Evaluation of the mineralizing capacity of varnishes by electrical impedance spectroscopy: an in vitro study

Avaliação da capacidade mineralizante de vernizes por espectroscopia de impedância elétrica: um estudo in vitro

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ABSTRACT
Background: To evaluate in vitro and compare a remineralizing capacity of three different types of varnishes. Material and Methods: The sample consisted of 24 third molars found in cycles of demineralization and remineralization (DES X RE) and distributed in three groups: Group 1 - fluoride varnish (NaF 5%; Duraphat, Colgate, São Paulo, Brazil), Group 2 - casein phosphopeptide varnish - amorphous calcium phosphate (CPP-ACP 10%) (Recaldent GC, Tokyo, Japan), Group 3 - 1% chlorhexidine varnish (Cervitec® Plus - Ivoclar Vivadent), applied to the mesial and distal as control. The mineral content of the enamel was evaluated using the CarieScan ProTM, at the central point of the sites at three times: before the DES X RE cycle, after it and after using the varnishes. Results: The results were obtained from the descriptive analysis of data using the Excel tool. In G1, the sodium fluoride varnish, Duraphat, showed the best effectiveness in the remineralization process, which occurred in 75% of the sites, followed by G2, which remineralized 50% of the sites and G3, which was less efficient, remineralizing only 31.25% of the sites. Conclusion: There is variation in the remineralizing capacity among the varnishes studied. The varnish based on 5% sodium fluoride, Duraphat, showed the best effectiveness in the remineralization process, also acting at a distance.

Keywords: Dental Caries, Chlorhexidine, Demineralization, Fluorides, Topical, Electric Impedance.

RESUMO
Objetivo: Avaliar in vitro e comparar a capacidade remineralizante de três tipos diferentes de vernizes. Material e Métodos: A amostra foi composta por 24 terceiros molares encontrados em ciclos de desmineralização e remineralização (DES X RE) e distribuídos em três grupos: Grupo 1- verniz fluoretado (NaF 5%; Duraphat, Colgate, São Paulo, Brasil), Grupo 2- verniz de fosfopeptídeo de caseína - fosfato de cálcio amorfo (CPP-ACP 10%) (Recaldent GC, Tóquio, Japão), Grupo 3- verniz de clorexidina 1%. (Cervitec® Plus - Ivoclar Vivadent), aplicados na mesial e a distal como controle. O conteúdo mineral do esmalte foi avaliado por meio do CarieScan ProTM, no ponto central dos sítios em três momentos: antes do ciclo DES X RE, após e após o uso dos vernizes.

Resultados: Os resultados foram obtidos a partir da análise descritiva dos dados por meio da ferramenta Excel. No G1, o verniz fluoretado de sódio Duraphat apresentou a melhor eficácia no processo de remineralização, que ocorreu em 75% dos sítios, seguido pelo G2, que remineralizou 50% dos sítios e G3, que foi menos eficiente, remineralizando apenas 31,25 % dos sites. Conclusão: Há variação na capacidade remineralizante entre os vernizes estudados. O verniz à base de fluoreto de sódio a 5%, Duraphat, apresentou a melhor eficácia no processo de remineralização, também atuando a distância.

Palavras-Chave: Cárie Dentária, Clorexidina, Desmineralização, Fluoretos Tópicos, Impedância Elétrica.

1 INTRODUCTION

A large part of the population has been affected by dental caries, being considered by the World Health Organization as a worldwide public health problem. This chronic disease affects dental tissues and has a high prevalence in the world population.[1,2]

Current views portray this disease as multifactorial. The presence of Streptococci from the mutans group in the biofilm is one of the factors that contribute to the etiology of this disease, dependent sucrose.[3] Due to the fact that Streptococcus mutans produce acids in biofilms, these become a considerable virulence mark of this bacterium.[4,5] The organic acids resulting from the fermentation of carbohydrates originating from the diet and metabolized by a specific microbiota clump between the biofilm and the enamel, initiating a process of demineralization of the hydroxyapatite crystals. The first clinical sign of the disease is the white spot, the macroscopic indicator of demineralization process.[6,7,8]

With the knowledge acquired about the etiology of caries disease, preventive and minimally invasive strategies, aiming at the conservation of dental structure, have replaced restorative dentistry. Different preventive techniques have been used in the treatment and control of injuries, such as plaque control methods (efficient oral hygiene), nutritional guidance, use of fluorides, fluorides combined with calcium and phosphate, such as casein phosphate-calcium phosphate amorphous (CPP-ACP) and also the use of antimicrobials, such as chlorhexidine in different concentrations: 1% with thymol (varnishes) or 0.12% (solutions for mouthwash).[3,4]

A method of prevention that has been used on a worldwide scale are varnishes, developed in order to improve the insufficiency of topical fluorides to remain in long-lasting contact with the enamel surface. Because they are effective and safe, their use has grown in the United States since the beginning of the its use in 1990.[2] Along with this,

many studies have shown its effectiveness in preventing caries, showing that this is due to the fact that these varnishes release fluoride contributing to the enamel remineralization process, also inhibiting the processes of demineralization of hard tissues dental.[5,9] In this sense, the casein phosphopeptide varnish - amorphous calcium phosphate was created from casein, which is a phosphoprotein in bovine milk, which has the ability to decrease the absorption of the enzyme glycosyl transferase, produced by Streptococcus mutans, directly interfering with bacterium adhesion to the dental structure.[10]

The aim of this in vitro study was to evaluate and compare the remineralizing capacity of three different types of varnishes, fluoride varnish (NaF 5%; Duraphat, Colgate, São Paulo, Brazil), casein phosphopeptide varnish - amorphous calcium phosphate (CPP-ACP 10%) (Recaldent GC, Tokyo, Japan) and 1% chlorhexidine varnish. (Cervitec® Plus - Ivoclar Vivadent). For this purpose, alternating current impedance spectroscopy was used, with the Cariescan Pro device, which numerically detects, from 0 to 100, the degree of demineralization of dental tissue.[11,12]

2 MATERIAL AND METHODS

2.1 PREPARATION OF DENTAL SAMPLES AND TREATMENT GROUPS

The sample consisted of twenty-four teeth, third human molars, acquired in public health services, through formal and clarified donation - Term of Donation of Biological Material. The Project was submitted for approval by the Research Ethics Committee of the Veiga de Almeida University, being approved by registration 86978218.6.0000.5291. After cleaning using periodontal instruments and professional prophylaxis with pumice and water for 10 seconds, they were kept in a 0.1% thymol solution for one week. After these procedures, the teeth were rinsed with deionized water and randomly distributed into three groups.

On each vestibular surface, two areas were insulated with hot glue, one mesial and the other distal, and the other surfaces were painted with red enamel (Colorama). Twelve hours after drying the enamel, the portions of glue were removed and the teeth divided into three groups: Group 1 (n = 8), the mesial surface received fluoride varnish (NaF 5%; Duraphat, Colgate, São Paulo, Brazil) and the distal received no additional treatment (control); Group 2 (n = 8), a mesial surface received application of casein phosphopeptide varnish - amorphous calcium phosphate (CPP-ACP 10%) (Recaldent GC, Tokyo, Japan); and the distal received no additional treatment (control) and Group 3 (n = 8), a mesial

surface received application of 1% chlorhexidine varnish. (Cervitec® Plus - Ivoclar Vivadent) and the distal received no additional treatment (control).

2.2 PREPARING DEMINERALIZING AND REMINERALIZING SOLUTIONS

For the in vitro experiment, the sample was cycled in des and remineralizing solution daily for a period of 8 days.

The des and remineralizing solutions used in the experiment were prepared at the Department of Biochemistry, State University of São Paulo, Bauru, as recommended by Queiroz.[12] For this, each component of the solutions was properly weighed on a precision scale (Adventurer Ohaus, China) and dissolved one by one in distilled-deionized water.

In preparing the demineralizing solution, the following components were used 1.28 mmol/L of Ca^{++}, 0.74 mmol/L of Pi (inorganic phosphate) and 0.03 μg F/mL, also was dissolved in 0.05 mol/L acetate solution at pH 5.0. For the remineralizing solution, it was used 1.5 mmol/L of Ca^{++}, 0.9 mmol/L of Pi, 150 mmol/L of KCl and 0.05 μg F/m, also was dissolved in 0.1 mol/L of Tris solution at pH 7.0. After homogenization of the solutions, the pH confirmation of the buffers was measured with the aid of a pHmeter (Nova Instrumentos, Piracicaba, Brazil).

2.3 QUANTITATIVE ANALYSIS OF MINERAL LOSS - CARIESCAN PROTM

Before and after the demineralization and remineralization cycles, the mineral content of the enamel was evaluated using CarieScan ProTM, analyzing the central point of the exposed surfaces.

The CarieScan ProTM is a handheld device that uses electrical impedance spectroscopy (EIS) to determine the electrical properties of the tooth. The device quantifies the levels of demineralization of the dental structure, being able to detect and monitor mineral losses early. The bioimpedance principle for the detection of carious lesions is based on the fact that the enamel has little porosity and a low amount of fluid, being, therefore, a bad conductor. When the evolution of mineral loss occurs, there is an increase in this porosity and fluids on the surface, generating a higher level of conductivity.

The device emits on the LCD display, measurements ranging from 0-100, according to the mineral loss. These measurements were noted on an individual form.

2.4 DEMINERALIZATION S X REMINERALIZATION CYCLES

The Des X Re cycle lasted eight days and took place in a greenhouse (Fanem, São Paulo, Brazil) at 37 ºC. The teeth were immersed in containers containing 50 ml of the solutions.

For demineralization, the teeth were immersed in a demineralizing solution (pH 5.0), for a period of four hours. After this, the teeth were rinsed with deionized distilled water and immersed in remineralizing solution for a period of twenty hours, with the solutions being replaced on the 4th day of the experiment.

2.5 APPLICATION OF VARNISHES

The varnishes studied were applied once a week for four weeks. After 5 minutes of drying the varnishes, all teeth were rinsed for approximately 10 seconds with deionized distilled water with the aid of a pissette and the teeth were kept in artificial saliva changed every 2 days.

3 RESULTS

The results were obtained from the basic descriptive analysis of data using the Excel tool. The mineralization level of each site was checked and quantified three times in a row using CarieScan Pro (Table 1).

Table 1: Average measurements for each site with CarieScan Pro - first measurement (baseline).

		CP1		CP2		CP 3		CP 4		CP5		CP 6		CP7		CP 8	
		Mesial	Distal	Mesial	Distal	Mesial	Distal	Mesial	Distal	Mesial	Distal	Mesial	Distal	Mesial	Distal	Mesial	Distal
First measurement Baseline	G1	2,00	5,33	1,67	6,00	6,00	4,33	6,00	6,00	6,33	1,00	4,00	6,00	2,67	0,00	0,00	7,33
	G2	1,33	4,67	6,00	7,33	1,67	6,00	5,33	6,33	2,33	3,67	5,33	5,67	2,67	5,67	3,67	6,00
	G3	1,33	2,00	4,00	3,33	0,00	0,00	2,67	4,67	4,33	3,67	3,67	6,33	0,67	5,00	3,67	4,67

After the initial measurement, the sample was cycled in a demineralizing solution and remineralizing (DES X RE cycle) daily, for a period of 8 days. At end new measurements were made with the Cariescan Pro, detecting this mineral loss at all sites (Table 2).

Table 2: Average measurements for each site with CarieScan Pro after DES X RE cycle.

		CP1		CP2		CP 3		CP 4		CP5		CP 6		CP7		CP 8	
		Mesial	Distal	Mesial	Distal	Mesial	Distal	Mesial	Distal	Mesial	Distal	Mesial	Distal	Mesial	Distal	Mesial	Distal
Average after DES X RE cycle	G1	46,33	50,00	64,67	41,00	50,00	50,00	50,33	50,00	100,00	100,00	55,00	32,00	57,67	50,00	37,00	35,00
	G2	51,67	56,00	52,00	38,33	88,00	50,00	50,00	50,00	50,00	50,00	60,67	50,67	30,33	72,33	51,00	56,00
	G3	93,67	47,33	95,33	100,00	74,67	50,67	71,00	20,00	77,33	45,00	97,33	53,00	24,33	53,33	50,00	79,33

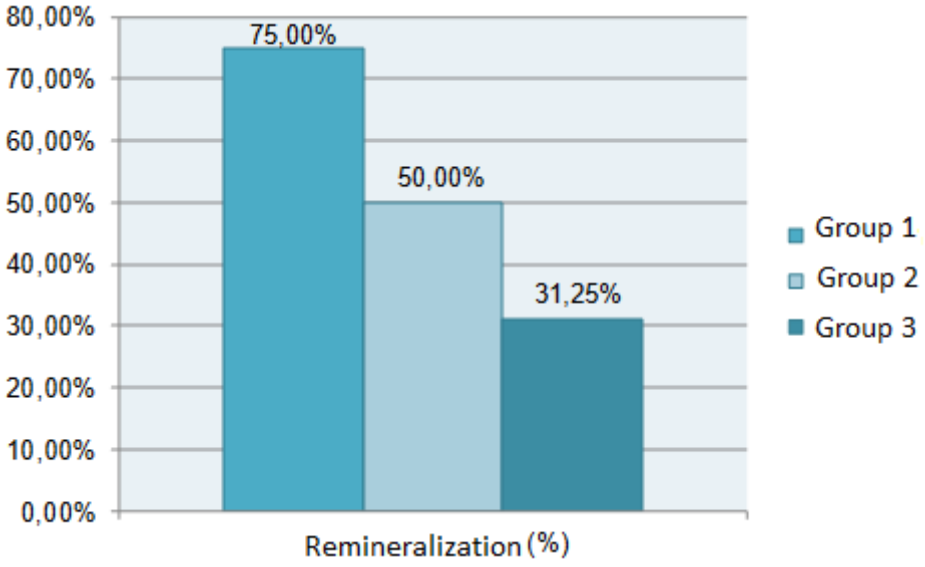
After the DES X RE cycle, the studied varnishes were applied at the mesial site of each group, the distal site being considered control (Table 3).

Table 3: Average measurements of each site with CarieScan Pro after applying the varnishes.

		CP1		CP2		CP 3		CP 4		CP5		CP 6		CP7		CP 8	
		Mesial	Distal	Mesial	Distal	Mesial	Distal	Mesial	Distal	Mesial	Distal	Mesial	Distal	Mesial	Distal	Mesial	Distal
Average after varnishes appications	G1	1,67	2,67	7,00	8,00	6,67	8,00	3,00	10,33	4,33	53,33	49,67	50,00	44,67	52,33	50,67	53,00
	G2	45,67	48,67	43,67	45,67	58,00	50,00	64,33	53,67	50,00	50,00	47,67	48,00	54,33	51,00	52,67	48,67
	G3	45,00	56,00	100,00	100,00	66,33	59,67	70,00	71,00	100,00	100,00	51,00	88,00	61,33	65,67	50,33	73,33

After the application of varnishes, there was variation in the capacity remineralizing between groups. In G1, the sodium fluoride varnish, Duraphat, showed the best effectiveness in the remineralization process, which occurred in 75% of sites, followed by G2, which remineralized 50% of the sites and G3, which less efficient, remineralizing only 31.25% of the sites (Figure 1).

Figure 1: Effectiveness remineralizing varnishes.



In G1, when comparing the averages between mesial sites (varnishes) and distal (control) of the teeth that compose it, it is evident that the 5% NaF varnish, Duraphat, promoted remineralization by 87.50%. of the mesial sites. However, 62.50% of the distal sites also showed mineral gain, which can be inferred that the varnish used in G1 has the ability to act even at a distance. In G2, casein varnish (CPP-ACP 10%) promoted the remineralization of 50% of mesial sites and 50% of the distal sites. The G3 also presented 50% of remineralization at mesial sites. However, in the G3, only 12.50% of distal sites (control) showed mineral gain (Table 4).

Table 4: Comparison between the sites where varnish was applied and the control sites in the third measurement.

		R*	NR**	R (%)	NR (%)
Studied Groups	Duraphat mesial	7	1	87,50%	12,50%
	Caseína mesial	4	4	50,00%	50,00%
	Cervitec mesial	4	4	50,00%	50,00%
	Duraphat distal	5	3	62,50%	37,50%
	Caseína DISTAL	4	4	50,00%	50,00%
	Cervitec distal	1	7	12,50%	87,50%

* Refers to places where remineralization occurred.
**Refers to places where there was no remineralization.

When mesial and distal sites were compared, in each group separately, the varnish used in G1 proved to be the most effective, since 100% of the mesial sites were more mineralized than the distal sites. Mineral gain was also observed at the distal site (Table 5). In the G2, discreet mineral gain was observerd. In this group, 37.50% of the mesial sites had a higher remineralization that the distal sites and both showed slight remineralization (Table 6). In G3, 1% chlorhexidine varnish promoted remineralization of mesial sites, 62.50% greater than distal sites. At the mesial site, the mineral gain was discreet, unlike the distal site, where there was mineral loss (Table 7).

Table 5: Mean and standard deviation of the values found at the mesial and distal sites after the DES X RE cycle and after applying the varnish (G1).

Mesial Sites		Distal Sites	
G1 - Mineralization mean and SD		G1 - Mineralization mean and SD	
After cycle DES X RE	After varnish	After cycle DES X RE	After varnish
57,6 (18,9)	20,9 (22,8)	51,0 (21,1)	29,7 (24,1)

Table 6: Mean and standard deviation of the values found at the mesial and distal sites after the DES X RE cycle and after applying the varnish (G2).

Mesial Sites		Distal Sites	
G2 - Mineralization mean and SD		G2 - Mineralization mean and SD	
After cycle DES X RE	After varnish	After cycle DES X RE	After varnish
54,2 (16,0)	52,0 (6,8)	52,9 (9,5)	49,4 (2,34)

Table 7: Mean and standard deviation of the values found at the mesial and distal sites after the DES X RE cycle and after applying the varnish (G3).

Mesial Sites		Distal Sites	
G3 - Mineralization mean and SD		G3 - Mineralization mean and SD	
After cycle DES X RE	After varnish	After cycle DES X RE	After varnish
54,2 (16,0)	67,9 (21,4)	56,0 (23,9)	76,7 (17,3)

4 DISCUSSION

The dental enamel goes through cycles of mineral gain and loss, demineralization and remineralization, respectively.[13] Demineralization occurs when sugars are metabolized by acidogenic bacteria present in the biofilm, leading to a drop in saliva pH that can reach less than 4.0. When acids come into contact with enamel, bonds break molecules of hydroxyapatite crystals, releasing large amounts of Ca^{+} (calcium) and $PO4^{-}$ (phosphate). The loss of minerals from the enamel structure decreases the crystal size and widens intercrystalline spaces by increasing porosity the same. In equilibrium conditions, the remineralization process occurs, when the acids produced stimulate the salivary glands, increasing the amount of it, which has the ability to neutralize them, buffer effect,due to components like proteins, phosphate and calcium.[14] The loss or gain of mineral over time will determine whether tooth decay will progress (lesion active), stagnate or regress (inactive lesion)[13,15]

In this work, the in vitro demineralization model was used, suggested by Queiroz et al[12], who in their work evaluated the effect of fluoride enamel demineralization and remineralization in bovine dentin. This technique is widely used and accepted. In this way, it was possible to standardize the conditions demineralization and remineralize the entire sample studied, and therefore quantitatively analyze the mineral loss of enamel before and after the use of varnishes studied.[12,13,16,17,18]

This study did not assess microbiological issues regarding caries dynamics dental, only the physical-chemical issues involved in it. The analysis quantitative analysis was performed with the CarieScan ProTM device, which uses electrical impedance spectroscopy (EIS) to quantify the levels of demineralization of the dental structure. This is able to detect and monitor losses minerals early. The bioimpedance principle for the detection of injuries are based on the fact that the enamel has little porosity and a low[12]amount of fluid and is therefore a poor conductor. When evolution occurs mineral loss, there is an increase in this porosity and fluids on the surface, generating a higher level of conductivity. This method, according to Kühnisch et al. it's easy and good, even for inexperienced operators.[19]

To prove the effectiveness of cycling, the quantitative analysis of the degree of demineralization of the specimens that made up the sample, before of submitting them to this in vitro demineralization model, DES X RE cycle. The results show that mineral loss occurred in all specimens.

There are different types of remineralizing treatments. The varnishes were developed with the purpose of prolonging the contact of its active agents with the dental enamel. In this study, the action of varnishes was analyzed and compared of 5% sodium fluoride, casein and chlorhexidine. After the DES X RE cycle, varnishes studied were applied at the mesial site of each group, the site being distal control. The applications of the studied varnishes were carried out once a week for four weeks. Then, the final measurements at all sites using CarieScan Pro.

The American Academy of Pediatric Dentistry recommends the professional use of fluoride varnish to prevent or reverse tooth enamel demineralization in children with moderate or high risk of developing dental caries.[20] According to Carvalho et al.[21] the professional application of fluoride varnish provides additional protection against caries to populations that consume fluoridated water and / or make use of fluoridated toothpaste. [20] The high concentration of F- in the product would promote the formation of fluoridated apatite on the enamel surface, which together with the gain of ions such as calcium and phosphate, characterize the remineralization process. In addition, there would be the formation of calcium fluoride (CaF_2) on its surface, a crystal that acts as a reservoir of F^- [25].

In this study, the sodium fluoride varnish, Duraphat, showed the best effectiveness in the remineralization process, which occurred in 75% of the sites where it was applied, followed by the casein-based varnishes, which remineralized 50% of the sites and chlorhexidine, which showed less efficiency, remineralizing only 31.25% of the sites where it was applied (Figure 1).

In G1, when comparing the averages between the mesial (varnish) and distal (control) sites of the teeth that comprise it, it is evident that 5% NaF varnish,[16] Duraphat, promoted remineralization by 87.50% of the mesial sites. However, 62.50% of the distal sites also showed mineral gain, and it can be inferred that the varnish used in G1 has the ability to act even at a distance. In G2, casein varnish (CPP-ACP 10%) promoted remineralization of 50% of mesial sites and 50% of distal sites. G3 also showed 50% remineralization at mesial sites. However, in G3, only 12.50% of the distal sites (control) showed mineral gain (Table 4).

The remineralizing action of fluoride varnish at the distal site, that is, far from the application site, could be explained by the study by Bayrak et al.[22] who claim that the use of fluorides is associated with the formation of a calcium fluoride precipitate on the surface that would promote the release of fluoride ions, contributing to remineralization

and serving as a reservoir for future acid attacks. The integration of fluoride as a dental mineral has been fundamental for the prevention of caries.[13]

Remineralization using non-fluoridated agents, such as phosphopeptide casein (CPP) with amorphous calcium phosphate (ACP), is also suggested, due to the formation of a protective layer that inhibits the demineralization process.[17] What resembles the precipitate that forms when fluorides are used.

Mohd Said et al.[16] obtained results similar to those of this study. For the authors, the topical varnish based on 5% sodium fluoride (Duraphat) promoted significant enamel remineralization in carious lesions. The CPP-ACP varnish (MI Verniz TM), XCP (Embrace TMVerniz) used by the authors did not show a better remineralization capacity for carious enamel lesions, when compared to Duraphat.

In contrast, the study by Memarpour et al.[23], which used non-invasive methods to help reduce the active white spot, casein-based varnish, CPP-ACP, was as efficient as fluorine varnish. In addition, the authors consider it an advantage of CPP-ACP to be able to help reduce the risk of caries without increasing the risk of fluorosis.

Thakkar et al[18] concluded that the casein phosphopeptide varnish of amorphous calcium phosphate with fluorine (CPP-ACP) significantly inhibited the enamel demineralization in permanent molars. They also concluded that the CPP-ACP varnish is efficient in preventing demineralization, as well as remineralization of enamel. It can be used as a preventive measure.

In this study, chlorhexidine-based varnish proved to be the least effective as a remineralizing agent for enamel. We can observe, from the results of this research, that the action of chlorhexidine-based varnishes in the control of caries disease is not due to its remineralizing action on enamel, but rather to its microbiological action, since its effectiveness in decrease in the population of Streptococcus mutans.

Chlorhexidine is classified as a broad bacterial bis-guanidine and currently the most potent chemotherapeutic agent against Streptococcus mutans and for this reason to tooth decay.[24] For Wang et al,[24] chlorhexidine and CPP-ACP may be effective in the management of primary dental caries. The authors demonstrated the effectiveness of casein varnish in remineralization and in increasing the size of hydroxyapatite crystals.

The study by Cochrane et al,[25] which compares different dental varnishes, in vivo, concluded that they release their ions for a relatively short period of time. It is estimated that the varnishes only remain in situ for up to 24 hours. This condition is different from the condition of this study, in vitro, since the applied varnishes were not subjected to any

type of stress that could lead to their loss, thus remaining in contact with the enamel surface for a longer time.[25] However , the findings of this in vitro study can serve as a reference when the clinician needs to choose an agent that has the ability to remineralize tooth enamel, thus being clinically relevant.

5 CONCLUSIONS

There was variation in the remineralizing capacity among the studied varnishes. Also, the varnish based on 5% sodium fluoride, Duraphat, showed the best effectiveness in the remineralization process, also acting at a distance.

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