UNIVERSIDADE DE SÃO PAULO
FACULDADE DE ODONTOLOGIA DE BAURU

CASSIANA KOCH SCOTTI

# Evaluation of the anti-caries effect of a glass ionomer sealant and a fluoride varnish

# Avaliação do efeito anticárie de um selante ionomérico e um verniz fluoretado

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Dissertação constituída por artigo apresentada a Faculdade de Odontologia de Bauru da Universidade de São Paulo para obtenção do título de Mestre em Ciências no Programa de Ciências Odontológicas Aplicadas, na área de concentração Dentística.

Orientador: Prof. Dr. Rafael Francisco Lia Mondelli

Co-orientador: Profª. Drª. Juliana Fraga Soares Bombonatti

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## DEDICATÓRIA

*Em gratidão, dedico este trabalho:*

*A* **Deus** *que abençoou com toda a sua graça a batalha e a vitória, proporcionando ao longo desses dois anos momentos e pessoas (anjos) inesquecíveis. A Ele que guiou meus passos e acalantou meu coração diante das dificuldades, mostrando que estas são traduzidas hoje em grandes oportunidades que me fizeram mais forte pra seguir o meu caminho de felicidade e equilíbrio na Sua sabedoria.*

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***Augusto Cury***

# ABSTRACT

# ABSTRACT

**Evaluation of the anti-caries effect of a glass ionomer-based sealant and a fluoride varnish**

**Objective:** The aim of this *in vitro* study was to evaluate the mineral loss of enamel treated with a glass ionomer-based sealant and a fluoride varnish after pH-cycling, through the analysis of the microhardness of the enamel as well as the evaluation of Ca/P/F ratio by Energy-dispersive X-ray spectroscopy analysis (EDS). **Methods:** Thirty six bovine enamel blocks were analyzed for microhardness and randomly assigned to one of the following three treatment groups (n = 12): G1 — Clinpro XT Varnish (3M-ESPE); G2 — Duraphat (Colgate - Palmolive); or G3 — No treatment. The specimens were subjected to pH-cycling for 7 days. Subsequently, they were analyzed by EDS, and the final evaluations of the microhardness at standard distances from the treatment material as well as the cross-sectional microhardness at standard distances from the outer surface of the enamel were performed. **Results:** The EDS findings revealed that, in G3, the loss of calcium and phosphorus was significantly higher than G1 and G2 after pH-cycling; however, there were no significant differences in the initial and final fluoride ion concentrations among the three groups. The values of the surface microhardness and cross-sectional microhardness in G1 and G2 were higher than those in G3, at different distances of the materials. **Conclusion:** Within the limitations of this study, it may be concluded that both the evaluated materials were able to partially inhibit enamel demineralization when subjected to a dynamic pH-cycling model.



**Key words:** Demineralization. Fluoride. Dental enamel.

# Resumo

# RESUMO

**Objetivo:** O objetivo deste estudo *in vitro* foi avaliar a perda mineral do esmalte bovino tratados com um selante ionomérico e um verniz fluoretado após ciclagem de pH, por meio da analise da microdureza de superfície do esmalte e seccional, além da avaliação da composição química de superfície por análise quantitativa em espectroscopia de energia dispersiva (EDS). **Métodos:** Trinta e seis blocos de esmalte bovino após polimento e análise da composição superficial em EDS, foram submetidos a analise da microdureza superficial e sequencialmente divididos aleatoriamente divididos em três grupos em função do tratamento empregado (n=12): G1) Clinpro XT-Varnish (3M-ESPE), G2) Duraphat (Colgate-Palmolive) e G3) Sem tratamento. Os espécimes foram submetidos à ciclagem de pH por sete dias. Posteriormente, a análise em EDS e microdureza superficial final foi realizada, onde para microdureza em distâncias padronizadas em relação ao material de tratamento, bem como a análise da microdureza longitudinal em distâncias padronizadas da superfície externa do esmalte. **Resultados:** A análise em EDS demonstrou que para o G3 a perda de cálcio e fósforo foi significativamente maior do que para o G1 e G2 após a ciclagem de pH, já para o íon flúor não houve diferenças significativas entre os grupos nas duas condições. A microdureza de superfície e longitudinal do esmalte nos permite inferir que maiores valores de dureza foram evidenciadas para G1 e G2 do que para G3, nas diferentes distâncias do material. **Conclusão:** Considerando as limitações deste estudo concluímos que ambos materiais testados foram capazes de inibir parcialmente a desmineralização do esmalte submetido a um modelo dinâmico de ciclagem de pH.



**Palavras-chave:** Desmineralização. Flúor. Esmalte dental.

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# 1 Introduction

# 1 INTRODUCTION

Despite the advances in our understanding of the multifactorial etiology of dental caries and its behavioral and opportunistic characteristics, white spot lesions still present a frequent problem in dental clinics (MARCENES et al., 2013; COSTA et al., 2012).

The appearance of white spot lesions characterizes the first clinical signs of caries activity and results in enamel demineralization because of the interaction of several factors that cause biofilm accumulation (ZERO et al., 1999; FEJERSKOV, 2005).

These lesions have specific characteristics and develop especially at the expense of the loss of calcium and phosphate ions. The loss of calcium and phosphate ions occurs by the action of organic acids such as acetic and lactic acids. The acids release hydrogen ions, which bond with the calcium and phosphate, leading to the molecular breakdown and disorganization of the prismatic structure of enamel, culminating in increased porosity of the hydroxyapatite crystals (FEJERSKOV, 2005).

The conditions for the formation of these lesions are the result of an ionic imbalance in the oral environment, characteristic of established disease, which drastically affects the quality of life (GORELICK et al., 1982).

This scenario is commonly seen in patients during orthodontic treatment, when teeth are potentially most affected by caries because of biofilm accumulation as well as the fact that the number of cariogenic microorganisms increased in this phase and maintenance of oral hygiene is more difficult (BENKADDOUR et al,. 2014; NASCIMENTO 2016; PERRINI F 2016).

In 2011, a study reported that the number of white spot lesions in patients undergoing orthodontic treatment significantly increased in the first 6 months of treatment and continued to grow at a slower pace 12 months after treatment (TUFEKCI et al,. 2011).

Some evidences suggest that the increased prevalence and severity of white spot lesions in patients during orthodontic treatment, with a reported potential to develop within 4 weeks of the initiation of treatment and a rate of incidence of 2–97%

(GORELICK et al., 1982; JULIEN et al., 2013). Other study in 2011 reports that prevalence of white spot lesions was 32% before fixed brace treatment and this values increased to 74% after treatment (ENAYA et al., 2011). These findings reflect a significant problem posed by white spot lesions during and after orthodontic treatment, considering that the sequelae are generally not reversed in their entirety.

This problem highlights the necessity for the promotion of oral health as key to reducing the incidence of caries among the population. Different approaches have been proposed to preventing the development of white spot lesions as well as contain their progression in the same conservative manner as the prior intervention (BEHNAN et al., 2010; WIEGAND et al., 2007; STECKSÉN-BLICKS et al., 2007).

Among the arsenal of materials available, materials that release fluoride ions in high concentrations, such as glass ionomer-based cements, neutral or acidic fluoride gels, glass ionomer-based sealants, and fluoride varnishes, have great potential to promote the remineralization of enamel through professional application (GREIG; CONWAY, 2012; TOOD et al., 1999; GONZÁLEZ-CABELZAS et al., 2012).

Topical application of fluoride using these different vehicles attempts to restore the normal conditions and ionic balance between the tooth structure and the oral environment. The presence of high concentrations of fluoride enables its adsorption onto the enamel surface, forming true reservoirs of calcium and fluoride ions as calcium fluoride ($CaF_2$), which contributes to the incorporation of the less-soluble fluoride into the enamel, thus making the glaze more resistant to new challenges by acids (BENSON et al,. 2013).

Based on these findings, several studies have reported that the application of these strategies in conjunction with the control of the etiological factors of the disease contributes to the arrest of active carious lesions and accelerates disease regression (ARRENDS, et al., 1986; REHDER NETO 2009; ROBINSON 2009)

Recently, an ionomer sealant, Clinpro XT Varnish (3M - ESPE), has been commercially introduced as a light-cured coverage able to release fluoride, calcium, and phosphorus. It has been indicated by the manufacturer for the remineralization of white spots and protection against the development of carious lesions around orthodontic appliances. However, scientific evidence supporting its short and long-term performance is scarce.

The persistence of this critical scenario and the potential impact of its resolution on current dentistry, in the social context, are significant factors in devising strategies for the promotion of oral health in order to prevent the formation and development of white spot lesions in patients at a high risk for caries through the application of fluorides using different vehicles.

Considering the importance of the issues described hitherto, there is an urgent need for developing strategies for the control of cariogenic activity during orthodontic therapy. Thus, the purpose of this *in vitro* study was to evaluate the anti-caries effect of a glass ionomer-based sealant and a fluoride varnish on enamel in order to test the null hypothesis that the ionomer sealant Clinpro - XT and the fluoride varnish Duraphat will not prevent enamel demineralization when challenged with a dynamic model of pH-cycling.

# 2 ARTICLE

# 2 ARTICLE

The article presented in this Dissertation was written according to the Angle Orthodontics instructions and guidelines for article submission (Annex A).

# EVALUATION OF THE ANTI-CARIES EFFECT OF A GLASS-IONOMER SEALANT AND A FLUORIDE VARNISH


## ABSTRACT

**Objective:** The aim of this *in vitro* study was to evaluate the mineral loss of enamel treated with a glass ionomer-based sealant and a fluoride varnish after pH-cycling, through the analysis of the microhardness of the enamel as well as the evaluation of Ca/P/F ratio by Energy-dispersive X-ray spectroscopy analysis (EDS). **Materials and Methods:** Thirty six bovine enamel blocks were analyzed for microhardness and randomly assigned to one of the following three treatment groups (n = 12): G1 — Clinpro XT Varnish; G2 — Duraphat; or G3 — No treatment. The specimens were subjected to pH-cycling for 7 days. Subsequently, they were analyzed by EDS, and the final evaluations of the hardness at standard distances from the treatment material as well as the cross-sectional hardness at standard distances from the outer surface of the enamel were performed. **Results:** The EDS findings revealed that, in G3, the loss of calcium and phosphorus was significantly higher than G1 and G2 after pH-cycling; however, there were no significant differences in the initial and final fluoride ion concentrations among the three groups. The values of the surface hardness and cross-sectional hardness in G1 and G2 were higher than those in G3 at different distances of the materials. **Conclusion:** Within the limitations of this study, it may be concluded that both the evaluated materials were able to partially inhibit enamel demineralization when subjected to a dynamic pH-cycling model.


**KEYWORDS**: Demineralization. Fluoride. Dental enamel.

## INTRODUCTION

Despite the advances in our understanding of the multifactorial etiology of dental caries and its behavioral and opportunistic characteristics, white spot lesions still present a frequent problem in dental clinics.[1,2]

This scenario is commonly seen in patients during orthodontic treatment, when teeth are potentially most affected by caries because of biofilm accumulation as well as the fact that the number of cariogenic microorganisms are substantially increased in this phase and maintenance of oral hygiene is more difficult.[3,4,5]

Some studies have also reported the increased prevalence and severity of white spot lesions in patients during orthodontic treatment, with a reported potential to develop within 4 weeks of the initiation of treatment and a rate of incidence of 2–97%[6,7]

This problem highlights the necessity for the promotion of oral health as key to reducing the incidence of caries among the population. Different approaches have been proposed to preventing the development of white spot lesions as well as contain their progression in the same conservative manner as the prior intervention.[8,9,10]

Among the arsenal of materials available in the market, materials that release fluoride ions in high concentrations, such as glass ionomer-based cements, neutral or acidic fluoride gels, glass ionomer-based sealants, and fluoride varnishes, have great potential to promote the remineralization of enamel through professional application.[11,12,13]

The persistence of this critical scenario and the potential impact of its resolution on current dentistry, in the social context, are significant factors in devising strategies for the promotion of oral health in order to prevent the formation and development of white spot lesions in patients at a high risk for caries through the application of fluorides using different vehicles.

Considering the importance of the issues described hitherto, there is an urgent need for developing strategies for the control of cariogenic activity during orthodontic therapy.

Thus, the aim of this *in vitro* study was to evaluate the anti-caries effect of a glass ionomer-based sealant and a fluoride varnish on enamel in order to test the null hypothesis that the glass ionomer-based sealant Clinpro XT and the fluoride varnish Duraphat will not prevent enamel demineralization when challenged with a dynamic model of pH-cycling.

## METHODS

### Experimental design

An *in vitro* study was conducted to evaluate the anti-caries effect of a glass ionomer-based sealant (Clinpro XT; 3M - ESPE) and a fluoride varnish (Duraphat; Colgate) through the analysis of the microhardness of enamel and quantitative analysis of the enamel composition by EDS. (Fig. 1).

### Selection and preparation of enamel blocks

Seventy blocks (3x6x3) were obtained from bovine incisors, which were selected after cleaning, removal of debris, and exclusion of units with cracks, fractures, hypocalcifications, and excessive wear of the incisal third.

The crowns were cut with a precision cutting machine (Isomet low-speed saw; Buehler, Lake Bluff, IL, USA) using two double-sided diamond disks (Extec Diamond Wafering blade; 5" x 0.015" x 1/2"; Extec Corp, Enfield, CT, USA). The cuts were processed at speed of 300 rpm under cooling with deionized water.

### Enamel polishing

The dentin surface was planed to obtain specimens of 2-mm thickness, with the surface enamel and dentin parallel to each other.

After the blocks were repositioned and mounted on a metallographic polisher (Aropol 2V; Arotec, Cotia, SP, Brazil), they were polished using #600 and #1200 grit sandpaper discs (CarbiMet paper discs; Buehler, Lake Bluff, IL, USA) sequentially.

The final polishing was performed using a felt disc with a 1-μm diamond suspension (Buehler, Lake Bluff, IL, USA) at a high speed under a weight of 172 g.

At each change of grit as well as at the end of the polishing process, the specimens were ultrasonicated in deionized water for 2 min using an ultrasonic device (USC 750; Unique Group, Indaiatuba, SP, Brazil) in order to remove any residue from polishing.

**Ca/P/F ratio by Energy-dispersive X-ray spectroscopy analysis (EDS).**

A micro-analytical technique employed to estimate quantitatively the amount of mineral of the enamel using a Scanning Electron Microscope (Personal SEM [PSEM] eXpress; Aspex Corporation) equipped with an energy-dispersive spectrometer. Thus, Ca/P/F ratios were analysed for the groups before and after pH-cycling.

**Hardness measurements**

Surface hardness (Knoop) was determined using a microhardness tester (Shimadzu HMV-2; Shimadzu Corporation, Kyoto, Japan) under a 25-g for 10 s, coupled to an image analysis software (CAMS-WIN; Newage Industries, Southampton, PA, EUA).

The hardness values were calculated from the arithmetic mean of five indentations in the central region of the specimens[14], at standard distances of 150 μm, 300 μm, and 450 μm from the area of treatment, with a separation of 100 μm between each indentation (Fig. 2). To establish the homogeneity of the samples, specimens with average surface hardness > 10% or < 10% 350 KHN were excluded.

**Treatment of specimens**

Twenty-four blocks (3x6x2) was divided into three areas, each of 2-mm width and 3-mm length, and twelve blocks (3x4x2) was divided into two areas. The area designated as the cross-sectional hardness control was also covered. Then, the central area of the specimen was secured with tape in order to restrict the treatment of the enamel with the test materials to the secondary experimental areas (Fig. 3).

Following treatment, the tape protecting the central area was removed and the specimens were stored for 24 h in relative humidity at 37°C.

**pH-cycling**

The specimens were subjected to a dynamic model of pH-cycling for 7 days at 37°C. During the first 5 days, the specimens were immersed in a demineralizing solution for 6 h followed by immersion in a remineralizing solution for 18 h (Fig. 4). On the final 2 days of the protocol, the specimens were immersed in the remineralizing solution[15].

The specimens were immersed in the solutions separately. Each specimen was stored in a plastic container to avoid the sum effect of the fluoride ions released by the materials. In order to ensure total immersion of the specimens in the solutions, the volume of the solution in each container was maintained at 30 ml. Following pH-cycling, the specimens were stored at 37°C ± 1°C.

At each solution exchange, the specimens were washed under running deionized water, and the moisture was removed using blotting paper, before they were transferred to the next solution, which would have been stored in an incubator for 1 h prior to transfer. The solutions had been validated by a pilot test prior to this experiment.

**Analysis of the final surface hardness (SHf)**

Following the same method used in the analysis of the initial hardness (SHi) after pH-cycling, the final surface hardness of each of the specimens was evaluated at standard distances of the indentations, relative to the position of the materials applied to the enamel surface, in order to calculate the percentage of surface hardness loss values (%SH = [(SHi–SHf)/SHi]x100).

**Analysis of the final cross-sectional hardness of enamel**

The final cross-sectional hardness was evaluated after the sectioning of the specimens in the longitudinal direction, which ensured the exposure of both the experimental and control areas.

The sectioned specimens were embedded in a polymerized resin (Transoptic Powder; Buehler, Lake Bluff, IL, USA) using an embedding machine (PRE-30S; Arotec) and grouped according to each experimental group. They were then subjected to sequential buffing, as previously described. Under these conditions, the cross-sectional hardness was evaluated under a 25-g load for 10 s in the depth of 20, 50, 90, 110, and 220 μm from the external surface of the specimen in material area and control area. The same protocol was used at standard distances of 150, 300, and 450 μm from the area of material (Fig. 5).

**Statistical Analysis**

The statistical analysis was determined by Statistc Program (SPSS - 17).

**Surface hardness**

The effects of treatments on the final surface hardness were compared from a Generalized Linear Model, which assumed a distribution Tweedie Family (150μm q = 1.5, 300μm q= 1.5 and 450μm q=1.2) for response and Identity to the link function, with the initial hardness added as covariate, and treatment as factors.

The deviance of normality hypothesis was performed using Q-Q Plot and the Kolmogorov-Smirnov test. The hypothesis of homoscedasticity was observed from Deviance chart versus Linear Predictor. Both were not violated.

The results indicated that both the effect of initial hardness and the treatment is significant at the final hardness. To perform pairwise comparisons of treatment effects was made a Sidak correction test for the degrees of freedom.

**Cross-sectional hardness of enamel**

To analyze the data proceeded to the one-repeated measures analysis, considering two intra-subject factors (depth and distance) and a factor between-subjects (Group). We opted for the univariate analysis. The residue normality

hypothesis was performed using Q-Q Plot and the Kolmogorov-Smirnov test. The homoscedasticity hypothesis the residue was checked from the residue graph versus predicted values.

The Mauchly test was used to check sphericity. As the sphericity assumption was violated for testing the effects of depth and depth interaction X distance, there was the need for correction of degrees of freedom for the Huynh-Feldt method.

The test results Huynh-Feldt correction for the degrees of freedom indicate that the effects of interaction depth X Distance and Group X Group are significant, or the groups behaved differently in different depths and distances. Thus, the effects groups within each depth and group effects within each distance were compared using a Sidak method to correct degrees of freedom.

**Energy-dispersive X-ray analysis (EDS)**

To evaluate the effect of material on the calcium level ANOVA was constructed, and the effect of material added as a factor and the initial level of calcium as a covariate. The assumption of homogeneity of variance between treatments was not rejected by Levene, meeting the ANOVA assumptions. The residue of the normality assumption was not rejected by Kolmogorv-Smirnov test. The homoscedasticity hypothesis residue also seems to have been violated. Thus, for pairwise comparisons was used Sidak correction test.

In the fluoride results, the effect of each treatment was evaluated from using the Kruskal-Wallis test for comparison of independent samples.

To evaluate phosphorus results were used the Kruskal-Wallis test for comparison of independent samples that demonstrated results significant. Thus, to compare groups were used Mann-Whitney test.

## RESULTS

**Surface hardness measurements**

The marginal means and error deviations of the surface hardness of the experimental groups are shown in Table 1. The percentages of surface hardness loss are shown in Table 2.

Following pH-cycling, the treated groups, G1 and G2, showed partial loss of enamel surface hardness, without any significant difference of surface hardness between the two groups at different distances of 150 μm (p=0,882), 300 μm (p=0,995) and 450 μm (p=0,998). However, G3 showed a more severe and statistically significant loss of surface hardness compared to G1 and G2, at different distances from the treatment material (p<0,001).

**Cross-sectional hardness of enamel**

The values of the cross-sectional enamel hardness at different depths of the enamel surface (20, 50, 90, 110, and 220 μm) and at different distances from the material in the experimental groups are shown in Figure 7.

**Energy-dispersive X-ray analysis (EDS)**

The means values and standard deviations of the evaluated elements at the initial condition and after pH-cycling are shown in Table 4.

In the fluoride analysis, Kruskal-Wallis test resulted not significant (p-value = 0.686), indicating that the three groups showed no significant difference in reducing fluoride levels.

The Phosphorus analysis demonstrated by Kruskal-Wallis test there was significant differences to Phosphorus (p-value <0,001). The Mann-Whitney demonstrate that no differences to Clinpro-XT and Duraphat groups (p-value 0,478). Furthermore, showed significant differences to both groups in comparison no treatment group (p-value<0,001) indicating that significant reduction of phosphorus levels.

The analysis of Calcium by Sidak test demonstrate significant differences to no treatment group (p-value < 0,001). However, no differences were observed in Clinpro-XT and Duraphat groups (p-value = 0,961).

## DISCUSSION

The present study was conducted to determine the efficacy of two materials — A glass ionomer sealant recently insert, Clinpro XT varnish, and the fluoride varnish Duraphat, whose efficacy has already been proved — for potential use in therapeutic strategies for the prevention of caries in patients undergoing orthodontic treatment. According to the results of this study, the null hypothesis that the tested materials cannot inhibit the demineralization of enamel upon dynamic cycling-pH challenge was rejected.

According to some studies, enamel hardness is related to the concentration of minerals in the enamel, indicating a high correlation of microhardness analysis with the results of microradiography analysis, which is considered to be the reference standard for the evaluation of mineral loss.[16,17]

In contrast, the surface hardness data should be treated with caution since the evaluation of microhardness has been considered as a poor method for the objective evaluation of subsurface lesions because these lesions keep the surface layer more mineralized. Therefore, for a more conclusive analysis of the enamel hardness, the cross-sectional hardness data should also be evaluated. Several reports have shown that although cross-sectional hardness does not reflect the extent of mineral loss, it is a reliable parameter for assessing the mechanical properties of the substrate.[18,19]

Thus, in the present study, the results of the surface hardness analysis as well as the cross-sectional hardness analysis showed that both materials were able to inhibit the demineralization of enamel to a greater extent in comparison to the group that received no treatment. However, there was no evidence of any difference in the efficacies of the two materials from each other.

On the other hand, the unexpected results of the cross-sectional hardness led us to infer that the unprotected area of the material showed different behavior as the area protected with the nitrocellulose lacquer base.

Surprisingly, the areas adjacent to the tested materials apparently suffered partial demineralization; however, we could not establish any association between the extent of demineralization and the proximity of the material, on the basis of the results of either the surface hardness or the cross-sectional hardness.

In the areas directly subjected to pH-cycling, the extent of demineralization in

G3 was observed to be greater at depths of 20 and 50 μm; this indicates that the cycling method of pH-cycling used in this study promoted demineralization to these depths. In the G1 and G2, although a more pronounced partial loss of hardness was observed at distances of 20 μm and 50 μm. However, the values of the enamel hardness were higher compared to G3.

One possible explanation for these results is that the fluoride varnishes and the glass ionomer-based sealants are vehicles for the topical application of fluoride at high concentrations.[20,21,22,23] The fluoride is not only deposited on the enamel surface, but also released into the medium, which increases the level of mineral saturation and results in the formation of fluoride deposits. Thus, upon acid challenge, ionic balance is maintained not only by the release of these deposits, but also by the interaction of the fluoride in the most superficial layers of the enamel with the dissolution of hydroxyapatite.[24, 25, 26, 27]

Furthermore, from the results of EDS analysis in this study, the most obvious finding was that the percentages of calcium and phosphorus ions in G3 (no treatment) were lower compared to those in G1 and G2 after the cariogenic challenge. This result confirms the dissolution of hydroxyapatite and the loss of minerals into the medium in the absence of remineralizing agents. In contrast, the percentages of fluoride ions in the different groups showed no differences, leading us to conclude that the fluoride present in the materials was apparently not incorporated into the enamel.

The fluoride varnish Duraphat (G2) was chosen for evaluation in this study because its efficacy in preventing caries has been reported by several studies.[28,29,30,31,32] However, most of these studies have suggested that frequent applications of the fluorinated coating are required for the best results.

Although we expected that the group of specimens treated with the fluoride varnish Duraphat (G2) would control demineralization better than the glass ionomer-based sealant Clinpro XT, given that the fluoride-release mechanism of the former more direct compared to that of the latter, the similarity of the results in G1 and G2 can be attributed to the unique protocol followed for the application of the Duraphat varnish; the varnish was not reapplied during pH-cycling and the initial coating might have loosened during the cycling.[29,30,31,33,34,35]

Regarding the results of G1, some studies claim that the addition of calcium and phosphorus ions to a glass ionomer-based material increases their availability for

binding the released fluoride ions.[36,37] Therefore, the composition of the resin might not lower its efficacy since it showed similar behavior as the fluoride varnish in the present study.

A few studies in literature also discuss the dosage of fluoride required to prevent enamel demineralization; while high initial doses are more effective in increasing the resistance to demineralization, they could block the penetration of calcium ions into the subsurface of the enamel. Therefore, studies reports that high initial doses are best employed for inhibiting the formation of new lesions, and lower initial doses are most effective for remineralization and control of the progression of the lesion.[38,39]

Based on our findings, the fluoride varnish and the glass ionomer-based sealant evaluated in this study released fluoride in doses sufficient for inhibiting the formation of new lesions during pH-cycling.

Corroborating the results of the present study, an *in vivo* study published in 2014 reported that the application of the glass ionomer-based sealant Clinpro XT increased the resistance of enamel to demineralization compared to other fluoride-releasing agents, when applied to the occlusal surface of the posterior teeth in children between the ages of 7 to 12 years.[40]

Furthermore, an *in vitro* study published in 2013 noted that the glass ionomer-based sealant ionomer Clinpro XT varnish showed greater efficacy for the remineralization of artificial caries lesions in extracted molars in comparison to other means of remineralization such as fluoride-release systems and calcium and phosphate-release systems.[40] According to a previous study, the glass ionomer-based sealants tested in the study — Clinpro XT being one among them — provided greater protection from demineralization compared to resin sealants and non-fluoride release systems.[41]

Thus, because carious lesions initiate the degradation of enamel at the ultrastructural level and that this process of mineral loss and dissolution of dental tissues can be inhibited or prevented at the earliest by the use of preventive strategies,[43, 44, 45 46, 47] we propose the application of the Clinpro XT sealant not as a superior option to other materials, but as an alternative to resolving the progress of incipient carious lesions around orthodontic appliances. However, despite the encouraging outcome of this study, these results should be interpreted with caution.

Thus, more detailed research on this topic is required to better understand and conclusively establish the efficacy of the glass ionomer-based sealant Clinpro XT varnish in inhibiting demineralization.

## CONCLUSIONS

Considering the results and limitations of this *in vitro* study, we can conclude that both the tested materials were able to partially inhibit enamel demineralization when submitted to a dynamic pH-cycling model.

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## FIGURE CAPTIONS

**Fig 1.** Schematic drawing of the experimental steps (1. Selection of teeth; 2. Section crowns; 3. Obtaining blocks; 4. Initial surface hardness and EDS; 5. Treatment of the specimens; 6. pH-cycling; 7. Final surface hardness and EDS; 8. Section of the blocks; 9. Cross-sectional hardness)

**Fig 2.** Schematic representation of the surface microhardness at standard distances

**Fig 3.** Schematic representation of the specimen division (1. Control area; 2. Unprotected area; 3. Material area)

**Fig 4.** Table showing the composition of the chemical solutions used for pH-cycling

**Fig 5.** Schematic representation of the cross-sectional hardness at different areas of the specimen showing the standard distances to the material as well as to the enamel surface.

**Fig 6.** Graphical representation of surface hardness results

**Fig 7.** Graphical representation of cross-sectional hardness results

**Fig 8.** Graphical representation of EDS results

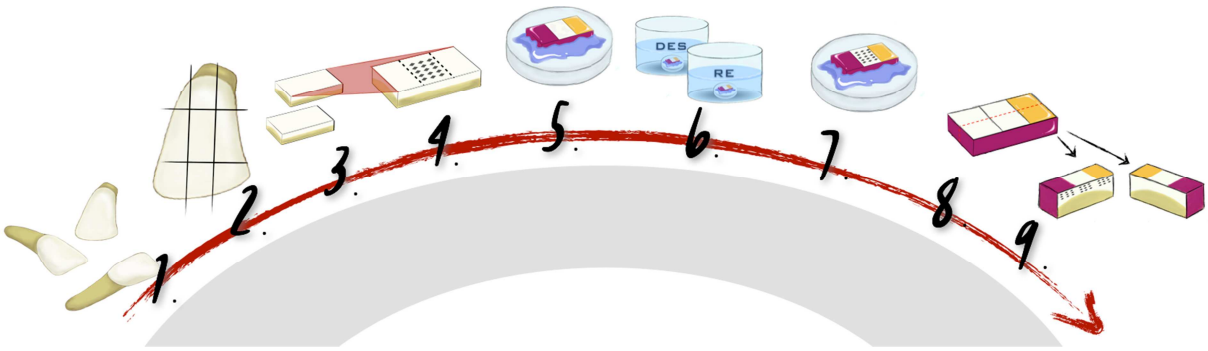


**Fig 1**

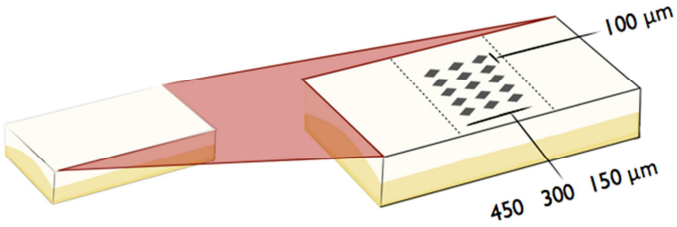


**Fig 2**

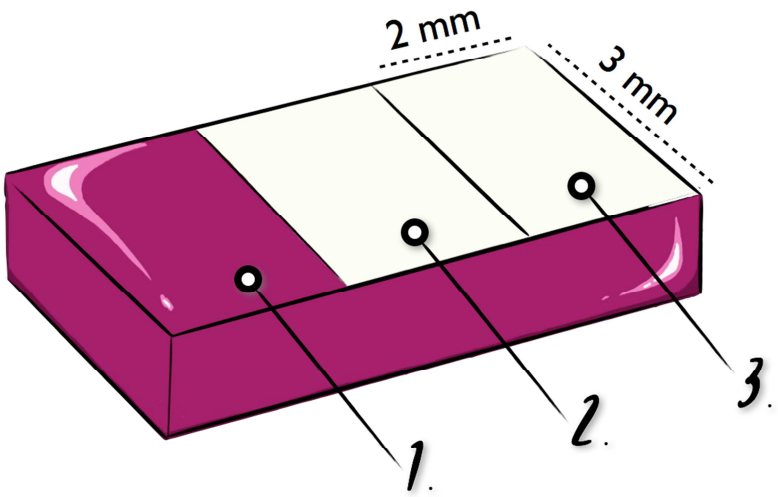


**Fig 3**

| | Chemical composition |
|---|---|
| **DES/RE Cycling** | Demineralizing solutions:<br>2,0mM Ca(NO3)2.4h2O,<br>2,0mM NaH2PO4.2H2O,<br>0,077mM acetate buffer,<br>0,02 ppm F.<br>Remineralizing solution:<br>1,5mM Ca(NO3)2.4H2O,<br>0,9mM NaH2PO4.2H2O,<br>150mM KCl,<br>0,1mol/l Buffer tris, 0,03 ppm F. |

**Fig 4**

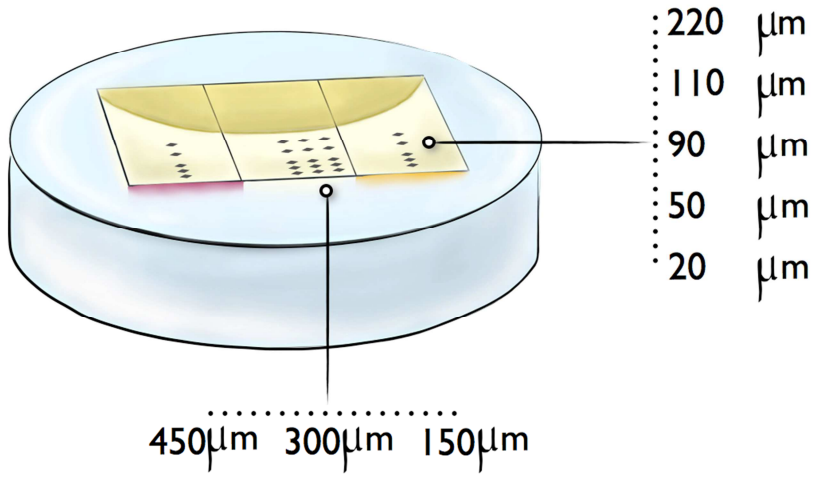


**Fig 5**

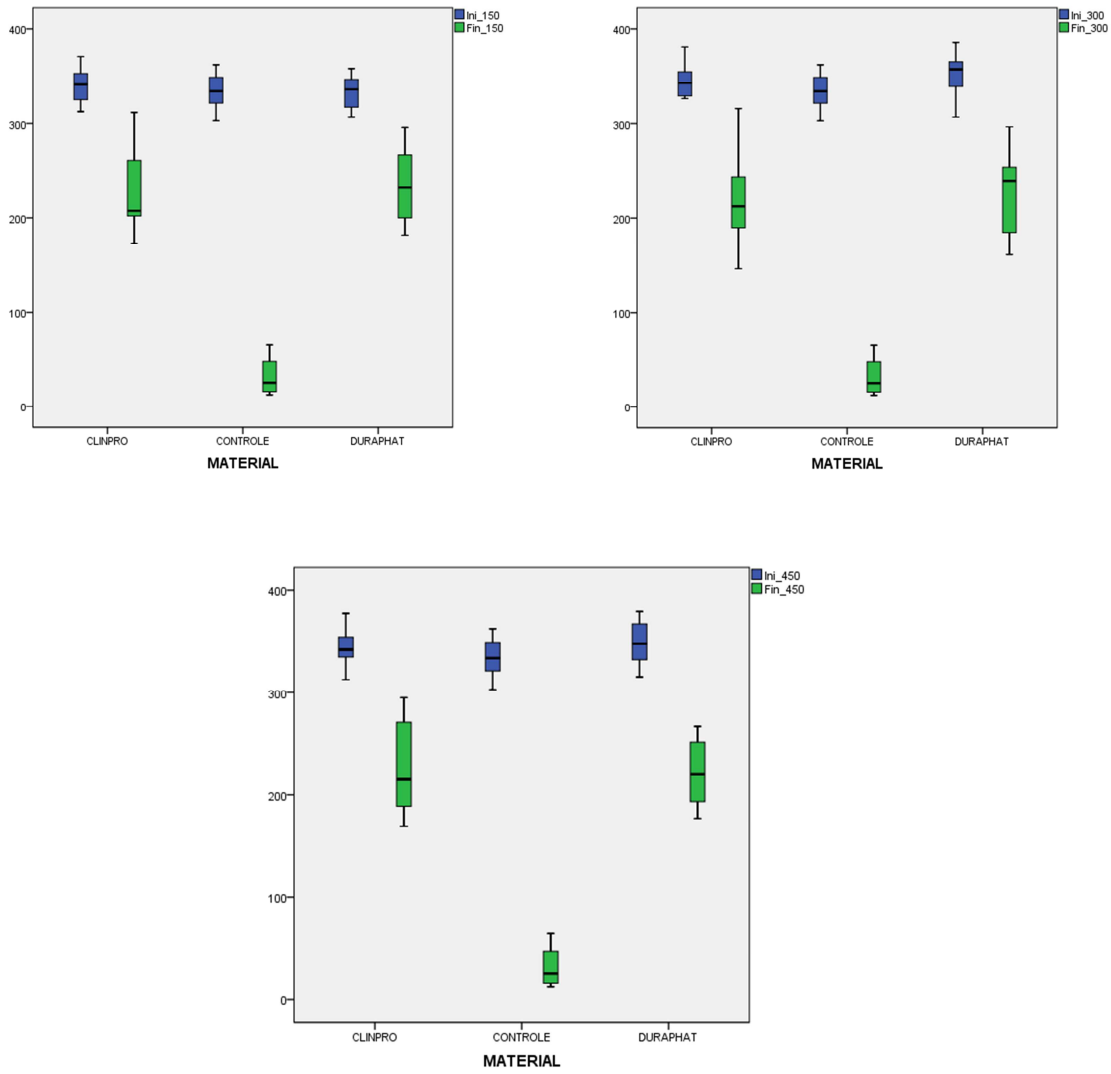
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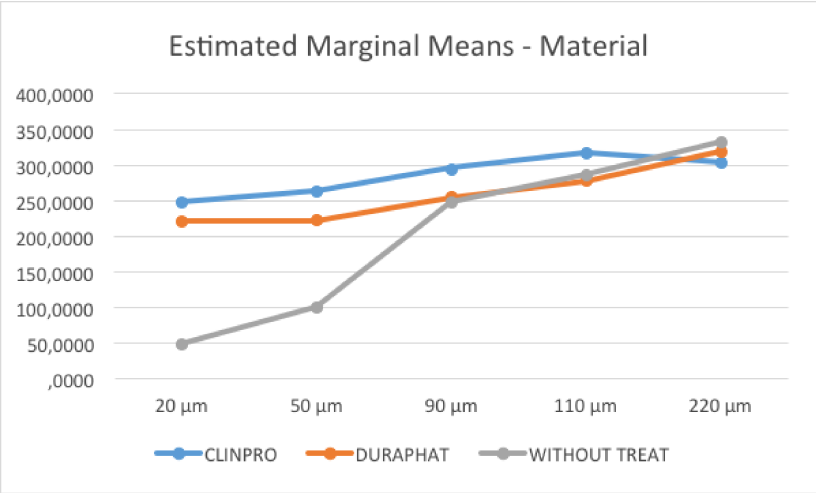
**Fig 6**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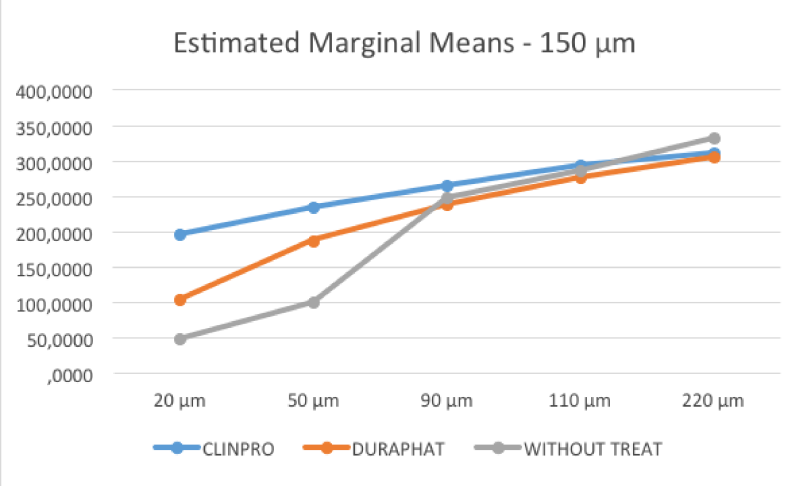
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CLINPRO
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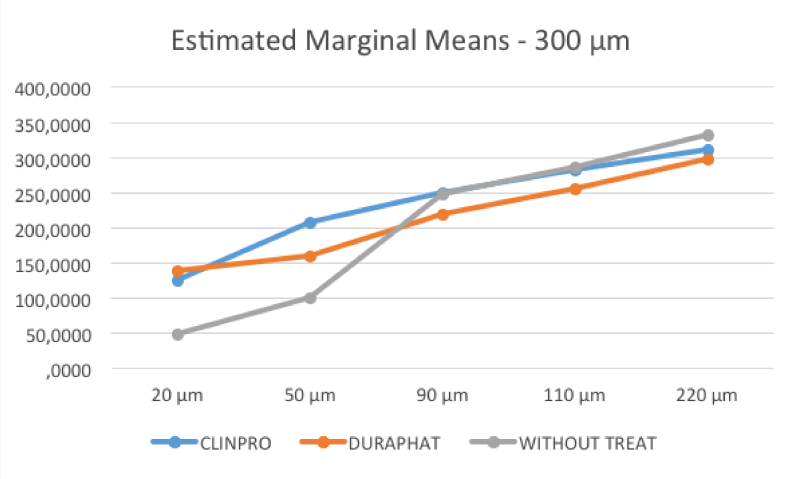
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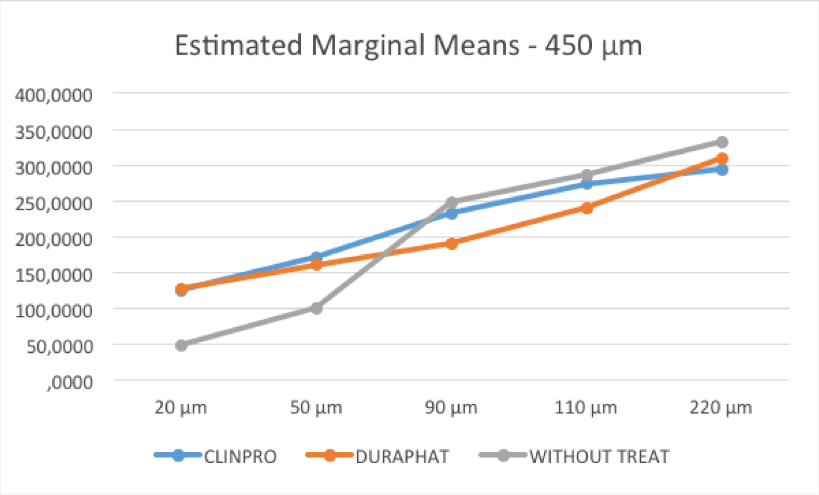
**Fig 7**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WITHOUT TREAT

**Fig 7**

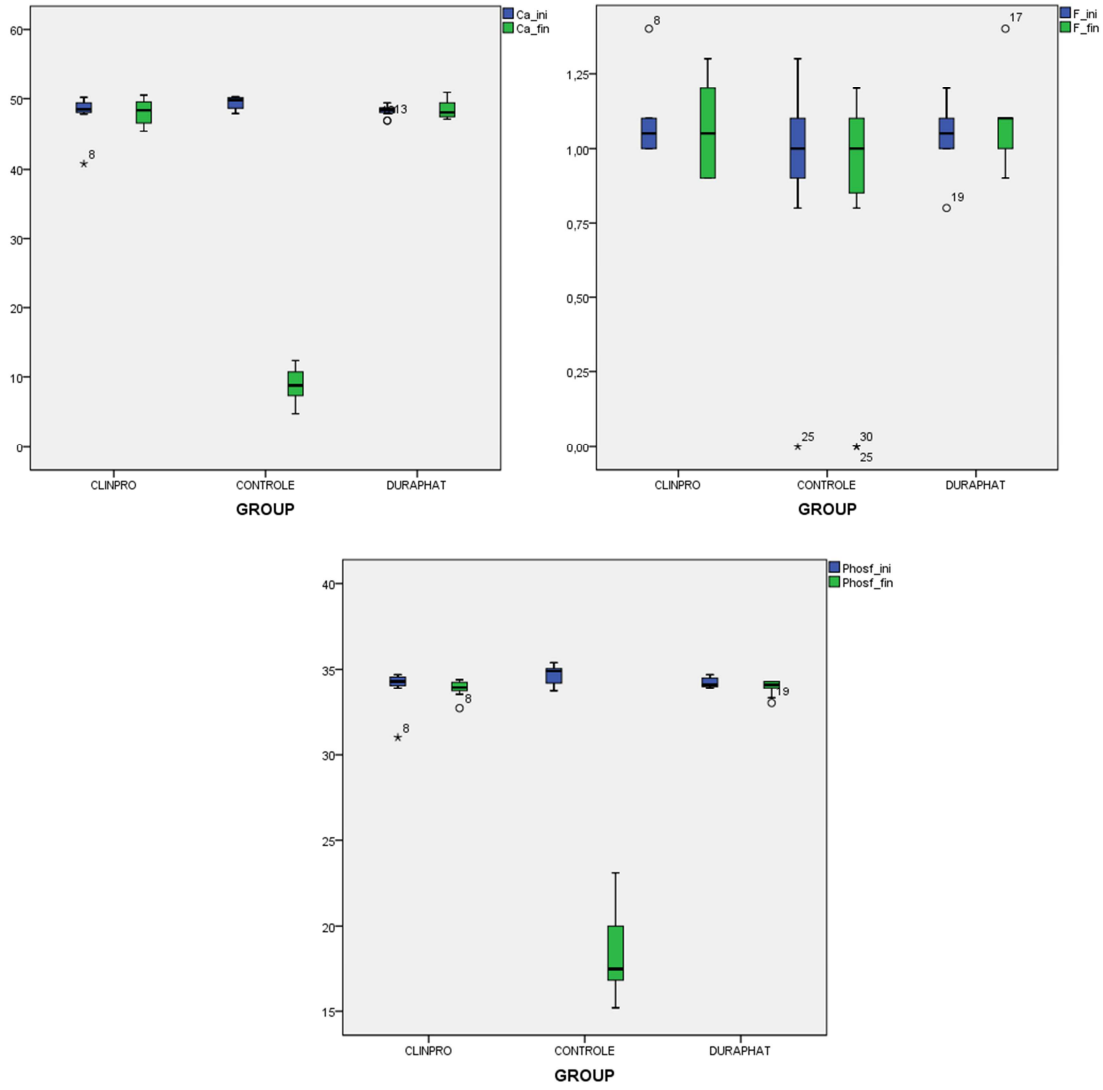
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**Fig 8**

**Table I.** The marginal means and error standard of the surface hardness of the experimental groups.

| Group | Mean | Std. Error | 95% Wald Confidence Interval | |
|---|---|---|---|---|
| | | | Lower | Upper |
| CLINPRO – 150 | 223,9379**A** | 14,18425 | 196,1373 | 251,7385 |
| DURAPHAT – 150 | 237,4002**A** | 14,62261 | 208,7404 | 266,0600 |
| CONTROLE -150 | 32,1930**B** | 3,32916 | 25,6680 | 38,7180 |
| CLINPRO – 300 | 218,32**A** | 14,88 | 189,14 | 247,50 |
| DURAPHAT - 300 | 222,77**A** | 15,26 | 192,84 | 252,69 |
| CONTROLE - 300 | 32,61**B** | 4,31 | 27,15 | 44,06 |
| CLINPRO – 450 | 222,98**A** | 14,08 | 195,38 | 250,57 |
| DURAPHAT - 450 | 219,82**A** | 13,92 | 192,53 | 247,11 |
| CONTROLE - 450 | 35,27**B** | 3,90 | 27,61 | 42,93 |

Covariates appearing in the model are fixed at the following values: Micro_150_ini=335,8926; Micro_300_ini = 344,16; Micro_450_ini =342,87

**Table II.** The percentages of surface hardness loss (%SHL)

| Grupos | % SHL | | |
|---|---|---|---|
| | 150 μm | 300 μm | 450 μm |
| **G1 – Clinpro XT** | 33,54% ±10,9 | 39,77% ± 18,38 | 35,68% ± 12,39 |
| **G2 - Duraphat** | 28,39% ± 13,88 | 35,14% ±15,27 | 37,56% ±10,77 |
| **G3 - Controle** | 90,88%±5,86 | 90,19%±5,70 | 90,86%±5,23 |

**Table III.** The means and standard deviations of the evaluated elements (Ca/P/F) at the initial condition and after pH-cycling

| Group | Ca initial | Ca final | P intial | P final | F initial | F final |
|---|---|---|---|---|---|---|
| **G1** | 48,50 ±2,49 | 48,15 **A** ± 1,73 | 34,05 ±0,99 | 33,89 **A** ±0,47 | 1,07 ±0,11 | 1,05 **A** ±0,14 |
| **G2** | 48,32 ±0,78 | 48,55 **A** ± 1,34 | 34,20 ±0,27 | 33,97 **A** ±0,42 | 1,04 ±0,09 | 1,07 **A** ±0,12 |
| **G3** | 49,39 ± 0,85 | 8,78 **B** ± 2,25 | 34,67 ±0,55 | 18,34 **B** ±2,52 | 0,93 ±0,32 | 0,85 **A** ±0,41 |

Different capital letters show statistically significant difference ($p<0.05$) in the vertical columns

# 3 Discussion

# 3 DISCUSSION

The present study was conducted to determine the efficacy of two materials — a glass ionomer-based sealant recently inserted, Clinpro XT varnish, and the fluoride varnish Duraphat, whose efficacy has already been established — for potential use in therapeutic strategies for the prevention of caries in patients undergoing orthodontic treatment. According to the results of this study, the null hypothesis that the tested materials cannot inhibit the demineralization of enamel upon dynamic pH-cycling challenge was rejected.

Thus, in the present study, the results of analysis of the surface hardness as well as the cross-sectional hardness showed that both test materials were able to inhibit the demineralization of enamel to a greater extent in comparison to the group that received no treatment. However, there was no evidence of any difference in the efficacies of the two materials from each other.

On the other hand, the unexpected results of the cross-sectional hardness led us to infer that the unprotected area of the material showed different behavior as the area protected with the nitrocellulose lacquer base.

Surprisingly, the areas adjacent to the tested materials apparently suffered partial demineralization; however, we could not establish any association between the extent of demineralization and the proximity of the material, on the basis of the results of either the surface hardness and cross-sectional hardness.

In the areas directly subjected to pH-cycling, the extent of demineralization in G3 was observed to be greater at depths of 20 and 50 µm; this indicates that the method of pH-cycling used in this study promoted demineralization to these depths. In the G1 and G2, although a more pronounced partial loss of hardness was observed at distances of 20 a 50 µm. However, the values of the enamel hardness were higher compared to G3.

One possible explanation for these results is that, because fluoride varnishes and glass ionomer-based sealants are vehicles for the topical application of fluoride at high concentrations (CURY J 2016; SALEHZADEH 2015; FEATHERSTONE 2008; YAMAZAKI 2007). the fluoride is not only deposited on the enamel surface, but also released into the medium, which increases the level of mineral saturation and results

in the formation of fluoride deposits. Thus, upon acid challenge, ionic balance is maintained not only by the release of these deposits, but also by the interaction of the fluoride in the most superficial layers of the enamel with the dissolution of hydroxyapatite (TEN CATE; FEATHERSTONE 1991; TODA 2008; TANIMOTO 2008; BUSALAF 2011).

Furthermore, from the results of EDS analysis in this study, the most obvious finding was that the percentages of calcium and phosphorus ions in G3 (no treatment) were lower compared to those in G1 and G2 after the cariogenic challenge. This result confirms the dissolution of hydroxyapatite and the loss of minerals into the medium in the absence of remineralizing agents. In contrast, the percentages of fluoride ions in the different groups showed no differences, leading us to conclude that the fluoride present in the materials was apparently not incorporated into the enamel.

Although we expected that the group of specimens treated with the fluoride varnish Duraphat would control demineralization better than the glass ionomer-based sealant Clinpro XT, given that the fluoride-release mechanism of the former more direct compared to that of the latter, the similarity of the results in G1 and G2 can be attributed to the unique protocol followed for the application of the Duraphat varnish; the varnish was not reapplied during pH-cycling and the initial coating might have loosened during the cycling (EVRENOL 1999; BELTRAN-AGUILAR 2000;MODEER 1984; PETERSSON 1991; SKOLD 1994;BENSON 2013).

A few studies in literature also discuss the dosage of fluoride required to prevent enamel demineralization; while high initial doses are more effective in increasing the resistance to demineralization, they could block the penetration of calcium ions into the subsurface of the enamel. Therefore, studies report that high initial doses are best employed for inhibiting the formation of new lesions, and lower initial doses are most effective for remineralization and control of the progression of the lesion (SEPPA 1983; MARGOLIS 1986).

Thus, we propose the application of the Clinpro XT sealant not as a superior option to other materials, but as an alternative to resolving the progress of incipient carious lesions around orthodontic appliances. However, despite the encouraging outcome of this study, these results should be interpreted with caution.

Thus, more detailed research on this topic is required to better understand and conclusively establish the efficacy of the glass ionomer-based sealant Clinpro XT varnish in inhibiting demineralization.

# 4 Final Considerations

# 4 FINAL CONSIDERATIONS

Based on the findings of this *in vitro* study, the fluoride varnish Duraphat and the glass ionomer-based sealant Clinpro XT Varnish promoted the partial inhibition of enamel demineralization by acid challenge. However, considering the limitations of this study, further long-term analysis and *in vivo* studies are required to determine the efficacy of these materials in the control of white spot lesions.

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# Annexes

# ANNEX A – Guidelines for Angle Orthodontist submissions:

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