UNESP - Universidade Estadual Paulista

"Júlio de Mesquita Filho"

Faculdade de Odontologia de Araraquara

Ana Claudia Pedroso de Barros

Remineralização de lesão de cárie inicial por meio da aplicação de diferentes produtos

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Orientador: Profa. Dra. Alessandra Nara de Souza Rastelli

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Dissertação para obtenção do grau de Mestre em Ciências Odontológicas

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Araraquara, 09 de maio de 2018.

DADOS CURRICULARES

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“Porque todo que é nascido de Deus vence o mundo; e esta é a vitória que vence o mundo, a nossa fé.”
I João 5:4[2]

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RESUMO

Ações preventivas têm resultado na diminuição das doenças orais infecciosas e não infecciosas, porém a prevalência de cárie permanece alta, assim como os casos de hipersensibilidade dentinária (HD) estão cada vez mais frequentes. Muitos produtos têm sido considerados na remineralização dos tecidos dentários, como aqueles que mimetizam hidroxiapatita, a base de cálcio e fosfato e flúor. O objetivo desta dissertação foi avaliar experimentalmente o efeito remineralizador de materiais bioativos (Bioglass® 45S5, Biosilicato® e F18) frente às lesões de cárie inicial (LCI) e observar o comportamento do F18 sobre hipersensibilidade dentinária em relato de caso. O estudo experimental avaliou in vitro o efeito remineralizador de materiais bioativos e produtos fluoretados sobre LCI em esmalte dental bovino. Os espécimes foram divididos em seis grupos (G1 - saliva artificial, G2 - flúor gel acidulado, G3 - verniz fluoretado, G4 - Bioglass® 45S5, G5 - Biosilicato® e G6 - F18) e tiveram sua superfície dividida em duas partes, sendo elas, esmalte hígido e desmineralizado para o grupo controle, e desmineralizado e remineralizado para os demais grupos. A indução de cárie foi feita por meio da aplicação de gel de metilcelulose e solução de ácido lático (pH=4,6) por 10 dias a 37°C. Então, foi feita a aplicação dos agentes remineralizadores e os espécimes armazenados em saliva artificial por 24 horas. Para as análises, o G1 foi considerado controle (positivo - hígido e negativo - desmineralizado), e os resultados de dureza Knoop mostraram que apenas o G2 não possuiu capacidade remineralizadora ($p>0,05$). Os demais grupos apresentaram essa capacidade, porém G5 e G6 apresentaram resultados mais expressivos ($p<0,05$). Essas informações foram confirmadas pelas imagens em MEV em aumentos de 1000 e 5000x, em que G5 e G6 apresentaram morfologia de superfície mais próxima em relação à superfície hígida. A análise de espectroscopia Raman foi realizada com laser de 632.8 nm, resolução de 10 x, varredura de 40 s e banda espectral de 50 a 1300 cm^{-1}. Então, observou-se que a intensidade do pico de fosfato (961 cm^{-1}) dos diferentes grupos foi influenciada pela variável profundidade ($p=0,02$), mas principalmente pelo tipo de tratamento ($p=0,001$). Concluiu-se que o G5 e G6 foram eficazes no tratamento remineralizador da superfície desmineralizada do esmalte dental. Os casos clínicos apresentam o efeito remineralizador do F18 em dois pacientes com lesões cervicais não cariosas e HD. Foram aplicados estímulos mecânicos e evaporativos, antes e após as aplicações do produto, e para quantificar a dor dos pacientes foi utilizada uma escala visual analógica (EVA). Foram realizadas três aplicações de 8 horas com intervalos de 48 horas entre elas. A partir da segunda aplicação foi possível observar melhoria na sintomatologia dolorosa, que atingiu seu ápice após a terceira aplicação, e manteve-se após acompanhamento de um mês. Após um ano a melhora ainda pôde ser observada. Nesse relato de caso pode-se observar o potencial do F18 como produto para tratamento de HD, no entanto, ensaios clínicos randomizados controlados devem ser realizados para comprovar estes benefícios frente aos demais produtos destinados a esta finalidade.

Palavras chave: Cárie dentária. Remineralização dentária. Materiais biocompatíveis. Vidro. Sensibilidade da dentina.

Barros ACP. Remineralization of initial caries lesion by means of the application of different products [dissertação de mestrado]. Araraquara: Faculdade de Odontologia da UNESP; 2018.

ABSTRACT

Preventive actions have resulted in a decrease in infectious and non-infectious oral diseases, but the prevalence of caries remains high, as well as the cases of dentine hypersensitivity (DH) are becoming more frequent. Many products have been considered in the remineralization of dental tissues, such as hydroxyapatite-based, calcium and phosphate-based and fluoride. The aim of this dissertation was to experimentally evaluate the remineralizing effect of bioactive materials (Bioglass® 45S5, Biosilicate® and F18) against initial caries lesion (ICL) and to observe the behavior of F18 on HD in a case report. The in vitro experimental study evaluated the remineralizing effect of bioactive materials and fluoride products on ICL in bovine dental enamel. The specimens were divided into six groups (G1 - artificial saliva, G2 – Acidulated phosphate fluoride, G3 - fluoride varnish, G4 - Bioglass® 45S5, G5 - Biosilicate® and G6 - F18) and had their surface divided into two parts, sound and demineralized enamel for the control group, and demineralized and remineralized enamel for the other groups. Caries induction was performed by applying methylcellulose gel and lactic acid solution (pH=4.6) for 10 days at 37°C. Then, the remineralizing agents were applied and the specimens were stored in artificial saliva for 24 hours. For the analysis, G1 was considered control (positive - sound and negative - demineralized), and the results of Knoop hardness showed that only G2 had no remineralizing effect ($p>0.05$). The other groups able to remineralize, but only G5 and G6 presented more expressive results ($p<0.05$). This information was confirmed by SEM images at 1000 and 5000x magnification, in which G5 and G6 presented a surface morphology closer to the sound surface. The Raman spectroscopy analysis was performed with 632.8 nm laser, resolution of 10x, scan of 40 s and spectral band from 50 to 1300 cm^{-1}. It was observed that the intensity of the phosphate peak (961 cm^{-1}) of the different groups was influenced by the depth variable ($p=0.02$), but mainly by the type of treatment ($p=0.001$). It was concluded that G5 and G6 were effective in the remineralizing treatment of the demineralized enamel surface. The case reports present the remineralizing effect of F18 in two patients with non-carious cervical lesions and DH. Mechanical and evaporative stimuli were applied before and after the application of the product and a visual analogue scale (VAS) was used to quantify the patients' pain. Three 8 hour applications were performed with 48 hour intervals between them. From the second application it was possible to observe an improvement in the pain symptomatology, which reached its apex after the third application, and remained with a follow-up of one month. After one year, the improvement could still be observed. In this case report it was able to observe that the potential of F18 as a product for the treatment of DH, however, randomized controlled clinical trials should be performed to prove these benefits against the other products intended for this purpose.

Keywords: Dental caries. Tooth remineralization. Biocompatible materials. Bioactive Glass. Dentin Sensitivity.

SUMÁRIO

1 INTRODUÇÃO

Materiais bioativos são uma classe de materiais capazes de se ligar, reagir e estimular tecidos vivos. Os vidros bioativos e cerâmicas de vidro mostram excelentes propriedades osteocondutoras e osteoindutivas e sua taxa de degradabilidade é alta. O material bioativo mimetiza a resposta celular, mas maximiza a reparação dos tecidos permitindo a adequada recuperação da forma e função dos tecidos [3-7].

Dentre os materias bioativos de interesse para a odontologia pode-se destacar o 45S5 Bioglass®. Esse foi desenvolvido pelo professor Larry Hench em 1969 é um composto inorgânico multicomponente altamente biocompatível composto pelos elementos sílica, cálcio, sódio e fósforo que são encontrados naturalmente no corpo humano[3,8]. Este material é considerado um avanço na engenharia de tecidos, pois pode interagir de forma benéfica com o tecido do hospedeiro para potencializar sua reparação e regeneração[6-7,9].

Em ambiente aquoso, o vidro bioativo inicia imediatamente uma reação superficial em três fases, lixiviação e troca de cátions, dissolução da rede de SiO_2 e precipitação de cálcio e fosfato para formar hidroxicarbonato apatita (HCA) biologicamente ativa, quimicamente e estruturalmente similar à apatita encontrada no tecido ósseo[3,5-6,8].

Durante sua ação, seu componente ativo, o fosfossilicato de sódio e cálcio, liga-se à superfície do tecido para iniciar o processo de remineralização e a medida que o vidro bioativo degrada, íons silício, cálcio e sódio e grupos fosfato são liberados no ambiente fisiológico. Com isso há um aumento no pH, e por meio de dissolução e precipitação dos íons cálcio, fosfato e silicato da superfície do vidro precipitam e formam uma camada rica em sílica policondensada que modula a formação de fosfato de cálcio e posteriormente cristaliza-se em HCA[3-5,8,10].

Além disso, há uma estimulação de genes que codificam fatores de crescimento e estimulam células osteogênicas a secretar matriz óssea[9]. Estudos apontam que sete famílias de genes envolvidos no processo de osteogênese são estimuladas, principalmente devido aos íons cálcio e sílica[5].

As maiores desvantagens dos vidros bioativos são a baixa resistência mecânica e à fratura[5], então para superar essas limitações, foram desenvolvidas as vitrocerâmicas[7].

A semelhança entre osso, dentina e esmalte levou à hipótese de que vidros e vitrocerâmicas bioativas poderiam ser eficientes na remineralização dos tecidos dentários afetados pela cárie ou erosão[7,9,11].

Estudos afirmam que a camada de HCA contribue para o processo de remineralização da superfície do dente, pois as partículas aderem à superfície do tecido, continuam a liberar íons e remineralizá-lo por até 2 semanas após a aplicação inicial[3]. Essa 'camada de interação' pode proporcionar oclusão permanente dos túbulos dentinários, proteger o esmalte contra desafio cariogênico, inibindo o processo de desmineralização[8,10-11], além de ter potencial para tratar a hipersensibilidade dental[3,6,8] e remineralizar superficies erodidas dentro de 24 horas após a aplicação do biomaterial[12].

Em 2004, Zanotto et al.[13] desenvolveram um pó cristalino de vitrocerâmica totalmente cristalizadas do sistema P_2O_5-Na_2O-CaO-SiO_2 chamado de Biosilicato® para o tratamento de (HD)[7,14]. Esse composto quaternário é biocompatível, apresenta potencial osteogênico e angiogênico e acelera o processo de osteoreparação em experimentos de cultura celular[11] e modelos animais[5].

Mais recentemente, em 2015, nova composição de vidro bioativo (SiO_2-Na_2O-K_2O-MgO-CaO-P_2O_5) foi desenvolvida pelo Laboratório de Materiais Vítreos (LaMaV - Universidade Federal de São Carlos - UFSCar, São Carlos, São Paulo , Brasil) e foi chamada de F18. Esse biovidro possui maior estabilidade e bioatividade que os demais biovidros, além de ser o único a permitir que se façam fibras contínuas com controle de diâmetro preciso, malhas e formas 3D bioativas[17]. Apesar destas excelentes propriedades e de seu alto potencial como produto para prevenção e reparo da estrutura dentária e para tratar a HD, até o momento, não existem estudos que comprovem sua eficácia em comparação com outros produtos disponíveis no mercado.

2 PROPOSIÇÃO

1. Avaliar a eficácia remineralizadora dos vidros bioativos sobre lesões de cárie inicial.
2. Apresentar casos clínicos que utilizaram o F18 como tratamento da HD.

3 PUBLICAÇÕES

Essa dissertação é composta por dois artigos resultantes de um estudo laboratorial controlado, randomizado e de medidas repetidas e relato de casos clinicos.

3.1 Publicação 1*

Remineralization of initial caries lesion by means of the application of different products.

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ABSTRACT

The aim of this study was to evaluate the remineralizing effect of bioactive glasses and fluorine products in vitro on lesions of initial caries in bovine dental enamel. The specimens were divided into six groups (G1 - control, G2 – Acidulated phosphate fluoride, G3 - fluoride varnish, G4 - Bioglass® 45S5, G5 - Biosilicate® and G6 - F18) and had their surface divided into two parts, sound and demineralized enamel for the control group, and demineralized and remineralized enamel for the other groups. Caries induction was performed by applying methylcellulose gel and lactic acid solution (ph=4.6) for 10 days at 37°C. Then, the remineralizing agents were applied and the specimens were stored in artificial saliva for 24 hours. For the analysis, G1 was considered positive (sound) and negative (demineralized) control, and the results of knoop hardness showed that only G2 had no remineralizing effect ($p>0.05$), whereas only G5 and G6 were able to effectively remineralize the lesions produced on the enamel surface. This information was confirmed by SEM images at 1000 and 5000x magnification, in which G5 and G6 presented a surface morphology closer to the sound surface. By means of the raman spectra it was possible to see that the phosphate peak (961 cm^{-1}) intensity of the different groups was influenced by variables depth ($p=0.02$), and mainly by the type of treatment ($p=0.001$). It was concluded that Biosilicate® and F18 were most effective in the remineralizing treatment of the demineralized enamel surface.

INTRODUCTION

Preventive actions in dentistry had provided a decline in oral infectious diseases, although the prevalence of caries remains high and is considered as worldwide public health problem. The etiology of caries is multifactorial, and the bacterial origin can be modulated by extrinsic (environmental, behavioral / cultural) and intrinsic factors (flow, composition and buffer capacity of saliva, hereditary / immunological and genetic aspects) that interact and contribute to its development [Galvão et al, 2015; Maia, 2013; Vargas-Ferreira et al, 2015; Vianna, 2015].

The early diagnosis allows the use of resources that make it possible to stop its progression, establishing a balance between the de-remineralization process of the dental tissue [Vianna, 2015]. However, if untreated, its continuous development may result in the loss of the dental element [Costa et al., 2009; Vargas-Ferreira et al, 2015].

Over time, many treatments have been proposed to treat early caries lesions as the application of fluorides, calcium and phosphate based products, microabrasion of the injured surface, among others [Maia, 2013; Vianna, 2015]. On the other hand, new materials are investigated to reduce mineral loss, maintain structural integrity and remineralize early caries

lesions such as biomimetic materials [Attin et al., 2007]. The preservation of dental tissues through non-invasive approaches is a concept of minimally invasive dentistry [Vianna, 2015].

Fluoride in different forms of topical application has been shown to be quite effective in inhibiting demineralization and favoring remineralization [Costa et al., 2009]. Most treatments are performed just with topical fluoride application for some minutes, which represents the minimum availability of chemical elements in hydroxyapatite [Maia, 2013], what could be a limiting factor. In this way, there is evidence in the literature about the superficial characteristics of fluoride remineralization [Attin et al., 2007; Curtis et al., 2010; Jardim et al., 2008] where non-cavitated lesions still be clinically and radiographically visible [Vianna, 2015].

Fluoride varnishes are also a preventive option to remineralize initial caries lesions. They allow the deposition and incorporation of fluoride ions to demineralized enamel [Jardim et al., 2008; Rehder Neto et al., 2009; Upadhyay et al., 2013], are easy to apply, well tolerated, and still remain adhered by long period of time and is independent of the patient's collaboration. The gold standard commercial fluoride varnish of the literature is Duraphat® (5% NaF, 2.26% F) because it promotes a high degree of mineralization on the surface of the demineralized enamel [Mohd Said et al., 2017].

Other materials, as the bioactive glasses (BG), gained evidence in minimally invasive dentistry. Most of the BG are based on the original formulation SiO_2-P_2O_5-CaO-Na_2O of 1969, called Bioglass® 45S5, gold standard of the current literature, initially developed for bone regeneration [Chen et al., 2017; Deng et al., 2013; Karakoti et al., 2006; Khoroushi, Keshani, 2013]. They are highly inorganic biocompatible biomaterials that react with physiological fluids, adhere in bone tissues, and are able to obliterate exposed dentinal tubules, inhibit demineralization and remineralize dental structures by interfacial apatite precipitation bond. This apatite layer is adhered to the dentinal tubules and resistant to acid challenge and brushing abrasion [Chen et al., 2017; Curtis et al., 2010; Deng et al., 2013; Karakoti et al., 2006; Khoroushi, Keshani, 2013]. Biosilicate® is a particular composition of a set of totally crystalline glass ceramics of the Na_2O-CaO-SiO_2-P_2O_5 system developed by the Laboratory of Vitreous Materials (LaMaV - Federal University of São Carlos - UFSCar, São Carlos, São Paulo, Brazil) for the use in the treatment of dentin hypersensitivity in 2003, obtaining success in clinical trials [Crovace et al., 2015; Souza et al., 2013; Tirapelli et al., 2010]. Subsequently, the ability to inhibit and reverse the progression of initial caries in the enamel [Rehder Neto et al., 2009] was observed. Particle size is very important because the remineralization rate is higher when induced by BG particles of nanometric size

[Vollenweider et al., 2007]. Thus, a new BG called F18 was also developed by the Laboratory of Vitreous Materials (LaMaV - Federal University of São Carlos - UFSCar, São Paulo, Brazil) in 2015. The new formula composed of silica, calcium, sodium, potassium, magnesium and phosphorus [Souza et al., 2013], preventing its early crystallization time availability (to form three-dimensional fibers) and have bactericidal effect (all others are bacteriostatic). In addition, these materials have alkalinity and accelerated ionic release, which can neutralize the acidity of a medium and reduce demineralization, ensuring the integrity of the dental structure throughout its life [Deng et al., 2013].

Emphasizing the importance of the treatment of caries lesions in their initial stage and considering the lack of data in the literature on this new application for bioactive glasses, researches that address this subject are necessary to analyze the behavior of the materials regarding the fluoride products (gold standard in the literature for this purpose).

The aim of this study was to evaluate in vitro remineralization of initial caries-like lesion on the surface of bovine teeth after application of bioactive materials and fluoride products.

MATERIALS AND METHODS (Apêndice A)

Experimental design

This is an in vitro study of repeated measures performed to analyze the remineralization effect of different products. This study was approved by the Ethics Committee on the Use of Animals (Process nº 31/2017 of University of São Paulo State - UNESP, Araraquara School of Dentistry). Bovine incisors without lesions, cracks or hypoplasia on the enamel surface were selected and stored in a 1% thymol solution for 15 days. Enamel blocks (4 x 4 x 3 mm) were obtained by means of a doble cut on the buccal surface of bovine incisors and after that, the tetth were embedded in a polyether resin (Redelease®, Lot 106540, São Paulo, Brazil). The specimens were planned and polished with 600, 1200 and 1500 grit paper then with diamond paste and impregnated felt disc, washed with deionized water and cleaned in an ultrasonic bath for 10 min. The exclusion criterion was the presence of lesions, cracks or hypoplasia on the enamel surface of the included specimens. The inclusion criterion was given by the analysis of the Knoop surface microhardness (KHN) of each specimen. Five indentations were made in the enamel surface with a distance of 100 μm between them using a microdrometer (HMV-2, Shimadzu, Japan, FAPESP Proc.: 99/06060-6) with a Knoop diamond under 25 g load for 10 s [Chinelatti et al., 2017]. The specimens that showed a KHN upper than 270 [Masuda et al., 2013] were included in this study. Forty eight (n=48) specimens were randomized into six Groups: saliva

(positive and negative control), acidulated phosphate fluoride gel, fluoride varnish, Bioglass® 45S5, Biosilicate® and F18 (Chart 1 and Table 1). Twenty one (n=21) were analyzed by means of scanning electron microscopy.

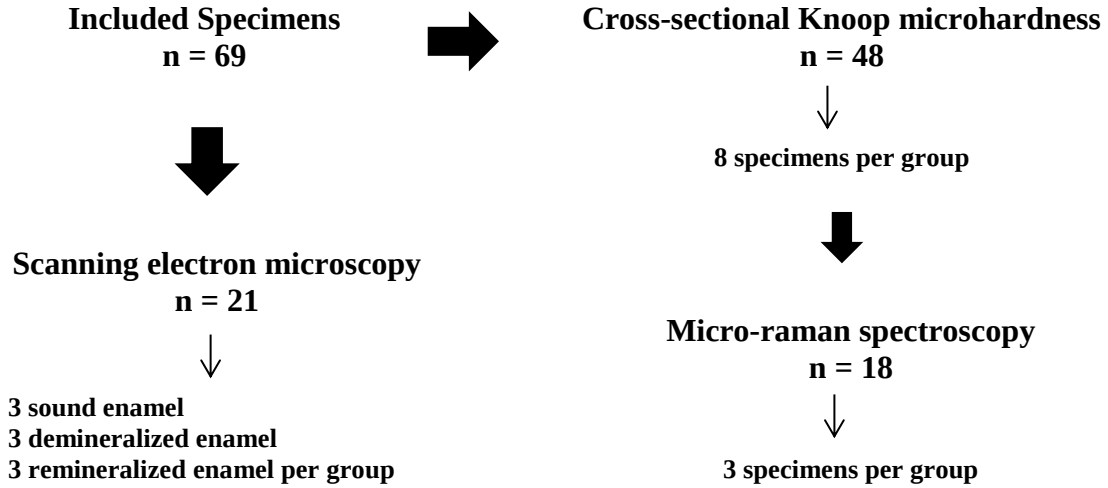


Chart 1. Especimens distribution.

Induction of artificial carious lesion

The specimens of the control group had a half of their surface protected where no treatment was performed. All specimens were covered with 0.5 cm of medium viscosity methylcellulose gel (Methocel® MC, Sigma®, batch number BCBR2107V) left in refrigerator for 12 hours at 4°C subsequently covered with 0.1 M lactic acid solution previously prepared with pH adjusted to 4.6 with NaOH and with sodium azide as a bacteriostatic agent. The containers were sealed and incubated at 37°C for 10 days [Buzalaf et al., 2010] and the gel was removed from the surface of the specimens by washing with deionized water and the surface was dried. Then, two specimens were randomly drawn and cutted into fragments of 100 µm (Microtome for metal cutting and histological processing system, EXAKT Technologies, Inc.) and analysed by means of microscopy of polarized light (MLP) with a microscope Leica DM 2500 (magnification of 20X) and the software Leica Application Suite v3.8 to verify the caries-like lesion induction .

Application of remineralizing agents

The specimens from the experimental groups had a half of their surface protected with a double layer of a nail polish varnish (demineralized area control) and the other one was treated according to the different remineralizing protocols (Table 1).

The treated specimens were stored for 24 h at 37°C in artificial saliva. Then, in order to be longitudinally analyzed, all specimens were segmented in two sides.

Sample Groups	Application Protocol	Composition	Manufactures
G1 – Control	Specimens were immersed in artificial for 24 h before the analisys.	Potassium chloride, magnesium chloride, sodium chloride, calcium chloride, CMC and sorbitol - pH 7.	Santa Paula Pharmacy
G2 – Acidulated phosphate fluoride gel	Applied on the exposed demineralized enamel surface with microbrush for 4 minutes [Chinelatti et al., 2017] without contact with artificial saliva.	Acidulated phosphate fluoride - 1,23%. Lot:16081166.	Nova DFL
G3 - Duraphat®	Applied on the exposed demineralized enamel surface with microbrush (waited one min), stored in artificial saliva and removed after 6 hours* [Mohd Said et al., 2017] by using surgical blade.	Sodium fluoride 5%, fluoride 2.26%. Lot:071401.	Colgate (Colgate-Palmolive)
G4 - Bioglass® 45S5	Individual trays were made with a silicone plate for each specimen to maintain the applied product in contact with the exposed demineralized enamel surface for 6 hours*. The powder of each bioglass was mixed with deionized water (1:10) [Tirapelli et al., 2010; Chinelatti et al., 2017] applied with a microbrush and removed by washing with deionized water for 30 seconds.	P_2O_5-Na_2O-CaO-SiO_2	Laboratory of Vitreous Materials - LaMAV, Federal University of São Carlos - UFSCAr.
G5 - Biosilicate®		Na_2O-CaO-SiO_2-P_2O_5, with additions of Li_2O and K_2O	
G6 - F18		SiO_2-Na_2O-K_2O-MgO-CaO-P_2O_5	

* The action time of the products was 6 hours to standardize the exposure time of the demineralized surface under the different remineralizing agents.

Table 1. Remineralizing agents protocol.

After, the specimens were seccionated (Isomet; Buehler Ltd, Lake Bluff, IL, USA) to separate the treated side from the demineralized side. The transversal surface was polished with 1500 grit paper, then cleaned in a ultrasonic tube for 10 min.

Cross-sectional Knoop microhardness (CSM)

Three indentations were made at seven enamel depths (10, 30, 50, 70, 90, 110 and 220 µm) until outer enamel surface. The first indentation sequence was made in the central region and the other two at a distance of 100 µm for both sides using a charge of 25 g for 10 s (Figure 1**).**

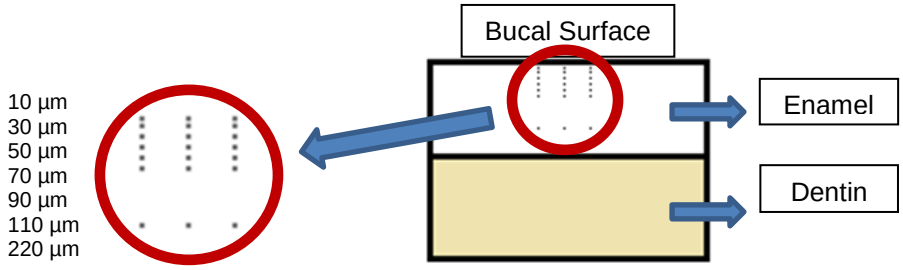


Figure 1. CSM squeme.

Micro-Raman Spectroscopy (RAMAN)

The specimens analysis was made using a Raman spectrometer (Lab RAM HR, Serial number 5/504, Horiba Jobin Yvon Inc.) equipped with 632.8 nm laser under a resolution of 10x. The analisys was performed for 40 seconds scanning the spectral band from 50 to 1300 cm^{-1} twice. A line map was made following the same depths as the CSH hardness analysis to analyze the mineral changes through the lesion. The phosphate peak area (961 cm^{-1}) was calculated using the software LabSpec 5 (Raman - Horiba Jobin Yvon Inc.) and graphics were ploted by using the software Origin Pro 9.

Scanning Electron Microscopy (SEM)

For the SEM analysis the specimens were dehydrated with increasing concentrations of ethanol (twenty minutes of 50%, 60%, 70%, 80% and 90% plus sixty minutes of 100%). The specimens were dried at 37ºC for 12 hours and sequentially attached to an aluminum stub and coated with gold for 2 minutes. Then, the enamel surface was examined using SEM (JSM-6610LV, JEOL, USA Inc) under magnification of 1000 and 5000x.

Statistical analysis

Repeated measures analysis of variance (ANOVA) with Greenhouse-Geisser (GG) correction was used. After, to confirm which treatment was the most effective as remineralizator, a mean comparison of the transversal data regardless the depth was performed by means of repeated measures ANOVA with Bonferroni correction before and

after the treatments. The statistical analysis was done by using the (IBM SPSS Statistics Version 19) with significance level of 5%.

RESULTS

Induction of artificial carious lesion

The images obtained by means of MLP (Figures 2 and 3) showed that the methylcellulose gel and lactic acid protocol was able to induce subsurface and erosion- like lesions.

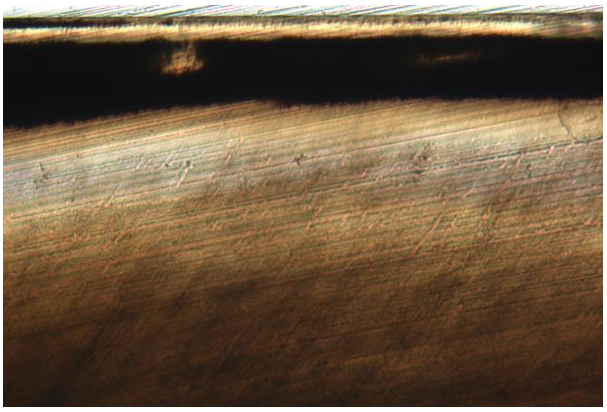


Figure 2. Induction of subsurface lesion.

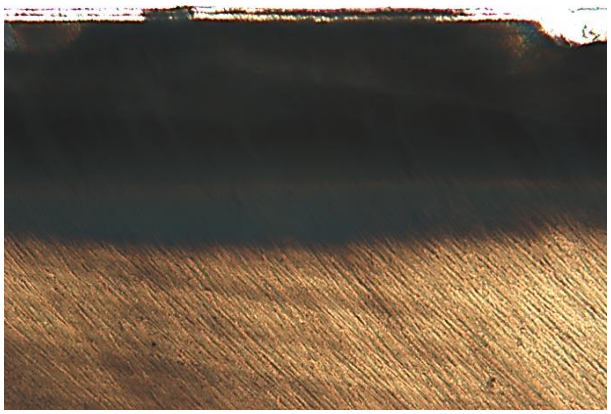


Figure 3. Induction of erosion lesion.

Cross-sectional Knoop microhardness (CSH)

Preliminary data analysis showed that the data did not present a normal distribution. Despite of this, it was decided to use repeated measures ANOVA due to the robustness of this statistical test.

The microhardness mean and the standard deviation (±sd) regarding the different treatments applied on the specimens are showed in Table 2. Initially it was verified if the specimens presented similar cross-sectional hardness. The ANOVA test showed adequate homogeneity between the test groups ($p=0.06$), but not between the control group and the test groups ($p=0.001$).

The effectiveness of remineralizing treatments was evaluated by ANOVA of repeated measurements with Greenhouse-Geisser (GG) correction. This test showed statistically significant differences for the interaction treatments*depth ($F_{GG}(21.949)=3.801$; $p<0.0001$; $\gamma^2_{partial}=0.124$; $\pi=1.00$); depth ($F_{GG}(3.658)=236.389$; $p<0.0001$; $\gamma^2_{partial}=0.595$; $\pi=1.00$) and treatment ($F_{GG}(6)=9.486$; $p<0.0001$; $\gamma^2_{partial}=0.261$; $\pi=1.00$). The variable that had more influence in the dental hardness was the depth (59%), while only 26% of the variation of the transverse hardness can be explained by the treatments performed.

The effect of the surface treatments on the CSH of the enamel regarding the analysis in different depths is described in Figure 4. The intra and intergroup statistical differences were determined by the overlapping of the extreme values of confidence interval (95% CI).

This graph (Figure 4) showed that G6 and G5 were the most effective remineralizing treatments, since the CSH of the lower depth layers presented values statistically similar to those observed in healthy enamel ($p>0.05$) and statistically different from the demineralized enamel ($p<0.05$). On the other hand, the G2, G3 and G4 did not present a remineralizing effect, since the hardness of the superficial layers was similar to that observed for the demineralized enamel ($p>0.05$) and statistically different from the healthy enamel ($p<0.05$).

Treatment	Detph													
	10um		30um		50um		70um		90um		110um		220um	
	Mean	±sd	Mean	±sd	Mean	±sd	Mean	±sd	Mean	±sd	Mean	±sd	Mean	±sd
+ Control	58.09	25.42	75.34	30.52	81.63	25.43	90.25	26.99	93.19	24.65	100.48	33.16	111.20	32.08
- Control	27.98	12.66	37.88	11.03	48.87	10.31	60.85	19.59	69.02	25.79	78.14	29.91	87.02	44.70
G2	32.97	28.83	38.68	23.55	46.15	21.40	58.62	28.47	66.37	27.12	74.77	30.46	105.95	23.38
G3	32.58	17.99	43.58	20.64	47.33	16.04	53.58	17.03	62.49	20.58	68.15	16.78	92.91	30.18
G4	39.39	12.60	53.33	19.12	71.49	27.38	82.37	29.37	84.55	27.09	98.95	42.97	146.40	48.46
G5	51.50	19.32	60.91	22.21	71.61	27.34	73.30	25.72	79.86	29.04	96.53	32.52	124.73	35.58
G6	56.15	14.71	56.42	16.82	69.27	20.89	69.62	27.31	68.80	26.97	76.60	26.86	109.42	28.12

Table 2 – Mean and standard deviation of dental enamel hardness in relation to treatments and depth of analysis.

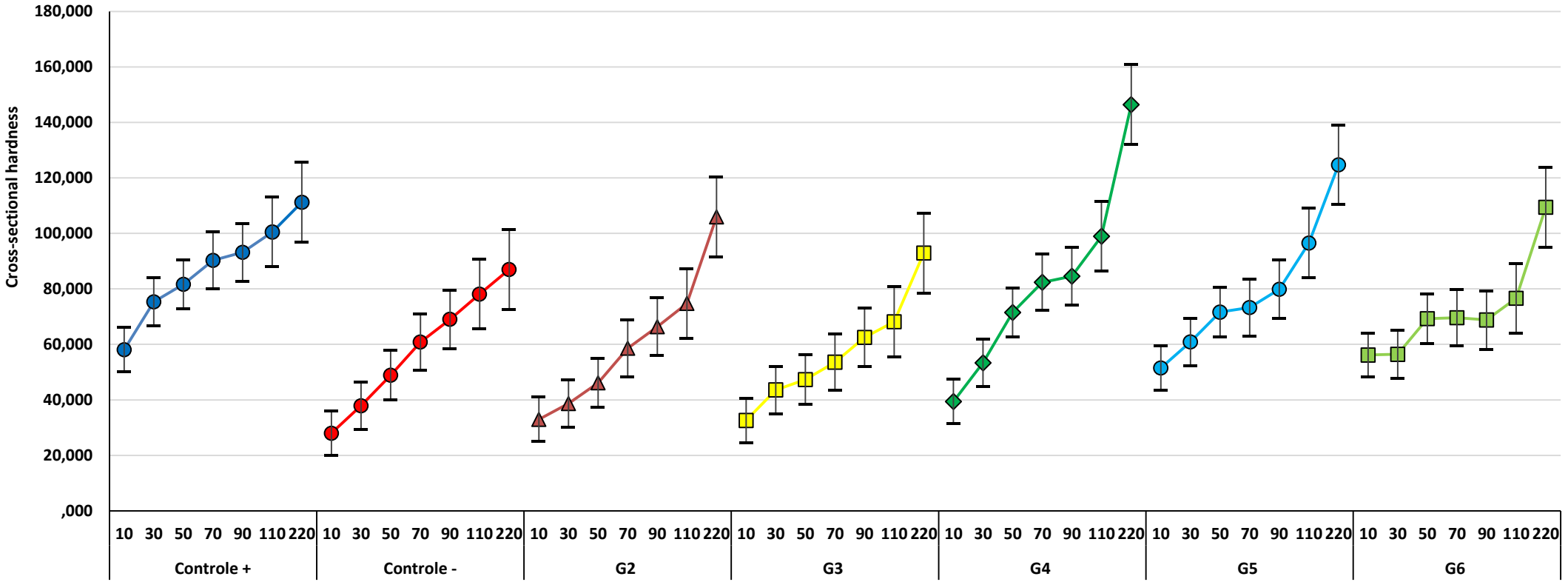
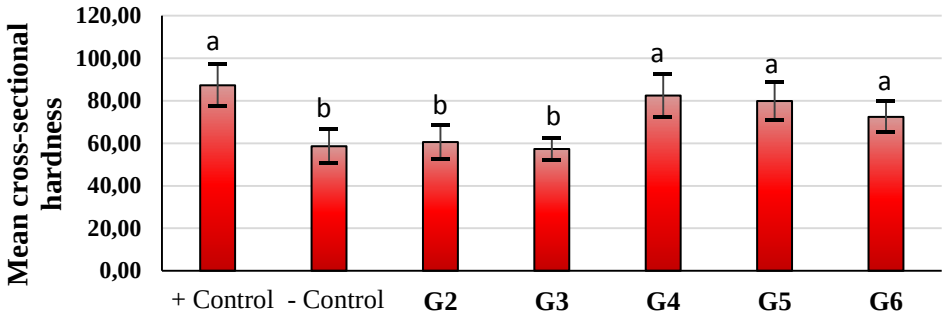


Figure 4 - The means and confidence intervals (95%CI) describe the effect of the surface treatments on the mean cross - sectional hardness of the enamel as a function of the depth of analysis.

In order to confirm which remineralizing treatment was most effective, the CSH average of the groups was also compared, regardless of the depth. The ANOVA test showed statistically significant differences between treatments ($F(6)=9.486$, $p<0.0001$) as seen in Figure 3. The multiple comparison test with Bonferroni correction confirmed the previous findings, in which the most effective remineralizing treatments were G4, G5 and G6, while G2, G3 did not show this effect (Figure 5).



[a,b] Different letters indicate statistical differences according to the multiple comparison test with Bonferroni correction for $p<0.05$.

Figure 5 – Mean and confidence interval (95%CI) of surface hardness regard the treatments (groups).

In addition, to determine the efficacy of the remineralizing treatments in function of the control group (between groups), the intra-group effect analysis was also performed (Table 2). The hardness values of the demineralized and demineralized and treated areas were compared by the ANOVA test. From this analysis it could be deduced that all the remineralizing treatments promoted an increase in the cross-sectional hardness of the enamel, except G2.

Group	Condition	Mean	± sd	Sig.*
Control	Sound	87.17	23.30	0.0001
	Desmineralized	58.54	19.21	
G2	Treated	60.50	18.52	0.11
	Desmineralized	52.50	16.02	
G3	Treated	57.23	12.55	0.0001
	Desmineralized	41.67	13.48	
G4	Treated	82.35	23.94	0.0001
	Desmineralized	47.51	10.03	
G5	Treated	79.78	21.31	0.0001
	Desmineralized	43.90	12.04	
G6	Treated	72.32	16.98	0.0001
	Desmineralized	45.25	14.26	

*Significant for $p<0.05$.

Table 2 – Results of the ANOVA test comparing the cross-sectional hardness of the non-demineralized (sound) and demineralized areas for the control and demineralized and treated (treated) and demineralized groups for each experimental group.

Scanning Electron Microscopy (SEM)

The SEM images were in agreement with the microhardness data. Firstly, SEM showed that there were clearly diferences between the enamel surface before (Figure 6 – a and b) after the demineralization process (Figure 6 – c and d) in the control group, where the smooth and intact surface enamel turned into an irregular and rough surface. Regarding the treated groups, there was a slight difference in the irregularity surface pattern between the demineralized enamel and G2, the enamel surface remains rough and porous (Figure 6 – c to f). Also, it was observed that the surface of G5 and G6 (Figure 6 – k to n) was more structurally like the suface of sound enamel (Figure 6 – a and b) both in 1000x and 5000x, than the surfaces of G4 that presented some porosity mainly on 5000x and G3 that was more likely demineralized enamel. These findings elucidated the remineralizing effect of the groups 4 to 6 (Figure 6 – i to n) and the presence of crystals or particles scattered on their enamel surface.

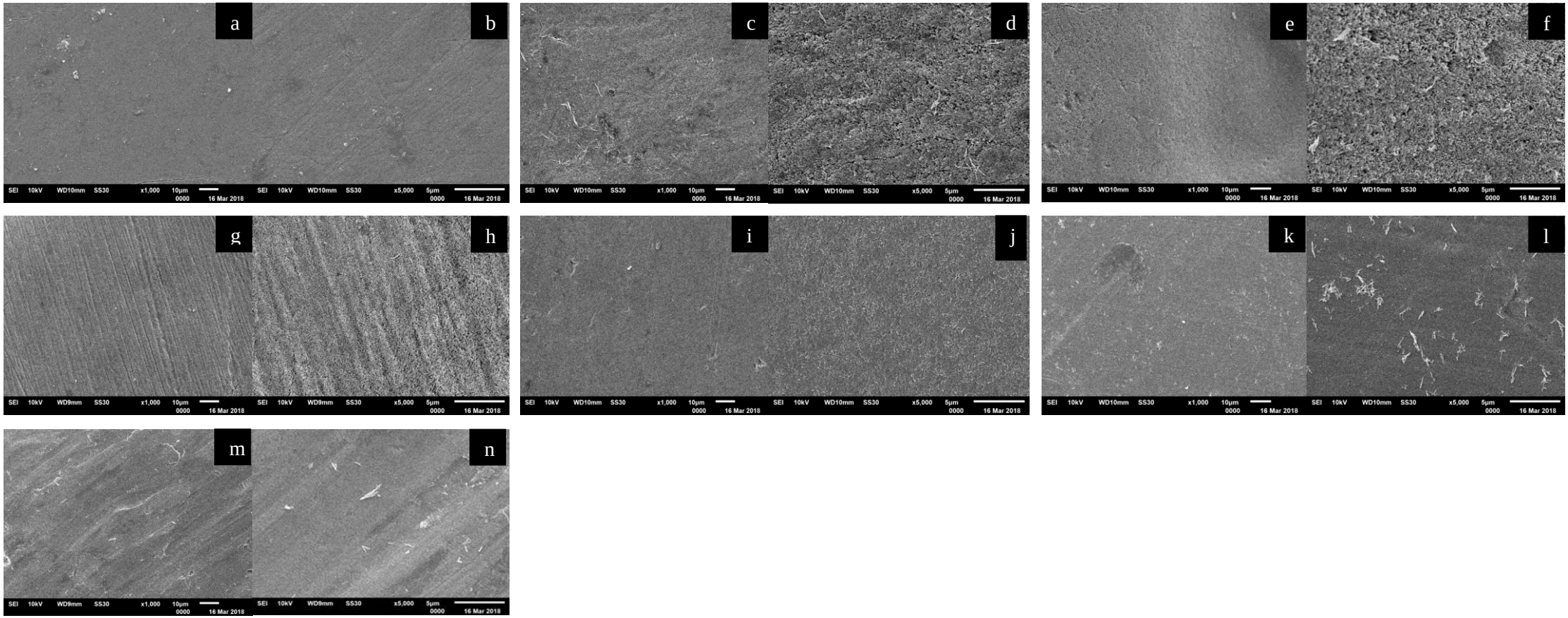


Figure 6 - (a) Sound enamel - 1 K, (b) Sound enamel - 5 K, (c) Demineralized enamel - 1 K, (d) Demineralized enamel - 5 K, (e) Group 2 – APF - 1 K, (f) Group 2 – APF - 5 K, (g) Group 3 - Duraphat® - 1 K, (h) Group 3 - Duraphat® - 5 K, (i) Group 4 – 45S5 Bioglass® - 1 K, (j) Group 4 – 45S5 Bioglass® - 5 K, (k) Group 5 - Biossilicate® - 1 K, (l) Group 5 - Biossilicate® - 5 K, (m) Group 6 – F18 – 1 K, (n) Group 6 – F18 – 5 K.

Micro-Raman Spectroscopy (RAMAN)

As the distribution of the data did not present normality, homogeneity of variables and sphericity, it was decided to use the statistical test of repeated measurements with Greenhouse-Geisser (GG) correction of ANOVA. The mean and standard deviation values of the Raman hardness are shown in Table 3.

Detph	Groups						
	+ Control	**- Control**	**G2**	**G3**	**G4**	**G5**	**G6**
10µm	1.07E+05	2.31E+04	2.05E+04	2.28E+04	2.74E+04	2.57E+04	3.16E+04
	4.59E+04	1.72E+04	9.74E+03	1.52E+04	1.71E+04	4.10E+03	1.88E+04
30µm	1.05E+05	1.94E+04	1.83E+04	1.83E+04	2.53E+04	1.92E+04	2.51E+04
	3.55E+04	1.30E+04	7.76E+03	1.34E+04	1.84E+04	3.09E+03	1.43E+04
50µm	9.70E+04	1.76E+04	1.70E+04	1.88E+04	2.28E+04	1.67E+04	2.09E+04
	3.65E+04	1.26E+04	8.92E+03	1.42E+04	1.69E+04	1.56E+03	1.05E+04
70µm	9.88E+04	1.80E+04	1.45E+04	1.63E+04	2.28E+04	1.53E+04	1.72E+04
	3.93E+04	1.25E+04	6.24E+03	1.20E+04	1.69E+04	1.46E+03	1.31E+04
90µm	1.02E+05	1.60E+04	1.40E+04	1.60E+04	2.21E+04	1.40E+04	1.59E+04
	4.39E+04	1.17E+04	5.24E+03	1.24E+04	1.75E+04	2.29E+03	1.27E+04
110µm	1.04E+05	1.54E+04	1.31E+04	1.65E+04	2.20E+04	1.35E+04	1.56E+04
	4.52E+04	1.06E+04	4.83E+03	1.27E+04	1.79E+04	1.11E+03	1.26E+04
220µm	1.07E+05	3.77E+04	1.23E+04	1.58E+04	2.24E+04	1.33E+04	1.44E+04
	4.13E+04	4.89E+04	4.41E+03	1.25E+04	2.01E+04	9.35E+02	1.11E+04

Table 3 – Effect of treatments on Raman spectra in function of depth for repeated measures expressed by mean and standard deviation.

The treatment*depth interaction had no statistically significant effect ($F_{GG}(10.098)=1.123$, $p=0.39$, $\gamma^2_{parcial}=0.33$, $\pi=0.43$). The main variables detph $F_{GG}(1.683)=4.811$, $p=0.02$, $\gamma^2_{parcial}=0.26$, $\pi=0.7$) and treatment ($F_{GG}(2.126)=7.927$, $p=0.001$, $\gamma^2_{parcial}=0.77$; $\pi=0.99$) significantly affected the phosphate amount over the detphs, being the treatment the variable that presented the greatest effect size and power of the test (77% and 0.99 respectively) as seen in Figure 7. Also, is possible to confirm the above data with the comparison of the groups in three different detphs (10, 70 and 220 µm) by means of RAMAN spectra (Figure 8), where is clear treatment effect followed by the detph.

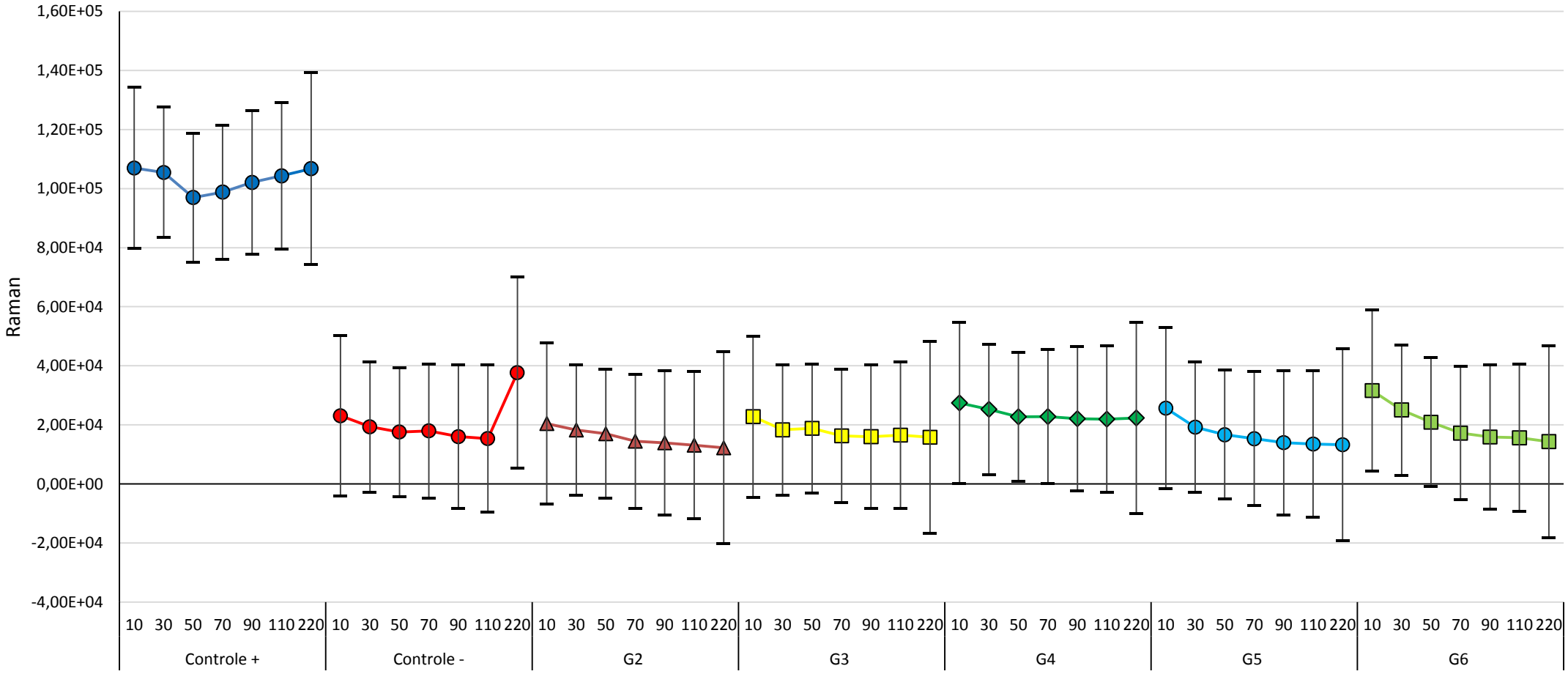
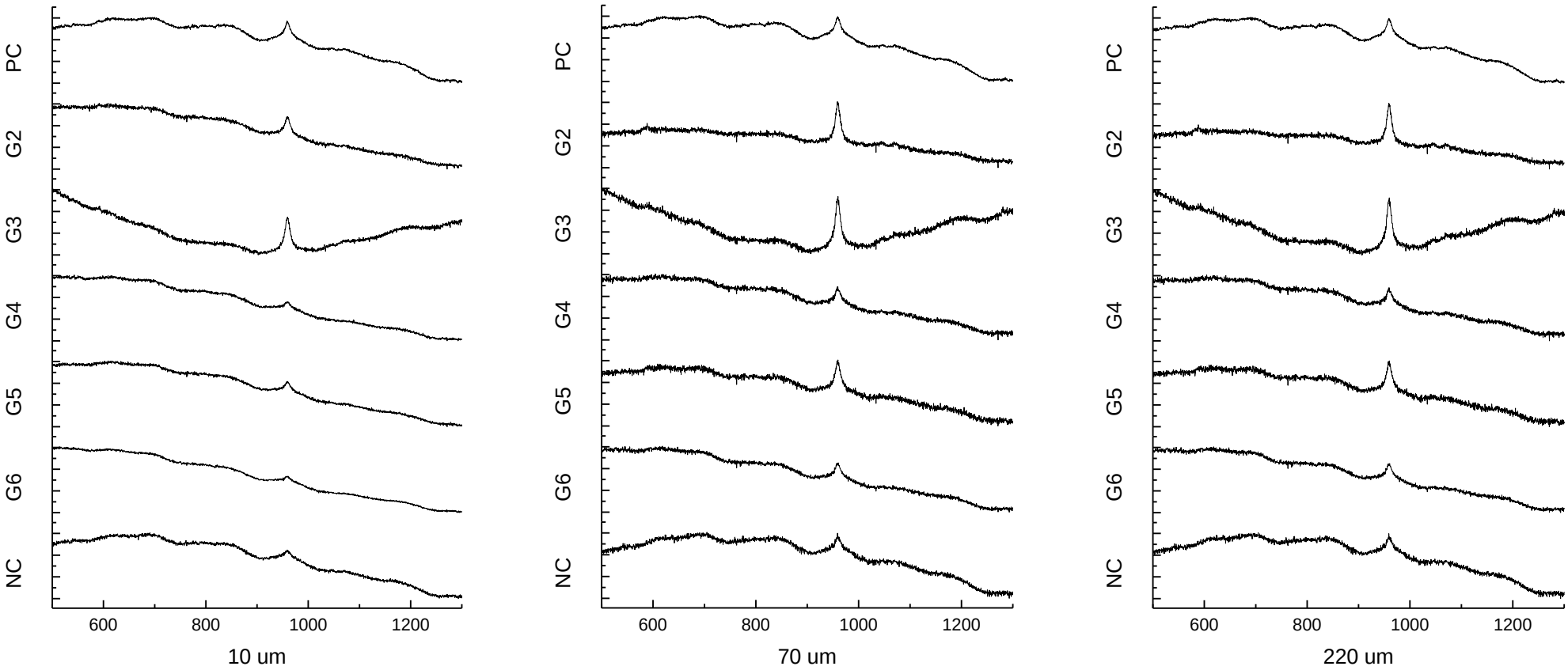


Figure 7 – The mean and confidence intervals (95%CI) describe the effect of the surface treatments on the average mineral content of the enamel as a function of the depth of analysis, evaluated by RAMAN.



PC –positive control (sound enamel); NC – negative control (demoneralized enamel).

Figure 8 –The RAMAN spectra behavior to each treatment at three different depths.

The ANOVA test showed statistically significant differences between the positive control group and all test groups and the negative control ($F(6)=7.729$, $p<0.001$) confirmed by the multiple comparison test with Bonferroni correction (Figure 7).

DISCUSSION

Dental caries remains a problem worldwide. Its development is due to an imbalance of the de-remineralization process in the enamel surface. The demineralization process occurs when the pH of the oral fluids is lower than 5.5 and a dissolution of the hydroxyapatite crystals of the enamel surface release calcium and phosphate to the oral cavity, when this process is not stopped it could result in caries. On the other hand, the remineralization process occurs when there is an increasing in oral pH and a saliva supersaturation, providing calcium and phosphate ions to the enamel surface, promoting the formation of hydroxyapatite cristals [Rehder Neto et al., 2009; Silva et al., 2012; Li et al., 2014; Upadhyay et al., 2013; Vargas-Ferreira et al., 2015; Damle et al., 2016; Palaniswamy et al., 2016; Nozari et al., 2017, Chinelatti et al., 2017; El-Wassefy, 2017; Featherstone et al., 2018; González-Cabezas, Fernández, 2018; Jagga et al., 2018; Kanwall et al., 2018].

The initial step of the caries development occurs just in a subsurface layer remaining the integrity of the enamel surface. Clinically, this initial caries lesion is seen as a white spot lesion, wich need to be remineralized, because it is well known that this is a reversible process [Huang et al., 2009; Majithia et al., 2016; Palaniswamy et al., 2016; El- Wassefy, 2017]. Despite of the intact surface, the microhardness of the enamel with initial caries is lower than the sound enamel due to the beggining of the mineral loss [Palaniswamy et al., 2016; Jagga et al., 2018].

The use of artifical caries-like lesion is interesting to better understand the demineralization process and structural changes in the enamel surface [Rehder Neto et al., 2009]. In this way, several enamel demineralization models and agents were proposed [Kamath et al., 2017], among them the use of methylcellulose gel and lactic acid [Buzalaf et al., 2010; Moron et al, 2013].

It is important to keep in mind that different protocols result in different types of lesions as well as the degree of the de-remineralization are directly related (the porosity and depth influence the mineral diffusion). Cariogenic challenge is a demineralizing procedure wich is modifyed by many factors as the pH and presence of calcium, phosphate and/or fluoride in the solution, time, volume and viscosity of the solution wich could lead to a subsurface or an erosion-like lesion [Buzalaf et al., 2010; Moron et al, 2013].

The method chosen for this study was the methylcellulose gel and lactic acid solution, considered simple to be done (few reagents) and is available to simulate the clinical lesions, although this procedure results in not homogeneous lesions due to the consistency of the methylcellulose gel and its purity, when more viscous and pure gel produce a less demineralized lesion, and it was observed on the enamel surface [Salomão et al., 2016]. The specimens were demineralized in pH 4.6 at 37 °C for 10 days [Buzalaf et al., 2010] in a methylcellulose/lactic acid system. This protocol resulted in subsuperficial and erosive-like lesions (Figure 3). This fenomenon can be explained because the demineralized period was longer then the study of Lippert et al. [2013], such as there was not a saturation of the acid solution as done by Lippert, Lynch [2014] (that induced caries-like lesions for 14 days) in order to respect the dental composition.

For treat the initial caries lesions several preventive treatments have been proposed, such as fluoride and BG [Rehder Neto et al., 2009; Li et al., 2014; Nozari et al., 2017, Chinelatti et al., 2017; El- Wassefy, 2017; Jagga et al., 2018; Kanwall et al., 2018]. In this study, the powder of the different BG and glass-ceramic was manual mixed in distiled water (1:10), then applied in the enamel surface and maintained in position with silicon trays for 6 h as varnish fluoride. After, fluoride varnish was removed with a surgical blade taking care for did not touch the enamel surface.

Fluorides are the gold standard remineralizing agent that can prevent and repair caries [Silva et al., 2012; Upadhyay et al., 2013; Nozari et al., 2017; Byeon et al., 2016; Damle et al., 2016; Majithia et al., 2016; Soares et al., 2017; Featherstone et al., 2018, González-Cabezas, Fernández, 2018], because they bonds on calcium and phophate ions forming fluoridated apatite, resistant to 4.5 pH [Huang et al., 2009; Rehder Neto et al., 2009; Narayana et al., 2014; Byeon et al., 2016; Damle et al., 2016; Soares et al., 2017; Jagga et al., 2018; Kanwall et al., 2018]. When applied, the fluoride ions sature the environment surround the tooth structure, interfering in the demineralization process. These ions adsorb the crystal surface and attract calcium and phosphate ions leading to a new mineral formation (remineralization), less soluble [Rehder Neto et al., 2009]. However, topical fluoride therapy just has a preventive caries effect if the supplying of calcium and phosphate ions are enough, but not eliminate caries totally [Mohd Said et al., 2017; Soares et al., 2017; Featherstone et al., 2018]

As showed in Figures 4 and 5, the fluoridated products (G2 and G3) had not a remineralize effect, and it could be explained by some reasons. Firstly, the fluoride gel remain in contact with the teeth for a short period than resulting in the formation of bonds in more

superficial portions of the enamel [Byeon et al., 2016] Then, according to Byeon et al. [2016], Majithia et al. [2016] and Silva et al. [2012] the enamel absorption of fluoride is proportional to the amount time of contact. Because of this, Duraphat® is well stablished as caries prevention since it can introduce have a greater concentration of fluoride and should remineralize more the enamel surface [Mohd Said et al., 2017], although it is not in agreement with the findings in Figure 5 and Table 2. Other reason could be the composition of the varnish, this product has a resin base [Majithia et al., 2016] that release slowly the fluoride ions with time and the period of 6 hours in contact with the enamel surface was not enough to remineralize the different detphs.

In agreement with the literature the different bioactive materials (G4, G5 and G6) could remineralize the eroded enamel surface (Table 2). Bioactive remineralizing materials are the BG and glass-ceramics [Tirapelli et al., 2011; Li et al., 2014; El- Wassefy, 2017; Chinelatti et al., 2017; Kanwall et al., 2018]. Their mechanism of action is based on the release of sodium changed for hydrogen cations (H^+ or H_3O^+) that result in the releasing of calcium (Ca^{2+}) and phosphate (PO_4^{2-}) ions. At this point, the pH increase and brings precipitation of Ca^{2+} and PO_4^{2-} ions from saliva, then a layer is formed structurally and chemically similar to biologic apatite, the hydroxycarbonateapatite (HCA). It is well known that bioglass adhere and can continuously deposit HCA as well as effective to treat dental caries, erosion and hypersensitivity [Rehder Neto et al., 2009; Tirapelli et al., 2010; Renno et al., 2013; Souza et al., 2013; Li et al., 2014; Narayana et al., 2014; Palaniswamy et al., 2016; Fernando et al., 2017; Soares et al., 2017; Jagga et al., 2018]. The 45S5 is the first bioglass used in dentistry, gold standard, consisted of calcium sodium phosphosilicate applied since bone defects until enamel and dentin remineralization [Souza et al., 2013; Palaniswamy et al., 2016; Fernando et al., 2017]. Rehder Neto et al. [2009] and Soares et al. [2017] observed an increase in surface microharness after remineralisation with 45S5 Bioglass®, attributing it to the precipitation of HCA forming a layer on the surface of the enamel.

In this way, coming to the non-surgical therapies for dental tissues the Biosilicate was developed to treat dentin hypersensitivity and it was effective [Tirapelli et al., 2010; Renno et al., 2013]. Recently, a new bioglass composition was developed, the F18, its new composition belongs to the system SiO_2–Na_2O–K_2O–MgO–CaO–P_2O_5 allows a more organized remineralization pattern due its continuous fibers with precise diameter [Souza et al., 2013].

Our findings confirm what was described above, however only the groups G6 and G5 had an effective remineralization as shown in Figure 4. The evolution of bioglasses, its

composition and manufacturing process, can explain the remineralize differences among the bioactive materials.

Despite the different demineralizing protocol, Chinelatti et al. [2017] compared the remineralizing effect of Biosilicate® and APF for cross-sectional Knoop hardness and the glass-ceramic presented a superior and continuos remineralizing effect for erosion and caries-like lesion. Moreover, Tirapelli et al. [2010] stated that it was required only 24 h to induce the precipitation of a homogeneous layer of HCA and the biomaterial benefited the hardness and morphology of the teeth. These statements embase the results presented in this study. It should be noted that the treated groups were in contact with the remineralizing products for only 6 hours, except APF for 4 minutes, storaged in artificial saliva for 24 hours, then in deionized water until the analysis moment.

In the SEM images of demineralized surface tooth (Figure 6 c-d) showed irregularities and the loss of structural characteristics compared with the sound surface enamel that showed an organised and smooth surface (Figure 6 a and b). Despite of the SEM imagens were related with the surface enamel, the information matches with the microhardness findings. The groups 3-6 showed a decrease in the micropores and, but only G5 and G6 had a recovery of the surface integrity. The images of the bioactive materials (Figure 6 i - n) have demonstrated amorphous crystals or particles scattered on the surface as founded by Soares et al. [2017]. The differences observed in the remineralized surface morphology between the fluoride varnish group and the bioactvive materials may be due for their different mechanisms of remineralization [Kamath et al., 2017].

The raman spectra of mineralized tissues contain several peaks that are associated with the vibration of specific chemical species in the tissues [Ko et al., 2005]. In this study just the dominant phosphate peak at 961 cm^{-1} was clear to calculate the peak area and compare the diferente groups, as seen in Figure 6. Although, the raman statistical analyses diverge of the microhardness analyses showing in Figure 7 that there was no difference between the treatments, just the positive control had higher mineral content by means of the calculating of the phosphate peak area. There is an interesting difference between the data presented by Figure 7 and 6. There was no statistical difference between the groups (Figure 7), while were differences among raman peaks intensities of sound, demineralized and remineralized enamel as seen by Ko et al.[2005] (Figure 8). It could be due to the changes promoted on hydroxyapatite crystallite orientation and morphology by the demineralization process, which reflects on raman spectra [Ko et al., 2005].

The literature states that raman spectroscopy has the potential to be specific and sensitive for detecting incipient carious lesions, because on the carious lesions the phosphate bands intensities are higher at the surface (until 10 μm) and decrease rapidly into the lesion until of 100–120 μm from the surface [Mohanty et al., 2013]. This statement disagrees with the findings by this study, as seen in Table 3 and Figure 7 and 8, where the phosphate peak increased as the depth increased. This fenomenon could reinforce the idea that the demineralizing protocol induced an erosive-like lesion.

According to Mohanty et al. [2013] at greater distances into the enamel the intensities of all the phosphate peaks converge to the sound enamel, however, the mineral content did not drop in the lesion body (15 to 75 μm) neither at 100 μm or more, instead of this, the mineral content increased (Figure 8).

Moreover, Table 3 and Figure 8 showed that between the variables, the treatment had influenced more in the mineral content than the depth, as well as the different spectra pattern of the fluoride groups (G2 and G3). This defference was not observed among positive control, G4, G5, G6 and negative control, and it could be due to differences on the products composition, mechanism of action or a remanescent of the fluoride material.

Lastly, it was possible to conclude that the bioactive materials, Biosilicate® and F18, showed a relevant remineralizing performance of a demineralized enamel surface, but F18 presented the closer results to the sound enamel. Also, it is important to keep in mind the limitations of this in vitro study, because it does not reproduce the complexity of an in vivo condition, there are differences between the human and bovine teeth and there are few studies describing these bioactive materials. Then, it could be interesting analyse the reproducibility of these findings in clinical trials to better understand their clinical implication, specially the novel bioglass F18.

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3.2 Publicação 2*

F18 Bioactive Glass: A New Perspective to Treat Dentine Hypersensitivity.

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F18 Bioactive Glass: A New Perspective to Treat Dentine Hypersensitivity.

Abstract

Several treatments were proposed to manage dentine hypersensitivity (DH) although it remains an oral health problem worldwide. The novel bioactive glass (F18) has been proposed as an alternative to treat and eliminate this painful condition not only during treatment but also having a long lasting effect. DH is defined as a pain response to thermal, chemical, tactile or osmotic stimuli due to the open tubules in the exposed dentine surface. Two female patients of different ages with high level of DH but different oral health conditions were examined. Tactile and evaporative tests were performed in the cervical third of all patients' teeth and the degree of sensitivity was analyzed using visual analog scale (VAS). Three applications of F18 were proposed as treatment. The protocol of application of F18 presented in this case report is simple to perform and yet effective to occlude exposed dentine tubules. These satisfactory results of the F18 application on DH could still be observed even after one year from the first application.

Key Words: Dentine hypersensitivity, bioactive glass, remineralizing agent.

Introduction

Dentine hypersensitivity (DH) has been described as a common clinical oral health problem that frequently can affect adult population around the world.[1-2] DH can be defined as a pain arising from exposed dentine in response to thermal, chemical, tactile or an osmotic stimuli. The prevalence of DH can vary depending on the type of population and also their lifestyle behaviors, oral self-care and the methods used to evaluate the clinical condition, ranging from 1 to 98%. This condition can greatly affect the patients' life quality, interfering constantly on their daily activities.[1-9]

Different mechanisms for DH have been proposed and described in the literature. Three main mechanisms have been cited and described[10], as follow: Direct Innervation (DI), Odontoblast Receptor (OR) and Fluid Movement/Hydrodynamic Theories (HT). However, there is not enough scientific evidence to sustain DI and OR theories. The first theory claims that the nerve's endings enter dentine through the pulp, extend to dentine-enamel junction and a mechanical stimuli directly transmit the pain. Although this theory haven´t support the existence of a nerve in the superficial dentine such as a sensitive response of Rashkov´s plexus regard the dental development stage.[11] The second one, states that the odontoblasts can act as nociceptors transmitting signals to the pulpal nerves, however, this theory has also been rejected since the cellular matrix of odontoblasts is not capable of exciting and producing

neural impulses. Furthermore, no synopsis has been found between odontoblasts and pulpal nerves.[10]

The most widely accepted theory for DH is the HT, which was first proposed by Brännström in 1964[12]. It is based on the concept of the movement of the fluid inside the dentinal tubules and states that the surface dentine tubules are open and exposed to the oral environment connecting them to the pulp.[5,9,12-14] In this way, DH can occur when an enamel loss causes the exposure of these dentine tubules present at the surface. Although, gingival recession is described as the primary cause for dentine tubules exposure, specially in the cervical region of the teeth. The same process happens when the dental root is exposed, because the protective layer of cementum is more easily removed leading to the opening of the dentine tubules. Other causes may include caries and non-caries lesions, chipped teeth, fractured or faulty restorations, specific restorative materials and cracked tooth syndrome.[5-9,13,15]

Several treatments have been proposed to alleviate or eliminate the pain caused by DH such as neural stimulus blockers, anti-inflammatory drugs, tubule occluding agents, tubule sealants, remineralizing agents and also laser therapy.[3,8-9,16-17] However, the most recent challenge in the treatment of DH is the development of materials and protocols that not only alleviate the pain temporarily but also eliminate the cause of the problem and/or present a more long-lasting effect.[3,6,8]

In vitro and clinical studies have demonstrated that bioactive glasses (BAG) and bioactive glass-ceramics are effective in the treatment of DH.[3,13,18-22] Their micro or nanosized particles seem to attach to the dentine stimulating mineralization through the deposition of calcium phosphate over the dentine tubules.[18] Clinical studies have shown that the Bioglass® 45S5 particles can provide adhesion to the dentine, inducing the formation of a hydroxycabonate apatite (HCA) layer providing the block of the tubules, thus relieving pain for a long duration.[8,13,23] The same was observed with the Biosilicate® which rapidly deposited HCA layer surrounding tissue inside the dentine tubules resulting in clinical reduction in sensitivity by promoting the occlusion of dentine tubules.[24] However, if their effects on DH can last for long periods of time has not yet been evaluated.

BAG easy crystallization during the fabrication process can restrict bioactive glasses application. The majority of bioactive glass compositions do not support repeated heat-treatments, since this procedure results in uncontrolled crystallization that normally degrades their mechanical properties and can substantially decreases their bioactivity.[25] This occurrence causes many problems in the manufacturing of shaped devices, fibers or scaffolds.

Consequently, for clinical application, the use of bioactive glasses has mainly been limited to particulates.

To overcome this phenomenon, a new BAG composition (SiO_2–Na_2O–K_2O–MgO–CaO–P_2O_5) called as F18 was recently developed at the Vitreous Materials Laboratory (LaMaV – Federal University of São Carlos - UFSCar, São Carlos, São Paulo, Brazil) that shows very high glass stability when compared to other BAG, coupled to high bioactivity, thus allowing one to make bioactive fibers, meshes and 3D shapes. This new composition allows the formation of continuous fibers with precise diameter of approximately 20 μm (±5.1 μm). [26,27,28,29] However, the ability for this new BAG composition to remineralize dentine and the application over the treatment of DH still needs to be evaluated.

Therefore, this article describes two case reports showing whether this new BAG could be an effective desensitizing agent for the treatment of DH over time.

Clinical parameters

The patients reported below had one common drawback that was sensitive teeth associated with gingival recession and exposure of cervical dentine (non-carious cervical lesions) or exposed root surface. To evaluate the level of pain a trained operator performed evaporative and mechanical tests as follows. An air application by means of dental syringe, close to the cervical enamel surface and a dental probe to scratch the cervical tooth surface on mesial to distal direction was used. In order to standardize this pain evaluation, all tests were conducted by the same operator and a similar air pressure and probe manipulation was persuaded. To determine the degree of DH a visual analog scale (VAS) was used and the patients were instructed to qualify their level of pain from 0 to 10, where the interval represented, e.g., 0 “no pain” and 10 “the worst pain possible”.[9,15]

Case Report 1

a) Patient Examination

The patient A. P. H., a 29-year-old woman reported to the Dental Clinic of the Araraquara School of Dentistry - UNESP, at the Department of Restorative Dentistry to an appointment, with a complaint of DH in her teeth. The patient related to have sensitivity in the upper and lower teeth, both to sweet, salty, cold and hot, and during speaking as well. A clinical examination was performed to observe the oral health conditions of the patient (Figure 1). It showed some cervical no carious lesions, and the sensitivity was triggered by evaporative test using cold air and tactile test using a probe with controlled pressure, both stimuli were applied for three seconds. The upper right teeth and upper and lower premolars

were the most reacted on the cervical area. Then, the patient signed the consent and authorization for the application of F18.

b) ***Patient History***

The patient's home care included three daily aggressive brushing using a soft toothbrush, daily flossing, and intermittent use of an anti-sensitivity toothpaste and occasional use of a chlorexidine rinse. The clinical photograph reflects the above findings, showing good gingival health with an absence of apparent supragingival plaque, however frequently associated with recession and some exposed dentine (Figure 1).

c) Bioactive-Glass Application:

An impression mold was taken from the oral cavity, and then silicone trays from upper and lower teeth were manufactured and adjusted. After receiving the trays, the patient was instructed regarding the application of BAG and the patient got an explanatory folder with all needed information. Three applications of the F18 (SiO_2–Na_2O–K_2O–MgO–CaO–P_2O_5), each during 8 hours, were performed overnight under intervals of 48 hours (the material was applied as a paste made of F18 particles – 10 μm mean particle size - and water in a 1:5 proportion). The patient returned to the Dental Clinic and the evaluation of the sensitivity was made by means of evaporative and tactile tests after each application. After the first application, the patient reported improvement in the sensitivity. These findings were observed after each next application, which showed a further improvement in the sensitivity and evidenced the effectiveness of the F18 in treating DH as shown in Tables 1-2 and Figure 2. The one year follow-up showed that the periodontal health remained satisfactory, although there were a slight increase in gingival recession, cervical no carious lesions and painful condition after one month sensitivity evaluation (Figures 2-3).

Case Report 2:

a) Patient Examination

The patient M. P., 46-year-old woman, after a chronic periodontitis treatment reported to the Dental Clinic of the Araraquara School of Dentistry – UNESP, at the Department of Restorative Dentistry to an appointment with a complaint of DH in her teeth which prevented her from receiving oral rehabilitation treatment. A clinical examination revealed evidence of early oral disease, absence of all second premolars and lower first molars, old restorations probably infiltrated or misfits and the sensitivity was triggered by evaporative and tactile tests as was done in patient 1. During the first sensitivity test, the patient reported high levels of pain. The patient related to have sensitivity in the most of the upper and lower teeth especially pronounced sensitivity in quadrants 2, 3 and 4, being the evaporative test the most

symptomatic. Afterward, the patient signed the consent and authorization for the application of BAG.

b) Patient History

The patient restored her periodontal health in order to proceed to the oral rehabilitation treatment, although the pain caused by DH yielded, the patient gave up of the rehabilitation treatment. Any stimulus (sweet, salty, cold, hot, brushing her teeth or even during oral breathing) were enough to trigger a disabling pain, what was harming her overall health. During the periodontal treatment, the patient was instructed about oral healthcare as brushing her teeth using a soft toothbrush and flossing after every meal. Several treatments based on fluoride varnishes and adhesive application were performed which promoted slight improvement, however, these results were not enough to go on with the treatment. The clinical photograph (Figure 4) showed that periodontal health had been restored, thus was possible to observe the sequels of the chronic disease as the gingival recession and exposed roots for example.

c) Application of the Bioactive Glass:

The BAG application was performed following the same protocol used for patient 1. The first application promoted a slight improvement on the sensitivity. Then, the patient reported a further improvement on DH after the third application showing the effectiveness of the F18. One month after the evaluation, a fourth application was made to maintain the sensitivity level low (Tables 3-4 and Figure 5). One year later, the patient reported a slight increase on gingival recession, loss of cervical restoration on lower right premolar (44), staining of the composite resin restorations, presence of apparent supragingival plaque and deficient oral health care (Figure 6). The patient related that the level of sensitive pain was high but not as before the treatment.

Discussion:

DH frequently occurs as a result of loss of dental enamel and cementum tissues or also as an exposure of dentinal tubules.[1,10,22,30] Different factors are involved in the intensity and degree of sensitivity and their perception can change for each individual.[19] Many predisposing factors can place people at risk for DH such as gingival recession, tooth wear, lifestyle behaviors, oral self-care habits, and the pH of the oral environment, which may be related to dietary as well as xerostomic conditions.[31]

Different mechanisms were proposed for the DH etiology (DI, OR and HT), although the most accepted theory is the HT. The concept of HT is based on the fluid movement of the

open tubules of an exposed dentine surface. When there is an cementum exposure, the same process is assumed.[5-9,13,15]

Many materials, substances and techniques have been used aiming to reduce or eliminate DH. They include the use of toothpastes containing potassium salts, fluoride, composite resins materials, laser, BAG among others. These materials and techniques usually exert their effects through sealing dentinal tubules or through disturbing the transmission of nerve impulses.[1-2,8,10,13,19]

In the present study, the novel BAG F18 was effective in reducing the DH. In both clinical cases, even with the different initial clinical oral conditions, the therapeutic response occurred within 24 hours after the first application and increased over time. The level of pain decreased gradually and the perception of the pain relief remained even after one year for both patients, even the VAS score of patient 2 has remained/increased in 68% for mechanical stimuli.

Patient 1 was a young adult worried about her dental aesthetic, however dental pain greatly impacted on her oral treatment options. Then, the application of F18 using a pre-established protocol was suggested. On the first application of F18, the patient stated that it was possible to feel “something happening with her teeth” because they were “tingling gently”. A continuous improvement was observed during the following applications as seen in Tables 1- 2 and Figure 2. After one year, Patient 1 reported that the pain sensation reappeared, however it was possible to observe an increase in gingival recession and non-carious cervical lesions on molars and premolars, which could contribute to the condition above.

On the other hand, the patient 2 had just completed a periodontal treatment when set off generalized teeth sensitivity. An oral rehabilitation treatment was planned for this case however the pain sensation increased and patient feared that the pain would get worst. Some approaches were tested such as the application of fluoride varnishes and adhesive systems, but no improvement regarding the relief of DH was observed. Thus, it was decided to apply F18 as an alternative to pain control. Following the same trend as patient 1, a gradual pain relief was observed for each F18 application. The one year follow up showed that the high painful sensitivity yielded mainly by mechanical stimuli due to a deficient oral healthcare and an increase in the gingival recession, root exposure and non-carious cervical lesions (Tables 3-4 and Figure 6). So, the modification in the oral environment could have led to infiltrated restorations such as the loss of composite restoration on lower right first premolar. This patient had cervical restorations in the majority of their teeth and this could have impacted the

dental sensitivity. Finally, the patient stated that despite of the high sensitivity level on VAS scale after one year it was not as disabling as before the beginning of the BAG treatment.

The findings on this study can be attributed to the F18 efficient remineralization potential due to its biocompatibility and high bioactivity leading to a decrease on patient pain sensation. According to Burwell, Litkowski & Greenspan, in 2009, BAG and glass-ceramics can induce osteogenesis in physiological systems.[32] Also, these materials are capable to provide the regeneration of enamel and dentine tissues. The mechanism of action of F18 is also to be similar to other BAG and glass-ceramics.[3] When F18 particles are exposed to an aqueous environment, they begin to dissolve and leach ions such as Ca, Na, K and P to the medium, leading to the raise of the surrounding pH and further to the depositing of a HCA, which is chemically and structurally similar to the mineral phase of enamel and dentine.[8,33] The localized increase in pH helps to precipitate the calcium and phosphate ions from the F18 particle, along with calcium and phosphorus found in saliva, to form a calcium phosphate (Ca-P) layer. This compound is nucleated on the hydroxyapatite wall of the dentine tubules (i.e. heterogeneous nucleation), where the interfacial energy is lower than that in aqueous solution (i.e. homogeneous nucleation).[8] Mineral salts from saliva may create crystal precipitates on the dentine surface. As the particle reacts with the medium and the deposition of calcium and phosphorus complexes continues, this layer crystallizes into HCA. The combination of the residual particles and the newly formed HCA layer results in the physical occlusion of dentinal tubules, which relieves DH.[34] In vitro studies have shown that this layer is resistant to acid challenges and is mechanically strong, as well as the bactericidal effect of F18 and its cristalization that occurs more slowly, producing fibers with a controlled diameter what leads to a better remineralization pattern.[26,28] Continuous release of calcium from this layer may help to maintain the protective effect on dentine and constant occlusion of the dentine tubules.[8]

For both patients the clinical conditions were inclined to get worst without any intervention. The application of F18 showed to diminish the initial painful condition and that could be maintained over time. After one year from the bioactive glass application the levels of pain were not as disabling as they were before the proposed treatment. So, it is possible to infer that the DH treatment with F18 was effective for both patients.

Conclusion:

The application of the novel F18 has been proved to be effective for the treatment of DH in patients with different oral health conditions. The therapeutic response was rapid and the decrease in DH pain levels were observed within 24 hours after the first application and

remained until after one year, making F18 a potential long-lasting alternative for treating DH, this wide spread clinical condition.

Tables

	Group of teeth														
	URM	ULM	LLM	LRM	URP	ULP	LLP	LRP	URC	ULC	LLC	LRC	URI	ULI	LLI	LRI
Initial assessment	10	5	0	2.5	10	0	7	10	10	0	2	0	5	0	2.5	0
Application 1	5	5	0	2.5	2	0	3	0	0	0	0	0	0	0	2.5	0
Application 2	1.5	0	0	0	0	0	0	0.5	0	0	0	0	0	0	0	0
Application 3	1.5	1	0	0	0.5	0	0	1	0	0	0	0	0	0	0	0
After 1 month	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Application 4	0	0.5	0	0	0	0	0	0.5	0	0	0	0	0	0	0	0
1 year follow up	1,5	1	0	0	0	0	9	1,5	0	0	0	0	0	0	0	0

Legend: UR: upper right; UL: upper left; LR: lower right; LL: lower left; M: molars; P: pre-molars; C: canine; I: incisors.

Table 1. Mean VAS score to tactile stimulus before and after bioactive glass application - Patient 1.

	Group of teeth															
	URM	ULM	LLM	LRM	URP	ULP	LLP	LRP	URC	ULC	LLC	LRC	URI	ULI	LLI	LRI
Initial assessment	5	2,5	0	5	10	0	5	10	5	0	0	0	0	0	2,5	0
Application 1	2,5	0	0	1	10	0	5	7,5	0	0	0	0	0	0	0	0
Application 2	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Application 3	2,5	0	0	0	2,5	0	1	1	0	0	0	0	0	0	0	0
After 1 month	0	0	0	1	1,5	0	0	1	0	0	0	0	0	0	0	0
Application 4	0	0	0	0	1,5	0	1	0	0	0	0	0	0	0	0	0
1 year follow up	5	0	1,5	4	8	3	5,5	1,5	0	0	0	0	0	0	0	0

Legend: UR: upper right; UL: upper left; LR: lower right; LL: lower left; M: molars; P: pre-molars; C: canine; I: incisors.

Table 2. Mean VAS score to evaporative stimulus before and after bioactive glass application - Patient 1.

.

	Group of teeth														
	URM	ULM	LLM	LRM	URP	ULP	LLP	LRP	URC	ULC	LLC	LRC	URI	ULI	LLI	LRI
Initial assessment	3	8	7,5	5	4	8	10	9	3	10	7	8	9,5	9,5	9	9,5
Application 1	2,5	6,5	6,5	6	3	10	10	10	3	10	8	10	3,5	8	10	10
Application 1	0	0	1,5	0	0	3	0	0	0	4	0	0	0	1,5	0	3
Application 1	0	0	0	0	0	0	2	0	0	3	0	2	0	0	1	0
After 1 month	2	1	1	0	0	3	3	2	0	5	0	0	1	0	0	0
Application 4	0	1	0	0	0	0	3	0	0	2	0	0	0	0	0	0
1 year follow up	2	7	10	9	3	10	10	9	4	6	10	10	5	7	9	10

Legend: UR: upper right; UL: upper left; LR: lower right; LL: lower left; M: molars; P: pre-molars; C: canine; I: incisors.

Table 3. Mean VAS score to mechanical stimulus before and after bioglass application - Patient 2.

	Group of teeth															
	URM	ULM	LLM	LRM	URP	ULP	LLP	LRP	URC	ULC	LLC	LRC	URI	ULI	LLI	LRI
Initial assessment	5	6	6,5	9	5	10	5	5	6	10	5	10	5	10	10	10
Application 1	3	5	6,5	5	5	10	8	8	10	10	5	8	5	10	8	8
Application 2	0	0	1	0	0	0	0	0	3	4	0	0	1	1,5	0	0,5
Application 3	0	0	0	0	0	0	0	0	3	4	0	0	1,5	1,5	1	1
After 1 month	2,5	1	1	0	0	0	0	0	0	5	0	0	7,5	10	1	0
Application 4	0	0	0	1	0	0	2	0	0	2	0	0	0	1	0	1
1 year follow up	2,5	4	2	6	2	6	6	3	1	10	8	7	6	9,5	6	8

Legend: UR: upper right; UL: upper left; LR: lower right; LL: lower left; M: molars; P: pre-molars; C: canine; I: incisors.

Table 4. Mean VAS score to evaporative stimulus before and after bioglass application - Patient 2.

Figures

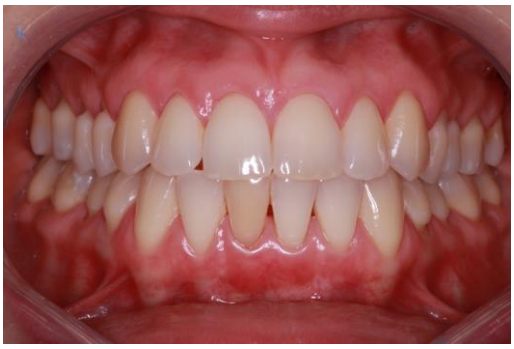

Figure 1. Initial clinical evaluation – Patient 1.

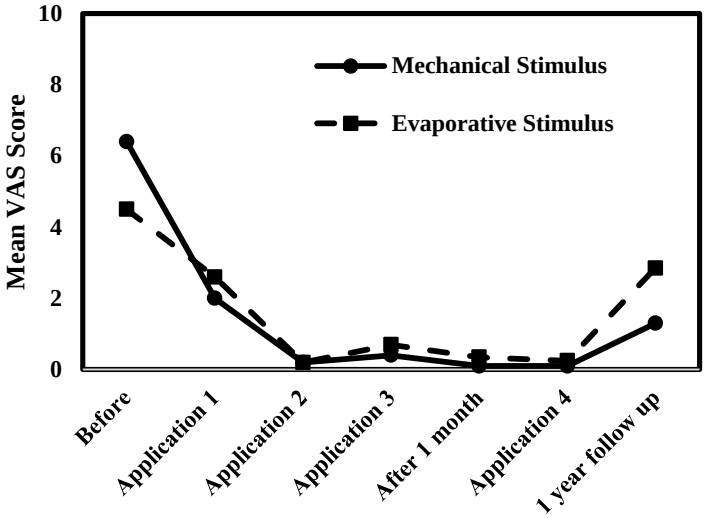

Figure 2. Mean VAS score overtime for patient 1 before and after treatment with bioactive glass for all teeth evaluated.

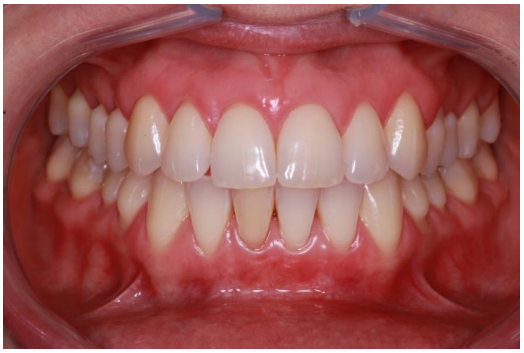

Figure 3. One year follow up – Patient 1.

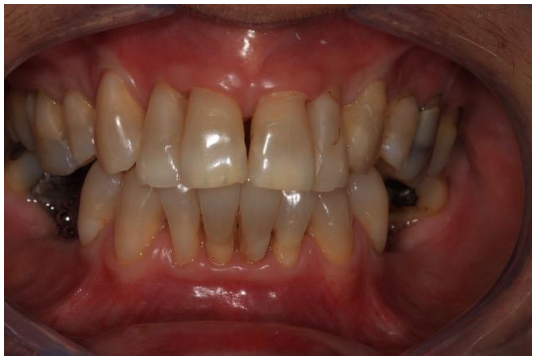

Figure 4. Initial clinical evaluation – Patient 2.

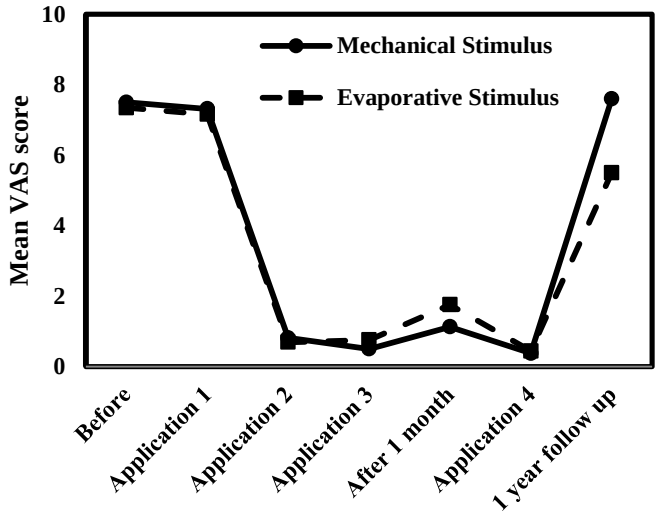

Figure 5. Mean VAS score overtime for patient 2 before and after treatment with bioactive glass for all teeth evaluated.

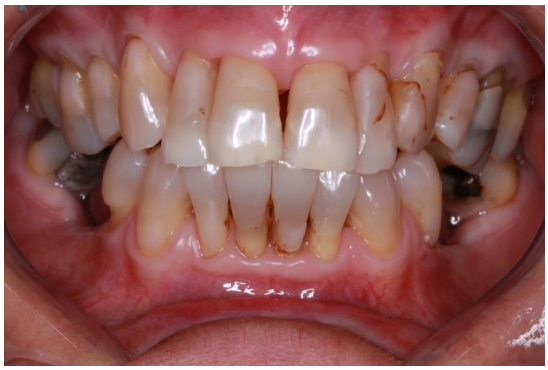

Figure 6. One year follow up – Patient 2.

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5 CONCLUSÃO

De acordo com a metodologia aplicada, pode-se concluir que:

- O vidro bioativo F18 foi o que obteve melhores resultados na remineralização da superfície dental desmineralizada apresentando valores de dureza próximos aos de um dente hígido, seguido pelo Biosilicato®.

- Foram observadas as primeiras impressões do efeito dessensibilizante do F18, apresentando resultado promissor na diminuição da sintomatologia dolorosa em longo prazo.

- Devido à escassez de dados na literatura sobre a proposta de utilização dos biomateriais apresentados nessa dissertação, em especial o F18, sugere-se que sejam realizados mais estudos laboratoriais assim como ensaios clínicos, para realmente compreendermos as limitações, implicações e possibilidades clínicas que esses materiais podem oferecer.

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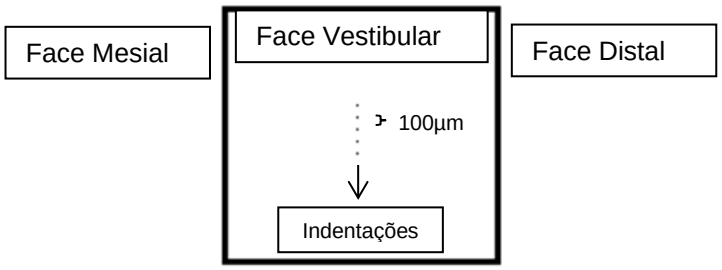
APÊNDICE A – Metodologia detalhada

A1. Delineamento do Estudo

Trata-se de um estudo experimental laboratorial (in vitro) aprovado pelo Comitê de Ética em Pesquisa Animal da Faculdade de Odontologia de Araraquara – UNESP (Processo nº 31/2017) (Anexo 1), cuja variável dependente foi alteração mineral do esmalte e a variável independente foi produtos remineralizadores (saliva, flúor gel acidulado, verniz fluoretado, Bioglass® 45S5, Biosilicate® e F18). A hipótese nula foi que não haveria diferença quanto à capacidade de mineralização entre os diferentes tratamentos. Para melhor padronização do estudo, após o polimento da superfície dos espécimes foi considerado como fator de exclusão a presença de lesões, trincas, hipoplasia ou mancha branca devido à natureza e hábitos alimentares do gado bovino. Por outro lado, o critério de inclusão foi dado pela análise da dureza Knoop superficial de cada um dos espécimes utilizando-se um microdurômetro Knoop com carga de 25g por 10s, sendo feitas cinco indentações com 100 µm de distância entre elas, e aqueles espécimes que apresentaram um valor de dureza acima de 270 KHN forão incluídos na pesquisa. Então, os espécimes selecionados foram randomizados e divididos em seis grupos experimentais. O número amostral por grupo para o teste de dureza Knoop longitudinal foi de 8 espécimes (n=48), desses foram sorteados três espécimes por grupo para espectroscopia micro-RAMAN (n=21). Para MEV foram assumidos três espécimes com esmalte hígido, três espécimes com esmalte desmineralizado e três espécimes remineralizados de cada grupo (n=21).

Figura A1.1. Análise de dureza Knoop superficial.



Fonte: Arquivo pessoal do autor.

A2. Preparo dos Espécimes

Dentes bovinos íntegros e sem anomalias foram selecionados por meio de exame sob lupa estereoscópica (aumento de 10x) para garantir que não apresentassem nenhum tipo de lesão, trincas, hipoplasia ou mancha branca na superfície e armazenados em solução de timol a 1% por 15 dias para descontaminação dos mesmos (Figuras A2.1, A2.2 e A2.3).

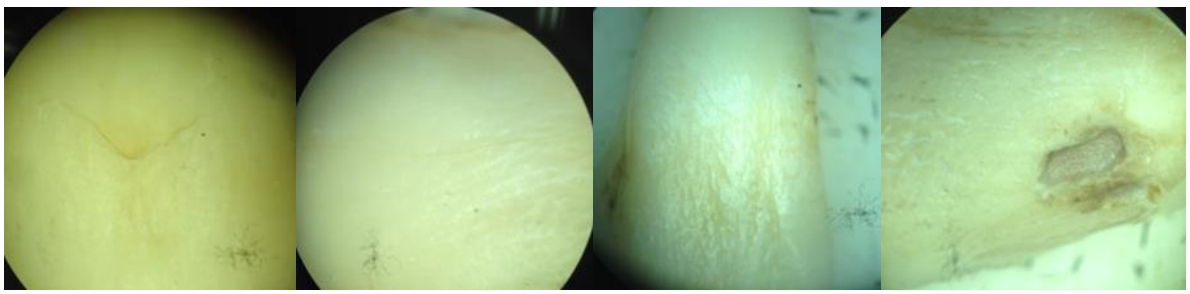
Figura A2.1 - Dente bovino.

Fonte: Arquivo pessoal do autor.

Figura A2.2 - Inspeção visual sob lupa esteroscópica (10X).

Fonte: Arquivo pessoal do autor.

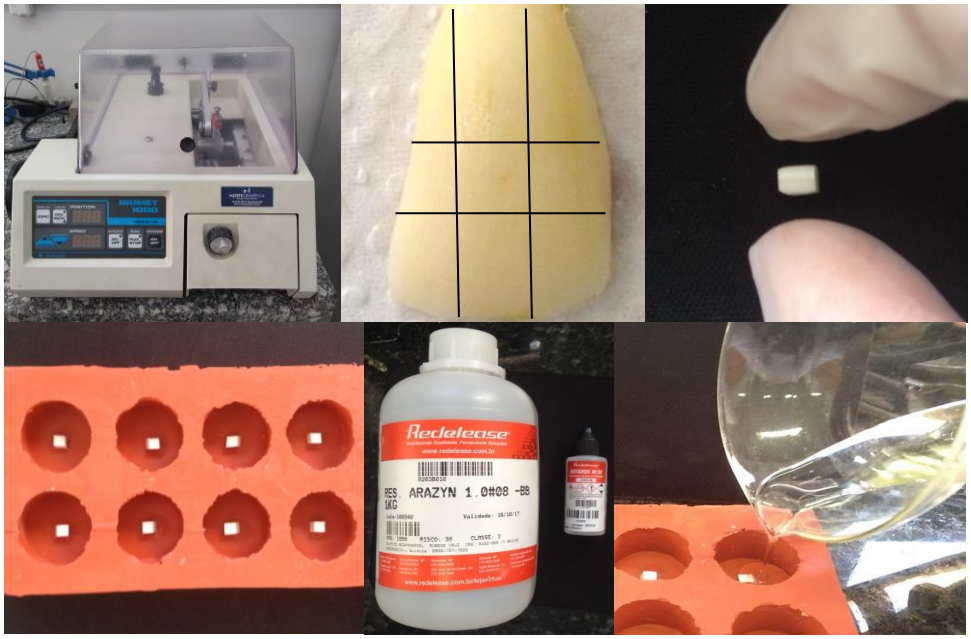
Figura A2.3 - Lesões, trincas, hipoplasia ou mancha branca.



Fonte: Arquivo pessoal do autor.

Os dentes selecionados foram posteriormente limpos com escova Robinson e pasta de pedra pomes + água e armazenados em água destilada a 4ºC (±1ºC) com trocas semanais. As porções radiculares foram seccionadas utilizando-se máquina de cortes (Isomet; Buehler Ltd, Lake Bluff, IL, USA), as porções coronárias foram fixadas em aparato de madeira com godiva e levadas a máquina de cortes sob-refrigeração, realizando-se secção dupla no sentido cérvico-incisal e outra no sentido mésio-distal para a obtenção dos fragmentos (blocos) de esmalte nas dimensões de 4x4 mm. A espessura dos fragmentos foi ajustada em 3 mm por meio de desgaste feito com disco de corte em peça reta na face dentinária. Os fragmentos selecionados foram posicionados em molde em silicona previamente confeccionado (SIQMOL TH-600, Siquiplás, São Paulo, SP, Brasil), e incluídos em resina incolor autopolimerizável (Kit Resina de Poliéster Arazyn 1.0#08-BB Lote 106540 e Gel coat com Catalizador Botanox M-50 Lote 024263, Redelease®, São Paulo, SP, Brasil) (Figura A2.4).

Figura A2.4 - Processo de corte de dente bovino em Isomet e inclusão com resina de poliéter.



Fonte: Arquivo pessoal do autor.

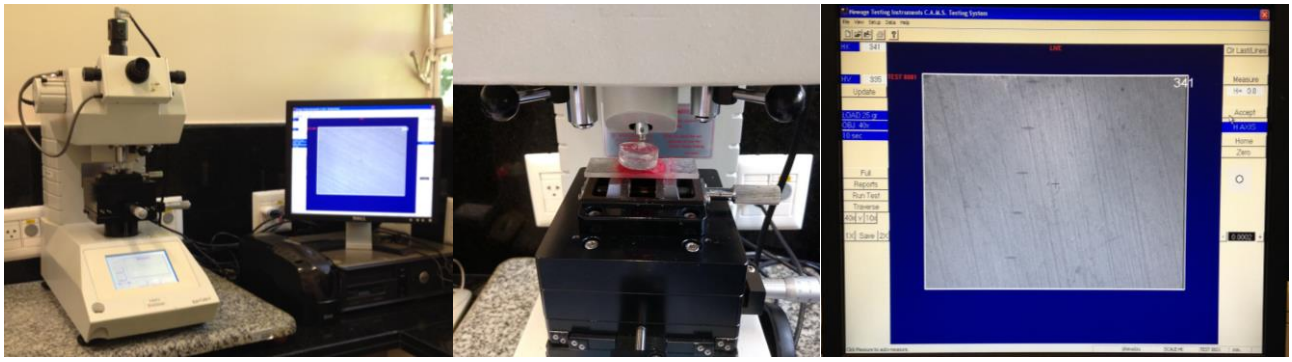
Então, a superfície dos espécimes foi planificada e polida com lixas d'água de granulação 600, 1200 e 1500 montadas em politriz (Panambra Struers DP-10, Panambra, São Paulo, Brasil) e, posteriormente, com pasta diamantada e disco de feltro (Figura A2.5).

Figura A2.5 - Processo de polimento e limpeza dos espécimes para análise de dureza superficial.

Fonte: Arquivo pessoal do autor.

Após o polimento, os espécimes foram limpos em uma cuba ultra-sônica com água destilada durante 5 min. Uma nova análise da superfície dos espécimes foi feita sob lupa estereoscópica e eles foram submetidos ao teste de dureza Knoop superficial para avaliação da dureza média de sua superfície (Figura A2.6).

Figura A2.6 - Análise de dureza superficial em microdurômetro knoop para seleção dos espécimes.



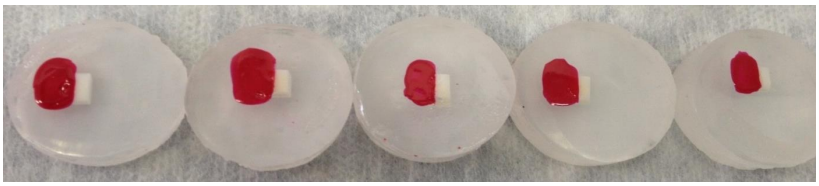
Fonte: Arquivo pessoal do autor.

A3. Indução da lesão artificial de cárie:

Previamente à indução da lesão de carie artificial em esmalte, os espécimes do grupo controle tiveram metade de sua superfície protegida aonde nenhum tratamento foi feito. Então, os espécimes foram colocados de forma individual em recipientes e cobertos com 0,5 cm de gel de metilcelulose de viscosidade média (Methocel® MC, Sigma®, Inc – lote: BCBR2107V), o qual foi deixado na geladeira

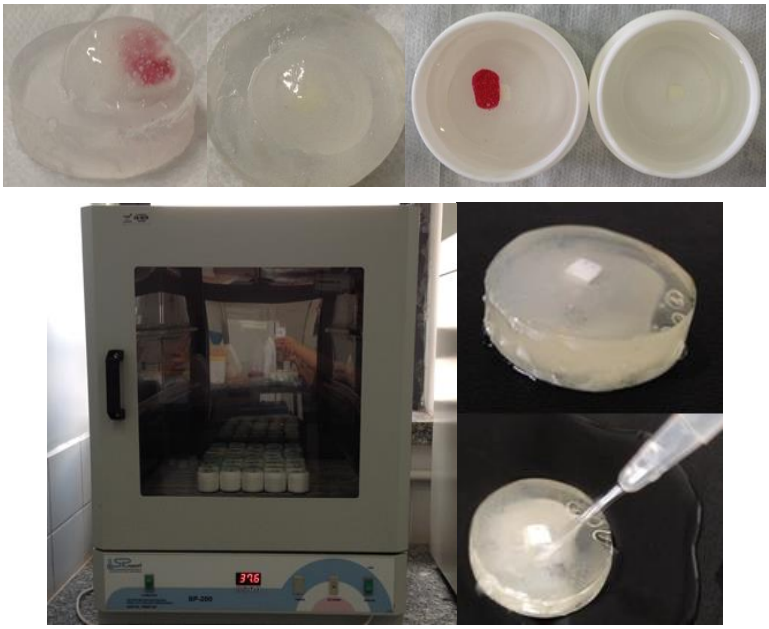
durante 12 horas para chegar à temperatura de 4°C e adquirir maior viscosidade a fim de, posteriormente, ser coberto com solução de 0,1 M ácido láctico (Ácido Lático 85%, 1 Kg, Lote: 1606001222, Via Farma SM Empreendimentos Farmacêuticos LTDA, Anápolis, Goiás, Brasil) previamente preparada com seu pH ajustado para 4,6 com NaOH e agente bacteriostático azida de sódio, e os recipientes foram selados e colocados em incubadora a 37°C durante 10 dias. O gel teve a finalidade de reduzir a velocidade de difusão do ácido e manter os íons perdidos pelo dente próximos a sua superfície, permitindo a precipitação superficial e produção da lesão de subsuperfície. Após o período de desmineralização, o gel foi removido da superfície dos espécimes lavando-os abundantemente com água destilada. Então, os espécimes dos grupos experimentais tiveram metade de sua superfície protegida com esmalte cosmético (controle da área desmineralizada) e a outra face exposta foi tratada de acordo com os diferentes protocolos remineralizadores.

Figura A3.1 – Proteção de metade da superfície hígida do grupo controle. Antes da indução de cárie.



Fonte: Arquivo pessoal do autor.

Figura A3.2 – Processo de indução de cárie com gel de metilcelulose e ácido lático, incubação por 10 dias a 37ºC e remoção do gel/ácido.

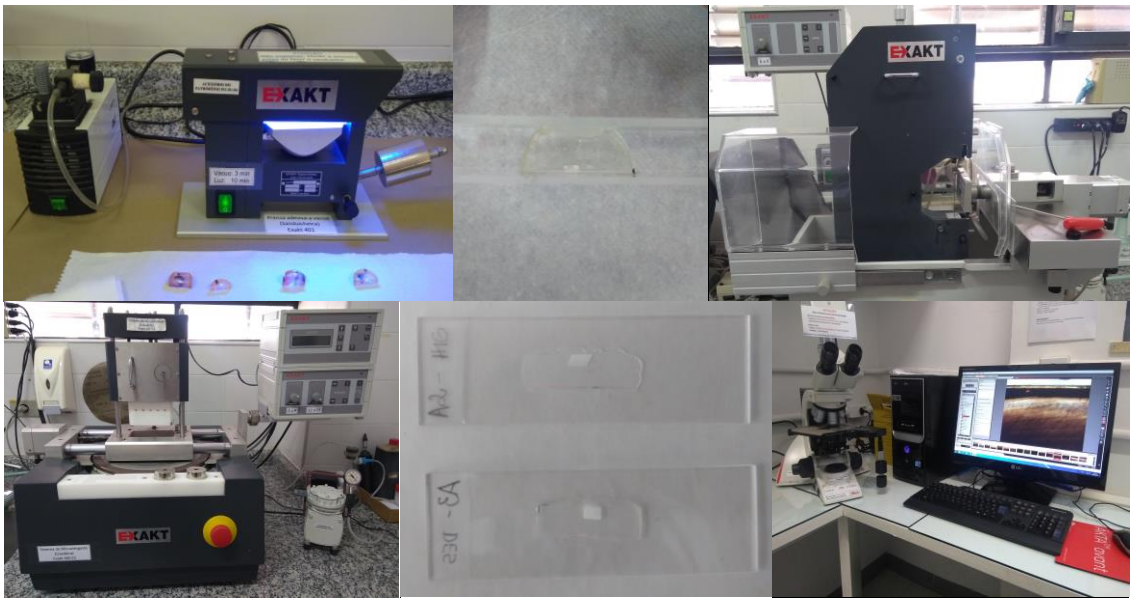


Fonte: Arquivo pessoal do autor.

A4. Microscopia de luz polarizada

Após o ciclo de indução de lesão de cárie artificial, dois espécimes foram analisados por meio de microscopia de luz polarizada para verificar a formação de lesão de subsuperfície. Os espécimes foram seccionados longitudinalmente em micrótomo especial (EXAKT – Micrótomo para corte de metais e sistema de processamento histológico) a fim de obter cortes de 100 µm (± 10). Os fragmentos ficaram imersos em água destilada e foram examinados sob microscópio de luz polarizada (Leica DM 2500) com aumento de 20x para verificar a formação de lesão de subsuperfície analisando a imagem por meio do software Leica Application Suite (LAS) Core versão 3.4.

Figura A4.1 – Processo de preparo dos dentes desmineralizados em EXAKT para microscopia de luz polarizada.

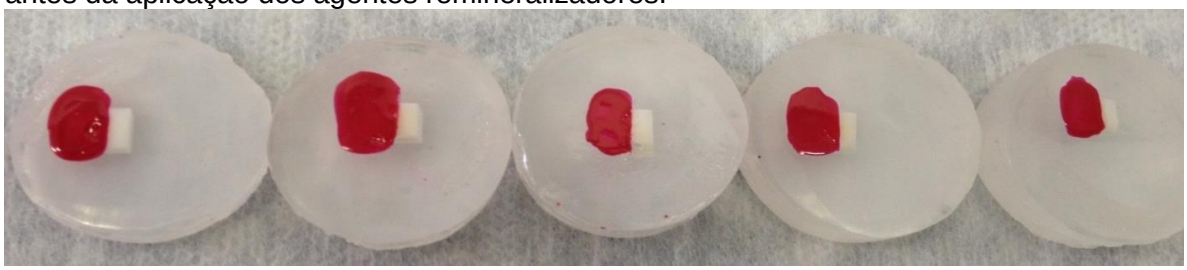


Fonte: Arquivo pessoal do autor.

A5. Protocolo de aplicação dos agentes remineralizadores

Os espécimes foram armazenados em saliva artificial após a aplicação dos diferentes produtos remineralizadores por 24 horas, então foram armazenados em água destilada para a realização das análises.

Figura A5.1 – Proteção de metade da superfície desmineralizada dos grupos experimentais antes da aplicação dos agentes remineralizadores.



Fonte: Arquivo pessoal do autor.

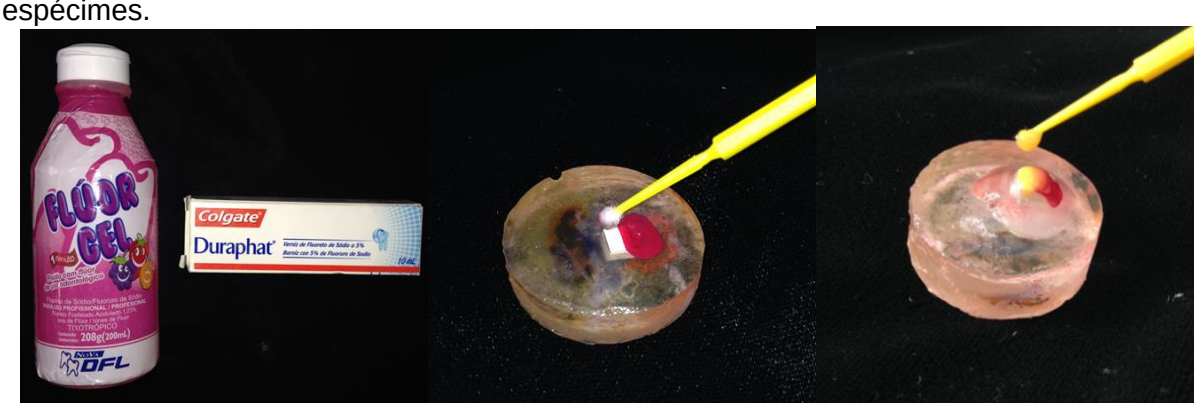
Quadro A5.1 - Protocolo de aplicação dos agentes remineralizadores.

Grupos amostrais	**Protocolo de aplicação**	**Fornecedores**
G1 – Controle	Os espécimes foram imersos em saliva artificial por 24 h antes da análise.	Farmácia de manipulação - Farmagna
G2 - Flúor gel acidulado	Aplicado na superfície do esmalte desmineralizado exposto com microbrush por 4 sem contato com saliva artificial.	Nova DFL
G3 - Duraphat®	Aplicado na superfície do esmalte desmineralizado exposto com microbrush (esperou-se um minuto para secagem do produto), armazenado em saliva artificial e removido após 6 horas* usando lâmina de bisturi.	Colgate (Colgate-Palmolive)
G5 - Biosilicate® G6 - Bioglass® 45S5 G7 - F-18	Moldeiras individuais foram feitas com placas de silicone para cada amostra a fim de manter o produto aplicado em contato com a superfície de esmalte desmineralizada exposta por 6 horas*. O pó de cada biovidro foi misturado com água deionizada (1:10) aplicado com microbrush e removido lavando com água deionizada por 30 segundos.	Laboratório de Materiais Vítreos – LaMAV, da Universidade Federal de São Carlos – UFSCAr.

*O tempo de ação dos produtos foi de 6 horas para padronizar o tempo de exposição da superfície desmineralizada sob os diferentes agentes remineralizadores.

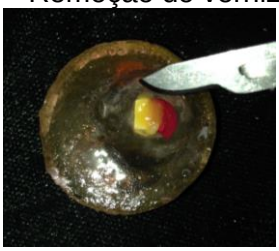
Fonte: Arquivo pessoal do autor.

Figura A5.2 – Aplicação do gel e verniz flouretados sobre a superfície desmineralizada dos espécimes.



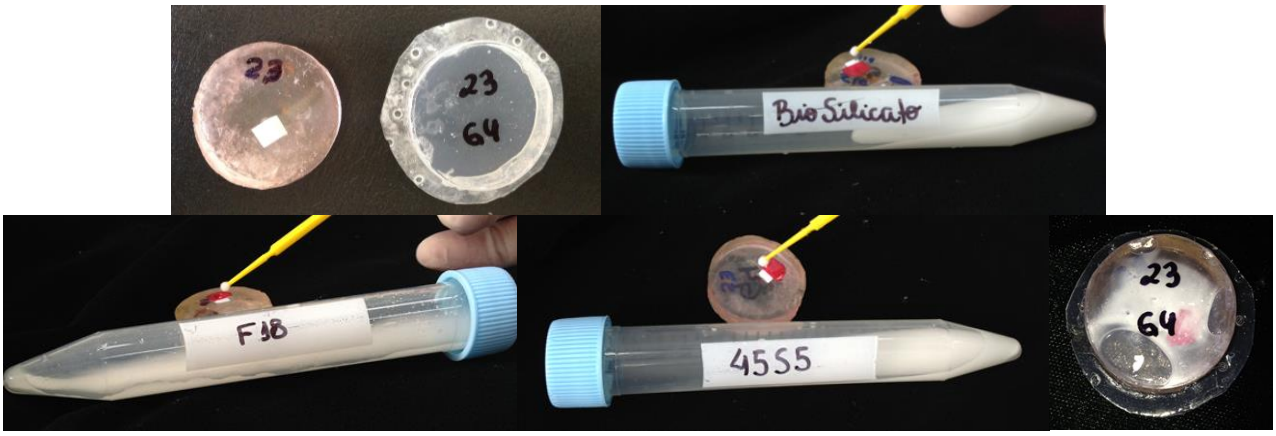
Fonte: Arquivo pessoal do autor.

Figura A12 – Remoção do verniz fluoretado.



Fonte: Arquivo pessoal do autor.

Figura A13 - Aplicação dos vidros bioativos e moldeira individual.

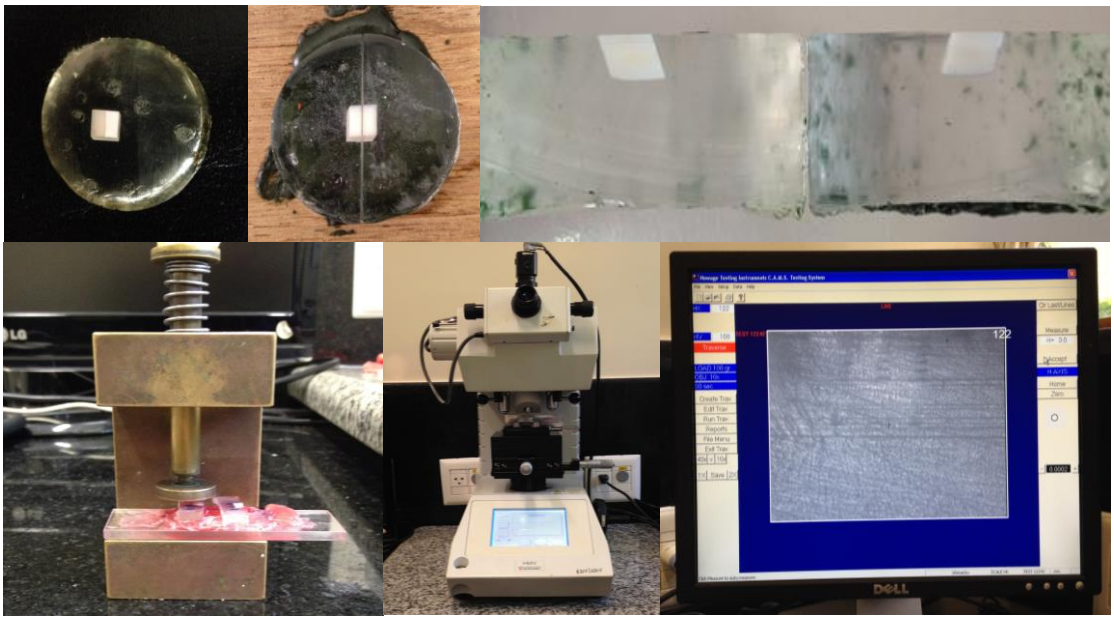


Fonte: Arquivo pessoal do autor.

A6. Análise da dureza Knoop transversal.

Para que o esmalte fosse analisado em toda a extensão (profundidade) da lesão artificial formada, os espécimes foram segmentados ao meio de forma a permitir análise longitudinal comparativa entre eles.

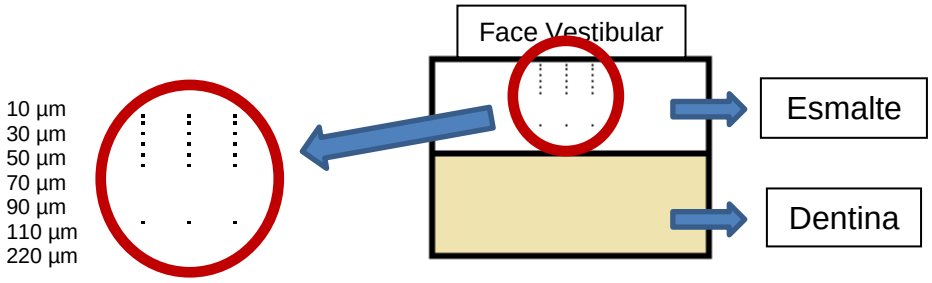
Figura A14 – Corte transversal dos espécimes e análise da dureza knoop longitudinal.



Fonte: Arquivo pessoal do autor.

Então, foram feitas três indentações em cada uma das profundidades do dente, sendo 10, 30, 50, 70, 90, 110 e 220 µm em relação à superfície externa do esmalte. A primeira sequência de indentações foi feita na região central e as outras duas a uma distância de 100 µm para ambos os lados da linha central em cada uma das metades de esmalte exposto usando uma carga de 25 gf por 10 s.

Figura A6.1. Análise de dureza Knoop superficial.



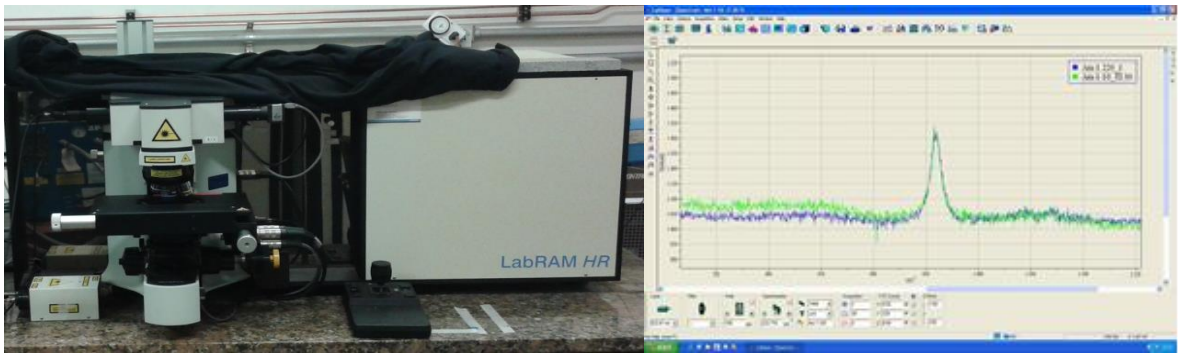
Fonte: Arquivo pessoal do autor.

A7. Espectroscopia micro-Raman

O micro-Raman foi utilizado como análise química qualitativa e quantitativa da composição do conteúdo mineral sobre a seção transversal dos espécimes. A análise quantitativa usando a espectroscopia Raman baseou-se no fato de que a intensidade de sua dispersão é proporcional ao número de moléculas presentes no volume do espécime que será avaliado, assim, a intensidade do pico e a área do pico foram utilizadas na análise quantitativa. Portanto, seria possível detectar as mudanças no conteúdo mineral comparando a intensidade do pico/área do pico,

desde que as condições experimentais permanecessem constantes (como tamanho do ponto do laser, volume do espécime sondado, potência do laser, etc.). Para tanto utilizou-se o Micro-Raman (Espectrômetro Raman modelo Lab RAM HR da Horiba Jobin Yvon Inc) equipado com laser de 632,8 nm, que obtém espectros de espalhamento Raman de 50 a 1300 cm^{-1}. Foi confeccionado um mapa de linhas seguindo as mesmas profundidades da análise da dureza Knoop transversal para analisar a alteração mineral através da lesão. As áreas dos picos foram calculadas utilizando-se o software LabSpec 5 Automation (Horiba Jobin Yvon Inc - Raman).

Figura A15 – Corte transversal dos espécimes e análise da dureza knoop longitudinal.



Fonte: Arquivo pessoal do autor.

A8. Microscopia Eletrônica de Varredura

Para tanto, os espécimes foram desidratados em soluções de etanol por vinte minutos em concentrações crescentes: 50%, 60%, 70%, 80%, 90% e, a seguir, por mais sessenta minutos em etanol a 100%. Os espécimes foram secos durante 12 horas em estufa e na sequência fixados em “stub” de alumínio e revestidos por ouro durante 2 minutos. Então, a superfície do esmalte foi examinada em microscopio eletrônico de varredura (JSM-6610LV, JEOL, USA Inc) com resolução de 1000 e 5000x.

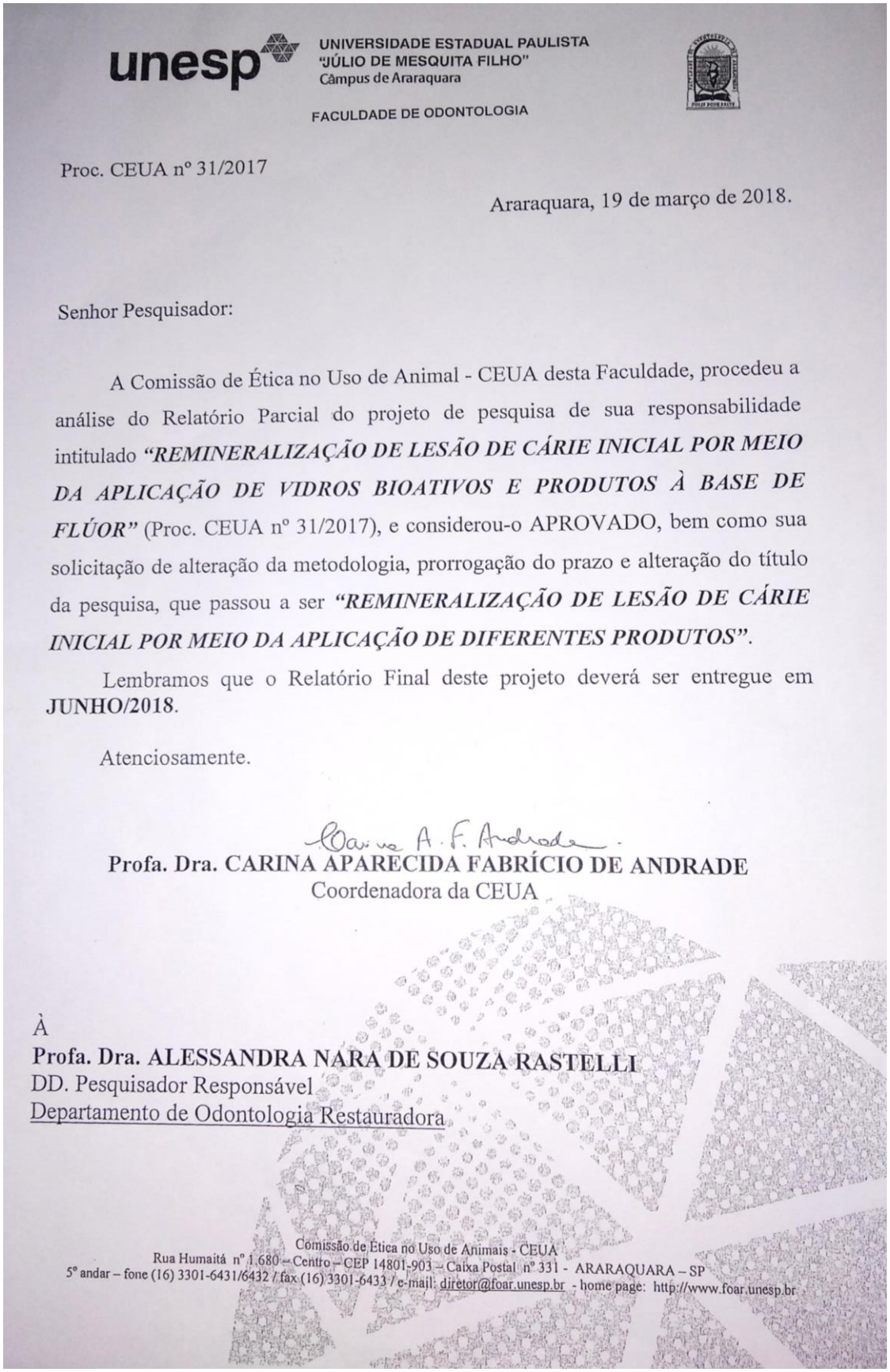
Figura A16 – Preparo dos espécimes para análise de microscopia eletrônica de varredura.

Fonte: Arquivo pessoal do autor.

A9. Análise estatística

Utilizou-se a análise de variância de medidas repetidas (ANOVA) com correção de Greenhouse-Geisser (GG). Em seguida, para confirmar qual tratamento foi o mais eficaz como remineralizador, uma comparação média dos dados transversais, independente da profundidade, foi realizada por meio de medidas repetidas ANOVA com correção de Bonferroni antes e após os tratamentos. A análise estatística foi feita utilizando o (IBM SPSS Statistics Version 19) com nível de significância de 5%.

ANEXO 1 – Aprovação pelo Comitê de Ética em Pesquisa Animal



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"JÚLIO DE MESQUITA FILHO"
Câmpus de Araraquara

FACULDADE DE ODONTOLOGIA

Proc. CEUA nº 31/2017

Araraquara, 19 de março de 2018.

Senhor Pesquisador:

A Comissão de Ética no Uso de Animal - CEUA desta Faculdade, procedeu a análise do Relatório Parcial do projeto de pesquisa de sua responsabilidade intitulado ***"REMINERALIZAÇÃO DE LESÃO DE CÁRIE INICIAL POR MEIO DA APLICAÇÃO DE VIDROS BIOATIVOS E PRODUTOS À BASE DE FLÚOR"*** (Proc. CEUA nº 31/2017), e considerou-o APROVADO, bem como sua solicitação de alteração da metodologia, prorrogação do prazo e alteração do título da pesquisa, que passou a ser ***"REMINERALIZAÇÃO DE LESÃO DE CÁRIE INICIAL POR MEIO DA APLICAÇÃO DE DIFERENTES PRODUTOS"***.

Lembramos que o Relatório Final deste projeto deverá ser entregue em **JUNHO/2018**.

Atenciosamente.

Carina A. F. Andrade

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Coordenadora da CEUA

À
Profa. Dra. ALESSANDRA NARA DE SOUZA RASTELLI
DD. Pesquisador Responsável
Departamento de Odontologia Restauradora

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Não autorizo a publicação deste trabalho até 09 de maio de 2020

Araraquara, 09 de maio de 2018.

Ana Claudia Pedroso de Barros