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ORIGINAL ARTICLE

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Influence of calcium lactate and sodium fluoride solution mouthwashes on enamel microhardness and dentin permeability during in-office bleaching

Influência das soluções enxaguatórias de lactato de cálcio e fluoreto de sódio na microdureza do esmalte e permeabilidade dentinária durante o clareamento em consultório

Laurianne Moreira GOMES[1], Mário Rodrigo TRINDADE[1], Fabiana Mantovani Gomes FRANÇA[1], Flávia Lucisano Botelho do AMARAL[1], Roberta Tarkany BASTING[1]

1 - Faculdade São Leopoldo Mandic. Campinas, SP, Brazil.

ABSTRACT

Objective: This study evaluated the influence of calcium lactate and sodium fluoride mouthwashes on enamel microhardness and dentin permeability during in-office bleaching. **Material and Methods:** For the microhardness evaluation, enamel blocks were randomly submitted to different treatments associated with in-office bleaching agent using 40% hydrogen peroxide (Opalescence Boost PF 40%/ Ultradent; 3 sessions x 40-minute each) (n = 10): 1) Control: application of bleaching agent; 2) Calcium lactate: 1-minute immersion before bleaching treatment; 3) Sodium fluoride: immersion for 1 minute before bleaching treatment; 4) Calcium lactate + sodium fluoride: 1 minute immersion in calcium lactate solution, followed by 1 minute immersion in sodium fluoride before bleaching treatment. Dentin discs received the same treatment protocols (n = 10) to evaluate permeability. Knoop microhardness and dentin permeability assessments were performed twice (at baseline and 48 hours after the end of bleaching treatment). **Results:** The generalized linear model considering the design of repeated measures over time showed that there was no significant difference between treatments ($p = 0.9577$) and between assessments ($p = 0.3267$) regarding Knoop microhardness. Kruskal Wallis and Dunn tests showed that calcium lactate immersion before bleaching treatment provided higher dentin permeability than other groups ($p = 0.0009$). **Conclusion:** The use of sodium fluoride solution and calcium lactate in association with in-office bleaching treatment did not influence the microhardness of tooth enamel, although calcium lactate may increase dentin permeability.

KEYWORDS

Tooth bleaching; Dentin; Sodium fluoride; Calcium lactate; Hardness tests.

RESUMO

Objetivo: Este estudo avaliou a influência dos enxaguatórios bucais com lactato de cálcio e fluoreto de sódio na microdureza do esmalte e na permeabilidade da dentina durante o clareamento em consultório. **Material e Métodos:** Para avaliação da microdureza, blocos de esmalte foram submetidos aleatoriamente a diferentes tratamentos associados a agente clareador em consultório com peróxido de hidrogênio 40% (Opalescence Boost PF 40% / Ultradent; 3 sessões x 40 minutos cada) (n = 10): 1) Controle: aplicação de agente clareador; 2) Lactato de cálcio: imersão de 1 minuto antes do tratamento clareador; 3) Fluoreto de sódio: imersão por 1 minuto antes do tratamento clareador; 4) Lactato de cálcio + fluoreto de sódio: 1 minuto de imersão em solução de lactato de cálcio, seguido de 1 minuto de imersão em fluoreto de sódio antes do tratamento clareador. Os discos de dentina receberam os mesmos protocolos de tratamento (n = 10) para avaliar a permeabilidade. As avaliações da microdureza Knoop e da permeabilidade dentinária foram realizadas duas vezes (no início do estudo e 48 horas após o término do tratamento clareador). **Resultados:** O modelo linear generalizado considerando o delineamento de medidas repetidas ao longo do tempo mostrou que não houve diferença significativa entre os tratamentos (p = 0,9577) e entre as avaliações (p = 0,3267) em relação à microdureza Knoop. Os testes de Kruskal Wallis e Dunn mostraram que a imersão de lactato de cálcio antes do tratamento clareador proporcionou maior permeabilidade dentinária do que os outros grupos (p = 0,0009). **Conclusão:** O uso de fluoreto de sódio e lactato de cálcio associado ao clareamento em consultório não influenciou a microdureza do esmalte dentário, embora o lactato de cálcio possa aumentar a permeabilidade dentinária.

PALAVRAS-CHAVE

Clareamento dental; Dentina; Fluoreto de sódio; Lactato de cálcio; Testes de dureza.

INTRODUCTION

Most of the bleaching agents used in office techniques use hydrogen peroxide as an active ingredient, which penetrates the dental structure and releases free radicals that oxidize the pigmented organic molecules that darken the dental element [1,2]. The diffusion of free radicals through the dental structure occurs due to their low molecular weight [3,4].

Despite the benefit regarding the efficacy of the color change promoted by the bleaching agents, changes in the mineral content of the dental substrate and the presence of superficial tooth loss and porosities can still be evidenced, being related to the type of bleaching agent, concentration, time of use and acidity of the gels [4-7]. In addition, they can lead to increased dentin permeability [8,9] when the gel comes into contact with dentin in situations or when there is gingival retraction or non-carious cervical lesions, which allow direct or even better diffusion pathways, thus setting patients on a seemingly higher risk for bleaching sensitivity [10].

The use of desensitizing agents containing fluorides, calcium and potassium nitrate have been indicated not only to reduce the effects of tooth sensitivity, but also to minimize the occurrence of mineral loss [11-14]. Fluoride acts by obliterating the dentinal tubules in the form of calcium fluoride deposits, thus blocking external stimuli, while potassium nitrate blocks nerve impulses, decreasing the painful stimulus, bringing some comfort to some patients [15].

It is recognized that a rinse with calcium lactate before the use of a rinse with sodium fluoride increases the anti-erosive effect of fluoride on the enamel [16-18], since there is an increase in the availability of calcium to lead to the formation of greater amount of calcium fluoride precipitates on the tooth surface [19]. In this regard, it is suggested that the use of this association could control or reduce the mineral loss of enamel promoted by the bleaching agent used. Furlan et al. [20] showed that daily mouthwash with sodium fluoride or mouthwash with calcium lactate prior to fluoridated mouthwash avoids increasing the degree of sensitivity of the teeth after whitening treatment, which could be justified by the reduction of dentin permeability and also due to reduction of superficial tooth loss. However, there are no studies that have evaluated this effect.

Thus, the objective of the present in vitro study was to evaluate the influence of the use of calcium lactate, sodium fluoride solutions and the association of calcium lactate + sodium fluoride during dental bleaching in the enamel microhardness and dentin permeability. The null hypotheses to be tested were that: H01) there would be no differences in enamel microhardness when using different treatments in association with the whitening treatment of the office with 40% hydrogen peroxide; H02) there would be no differences regarding dentin permeability when using different treatments in association with the office whitening treatment with 40% hydrogen peroxide.

MATERIAL & METHODS

Specification of the materials under study, experimental groups and application protocol of the bleach and association with the solutions

The bleaching gel with 40% hydrogen peroxide (Opalescence Boost, Ultradent) was used according to the protocol recommended in the studies by Furlan et al. [20] and de Paula et al. [21] (Table I). The solutions simulating mouthwashes (calcium lactate, sodium fluoride, calcium lactate + sodium fluoride) were manipulated in the research laboratory according to the specifications in Table I, following the protocol used by Furlan et al. [20].

The pH of the bleaching gel and solutions was measured in triplicate with a microelectrode (Model 2A14, Analytical Instrumentation Analyzer, São Paulo, SP, Brazil) and pH meter (Model MPA 210, MS Tecnopon Instrumentação, Piracicaba, SP, Brazil), obtaining the mean values shown in Table I.

The treatments applied in association with the bleaching were carried out at the times indicated in Table I, obtaining the following experimental groups: 1) control (only application of the bleaching agent); 2) calcium lactate; 3) sodium fluoride; 4) calcium lactate + sodium fluoride. In the group that received treatment with calcium lactate + sodium fluoride, the enamel blocks or dentin discs were immersed for 1 minute in the calcium lactate solution, followed by immersion for 1 minute in sodium fluoride prior to the bleaching treatment sessions.

To perform the whitening treatment, the gel used was manipulated according to the manufacturer's instructions, the same being applied to the enamel or dentin surface, with a layer of approximately 1 mm thick, covering the entire surface, for 40 minutes [20]. Then, the surface was washed with running water and air spray for 30 seconds, proceeding with air drying. The bleaching treatment was performed two more times, with each session performed every 48 hours.

Between the bleaching sessions, for all groups, the enamel blocks or dentin discs were stored separately in vials containing 13.5 ml of artificial saliva solution (1.5 mMol/ L Ca; 50 mMol/ L KCl; 0.9mMol/ L PO_4; 20 mMol/ L Tris buffer; pH = 7), recommended by Featherstone et al. [22] and modified by Serra & Cury [23], changing the solution every two days.

Enamel microhardness tests

Selection of teeth and preparation of specimens

Twenty-five sound human third molars obtained after approval of the project by the Ethics Committee (CAAE opinion number 08051519.8.0000.5374) were used. The teeth were stored frozen for up to six months, being thawed 24 hours in the refrigerator and cleaned with a Robinson brush, pumice stone, periodontal curettes and scalpel blades. Teeth were selected without cracks, wear and/ or fractures and the crown should be free of stains after observation in a stereoscopic magnifying glass (Eikonal Equipamentos Analíticos, EK3ST, São Paulo, SP, Brazil) with 20 x magnification.

The dental crowns were sectioned with a double-sided diamond disc (nº 7020 KG Sorensen, Cotia, SP, Brazil) and low-speed micromotor (Kavo do Brasil, Joinville, SC, Brazil) to obtain 3 mm x 3 mm enamel blocks from the buccal and lingual / palatal surfaces of each tooth. In total, 60 dental enamel blocks were obtained.

The enamel blocks were embedded in polyester resin (Maxi Rubber, Campinas, SP, Brazil), and the enamel surfaces were flatted in automated rotary electric polisher (Ecomet 250, Buehler Ltd., Lake Bluff, IL, USA) coupled with a head (Buehler Automet 250, Buehler Ltd., Lake Bluff, IL, USA) with aluminum oxide paper (Arotec S / A Ind. E Com., Cotia, SP, Brazil) with 600 and 1200-grit. Polishing was performed with

Table I - Specification of the bleaching agent, mouthwash solutions, composition of the products used and protocol of use

Bleaching/ Mouthwash solution	Commercial name (manufacturer, city, state, country) Lot number	Composition (weight percentage)	Protocol of use	pH
In-office bleaching	Opalescence Boost PF 40% (Ultradent Products, South Jordan, UT, USA) DO9FE	Hydrogen peroxide (40%), sodium fluoride (between 1 to 1.5%), potassium nitrate (<5%), potassium hydroxide (<5%)*	1 application for 40 minutes / 3 sessions, with one session every 2 days	7.24
Calcium lactate solution	Handled in the laboratory	150 mmol/L calcium lactate	Immerse in 20 mL of the solution for 1 minute before each bleaching treatment session	6.53
Fluoridated solution	Handled in the laboratory	12 mmol/L sodium fluoride	Immerse in 20 mL of the solution for 1 minute before each bleaching treatment session	5.69

*According to the MSDS of the material made available by the manufacturer a 0.3 μm alumina suspension (Alfa micropolish, Buehler Ltd, Lake Bluff, IL, USA) with felt cloths. The blocks were cleaned in an ultrasonic vat in deionized water for 10 min to remove any residue from the polishing procedure.

Baseline microhardness tests for selection of dental blocks and division between experimental groups

Baseline microhardness tests were performed on the 60 enamel blocks using a digital microhardness tester (Pantec HVS 1000 - Panambra - São Paulo / SP, Brazil) and a Knoop-type penetrator with a static load of 25 g for 5 seconds. Three microhardness indentations were carried out on each enamel surface of each block at random locations on the enamel surface, but separated by a distance of 200 μm. The values obtained in the baseline time were tabulated, excluding the lowest and highest values, obtaining average Knoop microhardness values for the selection of 40 enamel blocks between 350 to 450 Kg/ mm^2. The embedded enamel blocks were stored at 37 °C in a bacteriological oven in relative humidity.

Application of bleaching agent and treatments, and final microhardness tests

The blocks were separated into the four groups (n = 10): control, calcium lactate, sodium fluoride and calcium lactate + sodium fluoride, proceeding to the bleaching treatments and associations with the solutions. After the third session of in-office bleaching session and immersion for 48 hours in artificial saliva, the final enamel microhardness assessments were carried out according to the parameters previously described.

Dentin permeability tests

Selection of teeth and preparation of specimens

Forty sound third human molars obtained after approval of the project by the Ethics Committee (CAAE 19694719.4.0000.5374) were used, which were stored frozen until the moment of use, and cleaned with periodontal curettes and scalpel blades and washed with water.

A precision metallographic cutter (Isomet 1000, Buehler, Springfield, VA, USA) with high concentration diamond disc (Buehler, 102mm x 0.3mm) under cooling was used to obtain the dentin discs. The crowns were cut perpendicularly along the long axis of the tooth, 1.5 mm above the cement-cement junction. The occlusal surface of the sample was completely free of enamel. Then, both faces were sanded with 600 grit sandpaper for uniformity and smoothness of the surfaces.

The thickness of each sample was checked with a digital caliper (Mitutoyo Sul Americana LTDA, MIP/ E 103, Suzano, SP, Brazil). The final thickness of the dentin discs was 1.5 mm. Then, removal of the smear layer was performed with 37% phosphoric acid for 15s [24] and washed for 30s with water, on both sides. Acid conditioning allowed standardization of the opening of dentinal canaliculi and greater dentin permeability [25] for the first measurement of hydraulic conductance (L1), considered 100%, corresponding to the maximum filtration. Soon after, the occlusal surfaces of the specimens were sanded with 300 grit sandpaper for 30s to form a standardized smear layer [26,27].

Application of bleaching agent and treatments

The 40 dentin discs were separated between the groups (n = 10): control, calcium lactate, sodium fluoride and calcium lactate + sodium fluoride, proceeding to the bleaching treatment and associations with the solutions. After the treatments, the surfaces were again subjected to the permeability test.

Permeability tests

A permeability machine (THD, Odeme Dental Reseach, Luzerna, SC, Brazil) was used under 5 psi pressure, equivalent to 351.54 cmH_2O [28]. The dentin disc was mounted in the filtration chamber and the machine system was adjusted. The water penetrated under pressure the dentinal canaliculi towards the occlusal surface. This movement of the fluid was registered by the difference in the position of an air bubble in the glass capillary of the equipment. Three measurements were made, the first after 4 min and the next with a 3 min interval between them [29], using a digital caliper already attached to the permeability machine. The linear displacement in the glass capillary during a determined time allowed to calculate the amount of fluid that passed through the specimen using the following formula Q = (ri2l) / t. Where, Q ($\mu L/min^{-1}$) is the amount of fluid that passes through the specimen, l (cm) is the linear displacement in the glass capillary,

Table II - Mean (standard deviation) of Knoop microhardness (in kgf/ mm^2) according to groups and times

Groups	Inicial (before bleaching	Final (after bleaching)
	Mean (standard deviation)	
Control	364.90 (42.53)	369.07 (42.21)
Calcium lactate	364.53 (33.60)	362.47 (20.75)
Sodium fluoride	362.30 (35.14)	385.17 (54.43)
Calcium lactate + sodium fluoride	362.77 (41.82)	368.63 (30.98)

Table III - Kruscal Wallis and Dunn's tests for percentage (%) of dentin permeability according to each group

Groups	Minimum	First quartil	Median	Third quartil	Maximum
Control	-187.06	-19.44	-8.04 b	-1.02	9.94
Sodium fluoride	-226.04	-46.00	1.55 b	28.54	55.64
Calcium lactate	30.30	32.97	39.36 a	61.54	89.06
Calcium lactate + sodium fluoride	-167.26	-54.62	8.94 b	25.29	46.33

Statistical analysis showed significant differences among calcium lactate group (a) and the others (b).

t (min) is the time and ri (cm^2) is the internal radius of the glass capillary tube.

The hydraulic conductance (L) was calculated using a formula, considering the viscosity of the water and the thickness of the constant specimen, L = Q/ (AP): L is hydraulic conductance ($\mu L\ cm^{-2}\ min^{-1}\ cmH_2O^{-1}$), A ($cm^2$) is the dentin surface area and P (cmH_2O) is the pressure imposed.

The hydraulic conductance of each dentin disc was evaluated in two moments: initially right after acid conditioning (L1) and after bleaching treatment, associated or not with the mouthwash solution of each group (L2), when the specimen was assembled in exactly the same position. Using the following equation, the percentage of dentin permeability after the bleaching treatment was calculated, with each tooth being its own control: L (%) = [(L1 - L2) x 100]/ L1 [30], where L is the percentage of permeability, L1 is the hydraulic conductance after removal of the smear layer, and L2 is the hydraulic conductance after the bleaching treatment associated or not with the mouthwash solution of each group.

Statistical analysis

Descriptive and exploratory analyzes of the data were carried out. As the Knoop microhardness data did not meet the assumptions of an analysis of variance, a generalized linear model was used considering the design of repeated measures over time. For the dentin permeability data, exploratory analyzes showed that the data did not meet the assumptions of an analysis of variance (ANOVA). Then, the groups were compared by Kruskal Wallis and Dunn's nonparametric tests. The analyzes were performed using the R Core Team program [31] with a 5% significance level.

RESULTS

There was no significant difference between treatments (p = 0.9577) and between times (p = 0.3267) regarding microhardness (Table II). The interaction between the groups x time factors was not significant (p = 0.6938).

The group that received calcium lactate showed a significantly higher percentage of permeability (39.36%) than the other groups (p = 0.0009) (Table III). There were no significant differences in permeability between the other groups, despite the fact that the control group (which received only the bleaching treatment) showed a small reduction in permeability (-8.04%).

DISCUSSION

Although it is recognized that a rinse with calcium lactate followed by a rinse with sodium fluoride leads to a significant increase in the amount of fluoride present in the fluid of the oral cavity [19], and may also minimize or prevent erosion of the enamel [17,18], the treatments associated with tooth bleaching (calcium lactate, sodium fluoride and calcium lactate + sodium fluoride) did not influence the enamel microhardness, leading to the acceptance of the first hypothesis. On the other hand, calcium lactate allowed an increase in dentin permeability when used in association with the bleaching treatment, which treatment differed significantly from the others and led to the rejection of the second null hypothesis of the present study.

Fluoridated agents are deposited on the dental surface in the form of precipitates of calcium fluoride [32], which form a mechanical barrier that reduces dentin permeability [33]. Although there are no statistically significant differences regarding the permeability between the control and sodium fluoride groups, sodium fluoride prior to the bleaching treatment promoted the achievement of positive values, suggesting that the ability of calcium fluoride to remain on the dentin surface may have been affected by the application of the bleaching agent, which was applied after immersion in the fluoridated solution and remained on the surface for 40 minutes. It is possible that the action of hydrogen peroxide may have solubilized the deposited calcium fluoride salts, resulting in the fluoride's inefficiency in reducing the permeability of the substrate, despite not having the effect of inhibiting enamel demineralization [34] due to the absence of difference in microhardness tests.

It is suggested that an increase in the dentin permeability presented with the use of calcium lactate may have occurred because there is no deposition of calcium salts on the dentin surface due to the application of the bleaching agent, since the lactate solution may present greater solubility. Braga et al. [35] showed that the calcium lactate solution did not differ in terms of the effect of tubular occlusion provided by the sodium fluoride solution in controlling the permeability of hypersensitive dentin.

On the other hand, the association of calcium lactate + sodium fluoride associated with the bleaching treatment did not lead to differences in permeability in relation to the control group. In this respect, it is possible that the deposition of calcium fluoride salts - which would be increased by the action of lactate - was also minimized by the effect of the subsequent application of the bleaching agent, which led to a small increase in dentin permeability, but not significant as found in the group in which only calcium lactate was used. The fact that only sodium fluoride alone or associated with calcium associated does not have a significant effect on reducing dentin permeability can also be justified by the discrepancy between the size of dentin tubules when compared to the size of calcium fluoride globules, which are larger in diameter [36]. It can also be suggested that the deposition occurred only in a few tubules. Although dentin has the advantage of having three times the concentration of calcium fluoride deposits compared to enamel, the products are unstable and less absorbed by dentin [36].

When using only the bleaching agent (control group), there were no changes in the enamel microhardness due to the bleaching product used. Although some studies have shown that bleaching agents based on hydrogen peroxide for in-office use can reduce the enamel microhardness [37-39], this effect is related to the low pH of the agents [37,40,41] and protocol of use [39] used. The selected gel chosen for the study has a higher pH (pH = 7.24), preventing changes in mineral content from occurring, as observed in the control group. However, the use of the bleaching agent seems to have led to a small decrease in dentin permeability, which is justified by the negative median value (Table III). Despite the low concentration in the bleaching agent, the sodium fluoride present in the composition seems to have allowed slight obliteration of the dentinal tubules, which may have reduced its permeability by 8.04%. On the other hand, potassium nitrate, as well as potassium hydroxide - which is added to deprotonate acids and maintain the alkalinity of the product - appear to have no direct effect on tubular obliteration, as they do not act as agents that chemically bind compounds organic and inorganic dentin, not even forming deposits on the dentin surface.

CONCLUSION

The use of calcium lactate and/ or sodium fluoride solutions associated with the in-office bleaching treatment did not influence the enamel microhardness. Although sodium fluoride and calcium lactate + sodium fluoride treatments did not influence dentin permeability, calcium lactate solution during bleaching treatment increased dentin permeability.

Author Contributions

LMG: performed data acquisition, interpreted the data and wrote the paper. MRT: performed data acquisition, interpreted the data and wrote the paper. FMGF: revised the paper and wrote the final approved version to be submitted. FLBA:

data analysis, revised the paper carefully for important intellectual content and approved the final version. RTB: responsible for the conception and design of the study, interpretation of the data and writing the paper.

Conflict of Interest

The authors have no financial, economic, commercial, and/or professional interests related to the topics presented in the manuscript.

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Regulatory Statement

The projet was approved by the Ethics Committee (CAAE numbers 08051519.8.0000.5374 and 19694719.4.0000.5374).

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Roberta Tarkany Basting
(Corresponding address)
Faculdade São Leopoldo Mandic, Campinas, SP, Brazil.
Email: rbasting@yahoo.com

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