INFLUENCE OF THREE TREATMENT PROTOCOLS FOR DENTAL FLUOROSIS IN THE ENAMEL SURFACE: AN IN VITRO STUDY

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Palavras-chave: Fluorose Dentária. Esmalte Dentário. Clareamento Dental.

RESUMO

Introdução: A fluorose dentária é uma alteração do esmalte caracterizada por manchas opacas causadas pela alta exposição aos íons fluoreto durante o desenvolvimento dentário. **Objetivo**: Este estudo *in vitro* objetivou avaliar mudanças na superfície do esmalte em dentes humanos hígidos após três protocolos de tratamento para a fluorose dentária: microabrasão com ácido fosfórico a 37% e pedra-pomes, clareamento caseiro com peróxido de carbamida a 10% e a associação destas técnicas. **Métodos**: Trinta e oito espécimes (5×5×2 mm) com superfície em esmalte foram obtidos a partir de 19 terceiros molares, sendo que dois não receberam tratamento e, trinta e seis foram randomizados em três grupos (n= 12): MAB- microabrasão do esmalte; CP10- clareamento caseiro; e MAB+CP10- associação destas técnicas. A rugosidade superficial e microdureza foram realizadas antes e após os protocolos de tratamento. Dois espécimes representativos de cada grupo foram avaliados por microscopia eletrônica de varredura (MEV). A análise de variância e teste de Tukey foram utilizados para análise dos resultados (p< 0,05). **Resultados**: Todos os protocolos de tratamento promoveram um aumento da rugosidade superficial do esmalte (p< 0,02). MAB e MAB+CP10 mostraram um aumento significativo da microdureza do esmalte (p< 0,04), enquanto que CP10 mostrou uma menor microdureza comparado ao MAB e ao MAB+CP10 (p< 0,05). As imagens de MEV demonstraram uma superfície mais lisa do MAB e MAB+CP10 e um padrão irregular do esmalte erodido para o CP10. **Conclusão**: Os protocolos testados para tratamento da fluorose dentária testados modificaram significativamente a rugosidade, microdureza e micromorfologia do esmalte.

Keywords: Dental Fluorosis. Dental Enamel. Tooth Bleaching.

ABSTRACT

Introduction: Dental fluorosis is an enamel alteration characterized with opaque stains caused by high exposures to fluoride during the dentition development. **Aim**: This *in vitro* study aimed to evaluate changes in the enamel surface of sound human teeth after three treatment protocols for dental fluorosis: microabrasion with 37% phosphoric acid and pumice, home bleaching with 10% carbamide peroxide, and a combination of these techniques. **Methods**: Thirty-eight specimens (5×5×2 mm) with enamel surface were obtained from 19 third molars. Thirty six specimens were randomized into three treatment groups (n= 12): MAB- enamel microabrasion; CP10- home bleaching; MAB+CP10- a combination of these techniques and two specimens not received treatment. Surface roughness and microhardness analyses were performed before and after treatment protocols. Two representative specimens from each group were evaluated by scanning electron microscopy (SEM). Analysis of variance and Tukey's tests were used for data analysis (p< 0.05). **Results**: All treatment protocols promoted an increased in enamel surface roughness (p< 0.02). MAB and MAB+CP10 showed a significant increase in the enamel microhardness (p< 0.04), while CP10 showed a microhardness lower than MAB and MAB+CP10 (p< 0.05). SEM images demonstrated a smoother surface from MAB and MAB+CP10 and, an irregular pattern of enamel erosion from CP10. **Conclusions**: The treatment protocols for dental fluorosis tested significantly changed the enamel roughness, microhardness and micromorphology.

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INTRODUCTION

Dental fluorosis is an enamel alteration caused by successive exposures to high concentrations of fluoride during the period of permanent dentition development.[1,2] The severity of fluorosis varies according to the quantity of fluoride intake, time of exposure, and the stage of the amelogenesis, as well as being related to lifestyle.[3] Clinically, fluorosis is characterized by the presence of bilateral, diffuse, white and opaque stains that run horizontally across the enamel.[4] Another characteristic of this pathology is the presence of symmetry; homologous teeth tend to be affected.[3,5]

The occurrence of severe dental fluorosis compromises the appearance and aesthetics of teeth, causing embarrassment and difficulties to smile.[4] In addition, facial harmony plays important social and psychological roles in the individual's quality of life and social relationships.[4] This intrinsic condition can be treated by conservative methods, such as polishing, enamel microabrasion, dental bleaching, or the combination of these techniques. However, more invasive methods are also used, such as veneers in composite resin or porcelain, or ceramic crowns in the most severe cases.[6,7]

The microabrasion technique was first presented by Croll and Cavanaugh[8] (1986), who successfully removed opaque white stains from enamel using 18% hydrochloric acid and pumice under pressure with a wooden spatula.[6] Mondelli et al[9]. (1995) proposed a modified microabrasive technique, replacing the 18% hydrochloric acid from 37% phosphoric acid and pumice.[10] This technique is an aesthetic and conservative procedure for removal a thin layer of stains or defects localized in enamel surface by the action of abrasive agents, mechanical abrasion and, the acid penetration in the organic portion of tooth enamel. Other advantages of this technique is the availability of phosphoric acid in dental offices due to its common use in adhesive procedures and the reduced risks of accidental exposure compared to hydrochloric acid.[2,11,12]

Tooth bleaching techniques can be performed at home, where the patient uses low concentrations of carbamide (10–22%) or hydrogen peroxide (up to 14%) in custom trays or, in dental offices, where a professional can apply higher concentrations of hydrogen (15–38%) or carbamide peroxide (30–37%)[13] in the teeth surface. Additionally, 10% carbamide peroxide is the only bleaching gel considered safe, and that has received the American Dental Association (ADA) seal of approval.[14]

The treatment for dental fluorosis frequently associate tooth bleaching to enamel microabrasion in order to promote whiter and more uniform teeth, as well as reducing the contrast between the white stain lesions and the tooth surface around the stains.[15] This associated technique with 10% carbamide peroxide supervised home bleaching is the choice for treating white stains caused by fluorosis, showing excellent aesthetic results through a conservative treatment.[6,16] Thus, the aim of this *in vitro* study was to evaluate the changes in the roughness, microhardness, and micromorphology of the human enamel surface submitted to three treatment protocols for dental fluorosis: microabrasion with 37% phosphoric acid and pumice, home bleaching with 10% carbamide peroxide or the association of these techniques.

MATERIALS AND METHODS

Ethical considerations

This study was approved by the local Ethics and Research Committee of the Federal University of Paraíba, Brazil under protocol number 446/10 and Certificate of Ethics Appraisal (CAAE) number 0371.0.126.000-10.

Selection and preparation of specimens

Nineteen third molars donated by the Human Teeth Bank from Federal University of Paraíba (UFPB) were used. Teeth were examined under 40x amplification to detect cracks or defects and, those without structural defects were used in this study. Teeth were stored in 0.5% thymol solution (pH 7.0) at 4°C until beginning the experiment.

Roots were sectioned at cemento-enamel junction using a double-face diamond disc (EXTEC Corp., Enfield, CT, USA) and crowns were longitudinally sectioned into two equal enamel blocks using a low-speed diamond-edge saw under refrigeration (Labcut 1010, EXTEC Corp., Enfield, CT, USA). Thirty eight enamel blocks were obtained and the dimensions of each of them (5x5x2 mm) were measured with a digital calliper (resolution 0.01mm) (500-144B, Mitutoyo Corp., Japan). The enamel blocks were embedded in acrylic resin such that the enamel surface faced upward.

The enamel surface of the specimens were ground flat using water-cooled abrasive well in a sequence of 600 and 1200- grit silicon carbide papers in order to obtain a flat surface. Polishing was finalized with medium-grain diamond paste (Diamondac I, FGM Dental Products, Joinville, SC, Brazil) associated with felt discs (Diamond, FGM Dental Products, Joinville, SC, Brazil). Specimens were stored in distilled water at 37°C until beginning the treatments.

Randomization and treatment

Thirty-six enamel blocks were randomized into three treatment groups (n= 12): enamel microabrasion-(MAB); tooth bleaching with 10% carbamide peroxide-(CP10) and the association of both techniques- (MAB+CP10). Two

specimens were not treated and were used as a control group for scanning electron microscopy.

Specimens from MAB performed the microabrasion technique using a layer of microabrasive paste (approximately 2.0 mm) with equal parts of 37% phosphoric acid (Condac 37%, FGM Dental Products, Joinville, SC, Brazil) and ultrafine pumice (SS White LTDA; Rio de Janeiro, RJ, Brazil). A rubber cup (Microdont, KG Sorensen, Cotia, SP, Brazil) mounted on a slow-speed handpiece with a 10:1 gear reduction was used to abrade lightly the specimen surface. Paste excess was removed with sterile gauze and the specimens were rinsed for 20 s. This procedure was repeated 12 times, 10 s each, in a single treatment session. After applications, the abraded surface was polished with felt discs (Diamond, FGM Dental products, Joinville, SC, Brazil) and diamond paste (Diamond Excel, FGM Dental products, Joinville, SC, Brazil). Then, the treated specimens were rinsed, dried and stored in artificial saliva solution at 37°C (Phosphate potassium dibasic 4.35 g/L, Phosphate potassium monobasic 3.2 g/L, 70% Sorbitol, Sodium fluoride 0.044g/L, Potassium fluoride 0.62 g/L, Sodium chloride 5.85 g/L, Magnesium chloride 0.14 g/L, Calcium chloride 0.16 g/L, Sodium benzoate 5.0 g/L, Carboxymethycellulose 5.0 g/L in 1000 mL distilled water, pH 7.0)[17] during a week until start the surface roughness and microhardness tests.

Specimens from CP10 performed home bleaching with 10% carbamide peroxide (Whiteness Perfect 10%, FGM Dental products, Joinville, SC, Brazil). Custom trays were fabricated for each specimen using a 1-mm thick acetate plaque (FGM Dental products, Joinville, SC, Brazil) and a vacuum-formed process (Plastvac P7, Bioart, São Carlos, SP, Brazil). Bleaching agent was maintained in contact with specimen surface 4h/day during two weeks. After each time of application, bleaching gel was removed with air water spray for 30 s, cleaned, polished with felt discs, and polishing paste. The specimens were stored in artificial saliva at 37°C until next application.

The MAB+CP10 group performed both treatment techniques. However, home bleaching starts a week after the end of enamel microabrasion. The application of microabrasive paste with phosphoric acid and ultrafine pumice was performed during 10 seconds and repeated 12 times in a single treatment session; then the specimens were rinsed, polished with felt discs and diamond paste and stored in artificial saliva solution at 37°C for a week with daily exchanges. One week, after this procedure the bleaching treatment was performed with 10% carbamide peroxide during two weeks. At the end of the treatment protocols, specimens were stored in artificial saliva at 37°C during a week until beginning experimental tests. Three bleaching agent tubes and the phosphoric acid and pumice used in this study were randomly chosen for pH measurements with a digital pHmeter (Hanna Instruments, Woonsocket, RI, USA) (Table 1). Product specifications are listed in Table 2.

***Table 1**: Means and standard deviations for pH of different treatment groups.*

Product	pH
10% Carbamide peroxide	5.82 (0.03)[A]
37% Phosphoric acid	-0.74 (0.03)[B]
37% Phosphoric acid + Pumice	-0.07 (0.07)[B]

*Note:*Different uppercase letters in the same column represent significant difference between treatment groups (p< 0.05).*

***Table 2**: Composition and manufacturers.*

Product	Composition	Manufacturer
Condac 37%	37% Phosphoric acid, thickener, pigment and deionized water	FGM Dental products, Joinville, SC, Brazil
Extra fine Pumice	Pumice	SSWhite, São Cristovão, RJ, Brazil
Whiteness Perfect 10%	10% Carbamide peroxide, neutralized carbopol, glycol and deionized water	FGM Dental products, Joinville, SC, Brazil
Diamond Excel	Micronized diamond (2-4µm), lubricant base, thickener and emulsifier	FGM Dental products, Joinville, SC, Brazil

Surface Roughness Test

Surface Roughness (Ra) was measured using a profilometer (SJ 301 Mitutoyo, Kanagawa, Japan). Before starting the measurements, the profilometer was calibrated in a reference block (2.94 ± 0.10 µm).[11,18] For each specimen, three measurements in different directions (0h, 3h and 6h) were performed with a cutoff value of 0.25 mm and speed of 0.5 mm/s. The measurements were performed before and after treatments, obtaining the initial (Ra1) and final (Ra2) roughness means for each specimen.

Microhardness Tests

Microhardness measurements were performed with a Knoop diamond under a load of 50 g for 10 s using a microhardness tester (HMV-2, Shimadzu, Tokyo, Japan) before (T0) and after (T1) each treatment protocol. The distance from the first indentation to enamel block edge was approximately 500 µm.[19] Three indents were made on the enamel surface of each specimen at intervals of approximately 300 µm in a parallel direction and, means were transformed in Knoop Hardness Number (KHN).

Surface Morphology Analysis

Eight specimens were separated from Scanning Electron Microscope (SEM) analysis (JEOL-JSM 5600LV, Tokyo, Japan): two non-treated specimens and two from each treatment group. After gradual dehydration with ethanol (25%, 50% and 75% for 20 minutes; 95% for 30 minutes and 100% for 60 minutes), each specimen was mounted on an aluminium stub, sputter-coated with gold-palladium (BAL-TEC SCD 050, Balzers, Fürstentum, Liechtenstein) and photomicrographs of representative areas were taken at 2.000X magnifications.

Statistical Analysis

Data were statistically analyzed by paired T-test for comparison within the same treatment group and by the one-way analysis of variance (ANOVA) followed by Tukey´s *post-hoc* test for comparisons between independent groups to determine significant differences in the different periods of evaluation regarding enamel roughness surface, microhardness, and pH. Differences were considered statistically significant when $p < 0.05$.

RESULTS

Surface Roughness, Microhardness and pH assessments

The mean values for surface roughness are shown in Table 3. All treatment groups showed an increase in the enamel surface roughness after treatment protocols employed ($p < 0.03$). However, there were no significant differences for roughness between groups at baseline and one week after treatment ($p > 0.05$).

At baseline, there were no significant differences for means of microhardness between groups ($p > 0.05$). One week after treatment, while MAB and MAB+CP10 shown an increase of enamel microhardness ($p < 0.04$), it was observed a decrease of microhardness for CP10 ($p = 0.001$). Additionally, one week after treatment the values for microhardness were significantly higher from MAB and MAB+CP10 than CP10 ($p < 0.05$) (Table 4).

The means values for pH are shown in Table 1. All materials tested showed low pH and, the pH values were significantly lower from 37% phosphoric acid and the paste of 37% phosphoric acid with pumice than CP10 ($p < 0.05$).

Scanning Electron Microscopy

SEM images demonstrated different conditioning patterns of enamel surface. The enamel surface of the control samples appeared smooth in general, with some scattered clear scrapes due to the polishing procedure (Figure 1). Groups MAB and MAB+CP10 showed a smoother enamel surface, where prism endings were more (MAB) or less (MAB+CP10) evident (Figures 2 and 3). Bleaching with CP10 resulted an irregular pattern of enamel erosion, which resemble a type I acid-etching pattern (Figure 4).

***Table 3**: Mean and standard deviations of surface roughness (µm).*

Treatment Group (n= 12)	Surface Roughness		p within group
	Baseline	After treatment	
I- Microabrasion	0.14 (0.03)^{A}	0.19 (0.07)^{A}	0.02
II- Home bleaching	0.13 (0.02)^{A}	0.18 (0.05)^{A}	0.02
III- Associated techniques	0.12 (0.03)^{A}	0.15 (0.04)^{A}	0.03

**Different uppercase letters in the same column represent significant difference between treatment groups ($p < 0.05$).*

***Table 4**: Mean and standard deviations of microhardness (KHN).*

Treatment Group (n= 12)	Microhardness		p within group
	Baseline	After treatment	
I- Microabrasion	274.5 (43.6)[A]	337.5 (28.1)[A]	0.002
II- Home bleaching	267.0 (49.7)[A]	166.3 (51.1)[B]	0.001
III- Associated techniques	270.8 (66.8)[A]	328.8 (21.6)[A]	0.04

*Note: *Different uppercase letters in the same column represent significant difference between treatment groups (p< 0.05).*

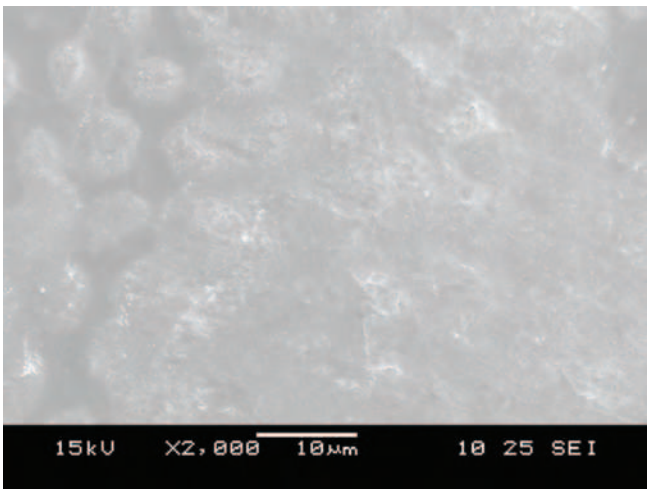

***Figure 1**: Enamel surface without treatment (control group) (2000x).*

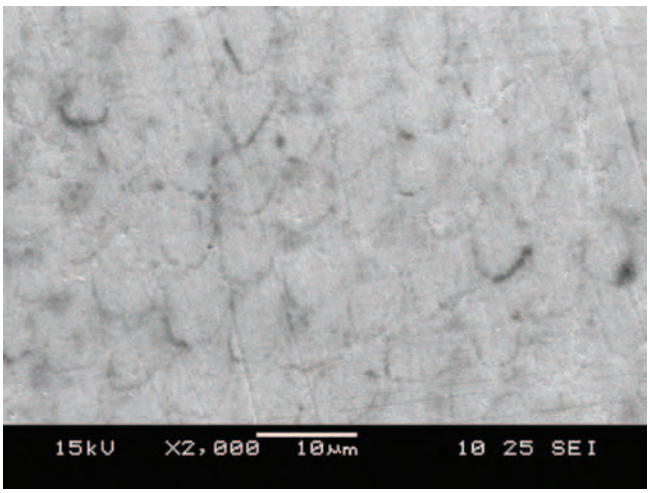

***Figure 2:** Enamel surface treated with microabrasion with 37% phosphoric acid and pumice (2000x).*

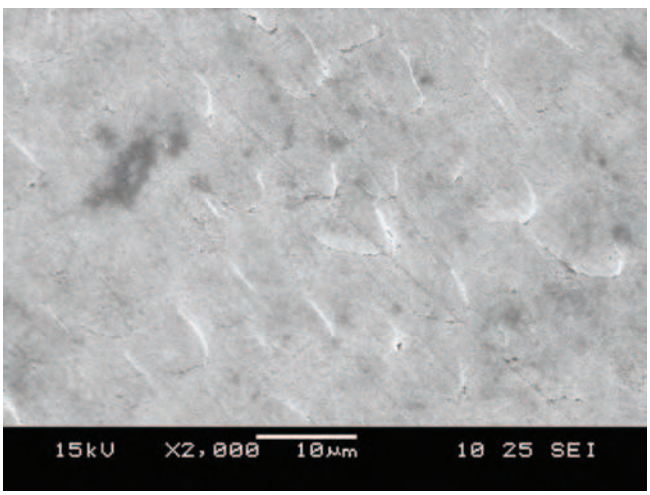

***Figure 3:** Enamel surface treated with microabrasion associated to home bleaching (2000x).*

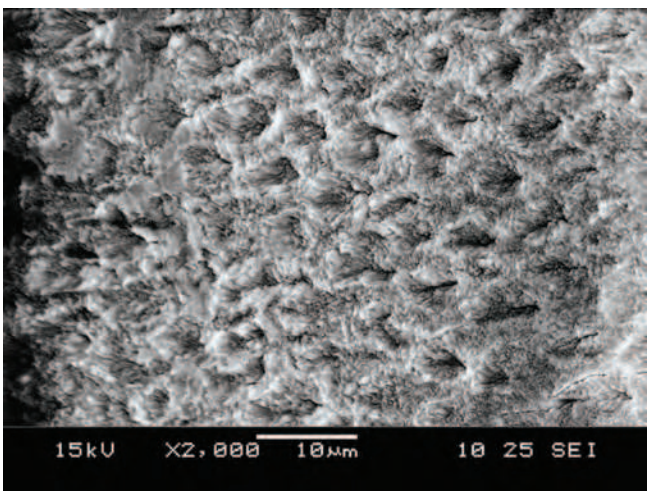

***Figure 4:** Enamel surface treated with home bleaching with 10% carbamide peroxide (2000x).*

DISCUSSION

Microabrasive procedures are considered conservative options for treating dental fluorosis, removing a thin layer of the enamel surface.[6,11,12,20] A microabrasive paste with 37% phosphoric acid and pumice has become a popular procedure due to its low cost, availability in dental offices, and lower aggressiveness compared to hydrochloric acid.[11,12] Studies have reported that this paste is effective in reducing fluorosis stains with minimal dental structure damage.[20-22] However, once that microabrasion removes enamel structure, causing teeth to become yellowish, the association of this technique with tooth bleaching is indicated to promote uniformity of teeth color.[22] A randomized clinical trial that evaluated the acceptability and efficacy of two treatment protocols for dental fluorosis reported that both

microabrasion (37% phosphoric acid and pumice) or the association of this technique with home bleaching (10% carbamide peroxide) were effective in reducing fluorosis stains, but in the associated technique the patients reported a major satisfaction with dental appearance.[6]

All treatment protocols performed in this study resulted in increase of enamel surface roughness.[11,12,23] The increase of roughness after microabrasion with phosphoric acid and pumice may be associated to the selective pattern of conditioning induced by the acid, which promotes a lower decalcification, leaving a surface more granular and irregular.[12] The microabrasive paste application promotes the compaction of mineralized tissue inside the organic area of the enamel due to simultaneous action of abrasion and acid erosion over prims.[10] Additionally, studies reported that some materials used for polishing the enamel as silicone tip (40µm) or aluminum oxide discs (14 µm-5 µm) reduce the surface roughness that was previously increased by microabrasion and it may be associated to the type of microabrasive used.[10,12,24] In this study, the use of 37% phosphoric acid with pumice promote a greater depth of demineralization and possibly the granulation of the diamond paste (2-4µm) used for polishing enamel was not able to reduce the extensive area demineralized by acid.

Studies have reported that bleaching gels with low concentrations of carbamide peroxide caused a significant increase in enamel surface roughness.[13,25,26] One reason for this may be the increasing of contact time of low concentrated bleaching gels with tooth surface, which promoted surface changes in concentration of ions calcium and phosphate, degradation of organic matrix, erosion, porosities, and depressions.[25,27] The gel used in this study was maintained in contact with enamel surface 4 hours/ day during two weeks. Additionally, the gel contains carbopol as thickening agent which has been suggested that can also adversely affect dental enamel.[25] Other studies did not detect alterations in the enamel surface roughness after treatments with 10% carbamide or 3.6%, 7.5% and 38% hydrogen peroxide.[17,28-30] A study that evaluated the effects of 7.5 and 13.5% hydrogen peroxide (HP) and 35% carbamide peroxide (CP) on the enamel surface reported that the exposition to an acidic CP bleaching agent (pH= 4.9) resulted in higher surface roughness compared to a mild (pH= 6.1) or alkaline (pH= 10.8) HP bleaching agents.[26] Furthermore, the topical application of fluoride on the enamel surface after bleaching with 35% HP prevented the increase of tooth enamel roughness.[31]

Groups treated with microabrasion or the associated technique with home bleaching showed an increase of enamel microhardness. The superficial abrasion of enamel after microabrasive treatments causes compression of mineralized tissue whitin the organic region´s of enamel, replacing the outer region free of prisms. Thus, the acidic and abrasive action of microabrasive compound probably modify the enamel prismatic structures, allowing the compressed mineral products to stay on the periphery, promoting the increase of microhardness.[23] Addictionaly, after microabrasive procedure, specimens were polished with felt discs and diamond paste and, studies reported that the increasing of microhardness after polishing occurs due to the compation of micronized diamond present in the diamond paste.[10,23] These findings are in agreement with our SEM data, where we could observe an enamel surface with a smoother and dense aspect after enamel microabrasion or with the associated technique. However, studies reported that topical application of fluoride after bleaching or microabrasive procedures can promote remineralisation due to calcium-phospate precipitation inside the porous enamel and increase the enamel hardness.[12,31,32]

The group treated with 10% carbamide peroxide showed a decrease of enamel microhardness. Several aspects related to bleaching agents might influence the enamel surface microhardness, such as peroxide concentration, time of application, pH or the incorporation of fluoride in bleaching agents.[25,32-35] The bleaching agent used has a pH of 5.8 and this pH could not have contributed significantly to enamel demineralization. One possible factor that may have contributed to the reduction of enamel microhardness was the contact time of fluoride free bleaching gel with enamel surface which may have disrupted the balance between demineralization caused by the bleaching agent and the remineralization caused by artificial saliva.[27,33,34] These findings are in agreement with our SEM analyses, where was observed an irregular surface with depressions, porosity, and increased depth of enamel grooves.[13] Other studies also observed these alterations and the enamel microhardness decrease when low carbamide peroxide (10 or 15%) concentrations were used for long treatment times.[25,27,33,34] Additionally, the *in situ* effects of low concentrated bleaching agents on human enamel surface demonstrated no morphologic or chemical changes. This may be attributed to the protective effects of saliva, which provided dilution, buffering capacity, and supply Ca and P ions for tooth remineralization.[13,30] However, when the specimens were stored in artificial saliva after bleaching with 38% hydrogen peroxide, the remineralization was not sufficient to restore tooth enamel microhardness.[17]

Within the clinical significance of this study was the evidence that the enamel microhardness and surface roughness were altered when protocols for treat dental

fluorosis were used. Thus, it is necessary to performed further *in vitro* or *in situ* studies using teeth with dental fluorosis in order to compare the surface properties of fluorotic enamel after microabrasive procedures. Addicitonaly, randomized clinical trials are necessary to compare the efficacy, safety and the remineralization effect of human saliva in the techniques available for dental fluorosis treatment.

CONCLUSIONS

Within the limitations of this *in vitro* study, it was concluded that the treatment protocols for dental fluorosis tested significantly changed the enamel roughness, microhardness and micromorphology.

REFERENCES

1.Moimaz SA, Saliba O, Marques LB, Garbin CA, Saliba NA. Dental fluorosis and its influence on children's life. Braz Oral Res. 2015;29. pii: S1806-83242015000100214. doi: 10.1590/1807-3107BOR-2015.vol29.0014.

2.Bassir MM, Rezvani MB, Ghomsheh ET, Hosseini ZM. Effect of Different Surface Treatments on Microtensile Bond Strength of Composite Resin to Normal and Fluorotic Enamel After Microabrasion. J Dent (Tehran). 2016 Nov;13(6):431-437.

3.García-Pérez Á, Irigoyen-Camacho ME, Borges-Yáñez SA, Zepeda-Zepeda MA, Bolona-Gallardo I, Maupomé G. Impact of caries and dental fluorosis on oral health-related quality of life: a cross-sectional study in schoolchildren receiving water naturally fluoridated at above-optimal levels. Clin Oral Investig. 2017 Mar 1. doi: 10.1007/s00784-017-2079-1.

4.Molina-Frechero N, Nevarez-Rascón M, Nevarez-Rascón A, González-González R, Irigoyen-Camacho ME, Sánchez-Pérez L, et al. Impact of Dental Fluorosis, Socioeconomic Status and Self-Perception in Adolescents Exposed to a High Level of Fluoride in Water. Int J Environ Res Public Health. 2017 Jan 12;14(1). pii: E73. doi: 10.3390/ijerph14010073.

5.Joshi N, Palaskar J, Joshi M, Kathariya R. Complete oral rehabilitation in a case with severe dental fluorosis. World J Clin Cases. 2014 Dec 16;2(12):938-42. doi: 10.12998/wjcc.v2.i12.938.

6.Castro KS, Ferreira AC, Duarte RM, Sampaio FC, Meireles SS. Acceptability, efficacy and safety of two treatment protocols for dental fluorosis: a randomized clinical trial. J Dent. 2014 Aug;42(8):938-44. doi: 10.1016/j.jdent.2014.01.011.

7.Bezerra-Júnior DM, Silva LM, Martins L de M, Cohen-Carneiro F, Pontes DG. Esthetic rehabilitation with tooth bleaching, enamel microabrasion, and direct adhesive restorations. Gen Dent. 2016 Mar-Apr;64(2):60-4.

8.Croll TP, Cavanaugh RR. Enamel color modification by controlled hydrochloric acid-pumice abrasion. I. technique and examples. Quintessence Int. 1986 Feb;17(2):81-7.

9.Mondelli, J, Mondelli RFL, Bastos MTAA, Franco EB. Microabrasão com ácido fosfórico. Rev Bras Odontol. 1995 mai./jun; 52(3): 20-22.

10.Bertoldo C, Lima D, Fragoso L, Ambrosano G, Aguiar F, Lovadino J. Evaluation of the effect of different methods of microabrasion and polishing on surface roughness of dental enamel. Indian J Dent Res. 2014 May-Jun;25(3):290-3. doi: 10.4103/0970-9290.138308.

11.Meireles SS, Andre Dde A, Leida FL, Bocangel JS, Demarco FF. Surface roughness and enamel loss with two microabrasion techniques. J Contemp Dent Pract. 2009 Jan 1;10(1):58-65.

12.Pini NI, Sundfeld-Neto D, Aguiar FH, Sundfeld RH, Martins LR, Locadino JR, et al. Enamel microabrasion: An overview of clinical and scientific considerations. World J Clin Cases. 2015 Jan 16;3(1):34-41. doi: 10.12998/wjcc.v3.i1.34. Review.

13.Alqahtani MQ. Tooth-bleaching procedures and their controversial effects: A literature review. Saudi Dent J. 2014 Apr;26(2):33-46. doi: 10.1016/j.sdentj.2014.02.002.

14.American Dental Association (ADA). Consumer products with the ADA Seal of Acceptance. Available in: <http://www.ada.org/ada/seal/adaseal_consumer_shopping.pdf2016>. Access in: 29 mar. 2017.

15.Bertassoni LE, Martin JM, Torno V, Vieira S, Rached RN, Mazur RF. In-office dental bleaching and enamel microabrasion for fluorosis treatment. J Clin Pediatr Dent. 2008 Spring;32(3):185-7.

16.Yildiz G, Celik EU. A minimally invasive technique for the management of severely fluorosed teeth: A two-year follow-up. Eur J Dent. 2013 Oct;7(4):504-8. doi: 10.4103/1305-7456.120661.

17.Franco LM, Machado LS, Salomão FM, Dos Santos PH, Briso AL, Sundfeld RH. Surface effects after a combination of dental bleaching and enamel microabrasion: An in vitro and in situ study. Dent Mater J. 2016;35(1):13-20. doi: 10.4012/dmj.2014-334.

18.Pachaly R, Pozzobon RT. Analysis of surface roughness of human enamel exposed to bleaching agent and submitted to brushing. Acta Odontol Latinoam. 2012;25(1):59-66.

19.Pinto CF, Oliveira Rd, Cavalli V, Giannini M. Peroxide bleaching agent effects on enamel surface microhardness, roughness and morphology. Braz Oral Res. 2004 Oct-Dec;18(4):306-11.

20.Ahmadi Zenouz G, Ezoji F, Enderami SA, Khafri S. Effect of Fluoride, Casein Phosphopeptide-Amorphous Calcium Phosphate and Casein Phosphopeptide-Amorphous Calcium Phosphate Fluoride on Enamel Surface Microhardness After Microabrasion: An in Vitro Study. J Dent (Tehran). 2015 Oct;12(10):705-11.

21.Celik EU, Y1´ld1´z G, Yazkan B. Comparison of enamel

microabrasion with a combined approach to the esthetic management of fluorosed teeth. Oper Dent. 2013 Sep-Oct;38(5):E134-43. doi: 10.2341/12-317-C. Epub 2013 Feb 7.

22.Higashi C, Dall'Agnol AL, Hirata R, Loguercio AD, Reis A. Association of enamel microabrasion and bleaching: a case report. Gen Dent. 2008 May;56(3):244-9.

23.Fragoso LS, Lima DA, de Alexandre RS, Bertoldo CE, Aguiar FH, Lovadino JR. Evaluation of physical properties of enamel after microabrasion, polishing, and storage in artificial saliva. Biomed Mater. 2011 Jun;6(3):035001. doi: 10.1088/1748-6041/6/3/035001.

24.Rodrigues MC, Mondelli RF, Oliveira GU, Franco EB, Baseggio W, Wang L. Minimal alterations on the enamel surface by micro-abrasion: in vitro roughness and wear assessments. J Appl Oral Sci. 2013 Mar-Apr;21(2):112-7. doi: 10.1590/1678-7757201302117.

25.Melo CF, Manfroi FB, Spohr AM. Microhardness and roughness of enamel bleached with 10% carbamide peroxide and brushed with different toothpastes: an in situ study. J Int Oral Health. 2014 Jul;6(4):18-24.

26.Azrak B, Callaway A, Kurth P, Willershausen B. Influence of bleaching agents on surface roughness of sound or eroded dental enamel specimens. J Esthet Restor Dent. 2010 Dec;22(6):391-9. doi: 10.1111/j.1708-8240.2010.00372.x.

27.Cvikl B, Lussi A, Moritz A, Flury S. Enamel Surface Changes After Exposure to Bleaching Gels Containing Carbamide Peroxide or Hydrogen Peroxide. Oper Dent. 2016 Jan-Feb;41(1):E39-47. doi: 10.2341/15-010-L.

28.Abouassi T, Wolkewitz M, Hahn P. Effect of carbamide peroxide and hydrogen peroxide on enamel surface: an in vitro study. Clin Oral Investig. 2011 Oct;15(5):673-80. doi: 10.1007/s00784-010-0439-1.

29.Smidt A, Feuerstein O, Topel M. Mechanical, morphologic, and chemical effects of carbamide peroxide bleaching agents on human enamel in situ. Quintessence Int. 2011 May 42(5): 407–12.

30.Nascimento WC, Gomes Ydo S, Alexandrino LD, Costi HT, Silva JO Jr, Silva CM. Influence of fluoride concentration and pH Value of 35% hydrogen peroxide on the hardness, roughness and morphology of bovine enamel. J Contemp Dent Pract. 2014 Jul 1;15(4):392-8.

31.Dionysopoulos D, Koliniotou-Koumpia E, Tolidis K, Gerasimou P. Effect of fluoride treatments on bleached enamel microhardness and surface morphology. Oral Health Prev Dent. 2017;15(2):169-175. doi: 10.3290/j.ohpd.a37929.

32.George L, Baby A, Dhanapal TP, Charlie KM, Joseph A, Varghese AA. Evaluation and comparison of the microhardness of enamel after bleaching with fluoride free and fluoride containing carbamide peroxide bleaching agents and post bleaching anticay application: An in vitro study. Contemp Clin Dent. 2015 Sep;6(Suppl 1):S163-6. doi: 10.4103/0976-237X.166835.

33.Moosavi H, Darvishzadeh F. The Influence of Post Bleaching Treatments in Stain Absorption and Microhardness. Open Dent J. 2016 Mar 25;10:69-78. doi: 10.2174/1874210616021000069.

34.Araujo NC, da Costa Soares MU, Nery MM, Sales WS, Gerbi ME. Effect of pH values of two bleaching gels on enamel microhardness. Gen Dent. 2013 Jul;61(4):55-8.