v. 3, n. 47, 2023

International Journal of Health Science

COMPARISON EFFECT OF TWO REMINERALIZING VARNISHES ON DEMINERALIZED ENAMEL *IN VITRO*

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Abstract: Introduction: Dental caries is a multifactorial disease, with a continuous, dynamic, and chronic process that affects dental structure; in its initial stages it is known as an incipient lesion or white spot; a lesion that can be reversible through the use of fluorinated varnishes. **Aim**: To compare the remineralizing effect of two varnishes, Duraphat Colgate® vs Fluor Protector Ivoclar™ on demineralized dental enamel under cyclical pH conditions. **Materials and methods.** 60 retained third molars were sectioned mesiodistally and 120 working surfaces were obtained, they were randomly divided into the following groups, Group Duraphat Colgate® (n=30), Group Fluoride Ivoclar™ Protector (n=30), Initial Lesion Group (IL n=30) and a Healthy Enamel group (n=30) used as control group. All groups were subjected to cyclical pH, alternating immersion in demineralizing (pH 4.4) and remineralizing (pH 7) solutions, applying the varnishes for 15 days. **Results**: The CDu group at 5 days of treatment had a median value of 354 HVN, at 10 days 352, and at 15 days 483 HVN, the FP group obtained 80 HVN at 5 days, 62 at 10 days, and 40 HVN at 15 days. The Ra values of the CDu group at 5 days 1.5±0.2mm, 10 days 0.6±0.2mm, and 15 days 0.9±0.1mm, the FP group at 5 days 0.6±0.4mm, at 10 days 1.6±0.1mm and 0.3±0.1mm at 15 days, if there were statistically significant differences between CDu and FP with a value of $p \leq 0.05$. **Conclusions**: The CDu group increased its hardness (483 HVN) even more than the HE group (315 HVN) at 15 days, in contrast, FP obtained the least hardness value of 40 HVN. The varnishes did remineralize and modify the roughness of all surfaces.

Keywords: Dental Enamel, Demineralization, Remineralization, Cyclic pH, Hardness, Roughness.

INTRODUCTION

Dental enamel has been considered the most mineralized and hard tissue of the of the human body, capable of withstanding the chewing process without presenting fractures. The secretory cells of the adamantine tissue after completing their formation disappear during dental eruption, which is why dental enamel cannot repair itself.

Dental enamel undergoes a chemical dissolution that promotes the loss of ions, which occurs due to organic acids from the metabolism of biofilm bacteria which decreases the pH to a critical level for hydroxyapatite crystals, or due to ingested acidic foods. in the diet, this process is known as demineralization. This process is reversed by the buffering capacity of saliva which, being supersaturated by calcium and phosphate ions, allows the reincorporation of said ions into the demineralized surface, beginning the process of remineralization.

The imbalance of the dynamic process of remineralization and demineralization originates an incipient carious lesion; the first clinical manifestation of dental caries. At this stage it is convenient to reverse or stop the incipient carious lesion through remineralization with topical sodium fluoride therapy. As fluoride ions have a high electronegativity, they tend to associate with other elements, diffusing towards the interior of the incipient demineralized lesion reacting with the remaining calcium and phosphate ions and forming fluor-hydroxyapatite crystals, which are more stable and resistant to acidic attacks, thus increasing the hardness of dental enamel.

The purpose of a pH model is the reproduction of the demineralization and remineralization process present in the oral cavity (Barrera Ortega C.C, 2022).

This type of model reproduces in vitro conditions of high-risk cariogenic activity to analyze the capacity of different materials that could interfere with this process, allowing for an effective way to evaluate the potential of anticaries products that contain fluoride in their composition. (Barrera-Ortega C.C, 2019)

Several remineralization agents have been introduced over time to increase the resistance of dental enamel, showing great anti-caries potential, since they can remineralize incipient enamel lesions; The most used agents for professional application in dental care are Duraphat Colgate® topical fluorides and Silane Fluoride varnish (Fluor Protector, Ivoclar™).

Lack of remineralization in early carious lesions can lead to cavitation of tooth enamel, for these reasons the present investigation aims to compare the remineralization of dental enamel using Duraphat Colgate® and Silane Fluoride varnish (Fluor Protector, Ivoclar™) in mandibular third molars with an initial lesion, following a cyclical pH model; in vitro.

MATERIALS AND METHODS

SAMPLE PREPARATION

The samples were obtained by maxillofacial surgeons extracted either for preventive or orthodontic purposes. 60 retained inferior third molars without caries or structural defects were selected and stored in deionized water in an incubator at 36°C until ready to use. The samples were then sectioned in a mesiodistal direction with a fine diamond disc (Germany Brasseler Diamond 910 <20,000 rpm, California, U.S.™) under constant irrigation, obtaining a total of 120 working surfaces, buccal and lingual. The work surfaces were then conditioned, removing soft tissue with Gracey curettes (7/8,11/12,13/14). The pulp space was blocked out with wax in order to obtain a flat working surface.

A 3X6 mm window was delimited on the middle third of each surface for the application

of the varnish. The rest of the tooth was covered with acid resistant varnish (Revlon™), a different color for each group. Prophylaxis was carried out on all enamel surfaces with a non-fluoridated prophylactic toothpaste.

INITIAL LESION

90 surfaces were subjected to an initial demineralization with 2mM CaCl2, 2 mM NaH2PO4, 0.05 M de CH3COOH at a pH of 4.4, corroborated by a pH-meter (Science Med SM-25CW Meter), for 96 hours in an incubator at 36°C.

After the 96 hours the samples were removed from the incubator and the following varnishes were applied following the manufacturer's instructions:

- Group CDu (n=30): The varnish Duraphat Colgate® was applied.
- Group FP (n=30): The varnish Fluor Protector, Ivoclar™ was applied.
- Control group (CG): With initial lesion and without varnish treatment.

PH CYCLE

The pH cycle consisted in a sequence of immersion in a remineralizing and demineralizing solutions:

- **For 3 hours**: Immersion of the samples in a demineralizing solution at a Ph of 4.4 at 36°C (2 mM $CaCl_2$, 2 mM NaH_2PO_4, 0.05 M de CH_3COOH).
- **For 21 hours**: Immersion of the samples in a remineralizing solution at a Ph of 7.0 at 36°C (1.5 mM $CaCl_2$, 0.15 mM KCl, 0.9 mM NaH_2PO_4).

For the remineralizing solution 1.5 mM $CaCl_2$, 0.15 mM KCl, and 0.9 mM NaH_2PO_4 was mixed in 1L of deionized water, adjusting the pH at 7.0, corroborated by a pH-meter (Science Med SM-25CW Meter).

The samples were rinsed with deionized water at every change in solution and application of a fluorated product.

The varnishes were applied every 24 hours with a microbrush, in between solutions, to groups CDu and FP. This cycle was followed for 5, 10 and 15 days.

- Healthy Enamel group (n=30): Without initial lesion, without treatment.
- Group CDu (n=30): With initial lesion, treatment every 24 hrs.
- Group FP (n=30): With initial lesion, treatment every 24 hrs.
- Control group (n=30): With initial lesion, without treatment.

LESION EVALUATION

After 5,10 and 15 days 10 samples were randomly selected from each group and stored in deionized water at a temperature of 36°C until ready to be tested.

To test the samples, they were fixed in acrylic squares with epoxy resin to avoid unwanted movement.

To test hardness, a Nanovea CB500® micro-indentator was used, locating the zones with minor convexity in the working surfaces. A total of 1200 indentations were made in the buccal and lingual surfaces.

Roughness was measured using an optic profilometer (Nexview-AMETEK®).

DATA ANALYSIS

The shapiro-wilk test was performed to analyze data distribution of hardness and roughness variables. For hardness tests non-parametric kruskall wallis tests were performed and multiple comparisons were made using the Wilcoxon paired comparisons test. For the roughness test, ANOVA and Tukey were performed as post-hoc. All statistical analyzes and graphs were performed with the GraphPad Software Prism 8.0® program for information processing.

RESULTS

HARDNESS

1200 indentations were made over the buccal and lingual surfaces using a Nanovea Micro-indentator, 10 indentations were made for each sample. The following configurations were made:

- Approximation velocity: 50 μm/min
- Contact charge: 50 mN
- Load: 10 N
- Charge rate: 5 N/min
- Discharge rate: 5 N/min

The indentations were determined using Vickers Hardness Calculations (n=120)

Graph 1 shows the box and whisker plot with the distribution of the hardness results (HVN) of the different experimental groups, where there were significant differences between HE and IL with FP at 5, 10 and 15 days, and the CDU experimental group at 5, 10 and 15 days had differences with FP at 5, 10 and 15 days of treatment.

ROUGHNESS

The surfaces of all the experimental groups were evaluated randomly in the 3 x 6 mm work window and 6 observations were obtained from each of the experimental groups: the average roughness (Ra) and its standard deviation.

DISCUSSION

Within the area of dentistry, remineralization based on fluorinated compounds has seen great progress in recent years, this being one of the first-choice treatments, since it increases the resistance of dental enamel and inhibits the process of cavities. In addition, they avoid the demineralization process (Min-Ji Kim, 2021), for this reason, in the present study, the remineralizing effect of two varnishes with fluorinated compounds was evaluated. Duraphat Colgate® with 22,600 ppm and Silane Fluoride (Fluor Protector, Ivoclar™) with 1000 ppm, applied to the surface of the dental enamel.

In previous studies (Min-Ji Kim, 2021; Sezici Yağmur Lena., 2021) the use of bovine teeth is mentioned, stating that their crowns are much larger and samples for research can be better prepared than in human teeth; however, in this study human mandibular third molars were acquired since the genetic, environmental and morphological characteristics of bovine teeth are different from those of human teeth and in this research it was sought to obtain a greater similarity to the oral cavity.

The process of a carious lesion begins with the demineralization of the dental enamel, which occurs at a pH of 5.5 and a critical pH of 4.4 where the risk of incipient lesion or dental caries is high. That is why, in this in vitro study, the samples were immersed in a demineralizing solution at a pH of 4.4 for 96 hours to cause an initial lesion; however, Sahiti et al. (SahitiJ Sai., 2020) mentions in his study that it`s enough to immerse the samples for only 72 hours; while other articles (Min-Ji Kim, 2021; Sezici Yağmur Lena., 2021), mention that an artificial lesion was caused; that is to say, a cavity was formed in the dental enamel, simulating demineralization as if it were an advanced caries process. In this investigation, a demineralizing solution was chosen since it is the most reported in various articles and at the same time it is similar to the demineralization that occurs naturally in the oral cavity.

Many methods have been reported to assess carious progression, including the cyclical pH model, in which the main variant is the time interval in which the specimen is subjected to demineralization and remineralization periods (Baik Alaa, 2021; Prado Sandra., 2018).

Ten Cate and Duijsters (Ten Cate JM, 1982), introduced a cyclical pH model in

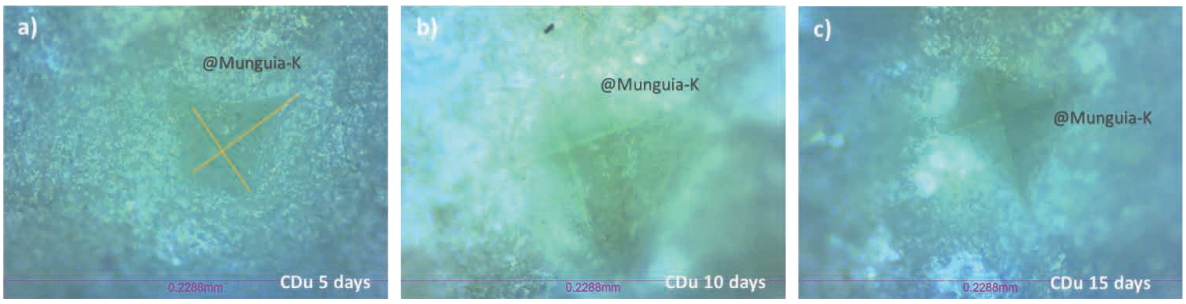


Fig 1. Shows the most representative images of the Duraphat varnish applied at 5, 10 and 15 days.

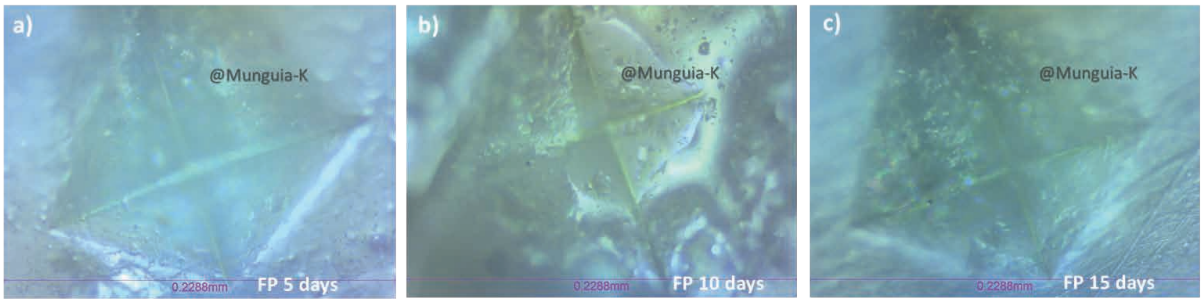
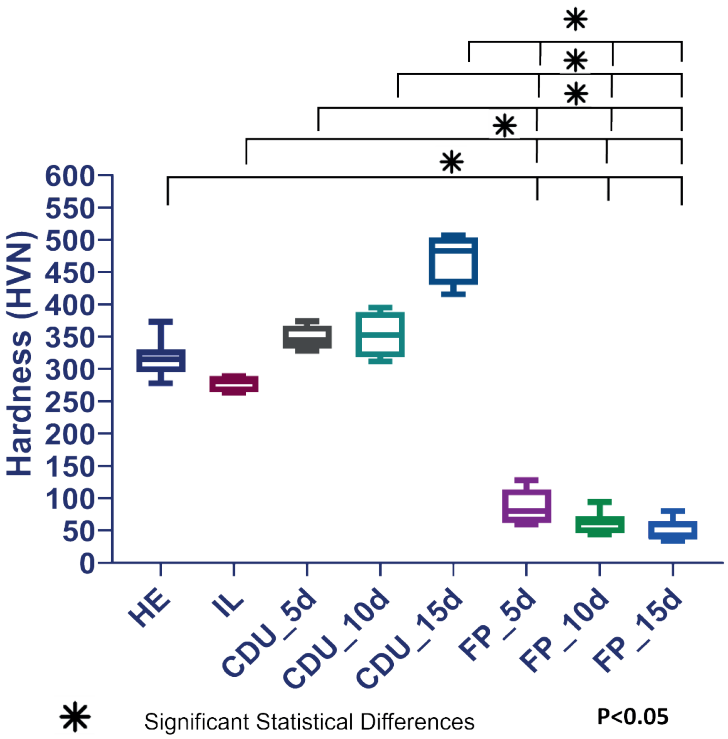


Fig 2. Shows the most representative images of the fluor protector varnish applied at 5, 10 and 15 days.

	HE	IL	CDU_5d	CDU_10d	CDU_15d	FP_5d	FP_10d	FP_15d
Sum of assigned range (W)	36.00	36.00	36.00	36.00	36.00	36.00	36.00	36.00
Sum of positive range	36.00	36.00	36.00	36.00	36.00	36.00	36.00	36.00
Sum of negative range	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Value of p (two tails)	0.0078	0.0078	0.0078	0.0078	0.0078	0.0078	0.0078	0.0078
Sig.(alpha=0.05)	Si	Si	Si	Si	Si	Si	Si	Si

Table 1 shows the multiple comparisons analysis, significant differences were found in hardness tests between the experimental groups (Chi2 =58.30, DF= 7, P<0.05).



Graph 1. Box and whisker graph with the results obtained for hardness of the experimental groups ES, LI, CDu and FP. direct source.

	Mean (± SD)							
	HE	**IL**	**CDu_5d**	**CDu_10d**	**CDu_15d**	**FP_5d**	**FP_10d**	**FP_15d**
Ra	0.82 (±0.34)	1.43 (± 0.37)	1.56 (± 0.24)	0.65 (± 0.249	1.00 (± 0.14)	0.61 (± 0.48)	1.60 (± 0.16)	0.36 (± 0.14)
Rq	1.077 (± 0.4933)	1.622 (± 0.4263)	2.1 (± 0.3133)	0.811 (± 0.2665)	1.298 (± 0.1391)	0.9555 (± 0.7628)	2.065 (± 0.2536)	0.4725 (± 0.1798)
Rz	7.337 (± 0.3662)	34.03 (± 2503)	20.06 (± 3.733)	7.59 (± 1.167)	13.4 (± 2.053)	14.99 (± 10.66)	34.81 (± 9.991)	5.663 (± 1.113)

Table 2. Shows the results obtained from the roughness tests of the experimental groups, in relation to their median roughness and standard deviation.

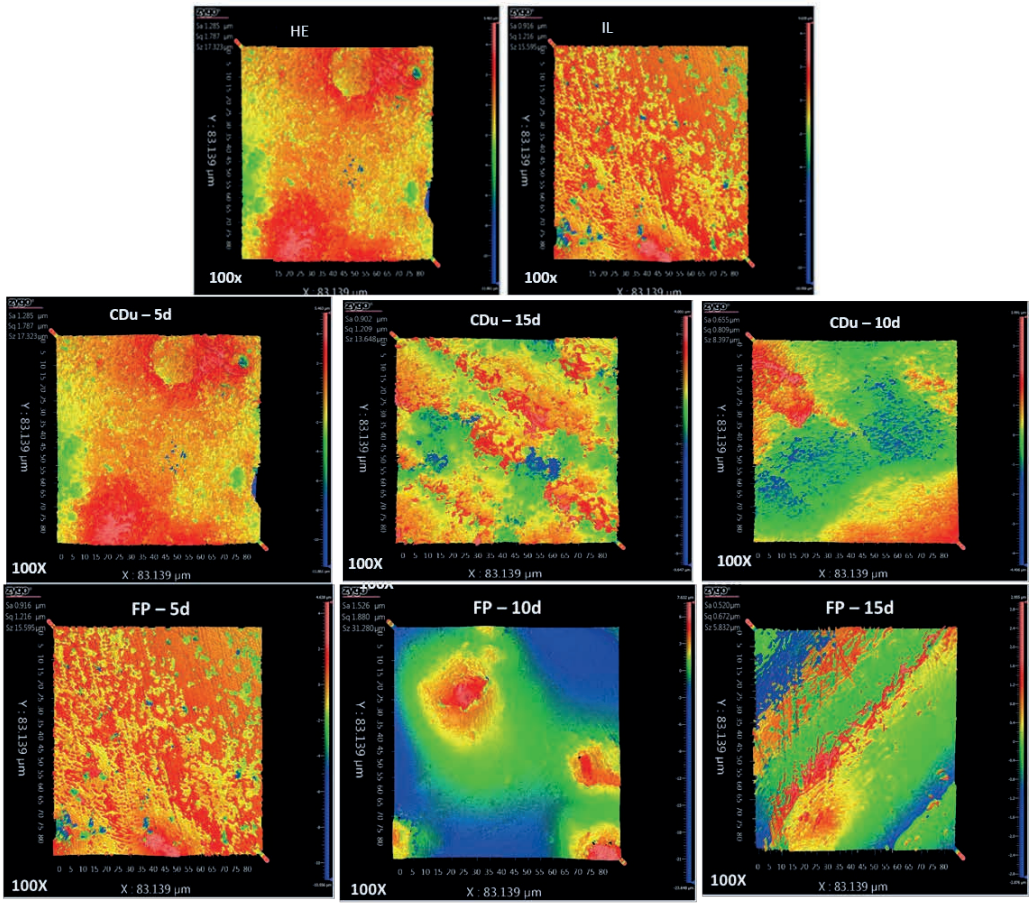
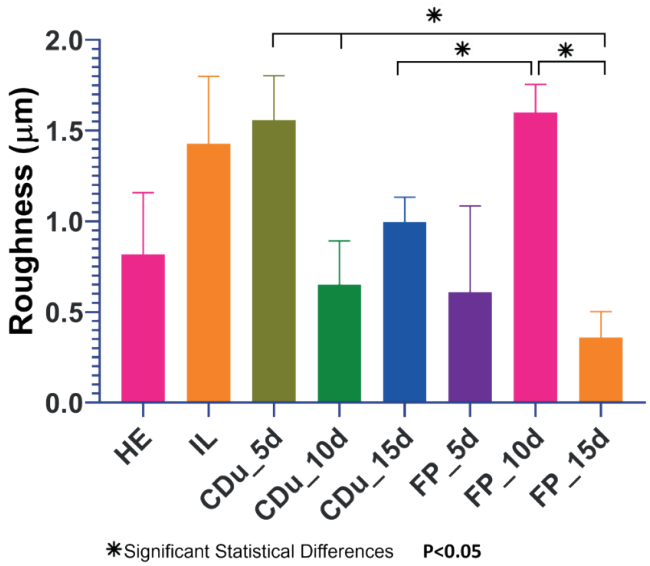


Figure 4. Surface roughness of dental enamel. Healthy enamel, Demineralization or initial lesion, CDu 5 days, CDu 10 days, CDu 15 days, FP 15 days, FP 10 days, FP 15 days.direct source.



Graph 2. Shows the bar graph with the distribution of the roughness results of the different experimental groups, where there were significant differences in the CDu group at 5 and 10 days, FP at 10 and 15 days, FP 15 days with CDu 5 days and FP 10 days with respect to the CDu group 15 days.

vitro demonstrating the ability of fluoride to promote remineralization of dental enamel, this model simulates remineralization and demineralization conditions that resemble the natural process of loss and gain of minerals involved in incipient lesion or caries of the tooth. In this research, the same cyclical pH model was used as it is the most reported in various articles, as well as being the most convenient to reproduce in a laboratory.

Within the cyclical pH model, remineralization is an important process, which is why in this study it was carried out with a remineralizing solution at a pH of 7.0 in which the samples were immersed for 21 hours after the application of the remineralizing agents, treated for a period of 15 days; On the other hand, some authors (SahitiJ Sai., 2020; Pinto Priscila., 2017; Santos Gabriela., 2021) carry out the remineralization process using artificial saliva, changing it every 24 hours before immersion of the treated samples for 7 days only; Likewise, Rodríguez (Rodriguez Maria Ana, 2018) used human saliva as a remineralizing medium, collecting daily fresh saliva from five volunteers, after which the different saliva samples were mixed and shaken to obtain a homogeneous solution. However, this process is not completely reliable since the oral conditions of each volunteer are different, which is why in this study a remineralizing solution was prepared instead. ((Rao Rahul., 2017; Montasser Mona., 2017).

Many methods have been reported in the literature that simulate the demineralization of the dental enamel surface under cyclical pH conditions in vitro. Torres A. ((Torres A., 2015), indicated that the dental enamel samples in demineralizing solution of lactic acid with a pH of 3, in a period of 3 hours artificially formed an incipient lesion; while Chicaiza and Navarrete, (Chicaiza, 2017), pointed out that the enamel samples in demineralizing solution of lactic acid with pH of 2 artificially formed the incipient lesion in a period of time of 50 min; however, when examining the dental enamel, they observed a total crumbling of the surface.

In the article by Santos (Attin T., 2017), he mentions that all the enamel samples used in the experimental study were demineralized with acid hydroxyethylcellulose, following the cyclical pH model.

Various studies ((Maden A., 2017; Murakami C., 2016; Haghgou E., 2017) mention the use of carbonated soft drinks, acids used for the demineralization of dental enamel surfaces. In a recent study Soraya Cheir et al. (Cheir Soraya., 2021) evaluated the effects of different soft drinks on dental enamel by measuring microhardness, showing a significant decrease, as well as gradual demineralization; affecting the enamel of primary teeth. Despite the different methods for the demineralization of the enamel surface, in this study a demineralizing solution was prepared daily at a pH of 4.4 in which the samples were immersed for 3 hours before the treatment of the fluorinated compounds. The solution was performed in this study in order to not affect or have drastic changes when following the cyclical pH model, since a solution was also made for remineralization; In addition, these solutions are the most reported in various articles (Rao Rahul., 2017)) and the most reproducible in the laboratory since the necessary equipment is available for the preparation of said solutions.

The adherence of sodium fluoride to non-demineralized dental enamel can provide a shield against attacks by bacterial acids and, therefore, prevent demineralization and promote remineralization (Baik Alaa, 2021), which is why in the present investigation the effect of two varnishes with sodium fluoride was studied.

The application of dental varnishes used as remineralizing agents depends on the type of

study to be realized and the way the author chooses to apply the varnish. *Dehailan L., Lippert F. et al.,* (Delhailan L., 2016) mentions that the quantity of the varnish to be applied must be the same on all samples of each experimental group. That is to say, the amount of Fluor Protector Ivoclar™ must be the same in relation to Duraphat Colgate®, however by studying the varnish Fluor Protector Ivoclar™ we found that it was hard to handle the proportions due to it`s low viscosity, in addition to it volatizing rapidly. On the other hand, the varnish Duraphat Colgate® presented higher viscosity which made it hard to measure the exact quantity for both experimental groups, that is why in this study only the manufacturer`s instructions were followed for each varnish.

Microhardness and hardness measurements are another method to determine the degree of demineralization and remineralization of dental enamel. Existing different measurement techniques such as the Knoop technique that was used in studies ((Rodriguez Maria Ana, 2018; SahitiJ Sai., 2020; Oliveira Regina., 2021), to test the microhardness of thin layers of ceramics or coatings. In this study, said technique does not agree with the procedure that was carried out. The Vickers hardness technique reported in other articles (Han Kim., 2018) was adequate to measure the hardness of dental enamel due to its ability to measure very fine materials and regions; thus, evaluating the ability of the material to resist the penetration of a diamond tip with a specific load and a predetermined time.

Roughness is frequently measured in various articles ((Min-Ji Kim, 2021; Shihabi Safaa., 2021; Gangwar A., 2019), with scanning electron microscopy, using it on dental enamel surfaces and observing whether or not there were changes in roughness according to remineralization; In this research, an optical microscope was used to analyze the roughness of the dental enamel surface; however, this technique has only been reported in articles where the roughness of the enamel is evaluated after braces have been removed ((López, 2019) and in other articles (Mohamed Shamel., 2019) where the roughness that occurred after dental whitening treatments is mentioned, for which the technique with optical profilometer is currently very accurate and fast to obtain the values of roughness of the dental enamel surface after the application of different remineralizing agents as was done in this investigation.

These results agree with Tewari A., et al. (Baik Alaa, 2021), who mentions that the Duraphat Colgate® varnish showed a significantly higher rate of initial lesion reduction compared to the control group, likewise their study suggested that this dental varnish is one of the dental varnishes with sodium fluoride with better results against the demineralization of enamel.

The Fluoride Protector group, Ivoclar™, was also compared in other articles where they mention their results in periods of time, which corroborates, like in this investigation, that this varnish has very rapid effects, being that in a period of 5 days , after its application it has very few or no significant differences on the reduction of demineralization of dental enamel; Therefore, the following days of treatment do not have a greater degree of hardness.

CONCLUSIONS

The group treated with the Duraphat varnish (Colgate®) after 5 days of treatment had a hardness (median) of 354 HVN, at 10 days 352 and at 15 days it increased to 483 HVN, the Duraphat varnish obtained higher hardness values, even when compared to the HE group (315 HVN). There were no significant differences between this group.

The group treated with the Fluor Protector

varnish (Ivoclar™) obtained a hardness of 80 HVN at 5 days, 62 at 10 days and 40 HVN at 15 days, well below the hardness values of healthy enamel (315 HVN), including from the initial lesion 281 HVN. There were no statistically significant differences between this group.

In the roughness test, the behavior of the group treated with Duraphat varnish (Colgate®) had a heterogeneous roughness, at 5 days of 1.5±0.2 µm, at 10 days 0.6±0.2 and at 15 days 0.9±0.1, with statistically significant differences between 5 and 10 days.

In the group treated with the Fluoride Protector varnish (Ivoclar™) its mean roughness was 0.6±0.4 µm after 5 days, 1.6±0.1 µm after 10 days and 0.3±0.1 µm after 15 days of treatment, with significant differences. between 10 and 15 days.

If there was a remineralization using the Duraphat varnish since its hardness increased as the days of treatment reached a hardness higher than that of healthy enamel (315 HVN).

The Ivoclar™ Fluor Protector varnish considerably decreased the hardness values compared to the initial lesion (HVN 281) with the lowest value at 15 days reaching up to 40 HVN.

DISCLOSURE STATEMENTS

"The authors do not have any financial interest in the companies whose materials are included in this article."

ACKNOWLEDGEMENTS

This work was supported by the DGAPA-PAPIIT IA-200421 and IA-202423 projects.

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