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LAURA NOBRE FERRAZ

USO DE CLAREADORES EXPERIMENTAIS COM DIFERENTES ESPESSANTES E DENTIFRÍCIOS REMINERALIZADORES NAS PROPRIEDADES FÍSICO-QUÍMICAS DO ESMALTE CLAREADO EM CONDIÇÕES DE FLUXO SALIVAR NORMAL E REDUZIDO: ESTUDO *IN SITU*

USE OF EXPERIMENTAL WHITENERS WITH DIFFERENT THICKENERS AND REMINERALIZING TOOTHPASTES IN PHYSICAL AND CHEMICAL PROPERTIES OF BLEACHED ENAMEL IN NORMAL AND LOW SALIVARY FLOW CONDITIONS: *IN SITU* STUDY

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Tese apresentada à Faculdade de Odontologia de Piracicaba da Universidade Estadual de Campinas como parte dos requisitos exigidos para a obtenção do título de Doutora em Clínica Odontológica, na Área de Dentística.

Thesis presented to the Piracicaba Dental School of the University of Campinas in partial fulfillment of the requirements for the degree of Doctor in Clinical Dentistry, in Restorative Dentistry.

Orientadora: Profa. Dra. Débora Alves Nunes Leite Lima

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A Comissão Julgadora dos trabalhos de Defesa de Tese de Doutorado, em sessão pública realizada em 07 de julho de 2020, considerou a candidata LAURA NOBRE FERRAZ aprovada.

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A Ata da defesa, assinada pelos membros da Comissão Examinadora, consta no SIGA/Sistema de Fluxo de Dissertação/Tese e na Secretaria do Programa da Unidade.

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RESUMO

Esse trabalho, através de dois estudos *in situ,* avaliou o efeito sobre o esmalte de géis clareadores com diferentes espessantes (Estudo 1 - Artigo 1) e dentifrícios com diferentes princípios ativos após o clareamento (Estudo 2 - Artigo 2) em pacientes com fluxo salivar normal ou reduzido. No Estudo 1, foram selecionados 14 voluntários com fluxo salivar normal e 14 voluntários com fluxo reduzido. Foram realizados 4 tipos de tratamento: sem clareamento (controle negativo), gel comercial de peróxido de carbamida (PC) 10% com carbopol (controle positivo), gel experimental de PC 10% com natrosol e gel experimental de PC 10% com aristoflex. Os participantes utilizaram um dispositivo palatino por 15 dias e o tratamento clareador foi realizado extra-oralmente por 4 horas durante 14 dias. Foram realizadas as análises de microdureza de superfície (SMH), cor (ΔE^*_{ab} e ΔE_{00}), rugosidade (Ra), microscopia eletrônica de varredura (MEV) e por energia dispersiva por Raio-X (EDS). As análises foram realizadas inicialmente e após o término do período experimental *in situ*. Para o Estudo 2, foram selecionados 12 voluntários para cada fluxo salivar, normal e reduzido. Esse estudo foi conduzido em 6 fases experimentais *in situ* de 24 horas. Os espécimes bovinos foram previamente clareados *in vitro* (1 sessão, peróxido de hidrogênio 35%, 45 minutos de aplicação) e em seguida foram fixados no dispositivo palatino. Durante o período *in situ* (24 horas) as amostras foram tratadas intra-oralmente 2 vezes com o dentifrício. Em cada fase um dentifrício foi avaliado: placebo, NaF, SnF_2, F/Sn/Quitosana, F/Arginina e F/Vidro Bioativo. As análises de SMH, Ra e cor (ΔE^*_{ab}, ΔE_{00}, ΔL, Δa, Δb) foram realizadas nos tempos baseline (T1), após o clareamento dental *in vitro* (T2) e após o período experimental *in situ* (T3). As analyses de MEV, EDS foram realizadas apenas em T3. As análises estatitisticas apropriadas para cada análise foram realizadas com nível de significância de 5%. Os resultados do Estudo 1 mostraram que diferenças foram encontradas entre os fluxos salivares em SMH para o natrosol e em Ra para o carbopol. Para o fluxo salivar normal, o natrosol e o aristoflex apresentaram maiores valores de SMH entre todos os grupos avaliados. Para o fluxo salivar reduzido o aristoflex apresentou o maior valor de SMH e para a Ra o aristoflex e o natrosol apresentaram os menores valores entre todos os grupos avaliados e não diferiram entre si. Na análise de EDS, o aristoflex apresentou maior relação Ca/P e diferiu do grupo placebo para o fluxo salivar normal. No Estudo 2, os resultados mostraram diferenças significantes entre os fluxos salivares em SMH e Ra para o

placebo e o NaF. Na relação Ca/P foram encontradas diferenças entre os fluxos para o dentifrício placebo. Para o fluxo salivar normal, a SMH em T3 foi igual em T1 independente do dentifrício utilizado. Para o fluxo reduzido, os dentifrícios a base de SnF_2, F/Sn/Quitosana e F/Vidro Bioativo resultaram em maior SMH em T3 e não diferiram estatisticamente de T1. O F/Vidro Bioativo apresentou os menores valores de Ra para ambos os fluxos salivares. Além disso, para a quantificação de Ca%, P%, Na%, o F/Vidro Bioativo apresentou os maiores valores, diferiu do grupo placebo e não apresentou diferença entre os fluxos salivares. Concluiu-se que o fluxo salivar e os espessantes interferiram nas alterações do esmalte após o clareamento. No fluxo salivar normal, o natrosol e o aristoflex apresentaram menores alterações no esmalte, enquanto que para o fluxo reduzido as menores alterações foi para aristoflex. O fluxo salivar e os dentifrícios interferiram na remineralização após o clareamento dental. Dentifrícios com vidro bioativo apresentaram a melhor performance no esmalte dental clareado para ambos os fluxos salivares.

Palavras-chave: Clareadores, Espessantes, Dentifrícios, Saliva.

ABSTRACT

This work, through two *in situ* studies, evaluated the effect on the enamel of bleaching gels with different thickeners (Study 1 - Article 1) and dentifrices with different active principles after bleaching (Study 2 - Article 2) in patients with normal and low salivary flow. In Study 1, 14 volunteers with normal salivary flow and 14 volunteers with low flow were selected. Four types of treatment were performed: without bleaching (negative control), commercial gel of carbamide peroxide (PC) 10% with carbopol (positive control), experimental gel of PC 10% with natrosol and experimental gel of PC 10% with aristoflex. The participants used a palatal device for 15 days and the bleaching treatment was performed extra-orally for 4 hours for 14 days. Analyzes of surface microhardness (SMH), color (ΔE^*_{ab} and ΔE_{00}), roughness (Ra), scanning electron microscopy (SEM) and by X-Ray dispersive energy (EDS) were performed. The analyzes were performed initially and after the end of the *in situ* experimental period. For Study 2, 12 volunteers with normal salivary flow and 12 volunteers with low flow were selected. This study was conducted in 6 experimental phases in situ of 24 hours. The bovine specimens were previously cleared *in vitro* (1 session, 35% hydrogen peroxide, 45 minutes of application) and then fixed in the palatal device. During the in situ period (24 hours) the samples were treated intraorally 2 times with the dentifrice. In each phase a dentifrice was evaluated: placebo, NaF, SnF_2, F/Sn/Chitosan, F/Arginine and F/Bioactive Glass. The SMH, Ra and color analyzes (ΔE^*_{ab}, ΔE_{00}, ΔL, Δa, Δb) were performed at baseline times (T1), after in vitro tooth bleaching (T2) and after the *in situ* experimental period (T3). SEM and EDS analyzes were performed only in T3. The appropriate statistical analyzes were performed with a 5% significance level. The results of Study 1 showed that differences were found between salivary flows in SMH for natrosol and in Ra for carbopol. For normal salivary flow, natrosol and aristoflex showed higher values of SMH among all groups evaluated. For low salivary flow, aristoflex had the highest SMH value and for Ra, aristoflex and natrosol had the lowest values among all evaluated groups and did not differ between them. In the EDS analysis, aristoflex showed a higher Ca/P ratio and differed from the placebo group for normal salivary flow. In Study 2, the results showed significant differences between salivary flows in SMH and Ra for placebo and NaF. In the Ca/P ratio, differences were found between flows for the placebo dentifrice. For normal salivary flow, SMH at T3 was equal at T1 regardless of the toothpaste used.

For the reduced flow, the dentifrices based on SnF2, F/Sn/Chitosan and F/Bioactive Glass resulted in greater SMH in T3 and did not differ statistically from T1. The F/Bioactive Glass showed the lowest Ra values for both salivary flows. In addition, for the quantification of Ca%, P%, Na%, the F/Bioactive Glass showed the highest values, differed from the placebo group and did not show any difference between salivary flows. It was concluded that the salivary flow and thickeners interfered in the enamel changes after bleaching. In the normal salivary flow, natrosol and aristoflex showed minor changes in the enamel, while for low flow the smallest changes were for aristoflex. Salivary flow and dentifrices interfered with remineralization after tooth bleaching. Dentifrices with bioactive glass showed the best performance in bleached dental enamel for both salivary flows.

Keywords: Bleaching agents, Thickeners, Dentifrices, Saliva.

SUMÁRIO

1 INTRODUÇÃO

O mecanismo de ação do clareamento envolve a difusão do peróxido de hidrogênio através do esmalte, o qual reage com os cromógenos responsáveis pela descoloração dental (Kwon & Wertz 2015). Devido a sua ação oxidante, o peróxido de hidrogênio é capaz de quebrar as macromoléculas de pigmento transformando-as em moléculas pequenas o suficiente para serem removidas da estrutura dental por difusão (Sulieman *et al.,* 2004; Kwon & Wertz 2015). O peróxido de hidrogênio (PH) é o princípio ativo dos agentes clareadores de dentes vitais, podendo ser aplicado diretamente na superfície do dente, ou ainda ser liberado a partir da reação química do peróxido de carbamida ($CH_6N_2O_3$), uma molécula estável, que ao entrar em contato com a superfície do dente se dissocia em PH (H_2O_2) e uréia ($(NH_2)_2CO$) (Kwon & Wertz 2015).

Embora o clareamento dental mostre-se eficaz quanto a alteração de cor na maioria dos casos, sua realização pode levar a alguns efeitos adversos no esmalte dental como: alterações morfológicas da superfície de esmalte (Vieira Junior *et al.,* 2018; Vilhena *et al.,* 2019), modificações na distribuição do cristal de hidroxiapatita (Vilhena *et al.,* 2019), aumento da porosidade (Eskelsen *et al.,* 2018), diminuição da microdureza superficial e subsuperficial (Vieira Junior *et al*., 2016). Além disso, a análise química da superfície relatou tanto a modificação na relação cálcio/fosfato como a perda de cálcio e fósforo (Vieira Junior *et al.,* 2018; Cavalli *et al.,* 2018), apoiando assim a hipótese de que o clareamento dental é capaz de induzir alterações estruturais no esmalte humano. Essas alterações estão possivelmente relacionadas a fatores como o pH, efeito oxidativo, concentração dos agentes clareadores ou podem estar relacionados com a composição dos agentes clareadores, como por exemplo, os agentes espessantes (Gouveia *et al.,* 2019).

Os espessantes presentes nos géis clareadores tem como função produzir géis cristalinos capazes de estabilizar emulsões e proporcionar uma viscosidade clinicamente aplicável (Oliveira et al., 2007). Tal fato evita a ingestão do gel pelo paciente durante a permanência do mesmo no meio bucal (Oliveira *et al*., 2007). O espessante mais comumente utilizado junto ao gel de peróxido de carbamida é o carbopol (polímero carboxipolimetileno). O carbopol apresenta características iônicas e baixa estabilidade de pH (Gouveia *et al*., 2016; 2019) é responsável por retardar a liberação do peróxido de hidrogênio para que o gel clareador seja eficaz por todo o

tempo de aplicação (Matis et al., 2000). A literatura mostra que o carbopol presente na formulação do gel clareador pode contribuir com a degradação da superfície do esmalte dental (Gouveia *et al*., 2019).

Outro espessante disponível é o natrosol (hidroxietilcelulose), um polímero que apresenta características não iônicas, ou seja, capaz de incorporar substâncias hidrossolúveis, mesmo que as mesmas sejam ácidas (Gouveia *et al*., 2016). Estudos *in situ* (Silva *et al*., 2018) e *in vivo* (do Carmo Públio *et al*., 2017) mostraram que o natrosol não é citotóxico (do Carmo Públio *et al*., 2017), não interfere na eficácia clareadora do esmalte (do Carmo Públio *et al.,* 2017; Silva *et al*., 2018) e resulta em menor alteração da rugosidade (Silva *et al*., 2018).

Um espessante que vem sendo estudado como substituto do carbopol nas formulações de géis clareadores é o aristoflex (ácido sulfônico acriloildimetiltaurato e vinilpirrolidona) (Gouveia *et al*., 2019). O Aristoflex é um polímero sintético pré-neutralizado, que permite a formação de géis cristalinos com boa consistência (Gouveia *et al*., 2019). As características mais relevantes deste espessante são a estabilidade em pH ácidos e a formação de gel aniônico, podendo atuar como um agente de viscosidade inerte a formulação (Gouveia *et al*., 2019). Na cavidade bucal, ele tem sido empregado na confecção de dentifrícios (Golding et. al., 2014) e nas concentrações de até 1%, não apresentando riscos toxicológicos a saúde humana. Assim, esse polímero poderia ser capaz de evitar possíveis alterações nas propriedades do esmalte dentário e, portanto, é considerado um espessante promissor para uso em novas formulações de géis clareadores (Gouveia *et al*., 2019).

Enquanto a realização do clareamento dental pode resultar em efeitos deletérios no esmalte, existem alguns fatores, como por exemplo a saliva, que agem na tentativa de prevenir e reverter as modificações do esmalte clareado (Zeczkowski *et al*., 2015). A exposição do esmalte dental à saliva ao final do tratamento clareador não resulta em diferença de microdureza superficial em comparação com os valores iniciais, indicando a recuperação completa da microdureza da superfície após o clareamento dental (Zeczkowski *et al*., 2015). A saliva age através do tampão fosfato e bicarbonato que dificultam o declínio nos valores de pH. Quando o pH está dentro dos limites fisiológicos, a saliva se encontra supersaturada com minerais cálcio e fosfato, favorecendo o processo de remineralização (Carpenter *et al*., 2013). Baseado nisso, alterações salivares que interfiram na qualidade da saliva e de seus

constituintes podem resultar em alteração da capacidade de remineralização do esmalte clareado.

A diminuição do fluxo salivar é acompanhada por alterações das características salivares, tais como pH, proteínas e concentrações de eletrólitos, viscosidade e níveis de imunoglobulinas (Jensen *et al.,* 2003). A hipofunção salivar seja qual for a etiopatogênese tem se mostrado uma condição comum por diversos fatores. A hipossalivação afeta aproximadamente 30% dos pacientes com idade entre 20 e 69 anos (Flink et al., 2008) e ainda não há estudos na literatura que avaliam a capacidade de remineralização do esmalte clareado sob a condição de fluxo salivar reduzido. As alterações salivares podem ocorrer devido a alguma disfunção congênita que leva a hipofunção das glândulas salivares ou tratamentos que envolvem o uso de medicamentos (Jensdottir *et al.,* 2013). Dados sugerem que cerca de 1.800 medicamentos prescritos e de venda livre têm possíveis efeitos colaterais xerogênicos, incluindo 8 dos 10 medicamentos mais prescritos (Papas et al., 2009). Além disso, a redução no fluxo salivar pode ser causada pela radioterapia da região de cabeça e pescoço (Jensdottir *et al.,* 2013). O câncer na região de cabeça e pescoço é uma doença com alta incidência (Lieshout *et al.,* 2014). De acordo com a *US National Cancer Institute,* aproximadamente 30 em 100.000 pessoas são acometidas pela doença. Para o tratamento, as glândulas salivares maiores e menores são frequentemente irradiadas incidentalmente (Jensen *et al.,* 2003). Como consequências, a diminuição objetiva da taxa de fluxo salivar ou hipossalivação são complicações comuns e persistentes (Al Nawas *et al.,* 2006).

Além da saliva, tem sido discutido o uso de fluoreto durante ou após o clareamento para reverter os efeitos deletérios no esmalte dental (Vieira Junior *et al.,* 2016, 2018). O flúor pode atuar como um agente remineralizador formando uma camada de fluoreto de cálcio na superfície do esmalte. Este depósito é subsequentemente dissolvido, permitindo que o flúor se difunda no esmalte, suporte a remineralização e aumente os valores de microdureza de superfície (Bayrak *et al.,* 2009). Dentifrícios são um excelente veículo para o fornecimento de flúor na cavidade bucal devido ao seu grande uso e disponibilidade. Além do dentifrício fluoretado, a literatura tem avaliado o uso de dentifrícios que possuem na sua formulação algum agente remineralizante capaz de favorecer a recuperação do esmalte clareado (Vieira Junior *et al.,* 2018; Scribante *et al.,* 2020). Os produtos que contêm íons de estanho, por exemplo, têm sido conhecidos por fornecer uma boa proteção contra a

desmineralização (Ferraz *et al.,* 2019). Dentifrícios contendo arginina e vidro bioativo também são conhecidos por influenciarem o processo de remineralização salivar (Deng *et al.,* 2013; Vieira Junior *et al.,* 2016, 2018).

Um composto que seria interessante avaliar a sua efetividade contra os efeitos deletérios do clareamento dental é a quitosana. A quitosana é um bio-polissacarídeo natural produzido pela desacetilação da quitina (Younes et al., 2015; Muxika *et al.,* 2017) e tem se destacado pela sua capacidade de formar camadas sobre a superfície (Younes et al., 2015). Sua carga molecular positiva é capaz de se ligar eletrostaticamente às superfícies com zeta potencial negativo, como os tecidos dentais duros e a película (Young *et al.,* 1997), o que resulta na formação de multicamadas sobre o esmalte. Essa camada de quitosana formada sobre o esmalte tem se mostrado mais estável em pH ácido e notavelmente mais resistentes, evitando o processo de desmineralização (Ferraz et al., 2019). Além disso, a quitosana tem se destacado pela sua interação com a saliva humana. A especulação do efeito da saliva sobre a quitosana mostrou que a interação dessa substância com o esmalte e com as proteínas da película adquirida, principalmente com a mucina, promove um fortalecimento da camada orgânica protetora (Carvalho e Lussi., 2014) o que possivelmente poderia resultar em uma maior remineralização do esmalte dental clareado.

Sendo o clareamento dental um crescente na prática clínica, o interesse pela realização do clareamento dental pode incluir pacientes com alteração no fluxo salivar. Associando a importância dos espessantes do gel clareador frente aos efeitos deletérios do clareamento e também conhecendo ação essencial da saliva e dos agentes remineralizadores no processo de clareamento dental, faz-se necessária a condução de estudos que avaliem a inter-relação destes fatores. Assim, esse trabalho se propôs a avaliar o efeito do gel experimental à base de peróxido de carbamida com os espessantes natrosol e aristoflex em pacientes com fluxo salivar normal e fluxo salivar reduzido. Ainda, foi avaliado o efeito de diferentes dentifrícios buscando uma opção de tratamento que possa ser realizada pós-clareamento tanto para pacientes que apresentam o fluxo salivar normal ou fluxo salivar reduzido.

De acordo com as normas para elaboração de teses e dissertações da FOP/UNICAMP, essa tese de Doutorado foi dividida em dois artigos científicos, sendo o primeiro artigo intitulado *“Bleaching gel formulated with aristoflex showed fewer changes in enamel surface in in situ low salivary flow conditions*” e o segundo artigo

foi intitulado como *“Bioactive glass dentifrice presents best performance on remineralization of bleached enamel under low and normal salivary flow in situ condition”.*

2 ARTIGOS

2.1 Artigo: Bleaching gel formulated with aristoflex showed fewer changes in enamel surface in *in situ* low salivary flow conditions.

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ABSTRACT

Objective: The purpose of this *in situ* study was to evaluate the effect of bleaching gels with different thickeners on enamel under normal and hyposalivatory conditions. **Materials and Methods:** Twenty-eight participants were assigned of which 14 had normal and 14 had low salivary flow. For each salivary flow, 4 types of treatment were performed with different thickeners: no bleaching (negative control), commercial gel of 10% carbamide peroxide (CP) with carbopol (positive control), 10% CP with natrosol and 10% CP with aristoflex. The volunteers used a palatal appliance for 15 days and the bleaching was performed extra-orally (4h/14 days). Analyzes of microhardness (SMH), color (ΔE^*_{ab} and ΔE_{00}), roughness (Ra), scanning electron microscopy (SEM) and Energy-Dispersive X-Ray Spectrometry (EDS) were performed. The SMH and Ra were analyzed by repeated measures and Tukey Kramer. For color and EDS data were analyzed by Mann Whitney's nonparametric, Friedman and Nemenyi tests ($p < 0.05$). **Results:** Salivary flow and thickeners did not influence the results of ΔE^*_{ab} and ΔE_{00}. Carbopol had the lowest SMH for low flow and the highest Ra and the lowest Ca% for normal and low flow. In normal flow, natrosol and aristoflex showed higher SMH. For low flow, aristoflex presented higher SMH and in Ra natrosol and aristoflex showed lower values. In Ca% and Ca/P aristoflex showed higher value and differed from carbopol for normal flow. **Conclusion:** For normal flow, 10% CP with natrosol and aristoflex showed fewer surface changes, and for low flow, the best results were for 10% CP with aristoflex.

Clinical Significance: Salivary flow and the bleaching gel thickeners influence enamel changes after bleaching. In the low salivary flow condition, the use of aristoflex thickener results in less deleterious effects on the enamel after bleaching. For normal

salivary flow, both natrosol and aristoflex result in minor changes to the bleached enamel.

Key words: Tooth bleaching, Thickeners, Salivar flow rate.

INTRODUCTION

In vitro tooth bleaching with carbamide peroxide can induce structural changes in human tooth enamel, such as changes in surface morphology [1], distribution of hydroxyapatite crystals [2], increase in roughness [3] decreased surface and subsurface microhardness [3,4], calcium loss and changes in the calcium/phosphate ratio [5]. These changes are possibly related to factors such as pH, oxidative effect, concentration of bleaching agents or may be related to the composition of bleaching gels, such as, the thickeners [6,7].

The most used thickener with carbamide peroxide gel, is carbopol (polymer carboxypolymethylene), however, the use of this thickener can be associated with changes in enamel after bleaching [7,8]. Therefore, it would be interesting to use experimental thickeners in an attempt to reduce the deleterious effects of tooth bleaching. An alternative would be the use of natrosol (hydroxyethylcellulose), a polymer with non-ionic characteristics, which can be used with acidic substances, because it has a stable pH [9]. Another option would be Aristoflex (Ammonium acryloyldimethyltaurate copolymer), that has a stable acidic pH, and forms an anionic gel capable of acting as an inert viscous agent in the formulation [7]. Because it is a new formulation, it is interesting to evaluate the effects of this thickener on tooth enamel.

While the tooth bleaching treatment results in deleterious effects on the enamel, human saliva has the capacity to prevent and reverse changes in dental tissue related to tooth bleaching [10]. Saliva has phosphates and bicarbonates that act to prevent the reduction in pH, and modulate the buffering capacity of saliva. When the pH is within the physiological limits, saliva is supersaturated with calcium, phosphate and fluoride, minerals that favor the remineralization process [11]. Based on this, salivary changes that interfere with the quality and the quantity of saliva constituents can possibly result in changes in the capacity of remineralization by saliva.

Salivary hypofunction, regardless of its etiopathogenesis, has been shown to be a common condition due to several factors and there are no studies in the literature that evaluate its association with tooth bleaching. Changes in salivary flow, such as hyposalivation, affects approximately 30% of patients aged between 20 and 69 years [12]. Changes in salivary flow are accompanied by changes in salivary characteristics, such as pH, proteins and electrolyte concentrations, viscosity and immunoglobulin levels [13]. Based on the foregoing factors, in conditions of hyposalivation, there is no way to guarantee the presence of all the salivary protection and remineralization that would be expected in a patient with normal salivary flow. Thus, it is extremely important to investigate the patient's salivary remineralization capacity after bleaching, in those with low salivary flow and to evaluate measures that reduce the deleterious effects caused by tooth bleaching.

The aim of this *in situ* study was to evaluate the effect of the application of experimental bleaching gels on dental enamel in participants with normal and low salivary flow. The hypotheses tested were the following: (1) the salivary flow would influence the physical and chemical properties of the enamel according to the bleaching gel used (2) experimental bleaching gels with different thickeners would result in less deleterious effects on the physical and chemical properties of the enamel after bleaching in participants with normal and low salivary flow.

MATERIALS AND METHODS

Experimental design

This *in situ* study tested the factors salivary flow (2 levels: normal salivary flow and low salivary flow) and bleaching gels (4 levels: no bleaching - negative control, commercial gel based on carbamide peroxide with carbopol - positive control, experimental gel based on carbamide peroxide with natrosol and experimental gel based on carbamide peroxide with aristoflex). This study was carried out using bovine enamel specimens (n=14). The analyses performed were surface microhardness (SMH) in two times (before and after), roughness (Ra) in two times (before and after), color analysis by reflectance spectrophotometry (ΔE^*_{ab} and ΔE_{00}), energy-dispersive X-Ray spectrometry (EDS) and scanning electron microscopy (SEM).

Volunteers and Ethical Aspect

This study was conducted according to the Declaration of Helsinki and approved by the local ethic committee in research (process No. 96044418.8.0000.5418). The number of volunteers was calculated using the G * Power 3.1.7 program considering a minimum power of 0.80 for the main effects, interaction with a significance level of 5% and average effect size according to Cohen *et al.*,[14], reaching the minimum number of 14 volunteers with low and another 14 with normal salivary flow, totaling 28 volunteers. Thus, 7 men and 7 women were selected for each type of salivary flow. The informed consent was obtained of all volunteers. The volunteers with low salivary flow (45-65 years) underwent head and neck radiotherapy during cancer treatment at least 2 years before the study because the hyposalivation caused by radiotherapy is not reversible by stimulation [15]. Thus, this is the safest alternative for carrying out this experiment, as this way you can control this variable and ensure that the volunteer in this study will not have variation in your salivary flow during the experimental period. The inclusion criterion for those participants was reduced salivary flow rate <0.8 mL/min in stimulated flow rate [16]. For the other participants with normal flow rate (25–35 years old), the inclusion criterion was stimulated flow rate >1.0 mL/min [16]. All other inclusion criteria had to be fulfilled by all participants: absence of active caries and periodontal disease, no using an orthodontic appliance, presence at least 50% of their teeth of up arch (8 teeth) for fixation of the palatal device, non-smokers, not pregnant or breast-feeding. None of the participants took any medication with an impact on salivary flow rate such as antidepressants, anxiolytics, antihypertensives or antiallergics.

Analysis of salivary parameters

For the selection of volunteers, stimulated saliva was collected from all volunteers. The volunteers were instructed to chew a piece (1g) of parafilm (Parafilm M, Pechiney Plastic Packaging Company, Chicago III, USA). The saliva produced in the first 30 s was discarded, and then, during the next 5 min, the volunteers were instructed to dispense the saliva produced in a metered test tube [17]. Collections were carried out between the hours of 9 am and 11 am or from 2 pm to 4 pm, and should also take place at least 1 hour after the meal and after oral hygiene to minimize the effects of daily variability in the salivary composition [18]. Immediately after the collections, the salivary flow was calculated by dividing the volume of saliva

(considering that 1 mL corresponds to 1 g) by the collection time. The salivary pH was also calculated using a peagameter (Orion 290A +, São Paulo, Brazil). For the calculation of the buffer capacity, 0.5 mL of stimulated saliva was added to 1.5 mL of 0.005 M HCl in a plastic tube that was at rest for 5 min to release CO_2 from the reaction of the saliva's bicarbonate buffer with the acid. Once this is done, the pH of this mixture was be determined in a previously calibrated parameter as an estimate of the salivary buffer capacity [19].

Preparation of specimens

Bovine teeth were stored in a 0.01% thymol solution at 4ºC for 30 days until use. Enamel and dentin specimens measuring 4x4x2mm (1 mm of enamel and 1 mm of dentin) were obtained using a metallographic cutter (IsoMet 1000, Buehler Ltd, Lake Bluff, IL, USA). Specimens measuring 2x2x2mm (1 mm of enamel and 1 mm of dentin) were additionally made to perform the reading of surface microhardness, since this analysis could interfere with the other analyzes in this study. For grinding, regularization, and polishing, decanting silicon carbide sanding discs were used (#1200/2500/p4000 - Buehler Ltd, Lake Bluff, IL, USA), and then, the test surfaces were polished with felts (TCT, TWI - Arotec, Cotia; SP, Brazil), associated with diamond pastes with decreasing granulation (1 and ¼ µm - Arotec, Cotia, SP, Brazil). After polishing, the specimens were placed in an ultrasonic machine for 10 minutes (Marconi, Piracicaba, Brazil) to remove residues in order to obtain a standardized enamel surface. At the end, the specimens were sterilized with ethylene oxide and stored in water at 4°C until use.

Sample Allocation

In order to reduce intra-voluntary variability, in palatal appliance, each volunteer received 4 specimens from each group measuring 4x4 mm and 2 mm of thickness (1mm of enamel and 1mm of dentin) for color analysis, roughness, scanning electron microscopy and energy-dispersive X-Ray spectrometry and 4 specimens measuring 2x2 mm and 2 mm of thickness (1 mm of enamel and 1 mm of dentin) for the analysis of surface microhardness. The specimen's randomization between groups was performed by microhardness analysis for specimens measuring 2x2x2mm and color analysis (L values) for specimens measuring 4x4x2mm so that there was no statistical difference between the initial values, in order to reduce the initial variability between

groups. After performing the analyzes proposed in that study, the average of the values obtained in the specimens was calculated for each analysis and at the end a value per analysis and per group was considered for each volunteer (n=14).

Preparing the experimental bleaching gels

A commercial gel made with carbopol thickener (Whiteness Perfect 10%, FGM, Joinville, Brazil) was used as positive control, which presents pH around 5.8 [7]. Bleaching gels with natrosol or aristoflex thickeners were prepared (Drogal Pharmaceutics, Piracicaba, SP, Brazil). The composition of each bleaching gel is available in Table 1. The natrosol thickener has a pH of around 5.85 [9] and the bleaching gel with natrosol has a pH of around 6.36. The aristoflex thickener has a pH of around 5.3 o the bleaching gel with aristoflex around 6.5 [7].

Table 1. Bleaching gels used in the study according to the manufacturer's information.

Type	Product (Manufactures)	Basic components	Thickener
Commercial gel (positive control)	Whitness Perfect 10% (FGM®)	10% carbamide peroxide, potassium nitrate, sodium fluoride, glycol humectant, deionized water.	Carbopol
Experimental gel	Bleaching gel 10% (Drogal Manipulation)	10% carbamide peroxide; 0,2 % sodium fluoride	Natrosol
Experimental gel	Bleaching gel 10% (Drogal Manipulation)	10% carbamide peroxide; 0,2 % sodium fluoride; 0,1% Nipagin; 7% glycerin; deionized water QSP 6g	Aristoflex® AVC

In situ phase

Plaster models were obtained by molding with alginate from the volunteer's upper arch (Hydrogum - Zhermack, Badia Polesine, Italy) and the palatal devices were made of acrylic resin.

The use of fluoride-free dentifrice during the experimental period was determined to avoid the use of any substance that could interfere with the saliva's remineralization capacity, because fluorides are able to reverse the deleterious effects of tooth bleaching [20]. The placebo dentifrice was manipulated (Drogal Pharmaceutics, Piracicaba, SP, Brazil) according the formulation: Glycerin, Silica, Carboximethyl cellulose, water, Methyl P, Saccharin, Titanium dioxide, Sodium Lauryl Sulfate, Mint oil. The use of other oral hygiene products was not allowed during the experimental period. The total study period was 1 phase of 15 days. In the 4 days prior to the start of the experimental phase, the volunteers also used non-fluoridated dentifrice to avoid the presence of residual fluoride in the saliva.

Intraoral appliances remained for 1 day in the volunteers' mouths before the beginning of the bleaching sessions, for the formation of acquired film. There were 14 daily treatment sessions. The volunteers were instructed to remove the palatal appliance and apply the bleaching gel according to the group. The negative control group did not receive a bleaching gel. A thin layer of bleaching gel was applied to the sample, which after 4 h was removed with flexible cotton tipped rods. Then the samples were washed with water and the devices were reinserted in the volunteers' mouth.

Color analysis

The readings were performed in a light chamber (GTI Mini Matcher MM1e, GTI Graphic Technology Inc., Newburgh, NY, USA) to obtain the standardization of ambient light. The equipment used was a spectrophotometer (CM 700D, Konica Minolta, Osaka, Japan), calibrated according to the manufacturer's recommendations. The values obtained were quantified in three coordinates of the CIE Lab System (L*, a*, b*). The analysis was performed at initial time and 24 hours after the *in situ* experimental period. The color change was calculated using the following equation: $\Delta E^{*}_{ab}=[(\Delta L^{*})^2 + (\Delta a^{*})^2 + (\Delta b^{*})^2]^{1/2}$ and by the ΔE_{00} formula [21,22]:

$$\Delta E_{00} = \left[\left(\frac{\Delta L'}{K_L S_L}\right)^2 + \left(\frac{\Delta C'}{K_C S_C}\right)^2 + \left(\frac{\Delta H'}{K_H S_H}\right)^2 + R_T\left(\frac{\Delta C'}{K_C S_C}\right)\left(\frac{\Delta H'}{K_H S_H}\right)\right]^{1/2}$$

Surface microhardness

The microhardness values were obtained by means of the arithmetic mean of 5 indentations carried out in the central region of the specimen with 100 µm of distance using a microdurometer and Knoop penetrator (HMV-2000, Shimadzu, Tokyo, Japan), with a load of 50 g for 5 s. For each volunteer, a microhardness value was obtained for each specimen and then the mean of these values was calculated to obtain a value per volunteer and per group. The surface microhardness analysis was performed at initial time and 24 hours after the *in situ* experimental period.

Roughness

The roughness (Ra) was analyzed using a profilometer tester (Surf-Corder 1700, Kosaka, Tóquio, Japão) at two times: initial time and and 24 hours after the end of the *in situ* period. Three different equidistant scans of 1.25 mm each were measured on the surface of each sample, with a cutoff of 0.25 mm and a velocity of 0.1 mm/s.

Energy-Dispersive X-Ray Spectrometry (EDS)

Five samples per group were randomly selected for analysis by energy-dispersive X-ray spectrometry (EDS). The samples were subjected to vacuum sputtering with carbon (Delton Vaccum, Desk II, Moorestown, NJ, USA). Then, the evaluation of the inorganic content present in the substrates were carried out by the MEV equipment (Jeol, JSM5600LV, Tokyo, Japan), with typical energies of the order 15kV, in 100x increase, with PHA deadtime varying between 20 and 25% [23]. The analysis was performed on dental enamel, in five regions per specimen selected at random, to determine the elemental levels (% by weight) of Ca and P, and the ratio between Ca and P were determined. For each sample, the results were described by means of the average percentage of the chemical elements found on the surface of the blocks.

Scanning Electron Microscopy (SEM)

Four specimens for each group were randomly selected after performing all analyzes and dehydrated in increasing degrees of ethanol in concentrations of 50%, 60%, 70%, 80%, 90% and 100% for 20 minutes. Then, were sputtered with gold (90 s, Baltec, MED / JEOL Ltd., Tokyo, Japan) and images of representative areas of the

specimens were obtained at 4000x using a scanning electron microscope (JSM 5600LV, JEOL, Tokyo, Japan).

Statistical Analysis

All analyzes were performed in the SAS program (SAS Institute Inc., Cary, NC, USA, Release 9.4, 2012) with a 5% significance level. Salivary flow was analyzed using Mann-Whitney test. The buffer capacity and pH where analyzed using Student's t-test. The variables ΔE^*_{ab}, ΔE_{00}, Ca, P, Ca/P were analyzed using Mann-Whitney non-parametric test comparing normal salivary flow and low salivary flow groups and Friedman and Nemenyi tests comparing different bleaching gels. The variables surface microhardness and roughness were analyzed using repeated measures mixed models and Tukey-Kramer test. The inverse transformation was applied to roughness, as indicated by the exploratory analysis.

RESULTS

Flow rate, salivary pH and salivary buffering capacity

Salivary flow rate of each participant as well as pH and buffering capacity values are given in Table 2. There was a significant difference between both groups for flow rate ($p<0,0001$) but not for the pH ($p=0,8268$) and buffering capacity ($p=0,6930$).

Table 2: Mean (standard deviation) of flow rate, salivary pH and salivary buffering capacity of both groups (normal and low flow rate) of the stimulated saliva (n=14).

	Flow rate, mL/min	pH	Buffering capacity
Normal flow	1.96 (0.29) *	7.50 (0.22)	4.80 (0.78)
Low flow	0.62 (0.12) *	7.52 (0.18)	4.67 (0.80)

* $p<0,0001$: statistically significant differences between groups (t test for independent data).

Color analyses

Based on color analyses (Table 3), for ΔE^*_{ab} and ΔE_{00}, no statistically significant differences were found between salivary flows under the same conditions of bleaching gel. For each salivary flow, the bleached groups differed statistically from the negative

control (p<0,0001) and did not differ statistically between themselves. The experimental gels differed statistically from the unbleached group (negative control) (p<0,0001) and did not differ from the carbopol group (positive control).

Table 3: Means (standard deviation) of surface color analyses (ΔE^*_{ab} and ΔE_{00}) as a function of salivary flow and bleaching gel (n=14).

Bleaching gel	ΔE^*_{ab}		ΔE_{00}	
	Salivary flow		Salivary flow	
	Normal flow	Low flow	Normal flow	Low flow
Unbleached	1.58 (0.75)Ab	1.56 (0.65)Ab	1.13 (0.50)Ab	1.17 (0.38)Ab
Carbopol gel	5.72 (1.48)Aa	5.36 (1.41)Aa	4.13 (1.05)Aa	3.82 (1.04)Aa
Natrosol gel	6.18 (1.39)Aa	5.91 (1.40)Aa	4.45 (0.93)Aa	4.13 (0.99)Aa
Aristoflex gel	6.07 (1.28)Aa	5.98 (1.12)Aa	4.34 (0.89)Aa	4.24 (0.80)Aa

Different letters (lowercase vertically) indicate significant differences (p≤0.05). The same letters (uppercase comparing horizontally) indicate no significant differences (p≥0.05).

Surface microhardness

The results of the analysis of surface microhardness are available in Table 4. All bleached groups showed a decrease in surface microhardness values after bleaching, regardless of the bleaching gel and salivary flow.

In normal flow after bleaching, the carbopol group (positive control) had the lowest values and was statistically different from all groups. The natrosol gel and aristoflex gel groups showed the highest values for the bleached groups and did not differ statistically between themselves.

In low flow after bleaching, the carbopol group (positive control) had the lowest values and was statistically different from all other groups. The natrosol gel group presented intermediate values and was statistically different from the other bleached groups. Among the bleached groups, the highest values after bleaching were found in the aristoflex group, which was statistically different from the other groups bleached.

Comparing salivary flows, in the low flow, natrosol gel group showed lower values after bleaching when compared to the same bleaching gel at the same time for normal flow. For the other groups, no statistically significant differences were found between salivary flows, under the same conditions of bleaching gel and time.

Table 4: Means (standard deviation) of surface microhardness (SMH) as a function of salivary flow and bleaching gel (n=14).

Bleaching gel	Salivary flow			
	Normal flow		Low flow	
	Before	After	Before	After
Unbleached	325.33 (9.68) Aa	323.79 (5.33) Aa	328.27 (12.82) Aa	327.16 (6.71) Aa
Carbopol gel	327.46 (10.36) Aa	271.33 (5.17) Bc	325.97 (11.94) Aa	264.45 (6.07) Bd
Natrosol gel	324.60 (7.99) Aa	304.19 (4.31) Bb	324.36 (12.02) Aa	*281.81 (14.06) Bc
Aristoflex gel	325.74 (11.30) Aa	305.64 (2.32) Bb	324.85 (11.99) Aa	306.08 (5.16) Bb

* differs from the group with normal salivary flow, under the same conditions of bleaching gel and time ($p \leq 0.05$). Different letters (uppercase in the horizontal comparing between the two times in the same salivary flow group and lowercase in the vertical) indicate significant differences ($p \leq 0.05$); p(salivary flow)=0.0729; p(bleaching gel)<0.0001; p(salivary flow x bleaching gel)=0.0007; p(time)<0.0001; p(salivary flow x time)=0.0168; p(bleaching gel x time)<0.0001; p(salivary flow x bleaching gel x time)=0.0077.

Roughness

The results of roughness (Table 5) show that all bleached groups presented an increase in the roughness values after bleaching, regardless of the bleaching gel and salivary flow.

In the normal flow after bleaching, no statistically significant differences were found between the bleaching gels used and all bleached groups differed from the unbleached group (negative control). In the low flow after bleaching, the carbopol gel group (positive control) showed the highest values and was statistically different from the all other groups. The natrosol gel and aristoflex gel groups had the lowest values and did not differ statistically between themselves.

Comparing salivary flows, in the low flow carbopol gel (positive control) showed higher values after bleaching when compared to the same bleaching gel at the same time for normal flow. For the other groups, no statistically significant differences were found between salivary flows, under the same conditions of bleaching gel and time.

Table 5: Means (standard deviation) of roughness (Ra) as a function of salivary flow and bleachin gel (n=14).

Bleaching gel	Salivary flow			
	Normal flow		Low flow	
	Before	After	Before	After
Unbleached	0.084 (0.004) Aa	0.083 (0.004) Ab	0.081 (0.008) Aa	0.080 (0.009) Ac
Carbopol gel	0.080 (0.004) Ba	0.124 (0.008) Aa	0.083 (0.006) Ba	*0.165 (0.005) Aa
Natrosol gel	0.081 (0.005) Ba	0.116 (0.004) Aa	0.080 (0.006) Ba	0.121 (0.011) Ab
Aristoflex gel	0.082 (0.006) Ba	0.109 (0.004) Aa	0.081 (0.006) Ba	0.107 (0.006) Ab

*differs from the group with normal salivary flow, under the same conditions of bleaching gel and time (p≤0.05). Different letters (uppercase horizontally comparing the two times in the same salivary flow group and lowercase vertically) indicate significant differences (p≤0,05). p(salivary flow)=0,2545; p(bleaching gel)<0,0001; p(salivary flow x bleaching gel)=0,0003; p(time)<0,0001; p(salivary flow x time)=0,0196; p(bleaching gel x time)<0,0001; p(salivary flow x bleaching gel x time)=0,0190.

Elemental Levels (wt%) and EDS

- Ca%

The values of the relative percentage weight of calcium (Ca%) are presented in Table 6. No significant differences were found between salivary flows for the same bleaching gel. In normal flow, statistically significant differences were found between the groups (p=0.0070). The bleached groups did not differ from the unbleached group (negative control), regardless of the bleaching gel used. Among the bleaching gels, the carbopol gel group (positive control) had the lowest Ca% values and it was statistically different from other bleaching gels, but it was not statistically different from the unbleached group (negative control). The natrosol gel and aristoflex gel groups showed the highest Ca% values and were not different between themselves and were not statistically different from the unbleached group (negative control). For low flow, no statistically significant differences were found between all groups (p = 0.0624).

- P%

The values of relative percentage weight of phosphorous - P% (Table 6) show that significant differences were found between the salivary flows for the aristoflex gel that presented higher values in low flow (p=0.0472). For the other bleaching gels, no statistically significant differences were found in relation to the salivary flow. In the

normal flow, statistically significant differences were found between the groups (p=0.0211). The carbopol gel and natrosol gel groups did not differ statistically from the unbleached group (negative control). The aristoflex gel group had the lowest P% values and was statistically different from the unbleached group (negative control). For the low flow, no statistically significant differences were found between all groups (p=0.9484).

- Ca/P%

The Ca/P% are presented in Table 6. No statistically significant differences were found between salivary flows for the same bleaching gel. In normal flow, statistically significant differences were found between the bleaching gels (p=0.0184). The carbopol group (positive control) did not differ statistically from the unbleached group (negative control). The aristoflex gel group showed the highest values and was different statistically from the unbleached group (negative control) and carbool group (negative control). For the low flow, no statistically significant differences were found between all bleaching gels (p = 0.3647).

Table 6: Mean (standard deviation) of elemental levels (wt%) for EDS analysis of enamel surface according to the treatment group as a function of salivary flow and bleaching gel (n=5).

Bleaching gel	Ca		P		Ca/P	
	Salivary flow		Salivary flow		Salivary flow	
	Normal flow	Low flow	Normal flow	Low flow	Normal flow	Low flow
Unbleached	71.11 (0.14)Aab	71.06 (0.23)Aa	28.28 (0.12) Aa	28.17 (0.24) Aa	2.51 (0.02)Ab	2.52 (0.03)Aa
Carbopol gel	70.85 (0.18)Ab	70.95 (0.25)Aa	28.15 (0.15) Aab	28.19 (0.32) Aa	2.52 (0.02)Ab	2.52 (0.03)Aa
Natrosol gel	71.34 (0.17)Aa	71.15 (0.19)Aa	28.06 (0.13) Aab	28.14 (0.24) Aa	2.54 (0.02)Aab	2.53 (0.03)Aa
Aristoflex gel	71.65 (0.64)Aa	71.21 (0.25)Aa	27.60 (0.68) Bb	28.10 (0.14) Aa	2.59 (0.09)Aa	2.54 (0.02)Aa

Different letters (uppercase comparing horizontally and lowercase vertically) indicate significant differences (p≤0.05).

Scanning Electron Microscopy (SEM)

The SEM images collected are available in Figure 1. A smooth and uniform enamel surface was observed in the unbleached groups for both salivary flows. Superficial changes were found in all bleached groups, regardless of the bleaching gel used and the salivary flow. The bleached groups did not present a demineralization pattern, however when compared to the control group, it was identified a surface full of

pores and depressions that varied in intensity according to the bleaching gel used and the salivary flow. The cabopol groups showed a higher pattern of roughness when compared to the other bleached groups. For the carbopol group, superficial differences were observed between salivary flows. The carbopol in low flow (Figure 1D) showed greater demineralization than the same bleaching gel for normal flow (Figure 1C). The groups bleached with natrosol showed intermediate surface changes when compared to the other bleached groups and also showed differences between salivary flows. The bleached group with natrosol in low salivary flow showed roughness to a greater extent over the all surface (Figure 1F), whereas for normal flow this demineralization was observed in specific places of the enamel (Figure 1E). The groups bleached with aristoflex showed fewer surface changes and less evidence of dissolution among all bleached groups (Figure 1G and 1H). In addition, aristoflex was the only bleaching gel that had the same surface characteristics in normal and low flow.

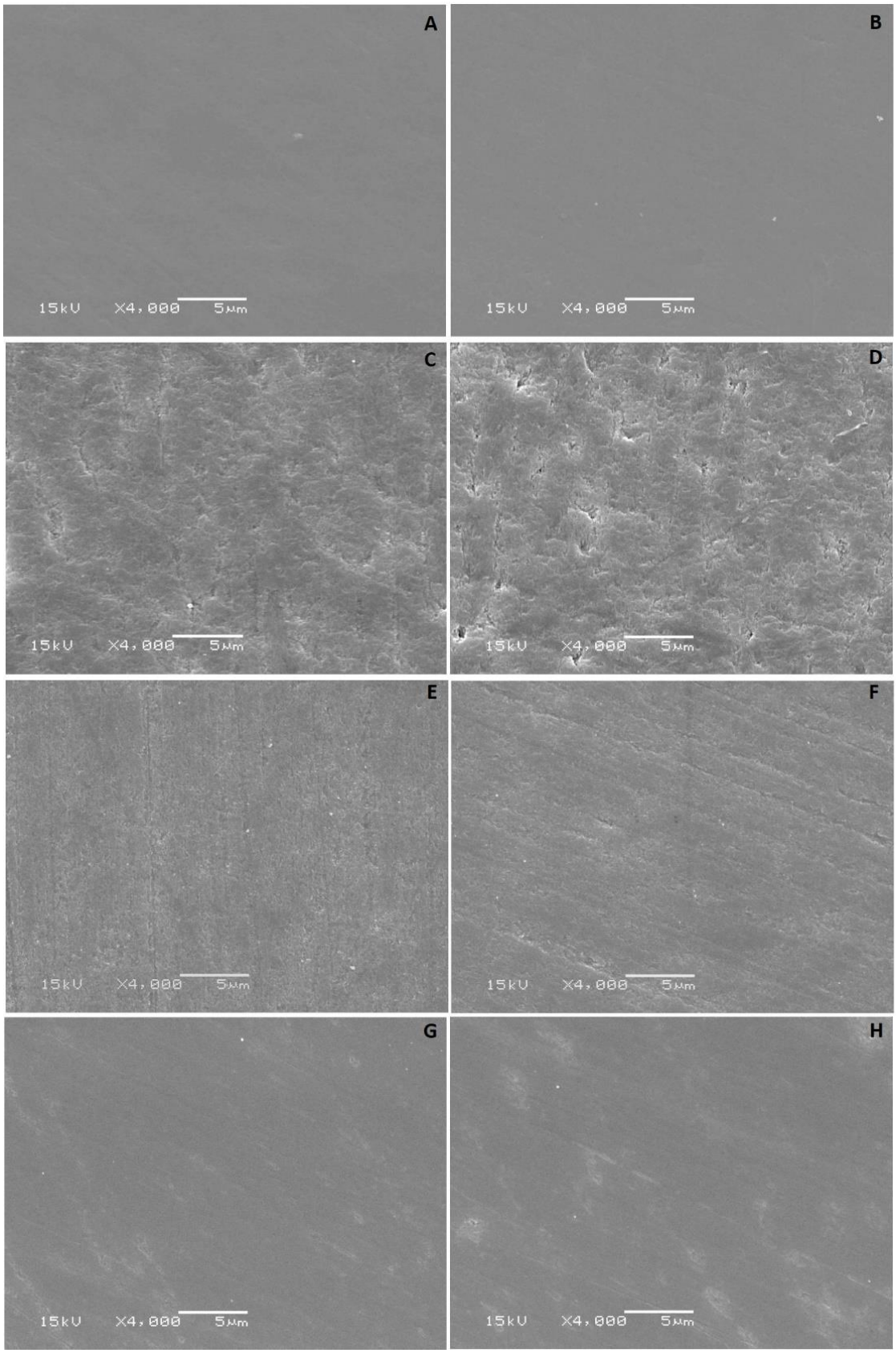

Figure 1: Representative SEM images (4000x) of the specimens according to the group: (A) unbleched in normal flow, (B) unbleached in low flow, (C) carbopol in normal flow, (D) carbopol in low flow, (E) natrosol in normal flow, (F) natrosol in low flow, (G) aristoflex in normal flow, (H) aristoflex in low flow.

DISCUSSION

The first hypothesis was partially accepted, because according to the bleaching gel used, the salivary flow influenced the results of the analysis performed in this study, except for color analysis. The second hypothesis evaluated was partially accepted, because for low salivary flow, the experimental bleaching gels with different thickeners resulted in less deleterious effects on the physical and chemical properties of the enamel, however, for normal salivary flow, no differences in roughness were found between the thickeners.

The salivary flows and the different thickeners used did not interfere in the bleaching efficacy because for all color analysis performed, the bleached groups did not differ statistically between them and differed from the unbleached group (negative control) (Table 3). These results were found due to the presence of carbamide peroxide in the formulation of all bleaching gels used. In addition, they all had the same bleaching agent in the same concentration, so that the only difference between them was the thickener. Carbamide peroxide releases hydrogen peroxide that dissociates into free radicals, which oxidize long-chain organic chromophore molecules responsible for the color of the dental tissue, thus promoting tooth bleaching [24].

In this study, for both salivary flows, the home bleaching with 10% carbamide peroxide led to a significant decrease in enamel microhardness (Table 4) and increase in enamel roughness (Table 5) irrespective of the thickener used, when compared with the no bleaching group. The change in physical properties was probably associated with the effects of demineralization, which are caused by diffusion of the hydrogen peroxide released by the dissociation of carbamide peroxide [25,26]. Furthermore, when the initial and final values were compared, saliva was not able to recover the enamel microhardness and roughness, irrespective of salivary flow. This result may be associated with the fact that thickeners may have interfered in the interaction between the dental surface and saliva. Avila *et al*., [6] showed that thickeners were able to interact with the dental structure due to their bioadhesive capabilities. This bioadhesive capacity is related to possible ionic bonds between the polymers, negatively charged centers forming a “film” of polymer deposited on the dental structure. This layer is capable of acting as a barrier [7] which may have prevented salivary remineralization, justifying some of the results found in this study.

For normal and low salivary flow, the carbopol-based bleaching gel (positive control) led to lower enamel microhardness values when compared with the other thickeners evaluated (Table 4). For low flow, carbopol led to the highest enamel roughness values and differed statistically from values of the other thickeners evaluated. In addition, differences in roughness were found between the two types salivary flows. In low flow, the carbopol led to the highest enamel roughness found after bleaching, and these differed statistically from the values found for the same thickener after bleaching in the normal flow condition (Table 5). The remineralization process modulated by saliva occurs irregularly, inducing the reorganization of enamel prisms that can cause an increase in roughness [8]. Moreover, the bioadhesiveness of carbopol and its interaction with the enamel surface result in the formation of a very strong, thick layer of polymers, showing the great affinity of this thickener for the dental structure [6]. Due to its strong calcium-binding capacity, carbopol causes inhibition of the incorporation of hydroxyapatite crystals during remineralization [27]. This could result in the need for a larger volume of saliva to promote enamel remineralization, thus justifying the worst results for low salivary flow.

Furthermore, the differences in the effect of carbopol found between the salivary flows may be associated with the fact that changes in salivary flow are accompanied by changes in salivary characteristics, such electrolyte concentrations [13,28]. The supersaturation of same electrolyte such as fluoride, calcium and phosphate in saliva is critical to the remineralization process [29]. In addition, the changes in the salivary flow can result in changes in the amount of protein in the saliva. Proteins such as statherin, histatins, cystatins, and proline-rich proteins that bind to hydroxyapatite to aid in controlling the crystalline growth of enamel by limiting mineral loss during demineralization, and allowing the penetration of minerals into the enamel for remineralization [30,31]. Thus, changes in salivary characteristics in patients with low salivary flow may result in a lower capacity for remineralization.

The EDS analysis supported the carbopol-related results. For Ca% in normal flow, although none of the thickener groups differed statistically from the unbleached group (negative control), the carbopol group (positive control) showed the lowest values and differed statistically from the other thickener groups. In addition, the carbopol group had the lowest Ca/P values among the thickeners evaluated (Table 6). In tooth bleaching, mineral dissolution occurs with loss of calcium and phosphorus [23,32,33]. The demineralization process begins with the loss of calcium ions from the

apatite crystals on the surface and alters the enamel microhardness [33]. The SEM images confirmed the results found for the carbopol thickener groups because they showed a higher level of superficial demineralization compared with the other thickeners evaluated. In addition, was possible to identify that in low salivary flow (Figure 1D) carbopol led to greater extent of demineralization than the same thickener used in normal salivary flow (Figure 1C).

For normal salivary flow the bleaching gels with natrosol and aristoflex showed the highest values of enamel microhardness and differed from the values shown in the carbopol group (Table 4). The microhardness results are in agreement with previous studies [7,8]. In Ca% analysis, the natrosol and aristoflex groups had the highest values and these did not differ between them in normal flow (Table 6). Aristoflex and natrosol have stable pH and are capable of forming gels with non-ionic characteristics [9,34], which probably resulted in a lower level of demineralization. In addition, the condition of normal flow promoted the availability of a saliva without changes in its characteristics, thus it was able to act normally and favor the remineralization of the enamel after bleaching.

Furthermore, in normal flow it was possible to observe higher Ca/P% values for natrosol and aristoflex groups. Besides that, the aristoflex group differed statistically from the carbopol (positive control) and unbleached groups (negative control) (Table 6). This result may indicate a recrystallization of the hydroxyapatite crystal that may have happened through the deposition of salivary calcium on the enamel surface in the remineralization process. Changes in the Ca/P ratio indicated changes in the inorganic components of hydroxyapatite [35]. Natrosol and aristoflex had the highest Ca% among the bleached groups. Enamel remineralization by incorporating calcium into the hydroxyapatite crystal could result in the alteration of other components present in the hydroxyapatite crystal. Aristoflex group had the lowest P% among the groups evaluated. These percentage values are relative to the area analyzed by weight. Due to the characteristics of the EDS methodology and since the amounts of all elements were presented in percentage of mass (%), a greater calcium gain can decrease the relative values of phosphorus, which exists in a small proportion in the hydroxyapatite crystal. Thus, an increase in the amount of calcium would lead to smaller proportions for P%, but this does not mean that the surface of the bleached enamel lost phosphorus ions when compared to the unbleached group. In addition, the decrease in the P% values presented by aristoflex in the normal flow may have been

caused by the location of the phosphorus in the hydroxyapatite crystal. The phosphorus is located in the inner portion of the hydroxyapatite crystal and the EDS analysis is a superficial analysis, thus resulting in a smaller proportion of P% on the enamel surface.

In low flow, natrosol did not have the same effect as it had in the normal flow, confirming the importance of interaction between salivary flow and this thickener. Although aristoflex and natrosol presented the lowest enamel roughness values in low salivary flow (Table 5), natrosol showed intermediate microhardness values among the thickeners evaluated (Table 4). In addition, a difference in microhardness was observed between salivary flows for this thickener. In the low flow, the use of natrosol resulted in lower microhardness when compared with its use in normal flow (Table 4). SEM images confirmed this data because it was possible to observe that low salivary flow showed a greater extent of demineralization, whereas in normal flow, demineralization was localized (Figure 1). Natrosol also interacted with the dental surface by forming a layer on the enamel. This layer is not resistant to low pH [6] as the pH variation of hydrogen peroxide and allows saliva to come into contact with the enamel surface [6]. Nevertheless, the natrosol is capable of forming complexes with calcium and/or phosphate ions [36], making these elements unavailable for remineralization [37]. In addition, changes in salivary flow are accompanied by changes in calcium and phosphate concentration in saliva as discussed above, resulting in a lower remineralization capacity. The association of these two factors could explain the results found for this thickener in low salivary flow.

For low salivary flow, the aristoflex showed the highest enamel microhardness values (Table 4). Moreover, aristoflex and natrosol in low flow showed the lowest enamel roughness among the thickener evaluated (Table 5). In addition to the characteristics of this gel, which may have resulted in minor surface changes after bleaching, these results may be related to the good interaction of this gel with the dental surface. Unlike the other thickeners evaluated in this study, aristoflex form bonds that are not strong enough or resistant to acid challenge [6] allowing the dental surface to be free for and available to salivary remineralization [7]. This fact justifies the best performance of this thickener relative to surface microhardness in low flow compared with the other thickeners evaluated. In addition, no differences were found between salivary flows. This result was confirmed in the SEM images because the aristoflex group was the only bleaching gel that did not show differences between

salivary flows (Figure 1). The combination of aristoflex and fluoride tended to reduce the loss of surface hardness [6]. As a positively charged molecule, aristoflex has the ability to attract fluoride ions present in saliva to interact with its positive locations, assisting in the completely beneficial interaction of fluoride with the enamel surface [6]. Due to this property, the presence of saliva and its fluoride ions, irrespective of the volume, was sufficient to promote the remineralization effects of the enamel.

CONCLUSION

Although differences between salivary flows have been found, the choice of gel seems to be more relevant. Regardless of the thickener and salivary flow, the bleaching treatment promotes changes in enamel properties, which seem to be more intensified in patients with low salivary flow and gels containing carbopol. Changes in enamel properties occur with less intensity for normal flow in gels containing natrosol or aristoflex and for low flow with gels containing aristoflex.

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2.2 Artigo: Bioactive glass dentifrice present best performance on remineralization of bleached enamel under low and normal salivary flow *in situ* condition.

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ABSTRACT

Objective: This *in situ* study evaluated different dentifrices on enamel after bleaching under normal and hyposalivatory conditions. **Materials and Methods:** 24 volunteers were selected: 12 with normal and 12 with low salivary flow. The study was conducted in 6 *in situ* experimental 6 phases of 24 hours: placebo, NaF, SnF_2, F/Sn/Chitosan, F/Arginine and F/Bioactive Glass. The specimens were previously bleached *in vitro*. Microhardness (SMH), roughness and color analyses (ΔE^*_{ab} and ΔE_{00}) were performed at baseline (T1), after bleaching (T2) and after *in situ* phase (T3). Scanning electron microscopy (SEM) and energy-dispersive X-Ray spectrometry (EDS) were at T3. The SMH and Ra were analyzed by Tukey Kramer. The color and Na% were analyzed by ANOVA in a subdivided plots and Tukey test. The EDS were analyzed by Mann's Whitney nonparametric, Friedman and Nemenyi tests ($p < 0.05$). **Results:** The low salivary flow had less capacity for remineralization of bleached enamel compared to normal flow. Comparing salivary flows, there was a significant difference in SMH, Ra and Ca% for placebo and NaF. For normal flow, SnF_2 resulted in greater SMH. For low flow, SnF_2, F/Sn/Chitosan and F/Bioactive Glass resulted in higher SMH in T3 and did not differ from T1. F/Bioactive Glass showed lower Ra for normal and low flow. F/Bioactive Glass had the highest values, differing from placebo for Ca%, P%, Na%. **Conclusion:** Salivary flow interferes with the remineralization capacity of the bleached enamel. Dentifrice with bioactive glass had the best performance in bleached enamel under low and normal salivary flow condition.

Clinical relevance: The deleterious effects caused by tooth bleaching on the enamel can be more intensified in patients with low salivary flow and the dentifrice that the patient uses can reduce these negative effects.

Key words: Tooth bleaching, Dentifrices, Salivary flow rate.

INTRODUCTION

Tooth bleaching is considered a safe, effective and conservative treatment, but it has been associated with changes as a reduction in surface microhardness, increased of roughness and alteration of enamel mineral content [1-4]. Despite this, some factors are responsible for enamel protection and recovery after tooth bleaching, like saliva. Saliva plays an important role in reducing of demineralization or to promote remineralization in tooth surfaces exposed to bleaching treatment [5]. In addition, saliva has the phosphate and bicarbonate buffer, which are responsible for neutralizing salivary pH. Also, saliva is supersaturated with minerals calcium and phosphorus, which act in the remineralization process [6].

Due to its importance, changes in its availability or even its absence of saliva in the oral cavity after bleaching can possibly interfere in the mineral recovery of the bleached dental enamel. Hyposalivation is a common condition that affects approximately 30% of patients aged between 20 and 69 years [7]. Changes in salivary flow can result in changes in salivary characteristics, such as pH, protein and electrolyte concentrations, viscosity, immunoglobulin levels and staining [8]. Thus, it is expected that under hyposalivation condition the saliva promotes less remineralization of the enamel surface after bleaching compared to the normal salivary flow condition.

In addition to saliva, the beneficial effects of dentifrices on bleached enamel have been reported [1,2]. Dentifrices have fluorides in their formulation that can act as a remineralizing agent [9]. Because of this, the use of fluoride during or after tooth bleaching to reverse the deleterious effects on tooth enamel has been recommended [9]. In addition to fluorides, dentifrices have other active agents presents in their formulation that can have a beneficial effect on bleached enamel.

Bioactive glass (calcium sodium phosphosilicate) based dentifrice is able to promote enamel remineralization [10] and have shown beneficial effects associated with bleached enamel [1,2]. The same happens with arginine-carbonate based dentifrice, which have been shown to be effective in preventing mineral loss when used before tooth bleaching [2]. Beside this, products containing tin ions have also been known to provide good protection against demineralization [11]. Another option may be the use of biopolymers, such as chitosan. Chitosan has shown anti-erosion effects

when used in combination with stannous ions and fluoride in a dentifrice [11]. Because of this, it would be interesting to evaluate its effectiveness on bleached enamel.

As tooth bleaching is an increasing practice in dental clinical for all patient profiles, including those who may have salivary hypofunction. Thus, the aim of this study was to evaluate the effectiveness of dentifrice with different active principles in the recovery of enamel after tooth bleaching under low salivary flow. The hypotheses tested were the following: (1) the salivary flow would influence the physical and chemical properties of the enamel according to the dentifrice used (2) the application of dentifrices with fluoride or remineralizing agents would result in the recovery of the deleterious effects of the bleached enamel for normal and low salivary flow.

MATERIALS AND METHODS

Volunteers and Ethical Aspect

This study was performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and approved by the local ethic committee in research (process No. 96037118.1.0000.5418). The number of volunteers was calculated in the G*Power 3.1.7. Program, considering a minimum power of 0.80 for the main effects, interaction with a significance level of 5% and average effect size according to Cohen *et al*., [12], reaching the minimum number of 11 volunteers per group. In order to have the same number of men and women participating in the study in each group, the selection of 12 volunteers with low and another 12 with normal salivary flow, totaling 24 volunteers was determined. Thus, 6 men and 6 women were selected for each type of salivary flow. Informed consent was obtained from all individual participants included in the study.

For volunteers with normal flow rate (25–35 years old), the inclusion criterion was stimulated flow rate >1.0 mL/min [13]. The volunteers with low salivary flow (45-65 years) underwent head and neck radiotherapy during cancer treatment at least 2 years before the study because the hyposalivation caused by radiotherapy is not reversible by stimulation [14]. Thus, this is the safest alternative for carrying out this experiment, as this way you can control this variable and ensure that the volunteer in this study will not have variation in your salivary flow during the experimental period. The inclusion criterion for those participants was reduced salivary flow rate <0.8 mL/min in stimulated flow rate [13]. None of the participants took any medication with

an impact on salivary flow rate, among them medications such as antidepressants, anxiolytics, antihypertensives or antiallergics. All other inclusion criteria had to be fulfilled by all participants: absence of active caries and periodontal disease, no using an orthodontic appliance, presence at least 50% of their teeth of up arch (8 teeth) for correct fixation of the palatal device, non-smokers, not pregnant or breast-feeding.

Analysis of salivary parameters

The collection of stimulated saliva was carried out for the analysis of salivary parameters and to enable the selection of volunteers to participate in the study. To minimize the effects of daily variability in the salivary composition, the collection of saliva was carried out between the hours of 9 am and 11 am or from 2 pm to 4 pm, and should also take place at least 1 hour after the meal and after oral hygiene [15]. The volunteers were instructed to chew unflavored parafilm (Parafilm Pechiney Plastic Packaging Company, Chicago, IL, USA) and dispense the saliva in a falcon tube (15 mL) for 5 minutes. From the stimulated saliva collected, the volume was measured and the flow rate (mL/min) was calculated by dividing the volume of saliva by the collection time [15]. Besides, saliva pH was measured directly using a peagameter (Orion 290A+, São Paulo, Brazil) previously calibrated. The buffering capacity was assessed in accordance with Ericsson [16]. For this analysis, 0.5 mL of stimulated saliva was added to 1.5 mL of 0.005M hydrochloric acid, the mixture was agitated for 30 s, and after letting it rest for 5 min for liberation of carbonic acid, the pH was measured.

Preparation of specimens

Enamel/dentin blocks measuring 4×4mm, with 2 mm of thickness (1 mm of enamel and 1 mm of dentin), were obtained, using a metallographic cutter (IsoMet 1000, Buehler Ltd, Lake Bluff, IL, USA). The enamel surface was planned and flattened using silicon carbide papers (#1200/2500/p4000 - Buehler Ltd, Lake Bluff, IL, USA) and felts (TCT, TWI - Arotec, Cotia; SP, Brazil) associated with diamond pastes with decreasing granulation (1 and ¼ µm - Arotec, Cotia, SP, Brazil) in a polishing machine, under water cooling (Arotec, São Paulo, SP, Brazil). Between each polishing step, the blocks were sonicated to remove debris in distilled water for 15 min (Marconi, Piracicaba, São Paulo, Brazil). Then, the specimens were sterilized with ethylene oxide and stored in water at 4°C until use.

Sample Allocation

The specimens were allocated into twelve groups. In order to reduce intra-voluntary variability, in palatal appliance, each volunteer received 4 specimens for color analysis, roughness, scanning electron microscopy and energy-dispersive X-Ray spectrometry and 4 specimens for surface microhardness. The specimen's randomization between groups were performed by microhardness analysis or color analysis so that there was no statistical difference between the initial values, in order to reduce the initial variability between groups. After performing the analyzes proposed in that study, the average of the values obtained in the specimens was calculated for each analysis and at the end a value per analysis and per group was considered for each volunteer (n=12).

Experimental Design

The study was conducted in 6 experimental phases of 24 h (see flowchart, Figure 1). Initially the specimens were subjected to bleaching *in vitro*. Then, the participants wore specimens *in situ* for 24 hours. During this period, the samples were treated intraorally with the test dentifrices and were exposed to saliva. At each phase a different dentifrice was evaluated. The dentifrices used in this study are available in Table 1. Sample analyzes were performed at baseline (T1), after bleaching (T2) and after the *in situ* phase (T3).

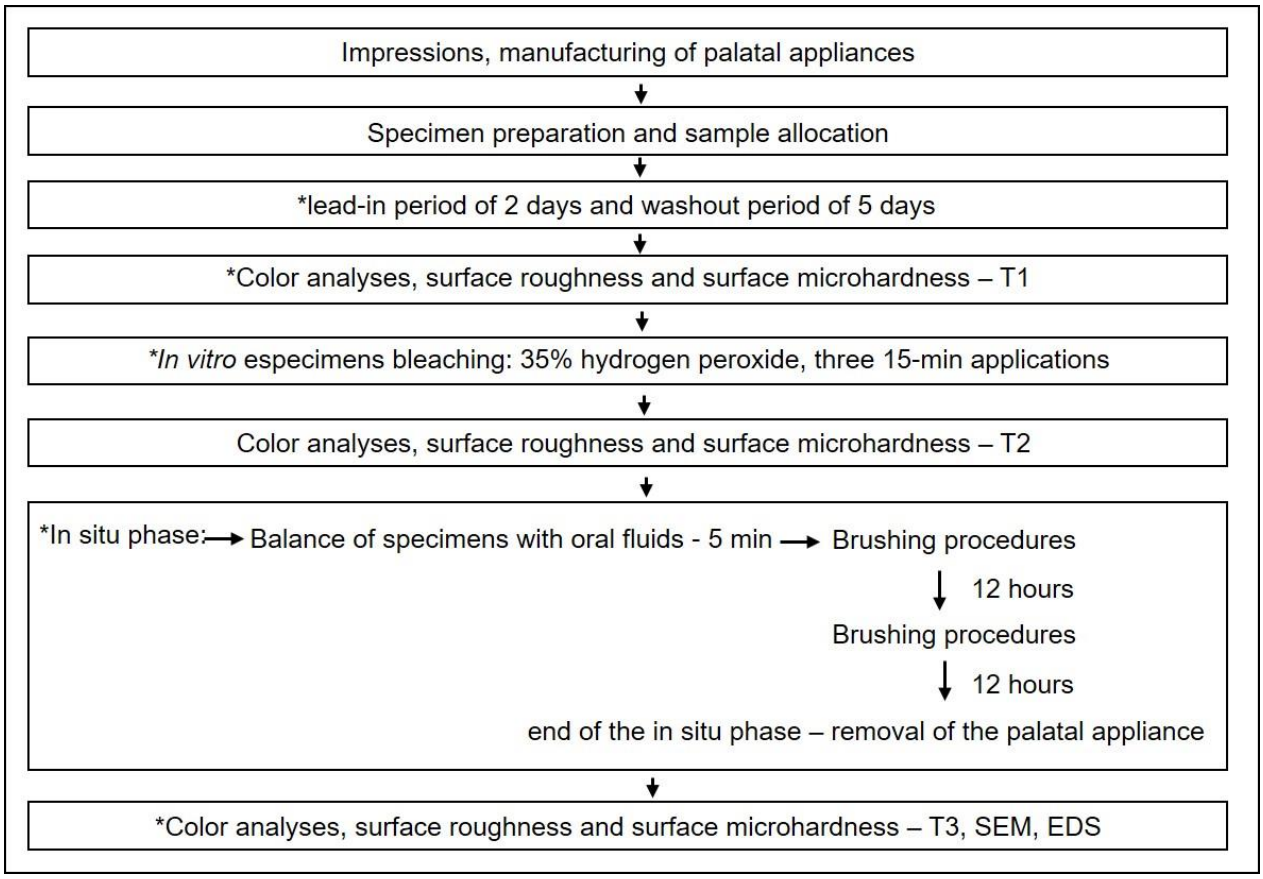

Figure 1: Flow chart of study procedures. The procedures marked by an asterisk were performed for each experimental phase.

Table 1: Products, Manufacturers, and Components of Dentifrices According to the Manufacturer's Information**.**

Dentifrice	Manufacturer	Active agent	Fluoride agent	Other components
Placebo - (PLA)	Drogal Manipulation	-	-	Glycerin, Silica, Carboximethyl cellulose, water, Methyl P, Saccharin, Titanium dioxide, Sodium Lauryl Sulfate, Mint oil
Colgate Total 12™ (NaF)	Colgate-Palmolive, São Bernardo do Campo, Brazil	-	Sodium Fluoride (NaF) 1450 ppm	Water, Triclosan, Sorbitol, Hydrated Silica, Sodium Lauryl Sulfate, PVM / MA Copolymer, Flavor Carrageenan, Sodium hydroxide, Sodium Saccharin, Titanium dioxide and Dipentene.
Oral-B® Pro-Gengiva™ (SnF_2)	Procter & Gamble Manufacturing GMBH, Gross GERAL, Hesse, Germany	-	Stannous Fluoride (SnF_2) 1100 ppm and Sodium Fluoride (NaF) 350 ppm	Glycerin, Silica, Sodium Hexametaphosphate, Propylene Glycol, PEG-6, Water, Zinc Lactate, Aroma, Trisodium Phosphate, Flavor, Sodium Lauryl Sulfate, Sodium Gluconate, Carrageenan, Sodium Saccharin, Xanthan Gum, Cinnamal, Titanium Dioxide, Stannous chloride
Elmex™ Protection Erosion ™ (F/Sn/Chitosan)	GABA International AG, Grabetsmattweg, Switzerland	Chitosan (0.5%), Stannous Chloride	Amine Fluoride (AmF) 700 ppm, Sodium Fluoride (NaF) 700 ppm	Glycerin.Hydrated, Silica, Sodium hexametaphosphate, Propylene glycol, PEG-6, Eau, Zinc lactate, trisodium phosphate, flavor, Sodium lauryl sulfate, Polyethylene, Sodium, Gluconate, Carrageenan, Sodium saccharin, Xanthan gum, Titanium dioxyde, red 40 aluminium lake
Colgate® Sensitive Pró-Relief™ (F/Arginine)	Colgate-Palmolive, São Bernardo do Campo,Brazil	8% Arginine	Sodium Monofluorophosphate (MFP) 1450 ppm	Water, Calcium carbonate, Sorbitol, Arginine bicarbonate, Sodium lauryl sulfate, Cellulose gum, Titanium dioxide, Tetrasodium pyrophosphate, Sodium bicarbonate, Benzyl alcohol, Sodium saccharin, Xanthan gum, Limonene
Sensodyne™ Repair & Protect Novamin™ Technology (F/Bioactive Glass)	SmithKline Beecham Consumer Heathcare, Berkshire, United Kingdom	5% Calcium Sodium Phosphosilicate	Sodium Monofluorophosphate (MFP) 1426 ppm	Glycerin, Silica, PEG-8, Titanium Dioxide, Carbomer, Cocamidopropyl Betaine, Sodium Methyl Cocoyl Taurate, Sodium Saccharin, d-limonene

Previously Procedures to the beginning of the experimental phase

Before beginning the study and between each phase, a lead-in period of 2 days and washout period of 5 days were planned. During the washout, the participants were instructed to use fluoride-free dentifrice. During the experimental phase so that there was no interference in the results on the effectiveness of saliva and effectiveness of dentifrice, it was also determined that volunteers should use fluoride-free dentifrice for oral hygiene. The use of other oral hygiene products was not allowed during the experimental period, except the use of dental floss.

Before the beginning of each experimental phase, the palatal device was made with acrylic resin by molding the volunteer's upper arch with alginate (Hydrogum - Zhermack, Badia Polesine, Italy).

In vitro bleaching

The bleaching was performed *in vitro*, with a gel based on 35% hydrogen peroxide (Whiteness HP, FGM, Joinville, Brazil) according to the manufacturer's instructions. The bleaching agent was applied to the enamel surface three times for 15 minutes each, totaling 45 min of application. The samples were then washed with distilled water and immediately attached to the palatal device using sticky wax. After that, the *in situ* phase was began.

In Situ Phase

After in vitro bleaching, the volunteers were instructed to wear their palatal appliance for 5 min to allow the specimens to balance with oral fluids [13]. Then, the brushing procedures started. The volunteers were previously calibrated to perform brushing procedures in particular regarding load, brushing speed, and duration to obtain the highest level of standardization among the volunteers. Velocity and duration were trained by using a timer and load was trained by using the respective load on an electronic scale. In addition, the volunteers received written instructions with the description and the times that the treatments should be carried out. All participants used stopwatches to perform the treatments. A new toothbrush was used per participant and experimental phase.

The volunteers received one soft-bristle toothbrush (Oral-B Indicator Plus 30 Soft, Gillette do Brasil Ltd, Manaus, Brazil) and a container with 1.5 g of the assigned

dentifrice. The volunteers were instructed to brush the buccal surface of their teeth for 25 s (without contact to the specimens) to create a mixture of dentifrice/saliva (slurry). Then, they remained with the appliances in position for 2 min to enable the contact of the specimens with the slurry. After this, the appliance was removed, and the volunteers brushed the specimen's surfaces for 20 s (1 movement/s) [17]. Finishing the specimen brushing, the device was washed with running water for 30 seconds and reinserted in the mouth. After 12 hours, brushing procedures were performed again as described. Appliances were worn intraorally for 24 h. During use of appliances participants were instructed not to eat, they were only allowed to drink water [13]. After this period, the specimens were removed and stored at 100% humidity in individual vials at 37°C to perform the analyzes. The same procedures were repeated in the subsequent phases, changing the dentifrice used.

Surface microhardness

The analysis of surface microhardness was performed in T1, T2 and T3 using a microdurometer and a knoop indenter (HMV-2000, Shimadzu, Tokyo, Japan). Five indentations were carried out with a load of 50g for 5 seconds in the central region of the block with 100 µm between them and the average was calculated.

Roughness

The roughness (Ra) was analyzed using a profilometer tester (Surf-Corder 1700, Kosaka, Tóquio, Japão) at T1, T2 and T3. Three different equidistant directions were measured on the surface of each specimen, with a cutoff of 0.25 mm, a reading length of 1.25 mm, and a velocity of 0.1 mm/s.

Color analysis

Color analysis was also performed at T1, T2 and T3. A spectrophotometer (CM 700D, Konica Minolta, Osaka, Japan) was used and previously calibrated according to the manufacturer's recommendations. The readings were performed in a light chamber (GTI Mini Matcher MM1e, GTI Graphic Technology Inc., Newburgh, NY, USA) to obtain the standardization of ambient light during the readings. The values obtained were quantified in three coordinates of the CIE Lab System (L*, a*, b*). The differences in the L*, a* and b* values between the initial reading and final were expressed in ΔL, Δa and Δb and the general color change was calculated using the following equation:

$\Delta E^*_{ab}=[(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$. Also, the color difference was calculated by the ΔE_{00} formula [18,19]:

$$\Delta E_{00} = \left[\left(\frac{\Delta L'}{K_L S_L}\right)^2 + \left(\frac{\Delta C'}{K_C S_C}\right)^2 + \left(\frac{\Delta H'}{K_H S_H}\right)^2 + R_T\left(\frac{\Delta C'}{K_C S_C}\right)\left(\frac{\Delta H'}{K_H S_H}\right)\right]^{1/2}$$

Energy-Dispersive X-Ray Spectrometry

At the end of the experiment, five samples per group were randomly selected for analysis by energy-dispersive X-ray spectrometry (EDS). The specimens were covered with a thin layer of carbon. The inorganic content present in the substrates was evaluated by the MEV equipment (Jeol, JSM5600LV, Tokyo, Japan) to determine the elemental presence of Na, P, and Ca. The equipment was configured and presented typical energies of the order 15kV, in an increase of 100x, with PHA deadtime varying between 20 and 25% [2]. For each sample, five points were randomly selected (300 µm2 for each point), and the mean values were calculated. The elemental levels (wt%) of Ca, Na, and P and the proportion between Ca and P were determined.

Scanning Electron Microscopy (SEM)

To observe the enamel surface, four specimens from each group were randomly selected at the end of the experiment. Specimens were plated with gold alloy, and photomicrographs of representative areas with a 4000 × magnification were performed by using scanning electron microscopy (JSM 5600LV, JEOL, Tokyo, Japan).

Statistical analysis

All analyzes were performed in the SAS program (SAS Institute Inc., Cary, NC, USA, Release 9.4, 2012) with a 5% significance level. The salivary flow was analyzed using Mann-Whitney test. The buffer capacity and pH were analyzed using Student's t-test. The microhardness and roughness were analyzed using repeated measures mixed models and Tukey-Kramer test. The inverse transformation of the square root was applied to roughness, as indicated by the exploratory analysis. The variables ΔE^*_{ab} baseline x after bleaching, ΔE^*_{ab} baseline x after experimental period *in situ*, ΔE_{00} baseline x after bleaching, ΔE_{00} baseline x after experimental period *in situ* and Na% were analyzed using ANOVA in a split-plot design and Tukey test. No known

distribution adjusted to Ca%, P% and Ca/P, therefore these data were analyzed using Mann-Whitney non-parametric test comparing normal salivary flow and low salivary flow groups and Friedman and Nemenyi tests comparing different dentifrices.

RESULTS

Flow rate, salivary pH and salivary buffering capacity

Salivary flow rate of each participant as well as pH and buffering capacity values are given in Table 2. There was a significant difference between both groups for flow rate ($p<0,0001$) but not for the pH ($p=0,8268$) and buffering capacity ($p=0,6930$).

Table 2: Mean (standard deviation) of flow rate, salivary pH and salivary buffering capacity of both groups (normal and low flow rate) of the stimulated saliva (n=12).

	Flow rate, mL/min	pH	Buffering capacity
Normal flow	1.84 (0.22) *	7.49 (0.17)	4.73 (0.79)
Low flow	0.59 (0.10) *	7.51 (0.20)	4.69 (0.81)

* $p<0,0001$: statistically significant differences between groups (t test for independent data).

Surface microhardness

The results of the analysis of surface microhardness are available in Table 3.

- Normal Flow:

When it was compared the times for the same dentifrice, T2 showed the lowest values of surface microhardness and differed statistically from T1 and T3 regardless of the dentifrice used. In addition, T1 and T3 showed the highest values of surface microhardness and did not differ statistically among themselves for all dentifrice groups.

Comparing the dentifrices at the same time, in T1 and T2 no differences were found for dentifrices groups. In T3 there were statistically significant differences between the groups. The group treated with SnF_2 showed the highest values compared with the dentifrices evaluated. The groups treated with pacebo, NaF,

F/Sn/Chitosan and F/Arginine showed had the lowest values and did not differ between themselves. The F/Bioactive Glass group had intermediate values and did not differ from the group with the highest values (SnF2) or from the groups with the lowest values (placebo, NaF, F/Sn/Chitosan and F/Arginine).

- Low Flow:

Comparing the 3 times for each dentifrice, lower values of microhardness are noted in T2, with a significant increase in these values in T3 for all treated groups. For the placebo group, there was no difference between times T2 and T3. The SnF_2, F/Sn/Chitosan and F/Bioactive Glass groups showed no differences between T1 and T3.

Comparing the dentifrice groups at the same time, in T1 and T2 no differences were found for dentifrices groups. For T3 was observed that the placebo group (negative control) had the lowest microhardness values and was statistically different from all dentifrices. The SnF_2, F/Sn/Chitosan and F/Bioactive Glass groups showed the highest microhardness values and did not differ between them. NaF and F/Arginine showed intermediate microhardness values and did not differ statistically between themselves.

- Salivary flow X Dentifrice X Time:

Comparing salivary flows, under the same dentifrice condition and time, in T3 it was observed that the placebo and NaF groups in low flow had lower microhardness values than the same dentifrice for normal flow.

Table 3: Mean (standard deviation) of surface microhardness (SMH) as a function of salivary flow and dentifrices (n=12).

Dentifrice	Salivary flow					
	Normal Flow			Low flow		
	T1	T2	T3	T1	T2	T3
PLA	325.64 (11.60) Aa	269.30 (10.07) Ba	322.49 (5.43) Ab	325.67 (11.67) Aa	265.19 (10.28) Ba	*270.34 (5.04) Bc
NaF	326.96 (10.85) Aa	267.75 (8.47) Ba	322.93 (5.04) Ab	326.89 (11.68) Aa	266.75 (7.61) Ca	*299.12 (5.26) Bb
SnF_2	326.45 (8.73) Aa	267.61 (6.27) Ba	337.52 (5.43) Aa	326.92 (11.66) Aa	265.40 (7.62) Ba	330.70 (5.73) Aa
F/Sn/Chitosan	326.22 (11.82) Aa	268.20 (7.48) Ba	321.98 (5.65) Ab	326.32 (11.92) Aa	263.90 (10.62) Ba	323.23 (5.89) Aa
F/Arginine	326.08 (9.38) Aa	263.05 (3.19) Ba	318.28 (9.79) Ab	326.44 (12.77) Aa	263.83 (8.80) Ca	308.22 (6.73) Bb
F/Bioactive Glass	326.76 (9.08) Aa	264.81 (6.86) Ba	330.32 (3.46) Aab	327.38 (12.34) Aa	264.52 (10.84) Ba	322.44 (6.57) Aa

T1: initial; T2: after bleaching; T3: after experimental period *in situ*. *It differs from the group with normal flow, under the same conditions as dentifrice and time ($p \leq 0.05$). Different letters (uppercase in the horizontal comparing between the three times in the same salivary flow group and lowercase in the vertical) indicate significant differences ($p \leq 0.05$). p(flow)<0.0001; p(dentifrice)<0.0001; p(flow x dentifrice)<0.0001; p(time)<0.0001; p(flow x time)<0.0001; p(dentifrice x time) <0.0001; p (flow x dentifrice x time)<0.0001.

Roughness (Ra)

The roughness data are available in Table 4.

- Normal flow:

When it is compared the times for the same dentifrice, the PLA, NaF, F/Sn/Chitosan and F/Bioactive Glass groups showed statistically significant differences between times T1, T2 and T3. For these groups in T1, the lowest values were observed, while T2 presented the highest values and T3 presented intermediate values of roughness. The SnF_2 group also showed statistically significant differences between T1, T2 and T3, however it was observed that the highest values of roughness were found in T3, while T2 presented intermediate values and T1 showed the lowest values for this dentifrice. The F/Arginine group, on the other hand, showed no statistically significant difference between T2 and T3 in which the highest values of roughness were observed compared to T1.

Comparing the action of dentifrices at the same time, in T1 and T2 no differences were found for dentifrices groups. In T3 was observed that the F/Sn/Chitosan and NaF groups did not differ from the placebo group. The SnF_2 group presented the highest values of roughness and differed statistically among all the other evaluated dentifrices. The F/Arginine group presented intermediate roughness values and differed statistically from all groups. F/Bioactive Glass, on the other hand, had the lowest values of roughness and was statistically different from the placebo group and all other evaluated dentifrices.

- Low flow:

When it is compared the times for the same dentifrice, for PLA, NaF and F/Arginine groups T1 it was statistically different from T2 and T3. For the same groups T2 and T3 they did not differ statistically between themselves and presented higher values of roughness when compared with T1. For the SnF_2 group, T1 presented the lowest values, T2 intermediate values and T3 presented the highest values of roughness for this dentifrice and all times differed statistically from each other. For the F/Sn/Chitosan group, a statistically significant difference was observed between all times, with T1 having the lowest values, T2 having the higher values and T3 having intermediate roughness values. The same behavior of F/Sn/Chitosan was seen for F/Bioactive Glass the values found in T3 were closer to those found in T1.

Comparing the action of dentifrices at the same time, in T1 and T2 no differences were found between dentifrices groups. In T3 the placebo group presented intermediate roughness values and did not differ statistically from the NaF and F/Arginine groups The SnF_2 group had the highest values and differed statistically between all groups. The F/Sn/Chitosan group differed statistically from all groups and showed low values of roughness, however it was not the lowest value found among all evaluated dentifrices. The lowest values of roughness were found in the F/Bioactive Glass group, which differed statistically from all other groups.

- Salivary flow X dentifrice X Time:

Comparing salivary flows, under the same dentifrice condition and time, in T3 it was observed that the placebo and NaF groups in low flow had higher roughness values than the same dentifrice for normal flow.

Table 4: Mean (standard deviation), of roughness (Ra) as a function of salivary flow and dentifrice (n=12).

Dentifrice	Salivary flow					
	Normal Flow			Low flow		
	T1	T2	T3	T1	T2	T3
PLA	0.079 (0.007) Ca	0.180 (0.009) Aa	0.118 (0.006) Bc	0.077 (0.010) Ba	0.177 (0.010) Aa	*0.159 (0.12) Ab
NaF	0.080 (0.007) Ca	0.176 (0.011) Aa	0.117 (0.009) Bc	0.077 (0.012) Ba	0.179 (0.009) Aa	*0.165 (0.008) Ab
SnF_2	0.073 (0.006) Ca	0.178 (0.010) Ba	0.254 (0.008) Aa	0.077 (0.008) Ca	0.176 (0.005) Ba	0.250 (0.011) Aa
F/Sn/Chitosan	0.077 (0.008) Ca	0.170 (0.006) Aa	0.119 (0.011) Bc	0.079 (0.011) Ca	0.172 (0.009) Aa	0.122 (0.008) Bc
F/Arginine	0.076 (0.008) Ba	0.173 (0.006) Aa	0.152 (0.006) Ab	0.076 (0.009) Aa	0.171 (0.007) Ba	0.152 (0.004) Bb
F/Bioactive Glass	0.076 (0.008) Ca	0.175 (0.010) Aa	0.093 (0.008) Bd	0.078 (0.007) Aa	0.173 (0.008) Ca	0.091 (0.010) Bd

T1: initial; T2: after bleaching; T3: after experimental period *in situ*. * It differs from the group with normal flow, under the same conditions as dentifrice and time ($p \leq 0.05$). Different letters (uppercase horizontally comparing the three times in the same salivary flow group and lowercase vertically) indicate significant differences ($p \leq 0,05$). p(flow)=0,0011; p(dentifrice)<0,0001; p(flow x dentifrice)=0,0209; p(time)<0,0001; p(flow x time)<0,0001; p(dentifrice x time)<0,0001; p(flow x dentifrice x time)<0,0001.

Color analyses

The data of the color analyzes are available in Table 5 for the comparison between baseline and after bleaching. In Table 6 are the data comparing the baseline with after *in situ* period.

- ΔL, Δa, Δb, ΔE^*_{ab}, ΔE_{00} - baseline x after bleaching:

No statistically significant differences were found between salivary flows and dentifrice treatments for all color analyzes performed when baseline and after bleaching times were compared.

- ΔL baseline x after *in situ* period:

No statistically significant differences were found between salivary flows under the same dentifrice treatment ($p \geq 0.05$).

In normal flow statistical differences were observed between dentifrice groups ($p=0.0005$). The SnF_2, F/Arginine and F/Bioactive Glass groups had the highest ΔL values and were not statistically different between them. The F/Sn/Chitosan group had the lowest values among all evaluated dentifrices. The placebo and NaF groups showed intermediate values and did not differ statistically from the groups with the highest values (SnF_2, F/Arginine and F/Bioactive Glass) or from the group with the lowest value (F/Sn/Chitosan).

In low flow, no statistically significant differences were found between dentifrice groups ($p=0.2830$).

- Δa baseline x after *in situ* period:

No statistically significant differences were found between salivary flows under the same dentifrice treatment ($p \geq 0.05$). In normal flow ($p=0.9941$) and in low flow ($p=0.2730$), no significant differences were found between the evaluated dentifrices.

- Δb baseline x after *in situ* period:

Statistically significant differences were found between salivary flows under the same dentifrice treatment. The F/Sn/chitosan group showed lower values of Δb in normal flow when compared to the same dentifrice in low flow ($p = 0.0102$).

In normal flow, statistically significant differences were found between ttothpastes groups ($p = 0.0009$). The placebo group had the highest values while the

F/Sn/Chitosan group had the lowest ∆b values. The groups NaF, SnF_2, F/Arginine and F/Bioactive Glass showed intermediate values and did not differ from placebo or F/Sn/Chitosan.

In low flow, no statistically significant difference was found between dentifrices groups (p=0.5563).

- ΔE^*_{ab} and ΔE_{00} baseline x after *in situ* period:

There was a significant difference between dentifrice treatment (p=0.0239) but not for the salivary flow (p=0.0814) and not for interaction between salivary flow and dentifrice (p=0.6930).

No statistically significant differences were found between salivary flows for the same dentifrice treatment. For normal salivary flow, statistically significant differences were found between the dentifrices. The SnF_2 group had the highest values and differed statistically from the placebo group which had the lowest values. The NaF, F/Sn/chitosan, F/Bioactive Glass, F/Arginine groups showed intermediate values and did not differ statistically from SnF_2 or the placebo group. The same results observed for normal flow were found in the low flow.

Table 5: Average (standard deviation) of color analysis between baseline x after bleaching as a function of salivary flow and dentifrice (n=12).

Dentifrices	ΔL		Δa		Δb		ΔE^*_{ab}		ΔE_{00}	
	Salivary flow		Salivary flow		Salivary flow		Salivary flow		Salivary flow	
	Normal Flow	Low flow	Normal Flow	Low flow	Normal Flow	Low flow	Normal Flow	Low flow	Normal Flow	Low flow
PLA	3.38 (0.75) Aa	3.37 (0.72) Aa	-0.66 (0.47) Aa	-0.60 (0.31) Aa	-5.36 (0.76) Aa	-5.30 (0.78) Aa	6.40 (0.99) Aa	6.36 (0.77) Aa	4.36 (0.73) Aa	4.34 (0.53) Aa
NaF	3.28 (0.67) Aa	3.23 (0.77) Aa	-0.49 (0.28) Aa	-0.64 (0.41) Aa	-5.32 (0.70) Aa	-5.05 (0.96) Aa	6.30 (0.81) Aa	6.11 (0.81) Aa	4.23 (0.52) Aa	4.14 (0.60) Aa
SnF_2	3.29 (1.14) Aa	2.88 (0.80) Aa	-0.51 (0.44) Aa	-0.48 (0.22) Aa	-5.55 (0.78) Aa	-5.28 (0.64) Aa	6.56 (0.92) Aa	6.07 (0.85) Aa	4.44 (0.62) Aa	4.10 (0.54) Aa
F/Sn/Chitosan	2.93 (0.91) Aa	3.00 (0.83) Aa	-0.58 (0.36) Aa	-0.74 (0.40) Aa	-5.44 (0.53) Aa	-5.46 (1.24) Aa	6.26 (0.73) Aa	6.33 (1.28) Aa	4.22 (0.48) Aa	4.41 (0.84) Aa
F/Arginine	3.05 (1.06) Aa	2.72 (0.88) Aa	-0.56 (0.36) Aa	-0.52 (0.41) Aa	-5.89 (0.55) Aa	-5.70 (1.06) Aa	6.71 (0.84) Aa	6.39 (1.15) Aa	4.55 (0.57) Aa	4.36 (0.82) Aa
F/Bioactive Glass	3.26 (0.90) Aa	2.96 (0.76) Aa	-0.71 (0.45) Aa	-0.69 (0.22) Aa	-4.82 (1.38) Aa	-5.30 (1.36) Aa	6.01 (1.04) Aa	6.20 (1.14) Aa	4.07 (0.71) Aa	4.22 (0.76) Aa

Equal letters (uppercase comparing horizontally and lowercase vertically) do not indicate significant differences (p≤0.05).

Table 6: Average (standard deviation) of color analysis between baseline x after *in situ* period as a function of salivary flow and dentifrice (n=12).

Dentifrices	ΔL		Δa		Δb		ΔE^*_{ab}		ΔE_{00}	
	Salivary flow		Salivary flow		Salivary flow		Salivary flow		Salivary flow	
	Normal Flow	Low flow	Normal Flow	Low flow	Normal Flow	Low flow	Normal Flow	Low flow	Normal Flow	Low flow
PLA	1.78 (0.62) Aab	1.65 (0.73) Aa	-0.77 (0.46) Aa	-0.87 (0.33) Aa	-5.76 (0.64) Aa	-5.78 (0.85) Aa	6.11 (0.72) Ab	6.12 (0.88) Ab	4.24 (0.56) Ab	4.33 (0.61) Ab
NaF	1.61 (0.44) Aab	1.76 (0.58) Aa	-0.65 (0.26) Aa	-0.83 (0.37) Aa	-6.49 (0.66) Aab	-6.18 (0.88) Aa	6.73 (0.68) Aab	6.51 (0.88) Aab	4.65 (0.42) Aab	4.54 (0.67) Aab
SnF_2	2.04 (0.64) Aa	1.51 (0.89) Aa	-0.73 (0.38) Aa	-0.78 (0.28) Aa	-6.78 (0.90) Aab	-6.41 (0.83) Aa	7.15 (0.89) Aa	6.70 (0.82) Aa	4.99 (0.56) Aa	4.70 (0.62) Aa
F/Sn/Chitosan	0.99 (0.51) Ab	1.28 (0.93) Aa	-0.74 (0.31) Aa	-0.89 (0.34) Aa	-7.00 (0.55) Bb	-6.08 (1.13) Aa	7.12 (0.59) Aab	6.35 (1.10) Aab	4.94 (0.42) Aab	4.54 (0.78) Aab
F/Arginine	2.38 (0.80) Aa	1.90 (1.42) Aa	-0.72 (0.33) Aa	-0.68 (0.38) Aa	-5.92 (0.55) Aab	-5.79 (1.15) Aa	6.46 (0.72) Aab	6.25 (1.37) Aab	4.44 (0.47) Aab	4.32 (1.00) Aab
F/Bioactive Glass	2.03 (0.44) Aa	2.08 (0.68) Aa	-0.86 (0.50) Aa	-0.78 (0.29) Aa	-5.82 (0.96) Aab	-5.88 (0.98) Aa	6.25 (0.96) Aab	6.34 (0.86) Aab	4.35 (0.61) Aab	4.39 (0.49) Aab

Different letters (uppercase comparing horizontally and lowercase vertically) indicate significant differences (p≤0.05).

Energy-Dispersive X-Ray Spectrometry

The Ca%, P%, Na% and Ca/P data are available in Table 7.

- Ca%:

Comparing salivary flows for the same dentifrice, statistically significant differences were found between normal flow and low flow only for the placebo group. Placebo showed lower Ca% values when compared to the same dentifrice in normal flow ($p=0.0090$). For the other groups, no significant differences were found between salivary flows for the same dentifrice ($p \geq 0.05$).

Comparing the dentifrices groups in normal flow, statistically significant differences were found between the groups ($p=0.0052$). The F/Bioactive Glass and SnF_2 groups had the highest Ca% values and did not differ statistically between themselves. The NaF, F/Sn/Chitosan and F/Arginine presented intermediate values and were statistically equal to the placebo group (lowest values) and to the SnF2 and F / Bioactive Glass groups (highest values)

Comparing the dentifrices groups in low flow, statistically significant differences were found between the groups ($p=0,0009$). The F/bioactive Glass group had the highest values while the placebo had the lowest Ca% values and these two groups were statistically different from each other. The NaF, SnF_2, F/Sn/Chitosan and F/Arginine groups did not differ statistically from the placebo group or from the F/Bioactive Glass group.

- P%:

Comparing salivary flows for the same dentifrice, statistically significant differences were found between normal flow and low flow for NaF ($p=0.0283$), SnF_2 ($p=0.0090$) and F/Arginine ($p=0.0472$). For these dentifrices the value of P% was lower in low flow when compared to normal flow. For the other dentifrices, no significant differences were found between salivary flows ($p \geq 0.05$).

Comparing the dentifrices groups in normal flow, statistically significant differences were found between the groups ($p=0.0128$). SnF_2 and F/Arginine groups had the highest values and did not differ between them. The placebo group had the lowest values and differed statistically from the SnF_2 and F/Arginine groups. The NaF, F/Sn/Chitosan and F/Bioactive Glass groups showed intermediate values and did not

differ statistically from the groups with the highest values (SnF_2 and F/Arginine) or from the groups with the lowest values (placebo).

Comparing the dentifrices groups in low flow, statistically significant differences were found between the groups (p=0.0055). The placebo group had the lowest values among all groups. The F/Sn/Chitosan groups and F/Bioactive Glass had the highest values and did not differ between them. The NaF, SnF_2 and F/Arginine groups showed intermediate values and did not differ statistically from the groups with the highest values (F/Sn/Chitosan and F/Bioactive Glass) or from the placebo group.

- Na%:

The p values for the Na% statistical analysis were: p(flow)=0.0022; p(dentifrice)<0.0001; p(interaction)<0.0001.

Comparing salivary flows for the same dentifrice, statistically significant differences were found for placebo and NaF groups. In low flow theses groups showed higher values than normal flow for the same dentifrice.

Comparing the dentifrices groups in normal flow, statistically significant differences were found between the groups. The placebo group had the lowest values among all dentifrices. The SnF_2 and F/Bioactive Glass groups showed the highest Na% values, did not differ between themselves and differed from the placebo group. The NaF, F/Sn/Chitosan and F/Arginine groups presented intermediate values and did not differ either from the groups with the highest values or from the placebo group.

Comparing the dentifrices groups in low flow, statistically significant differences were found between the groups. The placebo and NaF group had the lowest Na% values and it was statistically different from all other evaluated dentifrices.

- Ca/P:

Comparing salivary flows for the same dentifrice, statistically significant differences were found. The placebo group showed lower values in normal salivary flow when compared to the same dentifrice in low flow (p=0.0367). For the other dentifrices, no differences were found between salivary flows.

Comparing the dentifrices, no statistically significant differences were found between the groups for normal flow (p = 0.3090) and for low flow (p≥0.05).

Table 7: Mean (standard deviation) of elemental levels (wt%) for EDS analysis of enamel surface according to the treatment group as a function of salivary flow and dentifrice (n=5).

Dentifrices	Ca		P		Na		Ca/P	
	Salivary flow		Salivary flow		Salivary flow		Salivary flow	
	Normal Flow	Low flow	Normal Flow	Low flow	Normal Flow	Low flow	Normal Flow	Low flow
PLA	69,06 (0,38) Ab	67,42 (0,22) Bb	27,67 (0,25) Ab	27,39 (0,10) Ab	0,60 (0,18) Ab	0,20 (0,05) Bb	2,51 (0,02) Aa	2,44 (0,02) Ba
NaF	69,96 (0,78) Aab	69,96 (0,56) Aab	28,19 (0,10) Aab	27,97 (0,15) Bab	0,77 (0,08) Aab	0,20 (0,19) Bb	2,49 (0,02) Aa	2,52 (0,04) Aa
SnF_2	70,92 (0,17) Aa	70,96 (0,30) Aab	28,70 (0,58) Aa	27,88 (0,14) Bab	1,06 (0,09) Aa	0,96 (0,25) Aa	2,51 (0,01) Aa	2,52 (0,02) Aa
F/Sn/Chitosan	70,53 (0,75) Aab	70,71 (0,15) Aab	28,29 (0,23) Aab	28,40 (0,29) Aa	0,86 (0,16) Aab	0,87 (0,12) Aa	2,49 (0,03) Aa	2,49 (0,03) Aa
F/Arginine	70,76 (0,14) Aab	70,52 (0,23) Aab	28,48 (0,18) Aa	28,16 (0,31) Bab	0,86 (0,39) Aab	0,86 (0,20) Aa	2,44 (0,07) Aa	2,51 (0,03) Aa
F/Bioactive Glass	70,87 (0,22) Aa	71,16 (0,26) Aa	28,74 (0,19) Aa	28,42 (0,26) Aa	1,18 (0,07) Aa	0,89 (0,25) Aa	2,51 (0,02) Aa	2,51 (0,02) Aa

Different letters (uppercase comparing horizontally and lowercase vertically) indicate significant differences ($p \leq 0.05$).

Scanning electron microscopy

The SEM images collected (Figure 2) show that all groups showed superficial changes, but there was variation in the extend of these changes according to the group. Greater enamel demineralization with loss of interprismatic substance and increased porosity was observed for the placebo and NaF groups compared to the other groups (Figure 2A, 2B, 2C, 2D). Salivary flow interfered in the superficial characteristics of the placebo and NaF groups because the images show the presence of localized areas with intense demineralization for the low flow (Figure 2B and 2D), a fact that was not observed in the normal flow (Figure 2A and 2C). For the SnF_2, F/Sn/Chitosan and F/Arginine groups, it is possible to observe a very similar pattern between the three groups, with areas of smaller and more superficial demineralizations than those found in placebo and NaF (Figure 2E, 2F, 2G, 2H, 2I, 2J). The F/Bioactive Glass group, on the other hand, presented the smoothest and most uniform surface among all the groups evaluated with well-located and superficial demineralization areas. (Figure 2K and 2L). For the SnF_2, F/Sn/Chitosan, F/Arginine and F/Bioactive Glass groups, no differences were found between salivary flows.

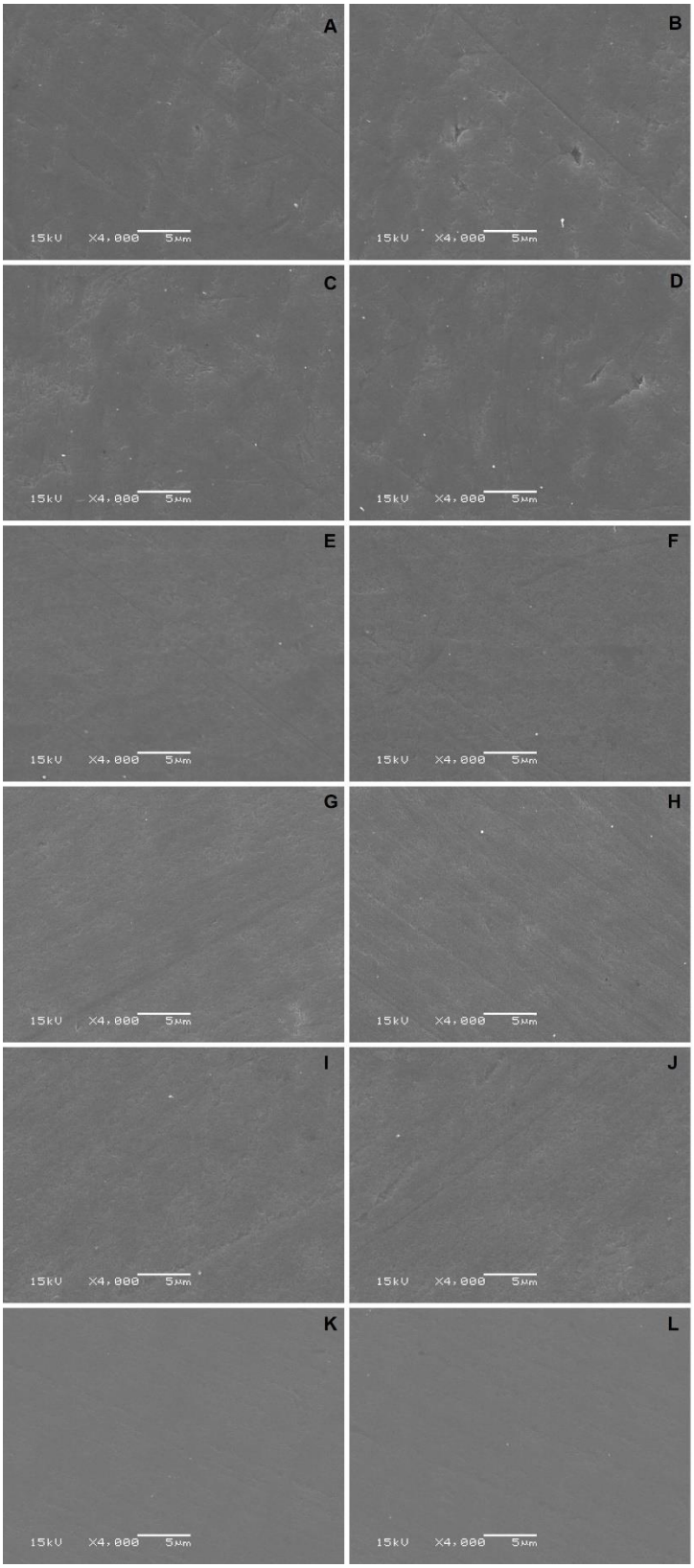

Figure 2: Representative SEM images (4000x) of the specimens according to the group: (A) placebo in normal flow, (B) placebo in low flow, (C) NaF in normal flow, (D) NaF in low flow, (E) SnF_2 in normal flow, (F) SnF_2 in low flow, (G) F/Sn/Chitosan in normal flow, (H) F/Sn/Chitosan in low flow, (I) F/Arginine in normal flow, (J) F/Arginine in low flow, (K) F/Bioactive Glass in normal flow and (L) F/Bioactive Glass in low flow.

DISCUSSION

The first hypothesis was partially accepted, because according to the dentifrice used, the salivary flow influenced the results of the analyzes performed in this study, except for color analysis between baseline x after bleaching. The second hypothesis evaluated was also partially accepted because for both salivary flows no dentifrice resulted in the recovery of roughness. In addition, for the low salivary flow only SnF_2, F/Sn/Chitosan and F/Bioactive glass dentifrices promoted the recovery of surface microhardness.

In this study, after the *in situ* period, the groups under normal flow condition did not show differences in surface microhardness compared to baseline regardless of the dentifrice used, indicating remineralization of enamel. Saliva is responsible for the enamel remineralization after tooth bleaching [5]. Remineralization is the process of replacing lost minerals through the organic matrix of the enamel to the crystals [20]. The contribution of saliva to the remineralization process points to the importance of monitoring salivary flow [20]. The salivary flow rate has been considered the most important single parameter to determine the protective effect of saliva, since practically all other salivary parameters depend on it [21]. The great differential of this study was to evaluate the condition of low salivary flow associated with tooth bleaching.

The results of this study showed that the salivary flow influence the enamel remineralization after bleaching. The surface microhardness after the *in situ* period for placebo group in low flow was not equal to baseline values and differed from the normal flow when the dentifrice was used. In the analysis of the volunteers' salivary parameters, it is possible to identify that the pH and buffer capacity were not changed, but only the salivary flow. Changes in salivary flow are accompanied by changes in salivary characteristics, such electrolyte concentrations [22,23]. The main electrolytes in saliva include sodium, potassium, calcium, magnesium, chloride, bicarbonate, phosphate, thiocyanate and fluoride [23]. The supersaturation of these minerals in saliva is critical to the remineralization process [20]. In this way, the possible decrease in electrolyte concentration caused by the decrease in salivary flow could result in a lesser capacity for salivary remineralization, justifying the results found in this study. The EDS data support this result, since the placebo in low flow had lower values of Ca%, Na% and a lower Ca/P ratio than the same dentifrice under normal flow

condition. Furthermore, it is important to note that for the Ca/P analysis, the placebo group was the only one that showed a difference between salivary flows.

Roughness after the *in situ* period when placebo dentifrice was used also was influenced by salivary flow. This result may be associated with salivary proteins. In addition to electrolytes, protein concentration in saliva is also influenced by salivary flow [23]. Proteins in the protective pellicle, such as statherin, histatins, cystatins, and proline-rich proteins remain on the surface, bound to hydroxyapatite, to aid in controlling crystalline growth of the enamel by allowing the penetration of minerals into the enamel for remineralization and by limiting mineral egress [24,25]. This control of precipitation and mineral egress enhances the stability of hydroxyapatite in the outer tooth structure [26]. The reduction of proteins in the low flow could result in a possible formation of a non-stable hydroxyapatite, which justifies the enamel roughness results found in this study. The surface characteristics of the placebo groups in SEM images are compatible with the roughness results presented. The placebo group in low flow, in addition to the demineralization pattern on the entire surface, it is possible to identify localized regions of profound mineral loss, which was not observed for this dentifrice in normal flow.

In addition to saliva, the decreased microhardness of the bleached enamel can be significantly reduced when fluoridated components are administered after bleaching [27], as fluoridation regimens with dentifrice [28]. Fluoride act as a remineralizing agent by forming a calcium fluoride layer on the enamel surface. This deposit is subsequently dissolved, allowing fluoride to diffuse into enamel and support remineralization and increase the microhardness values [29]. In this study, NaF was not effective after bleaching. In the normal flow the NaF group presented enamel microhardness after *in situ* period equal to baseline, however it did not differ from the placebo group. This suggests that the remineralization found in these groups is associated with the action of saliva and not with the action of NaF. For the enamel microhardness and enamel roughness in low flow, the effectiveness of NaF also was very limited because it differed from the placebo but it did not restore the values to levels found in baseline. In addition, for both analyzes, differences were found between the salivary flows and the low flow had a lower performance than the normal flow. It was possible to observe this difference between the salivary flows for NaF in SEM images because the low flow had localized and deep demineralization areas different from normal flow. This may have happened because the effect of NaF is apparently modulated by the amount of

saliva [21]. The efficacy of sodium fluoride containing preparations is based on the formation of CaF_2 -like particles on the surface. These precipitates appear to be more stable due to the impact of saliva and acquired pellicle [30]. This fact also justifies the difference between salivary flows in the analysis of P% and Na%. In addition, NaF was the only group that did not differ from placebo for Na%.

The combination of fluoride and tin showed the best results in this study for surface microhardness after the *in situ* period for both salivary flows. Tin containing products stand out in the remineralization of eroded enamel [11]. SnF_2 can interact and be incorporated into the eroded enamel [31], forming salts with calcium and phosphate [32]. This fact justifies the higher values of Ca%, P% and Na% presented in normal flow and the difference in relation to the placebo group. For low flow, SnF_2 promoted the recovery of enamel microhardness to baseline values. The tin ion was suggested to have a cross-linking action with proteins of previously formed acquired pellicle [33]. Thus, the interaction of the tin with the acquired pellicle and its remineralizing action was sufficient to compensate the losses with the decrease in salivary flow. This could explain the absence of difference between the salivary flows after the period *in situ* for surface microhardness and roughness. Also, no differences were found between salivary flows for Ca%, Na% and Ca/P analyzes. Despite this, the tin group showed the highest values of roughness, for both salivary flows after the period *in situ* and was statistically different from all other evaluated dentifrices. This may be associated with the fact that tin has the ability to deposit on dental tissues forming a mineral layer on the enamel [34]. In addition, products containing tin form amorphous deposits on the enamel surface and allow Sn to be incorporated into the enamel [31], which could have influenced the roughness results found.

In addition to the use of fluoridated products, the use of fluoride-containing remineralizing agents has been suggested by some researchers [35]. A F/Arginine dentifrice evaluated in this study has a combination of arginine and calcium carbonate and this high amount of inorganic components in the dentifrice can act to increase the microhardness of the enamel structure [36]. Nevertheless, in this study for normal flow, the enamel microhardness did not differ from the placebo group. This fact suggests that the remineralizing effects found in this group may be linked to the action of saliva and not the action of the dentifrice. EDS data support this result because this dentifrice did not differ from placebo for Ca%, Na% and Ca/P for normal flow.

For low flow, the F/Arginine dentifrice promoted a slight increase in enamel microhardness, but the values found before bleaching were not restored. Besides that, after the *in situ* period, the F/Arginine group differed from the placebo, but presented intermediate values in relation to the other groups. The combination of arginine and calcium carbonate can provide an alkaline environment that stimulates endogenous calcium and phosphate ions to precipitate in dental tissues [37]. Therefore, inorganic electrolytes contained in saliva as calcium, phosphate and fluoride can be important participants in the remineralization process [1], which possibly justifies its action, however reduced in low flow. The results of P% show that there were differences between the flows and the low flow presented lower results than normal flow. In addition, this dentifrice contains fluoride in the form of sodium monofluorophosphate (MFP). The hydrolysis of MFP is catalyzed by salivary enzymes [38] which may have been consequently affected by the decrease in salivary flow. The precipitation of ions on the surface caused by arginine may also have influenced the results of roughness, since for both salivary flows the F/Arginine showed intermediate enamel roughness after the *in situ* period.

The combination of chitosan with stannous and fluoride was effective for recovering the values of surface microhardness after the *in situ* period for low flow. Chitosan is a natural polymer capable of binding to proteins from saliva [39], fluoride [40] and other ions on the enamel surface, forming layers on the surface [41]. This layer could favor the dental remineralization process, justifying the microhardness results found in this study. For low flow, F/Sn/Chitosan presented the highest enamel microhardness and did not differ from SnF_2 or F/Bioactive Glass. Another important factor is that for microhardness, roughness, Ca%, P%, Na% and Ca/P the action of this dentifrice did not differ between salivary flows, it is, only the presence of saliva, regardless of the amount, was sufficient to promote remineralization of the surface. This can be justified by the fact that chitosan can be adsorbed onto salivary pellicle proteins [42]. In the presence of mucin, both chitosan and mucin interact to form firmly attached multilayers [43]. The surface layer made by chitosan can increase the retention of stannous [44], present in this dentifrice in the form of $SnCl_2$. As stannous is a metal, they have the ability to depose itself and to form stable salts with hydroxyapatite crystals, as abovementioned [32]. In the same way as in the SnF_2 group, tin deposition may have influenced the roughness values found for this

dentifrice, because intermediate roughness was observed for both salivary flows after *in situ* period.

The use of F/Bioactive Glass resulted in the recovery of surface microhardness to normal and low flow. These results are in accordance with previous studies that show the potential of bioactive glass to increase remineralization after tooth bleaching [35]. In low flow, this dentifrice showed the highest enamel microhardness after the *in situ* period and did not differ from SnF_2 and F/Sn/Chitosan. Beside this, there was no difference between salivary flows. Bioactive Glass is a ceramic material consisting of amorphous calcium sodium phosphosilicate which is highly reactive in water [45,46]. Water is the main component of saliva, constituting 99% of its volume [47]. This factor may explain the absence of differences between the salivary flows, because the presence of water in the low salivary flow was sufficient for the remineralizing action of bioactive glass. The Ca%, P%, Na% and Ca/P also showed no differences between salivary flows and the highest values between the groups evaluated with a difference from the placebo group for both flows.

Other important result that made F/Bioactive Glass stand out from other groups was the lowest values of roughness after *in situ* period for both salivary flows. This result was supported by SEM images because it is possible to identify that the F/Bioactive Glass groups had the smoothest and most uniform surface among all groups for both salivary flows. In the aqueous environment of the tooth, sodium ions from the bioactive glass particles release of calcium and phosphate ions from the glass which results in increased oral pH [48,49]. This increase in pH helps to precipitate the extra calcium and phosphate ions provided by the bioactive glass to form a layer of calcium phosphate that crystallizes in into carbonate-enriched hydroxyapatite [50]. The combination of the residual bioactive glass particles and the newly -formed carbonate-enriched hydroxyapatite layer results in remineralization of the enamel surface [35].

In the color analysis between baseline x after bleaching, it can be seen that the changes promoted by bleaching were the same for all dentifrice and salivary flows. In the comparison between baseline x after in situ period, no differences were found between salivary flows for each dentifrice in all analyzes performed except for F/Sn/Chitosan that showed differences between the salivary flows for the Δb. Also, the F/Sn/Chitosan in normal flow presented the lowest values of ΔL and the highest values of Δb among all evaluated dentifrices. The bleaching efficacy is evidenced by the increase in L* values and decrease in b* values, which represents a lighter and less

yellowish color for the tooth [51]. Based on this, the use of F/Sn/Chitosan dentifrice in the normal salivary flow resulted in a darker and more yellowish tooth when compared to the other evaluated dentifrices. This result may be associated with the formation of multilayers on the enamel surface by chitosan, which probably caused changes in the optical properties of the tooth. The normal flow may have resulted in the formation of a thicker layer and with a different structure from the layer formed by the low flow, justifying the results found for this group.

For normal and low flow, the SnF_2 group had the highest values of ΔE^*_{ab}, ΔE_{00} and was statistically different from the placebo group. This result may be associated with the superficial changes such as increased roughness and decreased microhardness presented in these groups. Changes in topography can promote a change in the surface associated with a change in the reflectance of light [52], because the observed color of the tooth is a combination of optical phenomena, including the light reflected from the enamel surface, light transmitted and the degree of light absorption of dental substrates [53].

However, the composition of dentifrices did not affect the result of the bleaching treatment, because all groups showed increasing values of L* and decreasing b* values. The mean values of total color change (ΔE^*_{ab}, ΔE_{00}) after *in situ* period were statically similar or greater than placebo group and greater than 4.2 units [54], the standard values suggested for clinical acceptability of color differences. The color results support a safe indication for the use of dentifrices after tooth bleaching.

CONCLUSION

The salivary flow and dentifrices with different active agents interfered in the enamel reminerealization after bleaching. The low salivary flow had a lower capacity for remineralization of the bleached enamel compared to normal flow, however, dentifrice can positively assist in this process, being the best results for bioactive glass-based dentifrice.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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3 DISCUSSÃO

A saliva humana se destaca por sua habilidade de reverter as modificações no tecido dental duro relacionadas ao clareamento dental (Attin et al., 2009). A literatura mostra que a exposição do esmalte dental à saliva humana por 24 horas ao final do tratamento clareador não resulta em diferença de microdureza superficial em comparação com os valores iniciais, indicando a recuperação completa da microdureza da superfície (Zeczkowski et al., 2015). Devido a essa função primordial da saliva ao término de um clareamento dental é importante que algumas características salivares possam ser analisadas de tal modo a elucidar os efeitos causados por uma possível variação dessas características.

A redução do fluxo salivar é uma alteração salivar muito comum e a sua associação com o clareamento dental ainda não havia sido investigada. A importância da avaliação dessa associação se dá pelo fato da hipossalivação afetar aproximadamente 30% dos pacientes com idade entre 20 e 69 anos (Flink *et al.,* 2008). A patogênese da hipofunção salivar pode ser atribuída a inúmeras causas, como algumas patologias (síndrome de Sjogren), medicamentos e radioterapia (Papas *et al.,* 2009). Dados sugerem que cerca de 1.800 medicamentos prescritos e de venda livre têm possíveis efeitos colaterais no fluxo salivar (Papas *et al*., 2009). Além desses fatores, um ponto importante a ser considerado é que quando o paciente procura o consultório odontológico para realização do tratamento clareador não são realizados testes que avaliem o seu fluxo salivar. Assim, o Cirurgião-dentista não consegue garantir que o paciente que está realizando o tratamento terá toda a proteção e recuperação da estrutura dental que se espera, sendo assim muito importante investigar se haveria algum tipo de gel clareador (Artigo 1) ou tratamento com dentifrício pós-clareamento (Artigo 2) que minimizasse os efeitos deletérios do clareamento para esse tipo de paciente.

Neste estudo, a composição da amostra se deu por voluntários que apresentaram o fluxo salivar normal ou reduzido. Para isso foi necessário a realização da análise dos parâmetros salivares dos voluntários onde foi determinado que os voluntários com fluxo normal deveriam apresentar uma taxa de fluxo salivar estimulado maior que 1,0 mL por minuto, enquanto que o voluntário de fluxo reduzido deveria apresentar uma taxa de fluxo salivar estimulado menor que 0,8 mL por minuto. Devido a isso, foram selecionados indivíduos saudáveis que possuíam a redução do

fluxo salivar devido ao tratamento radioterápico, pois ao contrário da hipofunção salivar relacionada à medicação, a hipossalivação causada por radiação não é reversível pela estimulação. Assim, essa foi a alternativa mais segura para a realização desse experimento, pois dessa maneira pôde-se controlar esse fator em estudo e assegurar que o voluntário não tivesse variação na sua taxa de fluxo salivar durante o período experimental proposto. Além do fluxo foram avaliados o pH e a capacidade tampão salivar. Como é possível ver na Tabela 2 do Artigo 1 e na Tabela 2 do Artigo 2, a única diferença entre os dois perfis de voluntários avaliados foi a taxa de fluxo salivar estimulado, garantindo assim que as diferenças encontradas nesse estudo foram decorrentes da diferença entre os fluxos salivares.

O Artigo 2 mostrou que o fluxo salivar interferiu nas propriedades físicas e químicas do esmalte após o clareamento com peróxido de hidrogênio 35% (Tabelas 3, 4, 7 e Figura 2). Para os voluntários com fluxo salivar normal quando o dentifrício placebo foi utilizado, após o período *in situ,* foi observado valores de microdureza de superfície igual aos valores baseline (Tabela 3). Esse fato mostra que, a saliva é capaz de reverter a perda de microdureza observada após o clareamento dental e assim promover a remineralização. O mesmo não foi observado para o baixo fluxo salivar. No baixo fluxo salivar, quando o dentifrício placebo foi utilizado, observou-se que após o período *in situ* não houve recuperação da microdureza de superfície aos valores baseline. Além disso, esse estudo confirmou a diferença entre os fluxos salivares para o dentifrício placebo após o período *in situ*. Resultado semelhante foi observado para a rugosidade de superfície no Artigo 2 (Tabela 4), mostrando diferença entre os fluxos salivares. O baixo fluxo apresentou maior rugosidade de superfície do que o fluxo normal para o dentifrício placebo após o período *in situ.*

Esses resultados são de grande relevância para a literatura, pois através dele pode-se sugerir que pacientes que apresentam a diminuição no fluxo salivar terão consequentemente a diminuição do potencial de recuperação das características de superfície após o clareamento dental. Isso se dá pelo fato de que a diminuição do fluxo salivar resulta na diminuição da concentração de eletrólitos na saliva como o sódio, potássio, cálcio, magnésio, cloro, bicarbonato, fosfato, tiocianato e flúor. A supersaturação desses minerais são críticas para o processo de remineralização (Humphrey & Williamson 2001). Dessa maneira, a diminuição na concentração de eletrólitos causadas pela redução do fluxo salivar possivelmente resulta em uma menor capacidade de remineralização. Os dados da análise de energia dispersiva por

Raios-X (EDS) apoiam esse resultado (Tabela 7), uma vez que o placebo em baixo fluxo apresentou valores mais baixos de Ca%, Na% e uma relação Ca/P menor do que o mesmo dentifrício em condições normais de fluxo. Além disso, é importante notar que, para a análise de Ca/P, o grupo placebo foi o único que mostrou diferença entre os fluxos salivares, demonstrando que a utilização de um dentifrício após o clareamento dental é um fator essencial e pouco discutido na literatura.

A concentração de proteínas também é influenciada pelo fluxo salivar (Dawes *et al*., 2004). As proteínas da película adquirida auxiliam no controle do crescimento cristalino do esmalte, permitindo a penetração de minerais no esmalte para remineralização e por limitação da saída de minerais (Scannapieco *et al*., 1993; Dowd *et al*., 1999). Esse controle de precipitação e saída mineral melhora a estabilidade da hidroxiapatita na estrutura dentária externa (Richardson *et al*., 1993). A redução de proteínas no baixo fluxo pode resultar em uma possível formação de hidroxiapatita não estável, o que justifica os resultados de rugosidade encontrados neste estudo. Nas imagens de microscopia eletrônica de varredura (MEV) do grupo placebo em baixo fluxo (Figura 2A), além do padrão de desmineralização em toda a superfície, também foi possível identificar regiões localizadas de profunda perda mineral, o que não foi observado para esse dentifrício no fluxo normal (Figura 2B).

Ao contrário do observado no Artigo 2, no Artigo 1, em relação aos fluxos salivares, foi observado que tanto para o fluxo normal como para o reduzido a exposição dos espécimes a saliva durante o perído *in situ* não foi suficiente para reverter completamente os efeitos deletérios do clareamento dental caseiro com peróxido de carbamida a 10%. Acreditamos que a diferença entre os dois estudos pode ser justificada pela diferença do produto clareador utilizado. No Artigo 2 foi utilizado o peróxido de hidrogênio a 35%, enquanto que no Artigo 1 foi utilizado o peróxido de carbamida 10%. Além disso, o protocolo de aplicação pode ter influenciado, pois apesar de no Artigo 2 ter sido utilizado um agente clareador em maior concentração, o tempo de aplicação foi de apenas 45 minutos, quanto que no Artigo 1 o peróxido de carbamida a 10% foi utilizado por 4 horas durante 14 dias consecutivos. Um outro fator importante em relação ao agente clareador é o fato de que o peróxido de carbamida se dissocia em peróxido de hidrogênio e uréia. A presença de erosões e porosidades no esmalte tem sido relacionada aos subprodutos, principalmente uréia e oxigênio, da reação oxidante dos agentes clareadores (Goldberg et al., 1983; Hegedus et al., 1999). A uréia tem a propriedade de desnaturar

proteínas presentes em porções orgânicas da estrutura dentária, com potencial de penetrar no esmalte e afetar não apenas a superfície, mas também a porção interprismática do esmalte (Goldberg et al., 1983). Portanto, a penetração da uréia pode contribuir para o aumento da permeabilidade do esmalte e alterações microestruturais (Sasaki et al., 2009). Tal fato pode ter resultado em maiores alterações no esmalte dental que, necessitariam de um período maior em contato com a saliva após o clareamento dental.

Os fluxos salivares apresentaram comportamentos diferentes nos Artigos 1 e 2 para a análises de EDS. No artigo 2 os fluxos salivares apresentaram diferenças estatísticas significantes quando o dentifrício placebo foi utilizado para a relação Ca/P. Já no Artigo 1 não foram encontradas diferenças entre os fluxos salivares na relação Ca/P. Isso pode ter acontecido pelo fato de que no Artigo 2, o período *in situ* teve o tempo de duração de 15 dias. O tempo longo de exposição a saliva pode ter compensado as modificações salivares decorrentes da redução do fluxo salivar, resultado na ausência de diferença entre os fluxos.

Tanto para o Artigo 1, quanto para o Artigo 2, um fator muito importante a ser levado em consideração é a cor, afinal ambos os estudos estão associados ao clareamento dental. O ideal seria que nem a utilização dos espessantes experimentais e nem os dentifrícios resultassem em alteração da cor do esmalte. Para avaliação de cor, além das análises de ΔL, Δa, Δb e ΔE^*_{ab}, foi realizada análise do ΔE_{00}. O ΔE^*_{ab} é a métrica mais utilizada em odontologia para avaliar diferenças de cores (Pecho *et al.,* 2019). No entanto, o espaço de cores do CIEL*a*b* assume peso igual para todas as coordenadas de cores (Mangine *et al.,* 2005). Estudos (Perez *et al.,* 2011; Pecho *et al.,* 2016) demonstraram uma discrepância na sensibilidade para diferentes coordenadas de cores no espaço de cores do CIEL*a*b*. Assim, foi proposto ΔE_{00}, que considera fatores paramétricos, para avaliar as diferenças de cores (Luo et al., 2001). Além disso, já foi demonstrado que ΔE_{00} apresenta uma melhor correlação com a percepção visual do que ΔE^*_{ab} (Pecho *et al.,* 2016, 2017).

Para o Artigo 1, em todas as análises de cor foi observado que os espessantes experimentais, natrosol e aristoflex, não diferiram do controle positivo (gel comercial fabricado com o espessante carbopol) (Tabela 3). Esse resultado mostra que em relação a alteração de cor resultante do clareamento dental é seguro a utilização dos espessantes experimentais avaliados. Esse resultado pode estar associado ao fato de que todos os géis clareadores utilizados nesse estudo possuíam o mesmo agente

clareador, na mesma concentração. No Artigo 2, os resultados de cor no tempo baseline x após o clareamento mostraram que não foram encontradas diferenças entre os grupos, ou seja, todos os grupos clarearam igualmente. No tempo baseline x após o período *in situ* foram encontradas diferenças entre os dentifrícios para o ΔL, Δb, ΔE^*_{ab} e ΔE_{00}. Apesar disso, pode-se afirmar que a composição dos dentifrícios não afetou o resultado do tratamento clareador, pois todos os grupos apresentaram valores crescente de L* e valores decrescentes de b*. Além disso, os valores médios da mudança total de cor (ΔE^*_{ab}, ΔE_{00}) após o período in situ foram estatisticamente semelhantes ou maiores que o grupo placebo e maiores que 4,2 unidades (Alghazali et al., 2012), o valor padrão sugeridos para aceitação clínica de diferenças de cores. Assim, os resultados de cor também suportam uma indicação segura para o uso de dentifrícios após o clareamento dental.

Em relação aos espessantes avaliados nesse estudo, o carbopol apresentou o pior desempenho para o fluxo salivar normal e para o fluxo salivar reduzido entre os espessantes avaliados nas análises de microdureza de superfície, rugosidade, energia sispersiva por Raio-X e microscopia eletrônica de varredura (Tabelas 4, 5, 6 e Figura 1). Derivado do ácido carboxílico, o carbopol apresenta características iônicas e baixa estabilidade de pH (Oliveira *et al*., 2007; Gouveia *et al*., 2016). Assim, esses achados importantes mostram que a utilização de espessantes experimentais nos géis clareadores pode ser uma boa opção na tentativa de diminuir os efeitos deletérios causados pelo clareamento dental. Outros resultados que merecem destaque relacionado ao espessante carbopol foram as diferenças estatísticas encontradas entre os fluxos salivares para a análise de rugosidade após o clareamento dental (Tabela 5). Na condição de baixo fluxo salivar, o gel clareador com o espessante carbopol resultou em maior rugosidade quando comparado com a utilização do mesmo gel para o fluxo salivar normal. Esse resultado pode estar associado a interação do espessante com a superfície dental. Os espessantes podem interagir com a estrutura dental devido às suas características bioadesivas. A capacidade bioadesiva está relacionada a possíveis ligações iônicas entre os polímeros formando um “filme” de polímero depositado na estrutura dentária. A interação do carbopol com a superfície dental resulta na formação de uma camada muito forte e espessa de polímeros na superfície, mostrando grande afinidade desse espessante pela estrutura dental (Avila *et al.,* 2017). Devido à sua forte capacidade de ligação ao cálcio, o carbopol causa inibição da incorporação do cristal de

hidroxiapatita na remineralização (van der Reijden *et al*., 1997). Isso poderia resultar na necessidade de uma quantidade maior de saliva para promover a recuperação do esmalte.

Para o fluxo salivar normal, o natrosol e o aristoflex não diferiram nas análises de microdureza de superfície (Tabela 4), rugosidade (Tabela 5), Ca%, P% e Ca/P (Tabela 6). Esses espessantes possuem estabilidade de pH e são capazes de formar géis com características não iônicas (Gouveia *et al*., 2016; Golding *et al*., 2014), o que provavelmente resultou em menores efeitos deletérios após o clareamento. Além disso, para esses grupos, devemos levar em consideração que a condição de fluxo salivar normal garante a disponibilidade de uma saliva sem alterações em sua composição possibilitando a remineralização salivar após o clareamento dental. Já para o fluxo salivar reduzido o natrosol apresentou microdureza de superfície maior que o carbopol (controle positivo) porém apresentou menor dureza que o aristoflex. Além disso, o natrosol em baixo fluxo apresentou diferença estatística entre o mesmo espessante para o fluxo normal após o clareamento. Esse resultado está associado à interação desse espessante com o esmalte e a formação de uma camada sobre a superfície (Ávila *et al*., 2017). Essa camada não é resistente a pH baixo (Ávila *et al*., 2017) como o pH do peróxido de hidrogênio, assim deixando a superfície livre para que ocorra a remineralização pela saliva (Ávila *et al*., 2017). No entanto, o natrosol é capaz de formar complexos com íons cálcio e fosfato (Vissink *et al*., 1985), tornando esses elementos indisponíveis para remineralização (Amaechi & Higham 2001). Também, as mudanças no fluxo salivar são acompanhadas por mudanças na concentração de cálcio e fosfato, conforme discutido acima, resultando em menor capacidade de remineralização. A associação desses dois fatores poderia explicar os resultados encontrados para esse espessante em baixo fluxo.

Já o Aristoflex apresentou os melhores resultados em todas as análises realizadas para a condição de fluxo salivar reduzido, além de não apresentar diferenças entre os fluxos salivares (Tabelas 4, 5, 6 e Figura 1). As imagens de MEV confirmam esse resultado porque o grupo aristoflex foi o único gel clareador que não mostrou diferenças entre os fluxos salivares (Figura 1G e 1H). Além das características deste gel que podem ter resultado em pequenas alterações superficiais após o clareamento, esses resultados podem estar relacionados à boa interação desse gel com a superfície dentária. Diferentemente dos outros espessantes avaliados neste estudo, o aristoflex forma ligações que não são fortes o suficiente ou

resistentes ao desafio ácido (Avila *et al*., 2017), permitindo que a superfície dentária esteja livre para remineralização salivar (Gouveia *et al*., 2019). Além disso, a combinação de aristoflex e flúor tende a reduzir a perda de dureza da superfície (Ávila *et al*., 2017). Como uma molécula carregada positiva, o aristoflex tem a capacidade de atrair íons fluoreto para interagir com seus locais positivos, auxiliando na interação benéfica total do fluoreto com a superfície do esmalte (Ávila *et al*., 2017). Devido a essa propriedade, apenas a presença de íons saliva e fluoreto, independentemente da quantidade, foi suficiente para promover os efeitos de remineralização do esmalte.

Visando a recuperação do esmalte, o uso de fluoretos e agentes remineralizantes na forma de dentifrícios também tem sido recomendado após a realização do clareamento dental (Wiegand *et al*., 2007). Observou-se nesse estudo que para a condição de fluxo salivar normal o uso de tais produtos seriam eficazes apenas se o agente ativo promovesse uma remineralização adicional ao promovido pela saliva, uma vez que apenas a presença da saliva sob fluxo normal foi capaz de promover a recuperação dos valores de microdureza após o clareamento e diminuir os valores de rugosidade. Dessa maneira, os únicos dentifrícios que forneceram proteção adicional ao oferecido pela saliva no fluxo salivar normal foram o SnF_2 e o F/Vidro Bioativo (Tabelas 3, 4, 7 e Figura 2).

Para o fluxo salivar reduzido, o uso de fluoretos e agentes remineralizantes pode ser uma opção na tentativa de recuperação do esmalte clareado devido à baixa ação da saliva nesses casos. Um fator que deve ser levado em consideração é se a ação do dentifrício é influenciada pelo fluxo salivar. Vemos que o NaF no baixo fluxo foi o único dentifrício que diferiu do fluxo salivar normal após o período *in situ* para as análises de microdureza de superfície (Tabela 3) e rugosidade (Tabela 4). Isso mostra que a ação desse dentifrício foi diminuída devido ao fluxo salivar. Esse resultado provavelmente está associado ao fato de que o efeito do NaF aparentemente é modulado pela quantidade de saliva (Scaramucci *et al.,* 2013). A eficácia de produtos contendo fluoreto de sódio é baseada na formação de partículas semelhantes a CaF_2 na superfície. Esses precipitados parecem ser mais estáveis devido ao impacto da saliva e da película adquirida (Ganss *et al*., 2007).

O dentifrício a base de estanho promoveu os maiores valores de microdureza e foi estatisticamente diferente do grupo placebo para o fluxo salivar normal (Tabela 3). Esse resultado pode estar ligado ao fato de que o SnF_2 pode interagir e ser incorporado ao esmalte erodido (Schlueter *et al*., 2009), formando sais com o cálcio e

o fosfato (Schlueter *et al*., 2013). Além disso, esse dentifrício apresentou os maiores valores de Ca%, P% e Na% e diferiu do grupo controle (Tabela 7). Entretanto o estanho apresentou os maiores valores de rugosidade após o período *in situ* entre todos os dentifrícios avaliados para ambos os fluxos salivares. Apesar disso, as imagens de MEV mostram que o SnF_2 apresenta irregularidades superficiais, porém não apresentam desmineralizações profundas ou o padrão de desmineralização dos cristais de hidroxiapatita conforme apresentado no grupo placebo (Figuras 2E e 2F). Acredita-se que esse achado pode estar relacionado com a capacidade de deposição do estanho sobre os tecidos dentais, formando uma camada mineral sobre o esmalte (Gans *et al*., 2010), resultado nos altos valores de rugosidade.

Os resultados encontrados para o SnF_2 no fluxo salivar reduzido podem estar associados ao fato de que o íon estanho pode se ligar com as proteínas da película adquirida (Veeregowda *et al*., 2011). Assim, a interação desse componente com a película adquirida e sua ação remineralizante foi suficiente para compensar a diminuição do fluxo salivar nos resultados de microdureza de superfície. Além disso, não foram encontradas diferenças entre os fluxos salivares nas análises de Ca%, Na% e Ca/P (Tabela 7). Entretanto, apesar de não apresentar diferença entre os fluxos salivares nas imagens de MEV (Figuras 2E e 2F), o estanho também demonstrou os maiores valores de rugosidade após o período *in situ* para o fluxo reduzido (Tabela 4). Da mesma maneira que no fluxo salivar normal, o alto valor de rugosidade para o fluxo salivar reduzido também pode estar relacionado com a capacidade de deposição do estanho sobre os tecidos dentais, formando uma camada mineral sobre o esmalte (Ganss *et al.,* 2010).

Em relação a efetividade do dentifrício a base de F/Sn/quitosana para o fluxo salivar reduzido, os resultados encontrados eram esperados devido a conhecida interação da quitosana com a saliva (Tabelas 3, 4, 7 e Figura 2). A quitosana é um polímero natural capaz de formar multicamadas na superfície dental (Ganss *et al*., 2011) através da sua ligação com as proteínas da saliva (Keegan *et al*., 2012), flúor (Lussi *et al*., 2015) e a outros íons presentes na superfície do esmalte. Ainda, a quitosana poder ser adsorvida em proteínas da película salivar (van der Mei *et al*., 2007). Na presença de mucina, a quitosana e a mucina interagem para formar multicamadas firmemente ligadas (Dedinaite *et al*., 2005). Dessa maneira, a presença da saliva, independente do fluxo, e a interação da quitosana podem ter favorecido o processo de remineralização dental, justificando os resultados encontrados para o

fluxo salivar reduzido (Tabelas 3, 4, 7 e Figura 2). Além disso, o dentifrício a base de F/Sn/Quitosana no fluxo reduzido também não diferiu do fluxo normal após o período *in situ* para as análises de rugosidade, Ca%, P%, Na% e Ca/P (Tabela 7). Ainda, para a análise de rugosidade o grupo F/Sn/Quitosana apresentou o segundo menor valor de rugosidade entre os dentifrícios avaliados (Tabela 4).

Já o F/Vidro Bioativo teve uma ação de destaque para ambos os fluxos salivares, pois além de recuperar os valores de microdureza após o período *in situ* e apresentar altos valores de microdureza (Tabela 3), esse grupo apresentou os menores valores de rugosidade após o período *in situ* entre todos os dentifrícios avaliados (Tabela 4). As imagens de MEV confirmam esse resultado pois é possível identificar uma superfície lisa, polida, regular e com menor desmineralização do que o grupo controle e do que todos os dentifrícios avaliados, sem diferença entre os fluxos salivares (Figuras 2K e 2L). Esse dentifrício também apresentou os maiores valores de Ca%, P% e Na% e diferiu do grupo controle para ambos os fluxos salivares (Tabela 7). Esses resultados estão de acordo com estudos anteriores que mostram o potencial do vidro bioativo em diminuir o efeito desmineralizador e aumentar a remineralização após o clareamento dental (Gjorgievska & Nicholson 2011; Vieira Junior et al., 2016,2018).

Um fator muito importante que deve ser levado em consideração é que o dentifrício a base de vidro bioativo também mostrou não depender dos constituintes da saliva para exercer sua ação no esmalte após o clareamento dental. Isso está relacionado ao fato de que o vidro bioativo é um material cerâmico que consiste em sódio-cálcio-fosfossilicato amorfo que é altamente reativo na água (Du *et al*., 2004; Jennings *et al*., 2004). Esse fator pode explicar a ausência de diferenças entre os fluxos salivares, porque a presença de água é suficiente para que o vidro bioativo possa exercer a sua ação remineralizante. No ambiente bucal aquoso, os íons sódio das partículas de vidro bioativo liberam íons cálcio e fosfato do vidro, o que resulta em aumento do pH (Andersson & Kangasniemi 1991; Hench & Andersson 1993). Esse aumento no pH ajuda a precipitar os íons extras de cálcio e fosfato fornecidos pelo vidro bioativo para formar uma camada de fosfato de cálcio que cristaliza em hidroxiapatita enriquecida em carbonato (Zhong *et al*., 2002). A combinação das partículas de vidro bioativas residuais e a nova camada de hidroxiapatita enriquecida com carbonato resulta na remineralização da superfície do esmalte (Gjorgievska & Nicholson 2010).

Baseado nos resultados encontrados nesse estudo, ficou evidente que a alteração do fluxo salivar é capaz de modular a ação dos espessantes e detifrícios sobre o esmalte dental, além de interferir na sua proteção e remineralização durante e após o clareamento. Assim, o tratamento clareador indicado para o fluxo salivar normal, o qual resulta em menor perda mineral e alterações superficiais é o peróxido de carbamida 10% associado ao natrosol ou aristoflex. Já para a condição de fluxo salivar reduzido o tratamento clareador indicado é o peróxido de carbamida 10% quando associado ao aristoflex. Quando objetivamos o tratamento e a recuperação do esmalte dental após o clareamento com peróxido de hidrogênio a 35% a utilização de dentifrício com vidro bioativo é o tratamento mais indicado para ambos os fluxos salivares.

4 CONCLUSÃO

Estudo 1: Os espessantes e o fluxo salivar interferiram nas alterações da superfície após o clareamento dental. O clareamento com gel de carbopol promoveu as maiores alterações superficiais para os dois fluxos salivares. Para fluxo salivar normal, os géis clareadores com aristoflex e natrosol apresentaram os melhores resultados para todas as análises realizadas. Sob condição de fluxo salivar reduzido, o clareador à base de aristoflex mostrou menores alterações morfológicas no esmalte.

Estudo 2: O fluxo salivar e os dentifrícios com diferentes princípios ativos interferem na reminerealização do esmalte após o tratamento clareador. O fluxo salivar pode influenciar a ação dos dentifrícios. O baixo fluxo salivar tem uma menor capacidade de remineralização do esmalte clareado em comparação com o fluxo salivar normal quando um dentifrício à base de placebo ou NaF é usado. O dentifrício à base vidro bioativo teve o melhor desempenho na recuperação dos efeitos deletérios no esmalte após o clareamento dental para ambos os fluxos salivares.

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APÊNDICE 1 - METODOLOGIA ILUSTRADA

- Artigo 1: Bleaching gel formulated with aristoflex shows less surface changes on enamel at low salivary flow in situ condition.

Delineamento Experimental

O estudo foi conduzido em 1 fase experimental de 15 dias. Foram selecionados 28 voluntários para participar do estudo (14 em condição salivar normal e 14 que apresentem hipossalivação).

Fatores em estudo

- Fluxo salivar (2 níveis): fluxo salivar normal e fluxo salivar reduzido

- Gel clareador (4 níveis): sem clareamento (controle positivo), clareamento com gel comercial à base de peróxido de carbamida associado ao carbopol (controle negativo), clareamento com gel experimental à base de peróxido de carbamida associado ao natrosol e clareamento com gel experimental à base de peróxido de carbamida associado ao aristoflex.

Variável de resposta: Microdureza knoop superficial (SMH), rugosidade superficial (Ra), análise de cor pela espectrofotometria de reflectância (ΔE^*_{ab} e ΔE_{00}) e espectroscopia de energia dispersiva por raio-X (EDS) e microscopia eletrônica de varredura (MEV).

Local de Realização da Pesquisa e Voluntários

Este estudo foi realizado em parceria com o Centro para Diagnóstico e Tratamento das Lesões Orais (Orocentro) da Faculdade de Odontologia de Piracicaba (FOP/Unicamp). O projeto foi aprovado pelo Comitê de Ética em Pesquisa da Universidade (CAAE 96044418.8.00005418). Todos os voluntários assinaram um Termo de consentimento livre e esclarecido (TCLE) antes da realização da triagem. Foram selecionados 28 voluntários, os quais foram divididos em dois grupos: 14

voluntários com baixo fluxo salivar (7 homens e 7 mulheres) e 14 voluntários com fluxo salivar normal (7 homens e 7 mulheres).

Os voluntários com fluxo salivar reduzidos convidados a participar do estudo foram pacientes que concluíram a radioterapia na região de cabeça e pescoço há no mínimo 24 meses, e que apresentam fluxo salivar reduzido. O critério de inclusão para esses participantes foi a redução do fluxo salivar <0,8 mL/min no fluxo estimulado (Pini *et al.,* 2018). Para os demais participantes com taxa de fluxo normal (25 a 35 anos), o critério de inclusão foi taxa de fluxo estimulada > 1,0 mL/min (Pini et al., 2018).

Os outros critérios de inclusão que foram adotados para pacientes com fluxo reduzido e também fluxo normal foram: ausência de lesões de cáries ativas, doença periodontal ou erosão dentária; sem uso de aparelho ortodôntico, boa saúde geral e bucal, apresentar no mínimo 50% dos seus dentes da arcada superior (8 dentes) para fixação do dispositivo palatino, não fumantes, não gravidez ou amamentação, e nenhum uso de antibiótico 2 meses antes do estudo. Nenhum sujeito de ambos os grupos poderia estar tomando medicamentos que possam causar uma redução do fluxo salivar.

Obtenção e preparo dos espécimes

As amostras foram obtidas a partir de dentes bovinos que, após a coleta, foram armazenados em solução aquosa de timol a 0,1% (Proderma, Piracicaba, São Paulo, Brasil). Os debris foram manualmente removidos com lâmina de bisturi e os dentes foram polidos com taça de borracha (KG Sorensen, Barueri, SP, Brasil) e pasta de pedra-pomes (SS White; Rio de Janeiro, RJ, Brasil) e água. Após isso, os dentes foram armazenados em água destilada até a sua utilização. Foi realizada a separação da coroa da porção radicular, com disco de diamante dupla face (KG Sorensen, Barueri, SP, Brasil) sob constante irrigação de jato de água em micromotor de baixa rotação (Dabi Atlante; Ribeirão Preto, SP, Brasil).

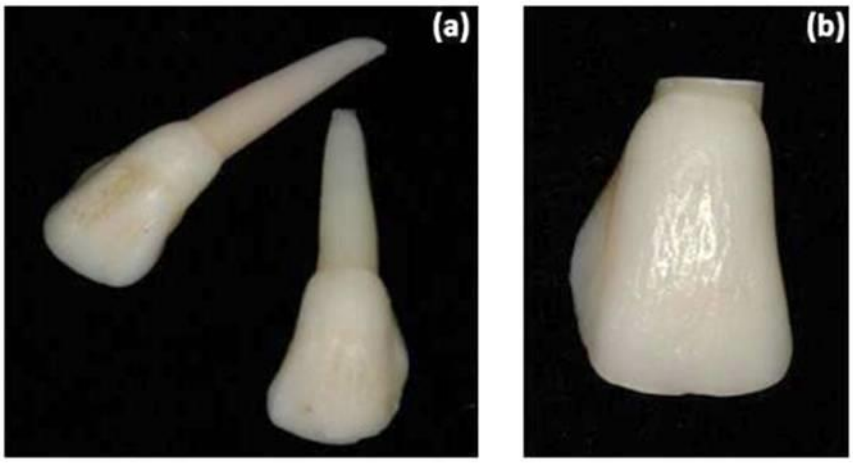

Figura 1: Incisivos bovinos utilizados no estudo: (a) dentes selecionados após limpeza e desinfecção; (b) porção coronária após a separação da porção radicular.

Em seguida, foram feitos outros cortes na porção coronária, nos sentidos mésio-distal e inciso-cervical em uma cortadeira metalográfica (Isomet 1000, Buehler, Illinois, USA), com disco diamantado de alta concentração (4" × 012 × ½, Buehler, Illinois, USA) para a obtenção dos fragmentos de esmalte e de dentina, respectivamente, com área de superfície de 16 mm^2 (4x4mm).

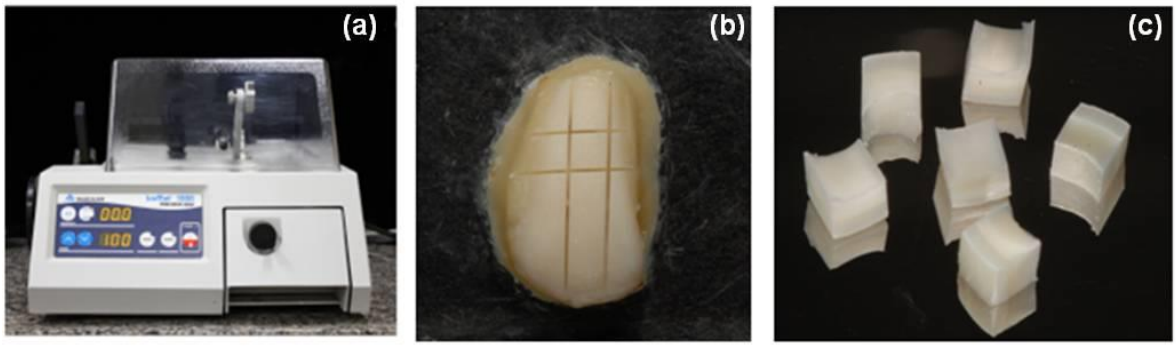

Figura 2: Obtenção dos blocos de esmalte/dentina: (a) Cortadeira Metalográfica de alta precisão; (b) cortes nos sentidos mésio-distal e inciso-cervical com distâncias de 4 mm; (c) bloco esmalte/dentina ($16mm^2$).

Para planificação e polimento das amostras, cada fragmento foi fixado em disco de acrílico com cera pegajosa de maneira que a superfície teste permaneceu paralela

a superfície do disco de acrílico. A superfície dos blocos foram planificadas com lixas de carbeto de silício (Sic), de granulação 500-, 1000-, e 2000-SiC (Buehler, Illinois, USA) sob irrigação constante, utilizando-se uma politriz giratória (Aropol E, Arotec, Cotia; SP, Brasil).

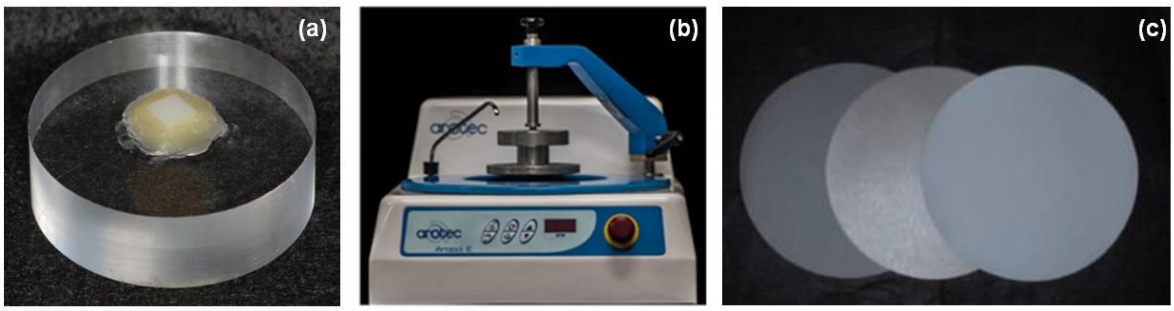

Figura 3: Planificação dos blocos: (a) bloco fixado com cera pegajosa em stub de acrilico; (b) politriz giratoria; (c) lixas de granulação 500-, 1000-, e 2000-SiC.

Por fim, realizou-se um polimento das superfícies a serem analisadas com feltros (TCT, TWI, FVC – Arotec, Cotia; SP, Brasil), associados a pastas diamantadas de granulação decrescente (6 µm, 3 µm e ¼ µm). Entre cada aplicação de lixa e feltro e ao final do polimento, as amostras foram lavadas em ultrassom (Marconi, Piracicaba, São Paulo – Brasil), por 15 minutos. Ao final, os blocos de esmalte apresentaram 2mm de espessura, sendo 1mm de espessura de esmalte e 1mm de dentina.

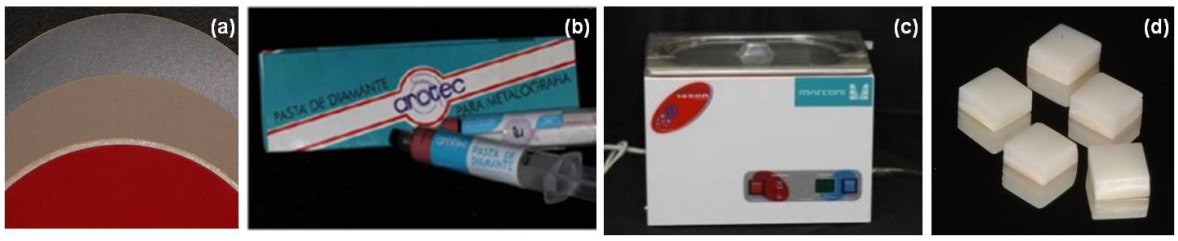

Figura 4: Polimento dos blocos: (a) discos de feltro TCT, TWI, FVC; (b) pasta diamantada; (c) cuba ultrassônica (d) blocos planificados e polidos.

Confecção dos dispositivos intrabucais

Modelos de gesso (ASFER, São Caetano do Sul, SP, Brasil) foram obtidos a partir dos moldes de alginato (Jeltrate – Dentsply, Petrópolis, RJ, Brasil) dos voluntários

para a confecção dos dispositivos intrabucais palatinos em resina acrílica (VIPI, Pirassununga, SP, Brasil). Cada dispositivo continha 4 linhas com nichos para adaptação de 8 espécimes em cada linha (4 espécimes de 4x4x2mm para as análises de cor, rugosidade e EDS e 4 espécimes de dimensões de 2x2x2mm para a análise de microdureza de superfície). Os espécimes foram posicionados horizontalmente, sendo que os espécimes de cada grupo foram posicionados em uma única linha. Cada dispositivo palatino continha 4 linhas com 8 espécimes em cada linha. Dentro do mesmo grupo os espécimes foram posicionados de maneira mais próxima possível, já para os espécimes de grupos diferentes foi deixado uma distância mínima de 5mm entre as linhas para evitar o contato do produto que foi utilizado em cada grupo. Os espécimes foram fixados no dispositivo com cera pegajosa (ASFER, São Caetano do Sul, SP, Brasil) de modo que sua superfície ficou no mesmo nível da resina acrílica. Ainda, para facilitar a aplicação do gel pelo paciente, os grupos foram divididos em cores diferentes e cada linha de espécime teve uma marcação colorida do grupo correspondente.

Procedimentos durante o período intrabucal / clareamento dos espécimes

Foi realizado 1 ciclo de 15 dias. Primeiramente o dispositivo permaneceu na boca do voluntário por 24 horas para a formação da película adquirida. O clareamento foi realizado durante os 14 dias subsequentes com aplicações de 4 horas diárias da seguinte forma: os dispositivos foram removidos da boca dos voluntários e secos com papel absorvente. O dispositivo palatino foi posicionado sobre o modelo de gesso da arcada superior do voluntário para que o mesmo se mantivesse estável durante a realização do tratamento. Os espécimes contidos em cada um dos dispositivos palatinos foram submetidos à aplicação de gel (de acordo com o grupo experimental), enquanto que os espécimes do grupo controle não receberam tratamento (sem clareamento). Os espécimes foram poscionados no dispositivo palatino de acordo com o grupo e sinalizados com a cor correspondente. A seringa do gel também estava devidamente adesivada com a cor do grupo para que não houvesse confusão na hora da aplicação do gel clareador. Os voluntários foram instruídos sobre manuseio e aplicação de gel, que foram aplicados e permaneceram em contato com os espécimes por 4 horas à temperatura ambiente. Além da instrução verbal dos voluntários, os mesmos receberam todo o procedimento por escrito. Foi disponibilizado ao voluntário

o telefone da pesquisadora responsável para que qualquer dúvida fosse sanada na hora da realização do clareamento dos espécimes. Após cada sessão, o gel foi removido com hastes flexíveis de algodão, os espécimes foram lavados com água, secos com papel absorvente e os dispositivos retornados para a boca dos voluntários.

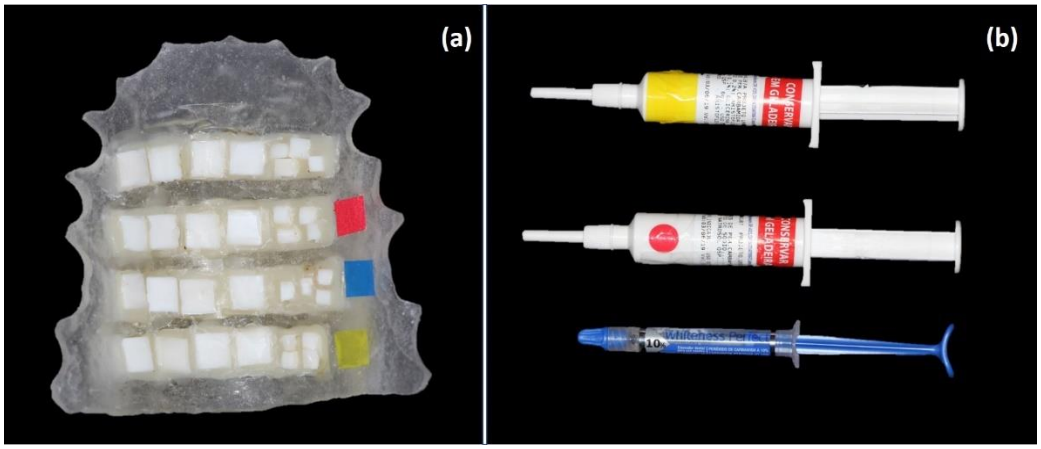

Figura 5: (a) Posicionamento dos espécimes no dispositivo palatino; (b) seringas de gel clareador identificadas por cor.

- Artigo 2: *Bioactive glass dentifrice presents best performance on remineralization of bleached enamel under low and normal salivary flow in situ condition.*

Delineamento Experimental

O estudo foi conduzido em 6 fases experimentais de 24 h. Foram selecionados 24 voluntários para participar do estudo (12 em condição salivar normal e 12 que apresentem hipossalivação).

Fatores em estudo

- Fluxo salivar (2 níveis): fluxo salivar normal e fluxo salivar reduzido

- Tempo: para a análise de microdureza de superfície e rugosidade: baseline (T1), após o clareamento dental (T2) e após o período *in siu* (T3).

- Dentifrícios (6 níveis): sem escovação (controle positivo), e 5 dentifrícios com diferentes princípios ativos: placebo, NaF, SnF_2, F/Sn/Quitosana, F/Arginina and F/Vidro Bioativo.

Variável de resposta: Microdureza knoop superficial (SMH), rugosidade superficial (Ra), análise de cor pela espectrofotometria de reflectância (ΔL (L*final- L*inicial), Δa (a*final- a*inicial), Δb (b*final- b*inicial), ΔE^*_{ab} e ΔE_{00}) e espectroscopia de energia dispersiva por raio-X (EDS) e microscopia eletrônica de varredura (MEV).

Local de Realização da Pesquisa e Voluntários

Como o estudo 1, essa pesquisa também foi realizada em parceria com o Orocentro. Os mesmos voluntários selecionados para o estudo 1 foram convidados a participar do estudo 2. Esse estudo também foi aprovado pelo Comitê de Ética em Pesquisa dessa universidade (CAAE 96037118.1.0000.5418).

Obtenção e preparo dos espécimes

A confecção dos espécimes foi a mesma descrita para o estudo 1.

Dispositivos Intrabucais

A confecção dos dispositivos intrabucais foi a mesma descrita para o estudo 1, porém cada dispositivo teve espaço para que fossem fixados 8 espécimes (4 espécimes para as análises de cor, rugosidade e EDS; e 4 para a análise de microdureza de superfície).

Procedimentos prévios ao início da fase experimental

Foi determinado que os voluntários deveriam utilizar para a sua higiene bucal um dentifrício placebo para evitar que houvesse interferência do flúor residual da saliva na remineralização dos espécimes do dispositivo palatino. Foi liberado também o uso de fio dental. O uso de outros produtos de higiene bucal não foi permitido durante o período experimental. O período total do estudo foi de 6 fases de 24 horas. Em cada fase, um dentifrício diferente foi usado pelo voluntário.

Clareamento dos espécimes

O clareamento dental foi realizado fora da boca, apenas nos blocos dentais bovinos, com um gel a base de peróxido de hidrogênio a 35% (Whiteness HP, FGM, Joinville, Brasil), de acordo com as instruções do fabricante. O gel foi aplicado na superfície do esmalte três vezes por 15 min cada, totalizando 45 min. Após o término do clareamento dental foi realizada a análise de cor, microdureza de superfície e rugosidade e em seguida iniciou-se o período experimental *in situ*.

Procedimentos durante o período *in situ*

Imediatamente após a análise de cor, microdureza de superfície e rugosidade pós-clareamento *in vitro*, os dispositivos foram introduzidos na cavidade bucal dos voluntários e permaneceram em posição pelo tempo de 24 h. O dispositivo só poderia ser removido no momento das refeições, no qual deveria ser acomodado sobre gaze úmida (Rios *et al.,* 2008).

O tratamento dos espécimes com o dentifrício foi realizado 2 vezes (imediatamente após a inserção do dispositivo em boca e 12 horas após a primeira escovação). Para escovar os dentes e os espécimes durante o período experimental, os voluntários utilizaram escova dental extra macia. Foi fornecido aos voluntários 1,5g de dentifrício para a realização da escovação (Pini et al., 2018). Com o dispositivo intrabucal em posição, inicialmente, os voluntários iniciaram a escovação sobre a superfície vestibular dos seus próprios dentes, por 25 s, para produzir a suspensão à base de dentifrício e saliva. Em seguida, os voluntários permitiram o contato dos espécimes com a suspensão (dentifrício e saliva) por 2 min e então, removeram o dispositivo para realizar a escovação nos espécimes por 20s, simulando o hábito de escovação (Strenhagen et al., 2014; Pini et al., 2018). Finalizando o tratamento dos espécimes, o dispositivo foi lavado com água corrente por 1 minuto e reinserido na boca. Os voluntários foram corretamente instruídos em relação a escovação e orientados a não exercer pressão sobre a amostra.

Todos os participantes foram treinados para os procedimentos, em especial para a escovação dos espécimes, a fim de que fosse obtido o melhor nível de padronização do estudo. Além disso, os participantes receberam instruções escritas, com a descrição e os horários dos tratamentos que eles deveriam realizar. Eles

também foram instruídos a utilizar cronômetros para a realização dos tratamentos. No início de cada fase experimental, os voluntários receberam seu dispositivo intrabucal acompanhado do dentifrício e escova dental os quais eles iriam utilizar.

Ao final das 24 h de utilização do dispositivo intrabucal, foram realizadas as análises em cada espécime.

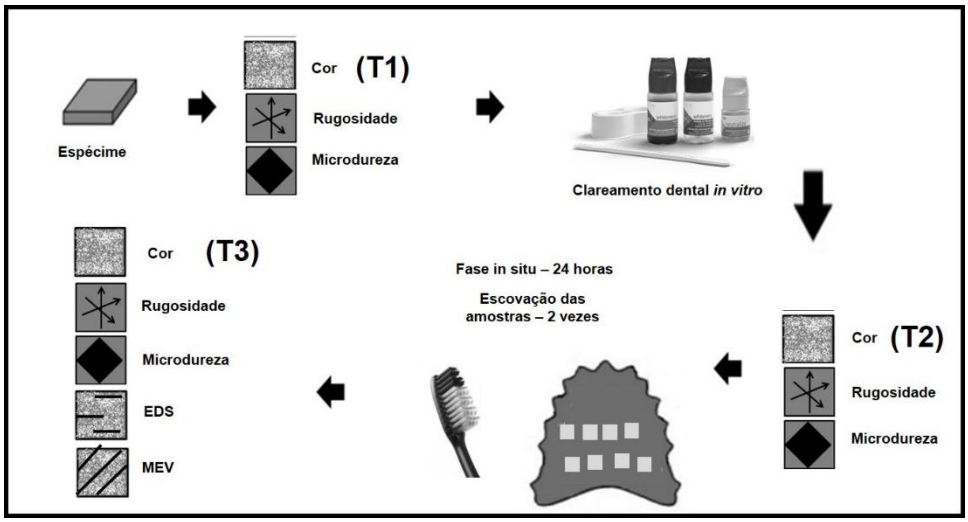

Figura 6: Delineamento experimental do estudo 2. (T1) análises iniciais, (T2) análises após o clareamento, (T3) análises após o perído *in situ*.

- Análises: comuns ao Estudo 1 e Estudo 2

Análise Salivar

Os voluntários foram instruídos a mastigar um pedaço (1g) de parafilme (Parafilm M, Pechiney Plastic Packaging Company, Chicago III, EUA). A saliva produzida nos primeiros 30 s foi descartada e, durante os 5 minutos seguintes, o voluntário foi instruído a dispensar a saliva produzida em um tubo de ensaio milimetrado. As coletas foram realizadas entre as 9:00 e as 11:00 ou entre as 14:00 e as 16:00, e também devem ocorrer pelo menos 1 hora após a refeição e após a higiene bucal para minimizar os efeitos da variabilidade diária na composição salivar Imediatamente após as coletas, o fluxo salivar foi calculado dividindo-se o volume de saliva (considerando que 1 ml corresponde a 1 g) pelo tempo de coleta.

As análises de pH e capacidade tampão da saliva foram realizadas em um peagâmetro (Orion 290A+, SP, Brasil) calibrado para os padrões de pH 7,0 e 4,0. Para a análise de capacidade tampão da saliva, 0,5 mL de saliva estimulada foi adicionada a 1,5 mL de HCl 0,005 M em um tubo plástico, que após misturados, foram mantidos em repouso por 5 minutos para a liberação de CO2 oriundo da reação do tampão de bicarbonato da saliva com o ácido. Feito isso, o pH dessa mistura foi determinado.

Microdureza de superfície

Foram realizadas 5 endentações a 100 µm de distância na região central do bloco dental utilizando o microdurômetro HMV-2000, Shimadzu, Tokyo, Japan com penetrador tipo Knoop, com carga de 50 g por 5 s.

Rugosidade

Para a análise de rugosidade foi utilizado um rugosímetro (Surf-Corder 1700, Kosaka, Tóquio, Japão). Para padronizar a posição da amostra foi feito uma marcação pontual na lateral do espécime com uma ponta diamantada esférica nº 1011 (Figura 7a). Dessa maneira, a primeira leitura da rugosidade foi feita com a marcação posicionada em direção ao operador (0°). As duas seguintes leituras foram feitas girando a amostra no sentido horário nas posições de 120º e 240° respectivamente. Para que a amostra fosse posicionada na angulação correta, no stub acrílico onde as amostras foram fixadas foi realizado uma marcação com referente as devidas angulações (Figura 7b). A leitura foi realizada com cut-off de 0,25 mm, comprimento de leitura de 1,25 mm e velocidade de 0,1 mm/s.

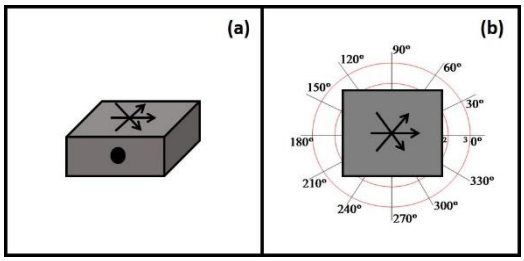

Figura 7: (a) Marcação na lateral do espécime feita com ponta diamantada esférica 1011, (b) Posicionamento do espécime para as leituras de rugosidade.

Análise de cor

Do mesmo modo que para a análise de rugosidade, a marcação esférica feita na lateral do bloco serviu como um guia para que fosse padronizado a posição da leitura da cor. As amostras foram colocadas em um dispositivo de teflon (porta amostra) com a marcação virada para o operador. O porta amostra foi posicionado dentro de uma câmara de luz (GTI Mini Matcher MM1e, GTI Graphic Technology Inc., Newburgh, NY, USA) para que fosse obtido a padronização da luz do ambiente em que foram realizadas as leituras (Figura 8). O equipamento utilizado foi um espectrofotômetro Konica Minolta CM-700d (Figura 8). O CM-700d é um espectrofotômetro portátil, desenhado para avaliar a cor de várias amostras de tamanho pequeno a grande, incluindo objetos com superfícies planas, com formas ou curvas. Este espectrofotômetro é um equipamento confiável e de alta precisão. A calibração do equipamento foi feita segunda as recomendações do fabricante. Com o equipamento posicionado sobre a amostra foram realizadas três leituras de cor. Os valores obtidos foram quantificados em três coordenadas do Sistema CIEL*a*b*. Cada amostra teve três valores de leitura, assim foi calculado a média desses valores para que cada amostra obtivesse um valor representativo de cada coordenada (L*, a* e b*).

As diferenças nos valores L*, a*, e b* entre a leitura inicial e final foram expressas em ΔL (L*final- L*inicial), Δa (a*final- a*inicial), Δb (b*final- b*inicial). ΔL corresponde a diferença entre mais claro e escuro (+ = mais claro, - = mais escuro), Δa é a diferença em vermelho e verde (+ = mais vermelho, - = mais verde) e Δb a diferença em amarelo e azul (+ = mais amarelo, - = mais azul). A mudança de cor geral foi calculada usando a seguinte equação: $\Delta E^*_{ab} = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$.

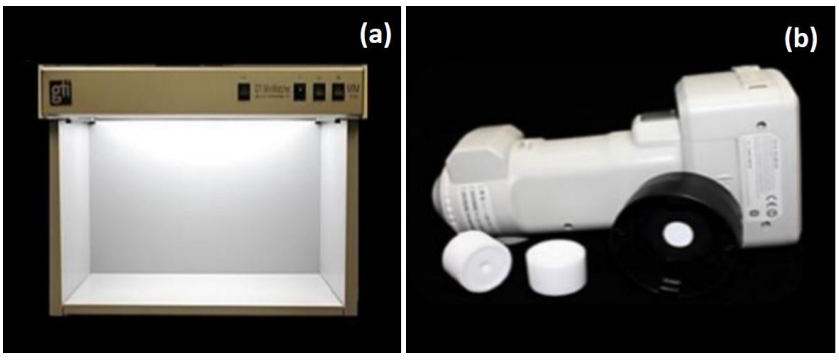

Figura 8: a) Cabine de luz GTI Mini Matcher MM1e; b) Espectrofotômetro Konica Minolta CM-700d.

Microscopia eletrônica de varredura (MEV)

Foram selecionadas aleatoriamente 4 amostras representativas de cada grupo para a avaliação em microscopia eletrônica de varredura (MEV). Os espécimes foram desidratados em graus crescentes de etanol. Para isso as soluções foram confeccionadas nas concentrações de 50%, 60%, 70%, 80%, 90% e 100%. As amostras ficaram imersas em cada solução pelo tempo de 20 minutos. Após isso, foi realizado a imersão por 60 minutos em etanol a 100%. Finalizando, os spécimes foram secos à temperatura ambiente durante 12 horas. Após o correto preparo dos espécimes, os mesmos foram fixados em stubs acrílicos e foram submetidos a vácuo em um pulverizador catódico (SCD 050, Balzers Union Aktiengesellschaft, Balzers, Liechtenstein) para depositar uma fina camada de ouro, equivalente a 10 6 mm, para aumentar a reflectância da superfície. Em seguida, imagens com magnitude de 4000x de áreas representativas foram obtidas usando um microscópio eletrônico de varredura (Figura 9) (JSM-5600LV, JEOL, Tóquio, Japão).

Figura 9: Microscópio eletrônico de varredura JSM-5600LV.

Espectroscopia de energia dispersiva por Raios-X (EDS)

Foi utilizado o microscópio Vantage (Aquisition Engine Company, Tóquio) com o software Easymicro Voyager digital, versão 5.2. As amostras foram submetidas em vácuo à pulverização catódica (Delton Vaccum, Desk II, Moorestown, NJ, EUA), para cobertura do espécime com uma fina camada de carbono. Então, a avaliação do

conteúdo inorgânico presente nos substratos foi realizada pelo microscópio eletrônico de varredura (Jeol, JSM5600LV, Tóquio, Japão), com energias típicas de ordem 15kV, em aumento de 100x, com PHA *deadtime* variando entre 20 e 25%. A análise foi feita no esmalte dental, em cinco regiões por espécime selecionadas aleatoriamente, para determinar a presença elementar de P e Ca. Os níveis elementares (% em peso) de Ca e P e a proporção entre Ca e P foram determinados.

ANEXOS

ANEXO 1– Certificado de aprovação no comitê de ética Estudo 1

COMITÊ DE ÉTICA EM PESQUISA
FACULDADE DE ODONTOLOGIA DE PIRACICABA
UNIVERSIDADE ESTADUAL DE CAMPINAS

CERTIFICADO

O Comitê de Ética em Pesquisa da FOP-UNICAMP certifica que o projeto de pesquisa "Avaliação de gel clareador contendo natrosol em condições de hipossalivação", CAAE 96044418.8.0000.5418, dos pesquisadores Débora Alves Nunes Leite Lima, Laura Nobre Ferraz, Alan Roger dos Santos Silva e Ajudarte Lopes, satisfaz as exigências das resoluções específicas sobre ética em pesquisa com seres humanos do Conselho Nacional de Saúde – Ministério da Saúde e foi aprovado por este comitê em 11/09/2018.

The Research Ethics Committee of the Piracicaba Dental School of the University of Campinas (FOP-UNICAMP) certifies that research project "*Evaluation of bleaching gel containing natrosol under hyposalivation conditions*", CAAE 96044418.8.0000.5418, of the researcher's Débora Alves Nunes Leite Lima, Laura Nobre Ferraz, Alan Roger dos Santos Silva and Ajudarte Lopes, meets the requirements of the specific resolutions on ethics in research with human beings of the National Health Council - Ministry of Health, and was approved by this committee on September, 11 2018.

Profa. Fernanda Miori Pascon
Vice Coordenador
CEP/FOP/UNICAMP

Prof. Jacks Jorge Junior
Coordenador
CEP/FOP/UNICAMP

Nota: O título do protocolo e a lista de autores aparecem como fornecidos pelos pesquisadores, sem qualquer edição.
Notice: The title and the list of researchers of the project appears as provided by the authors, without editing.

ANEXO 2 – Certificado de aprovação no comitê de ética Estudo 2

COMITÊ DE ÉTICA EM PESQUISA
FACULDADE DE ODONTOLOGIA DE PIRACICABA
UNIVERSIDADE ESTADUAL DE CAMPINAS

CERTIFICADO

O Comitê de Ética em Pesquisa da FOP-UNICAMP certifica que o projeto de pesquisa "Avaliação do potencial remineralizador de diferentes dentifrícios após clareamento em condições de hipossalivação", CAAE 96037118.1.0000.5418, dos pesquisadores Débora Alves Nunes Leite Lima, Laura Nobre Ferraz, Alan Roger dos Santos Silva e Ajudarte Lopes, satisfaz as exigências das resoluções específicas sobre ética em pesquisa com seres humanos do Conselho Nacional de Saúde – Ministério da Saúde e foi aprovado por este comitê em 11/09/2018.

The Research Ethics Committee of the Piracicaba Dental School of the University of Campinas (FOP-UNICAMP) certifies that research project "Evaluation of the potential remineralization of different dentifrices after bleaching under hyposalivation conditions", CAAE 96037118.1.0000.5418, of the researcher's Débora Alves Nunes Leite Lima, Laura Nobre Ferraz, Alan Roger dos Santos Silva and Ajudarte Lopes, meets the requirements of the specific resolutions on ethics in research with human beings of the National Health Council - Ministry of Health, and was approved by this committee on September, 11 2018.

Profa. Fernanda Miori Pascon
Vice Coordenador
CEP/FOP/UNICAMP

Prof. Jacks Jorge Junior
Coordenador
CEP/FOP/UNICAMP

Nota: O título do protocolo e a lista de autores aparecem como fornecidos pelos pesquisadores, sem qualquer edição.
Notice: The title and the list of researchers of the project appears as provided by the authors, without editing.

ANEXO 3 – Verificação de originalidade e prevenção de plágio

Tese Doutorado

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ANEXO 4 – Comprovante de submissão no periódico Journal of Dentistry

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Manuscript Draft

Manuscript Number:

Title: Bleaching gel formulated with aristoflex showed fewer changes in enamel surface in in situ low salivary flow conditions.

Article Type: Full Length Article

Keywords: tooth bleaching; thickeners; salivar flow rate.

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Corresponding Author's Institution: Piracicaba Dental School, University of Campinas

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Abstract: Objective: The purpose of this in situ study was to evaluate the effect of bleaching gels with different thickeners on enamel under normal and hyposalivatory conditions. Materials and Methods: Twenty-eight participants were assigned of which 14 had normal and 14 had low salivary flow. For each salivary flow, 4 types of treatment were performed with different thickeners: no bleaching (negative control), commercial gel of 10% carbamide peroxide (CP) with carbopol (positive control), 10% CP with natrosol and 10% CP with aristoflex. The volunteers used a palatal appliance for 15 days and the bleaching was performed extra-orally (4h/14 days). Analyzes of microhardness (SMH), color (ΔE*ab and ΔE00), roughness (Ra), scanning electron microscopy (SEM) and Energy-Dispersive X-Ray Spectrometry (EDS) were performed. The SMH and Ra were analyzed by mixed models for repeated measures and Tukey Kramer. For color and EDS data were analyzed by Mann Whitney's nonparametric, Friedman and Nemenyi tests ($p < 0.05$). Results: Salivary flow and thickeners did not influence the results of ΔE*ab and ΔE00. Carbopol had the lowest SMH, the highest Ra and the lowest Ca% among all groups. In normal flow, natrosol and aristoflex showed higher SMH. For low flow, aristoflex presented higher SMH and in Ra natrosol and aristoflex showed lower values. In Ca% and Ca/P aristoflex showed higher value and differed from carbopol for normal flow. Conclusion: For normal flow, 10% CP with natrosol and aristoflex showed fewer surface changes, and for low flow, the best results were for 10% CP with aristoflex.
Clinical Significance: Salivary flow and thickeners influence enamel changes after bleaching. In the low salivary flow condition, the use of aristoflex thickener results in less deleterious effects on the enamel after bleaching. For normal salivary flow, both natrosol and aristoflex result in minor changes to the bleached enamel.

ANEXO 5 – Comprovante de submissão no periódico Clinical Oral Investigation

Clinical Oral Investigations

Bioactive glass dentifrice presents best performance on remineralization of bleached enamel under low and normal salivary flow in situ condition.

--Manuscript Draft--

Manuscript Number:		
Full Title:	Bioactive glass dentifrice presents best performance on remineralization of bleached enamel under low and normal salivary flow in situ condition.	
Article Type:	Original Article	
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Funding Information:	Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Finance Code 001)	Dr. Laura Nobre Ferraz
	Fundação de Amparo à Pesquisa do Estado de São Paulo (Process 2018/24446-1)	Dr. Débora Alves Nunes Leite Lima
Abstract:	Objective The purpose of this in situ study was to evaluate different dentifrices on enamel after bleaching under normal and hyposalivatory conditions. Materials and Methods Twenty-four participants were assigned of which 12 had normal and 12 had low salivary flow. The study was conducted in 6 in situ experimental phases: placebo, NaF, SnF 2 , F/Sn/Chitosan, F/Arginine and F/Bioactive Glass. The specimens were previously bleached in vitro . Microhardness (SMH), roughness and color analyses (CIELAB and ΔE 00) were performed at baseline (T1), after bleaching (T2) and after in situ phase (T3). Scanning electron microscopy (SEM) and energy-dispersive X-Ray spectrometry (EDS) were at T3. The SMH and Ra were analyzed by Tukey Kramer. The color and Na% were analyzed by ANOVA in a subdivided plots and Tukey test. The EDS were analyzed by Mann's Whitney nonparametric, Friedman and Nemenyi tests ($p < 0.05$). Results The low salivary flow had less capacity for remineralization of bleached enamel compared to normal flow. Comparing salivary flows, there was a significant difference	