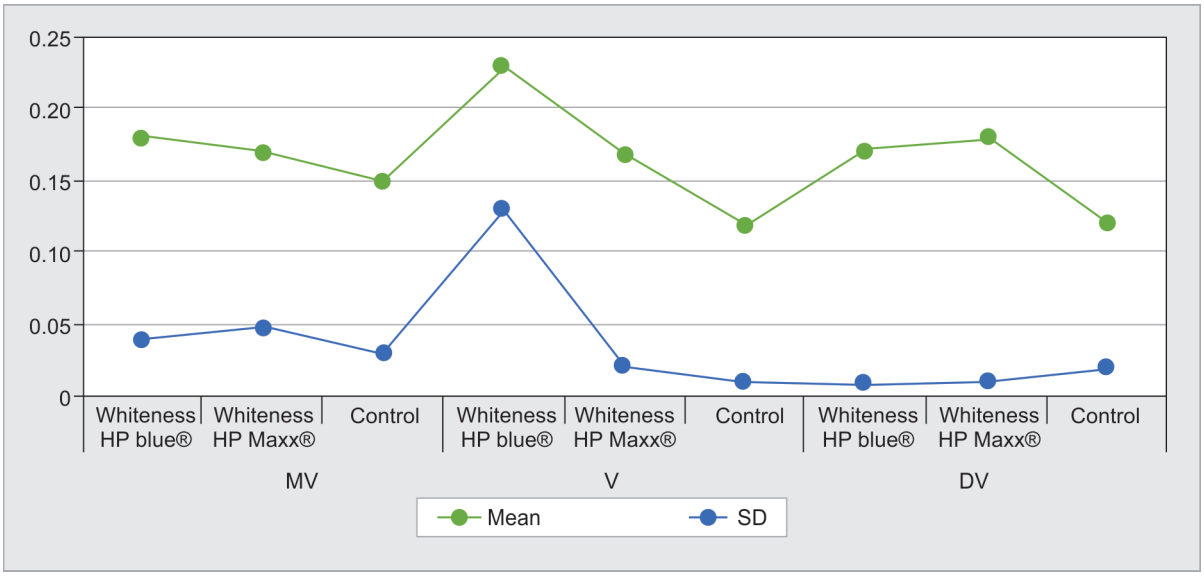


Fig. 3: Analysis of surface microroughness of the bovine enamel subjected to hydrogen peroxide with and without calcium gluconate according to different surfaces

Table 2: Comparison of surface microroughness of bovine enamel subjected to hydrogen peroxide with and without calcium gluconate according to different surfaces

Tooth surface	*Group*	*Mean*	*SD*	*Min*	*Max*	*p*
MV	Whiteness HP Blue	0.18	0.04	0.12	0.24	0.05
	Whiteness HP Maxx	0.17	0.05	0.11	0.23	
	Control	0.15	0.03	0.09	0.18	
V	Whiteness HP Blue	0.23	0.13	0.08	0.44	0.001
	Whiteness HP Maxx	0.17	0.02	0.14	0.2	
	Control	0.12	0.01	0.11	0.16	
DV	Whiteness HP Blue	0.17	0.01	0.15	0.18	0.001
	Whiteness HP Maxx	0.18	0.01	0.17	0.21	
	Control	0.12	0.02	0.08	0.15	

All specimens were measured in µm. MV, mesiovestibular surface; V, vestibular surface; DV, distovestibular surface
Whiteness HP Blue: H_2O_2 at 35% with calcium gluconate
Whiteness HP Maxx: H_2O_2 at 35% without calcium gluconate
Control: Physiological serum
*ANOVA test
Significance level $p < 0.05$

Table 3: *Posthoc* analysis of surface microroughness subjected to hydrogen peroxide with and without calcium gluconate

Groups assessed		*Mean differences*	*Confidence intervals*		*p*
Whiteness HP Blue	Whiteness HP Maxx	0.012	–0.042	0.066	0.832
	Control	0.060	0.005	0.114	0.032
Whiteness HP Maxx	Whiteness HP Blue	–0.012	–0.066	0.042	0.832
	Control	0.048	–0.006	0.102	0.090
Control	Whiteness HP Blue	–0.060	–0.114	–0.005	0.032
	Whiteness HP Maxx	–0.048	–0.102	0.006	0.090

All specimens were measured in µm
Whiteness HP Blue: H_2O_2 at 35% with calcium gluconate
Whiteness HP Maxx: H_2O_2 at 35% without calcium gluconate
Control: Physiological serum
*Tukey test
Significance level $p < 0.05$

mainly being the result of superficial pigmentation of the tooth generally caused by excessive consumption of substances with a high content of colorants such as coffee, tea, or tobacco, among others. Intrinsic factors include dentinogenesis imperfecta, fluorosis (congenital), tetracycline and fluoride stains pre-eruption, trauma, and tetracycline use post-eruption.[10]

Dental whitening is a conservative and efficient esthetic treatment option compared to other treatments that involve the wear of dental tissues, whether with veneers or porcelain crowns.[11–14] The present investigation measured the surface microroughness of the enamel of bovine teeth bleached with 35% hydrogen peroxide with and without calcium gluconate. Microroughness was measured with a Mitutoyo SJ-201 digital roughness meter demonstrating that 35% hydrogen peroxide with and without calcium produced the same enamel surface microroughness while a lightening agent with 35% hydrogen peroxide without calcium showed the same effect as the control group (physiological serum).

China et al.[15] evaluated the effect of whitening with substances containing added calcium combined with fluoride gels and measured microhardness and surface roughness. The control group underwent whitening without fluoride. Measurements were made before and after treatment and the samples were stored in distilled water at 37°C. They concluded that treatments with 35% hydrogen peroxide reduced microhardness, while hydrogen peroxide with calcium increased enamel hardness. Finally, the fluoride substances were not found to alter the roughness of the surface of the bleached enamel. These results disagree with ours since we did not find significant differences between the two groups ($p > 0.05$). On the other hand, Moura et al.[16] evaluated the enamel mineral content and surface microhardness before and after bleaching treatment with 10% carbamide peroxide containing calcium or amorphous calcium phosphate. Thirty-six bovine specimens underwent bleaching treatment with G1 Opalescence PF 10%, G2 NiteWhite, and G3 Opalescence with calcium. They found a significant decrease in microhardness after bleaching treatments in all the study groups, with no differences among the bleaching gels. It was concluded that bleaching reduces enamel microhardness, regardless of the presence of calcium, but there was no significant change in the enamel Ca/P ratio after bleaching with each gel tested. This indicates that bleaching gels have erosive potential, causing enamel softening without promoting surface loss, regardless of the presence of calcium.

According to Pimenta et al.,[17] amorphous calcium phosphate (ACP) bleach has an uncertain effect on the enamel surface. Therefore, it is important to evaluate the possible effects of different bleaching agents of this type on the enamel surface. Similar to our results, no beneficial effects were observed when adding calcium to the formulas of the blocking substances on the enamel. In another study, Alencar et al.[18] reported that while teeth whitening aims to improve appearance, it may lead to some side effects on enamel morphology. They evaluated the effect of strontium chloride 10% and potassium nitrate 5% with fluoride on the bovine enamel and found significant differences in the microroughness of the enamel surface ($p < 0.05$) between the experimental groups and the control group ($p < 0.05$). These results and ours also coincide with the study by Wiegand et al.[19] who evaluated, *in vitro*, the effect of different seven external bleaching agents on the enamel (A: Whitestrips, B: Rapid White, C: Opalescence 10%, D: Opalescence PF 15%) and bleaching agents in office (E: Opalescence extra, F: Opalescence quick, G: Opalescence extra boost). The statistical analysis did not reveal any significant differences among enamel roughness in groups E, F, G, and the control H. However, with the exception of Rapid White whitening treatment, abrasion of the bleached enamel by toothbrushing appears be clinically less relevant.

The importance of the present study was that it provides knowledge to oral health professionals about the effect of hydrogen peroxide-based thinning agents with and without calcium gluconate on the surface microroughness of the dental enamel. It also provides practical applicability regarding the use of a whitening agent with optimal results. Nonetheless, it is necessary to know the mechanism of action of these products, and also determine which whitening gel is more effective and efficient in meeting the treatment objective and producing a less effect on surface roughness.

However, one of the main limitations of this study was that the sample included only bovine teeth. These samples were used because the use of human organs is scarce and very controlled due to current bioethical regulations. It would be interesting to carry out research evaluating the effect of thinning agents on roughness using dispersed energy microscopy to analyze the amount of calcium and phosphorous minerals lost or gained on the tooth enamel after the application of whitening agents. Dental whitening is currently one of the procedures most commonly used by patients; therefore, dentists are investigating the possible side effects these products produce on enamel, dentin, and even on the pulp. In order to achieve optimal results for both patients and dentists, it would be very useful for the dental material industry to provide information on the composition and concentration of the products available in the market. Finally, the pH of the whitening agents should be determined before use in the treatments.

Conclusion

In summary, Whiteness HP Blue: H_2O_2 at 35% with calcium gluconate and Whiteness HP Maxx: H_2O_2 at 35% without calcium gluconate present the same surface microroughness of the adamantine tissue and that Whiteness HP Maxx has the same effect as the control group. Finally, there were no statistically significant differences in surface microroughness on comparing the Whiteness HP Maxx group with the control group and the Whiteness HP Blue group.

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