The effect of different fluoride varnishes on the release of calcium ions from hydroxyapatite discs: An ion-selective electrodes study

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ABSTRACT

Introduction: Recently, various types of fluoride varnishes have been developed, each with its own recommended concentration, potentially active ingredients, and flavoring agents, leading to additional preventive benefits. Differences in fluoride release patterns can potentially enhance or reduce the efficacy of fluoride varnishes. Numerous clinical trials have proven their ability to prevent and arrest dental caries. The aim of this study was to investigate the apatite demineralization process under the effect of different fluoride varnishes using ion-selective electrodes (ISE), in an attempt to comprehend their anti-caries mechanism. **Methods**: Four different fluoride varnishes (Fluor Protector S, Duraphat, ClinPro White, MI Varnish) were used to measure their effect on the demineralization process of hydroxyapatite (HAP) discs in 60ml pH 4.0 acetic solutions. The HAP discs were treated with these varnishes after 4-hours demineralization and then immersed back into the same solutions for further demineralization to observe the effect of the varnishes. Throughout the experiment, the calcium ISE was used to monitor calcium concentration. **Results**: Prior to the intervention, the loss of mineral mass from hydroxyapatite discs increased linearly over time. Following treatment, calcium release almost completely stopped, with ClinPro White exhibiting the most substantial inhibition within four hours (100%) while Duraphat, MI varnish, and Fluor Protector S showed 99.02%, 92.1%, 87.39%, respectively. Percentage changes indicated that ClinPro White was the most effective in minimizing calcium dissolution, whereas Fluor Protector S had the smallest impact. The calcium dissolution rates plotted against log fluoride content revealed significant variability among the varnishes, reinforcing ClinPro White's superior protective capabilities. **Conclusion**: Treatment with fluoride varnishes treatment was shown to be effective in inhibiting the demineralization of apatite regardless of fluoride concentration and the additional active ingredients incorporated in some fluoride varnishes.

Keywords: Demineralization, dental caries, fluoride varnishes, ion-selective electrodes, remineralization.

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INTRODUCTION

A goal of modern dentistry is the non-invasive management of non-cavitated caries lesions through remineralization strategies that repair the enamel with fluorapatite or fluorhydroxyapatite. Without doubt, fluoride supplements have contributed to a decrease in the prevalence and severity of dental caries in most industrialized countries over the past two decades. Intensive laboratory and epidemiological research into the mechanisms underlying caries prevention indicates that fluoride's predominant effect is topical.[1-3] Notably, this effect is strongest when fluoride is continuously available in solution[4], resulting in less mineral loss during acidic challenges.[5-7]

The use of topically applied fluoride has increased over recent decades, and fluoride-containing varnishes are widely used today. Recently, a range of novel calcium-phosphate-based remineralization delivery systems has been developed for clinical application and incorporated into products such as fluoride varnishes. Differences in fluoride release patterns can potentially enhance or reduce the efficacy of fluoride varnishes.[8,9]

Numerous clinical trials have demonstrated the effectiveness of fluoride varnishes in preventing and arresting dental caries. This study focused on evaluating the efficacy of four different fluoride varnishes in inhibiting the demineralization process of hydroxyapatite (HAP) discs and determining whether one varnish demonstrates superior effectiveness. In this study, HAP discs were used as tooth analogues, as described by Kosoric et al.[10] This study aimed to analyze the effect of these varnishes on the calcium ion release from HAP discs using an ion-selective electrode (ISE) approach.

METHODS

Ion Selective Electrode (ISE)

Ion-selective electrodes (ELT8041, Nico2000 Ltd., Middlesex, UK) are solid-state electrodes consisting of saturated crystalline membranes or polyvinyl chloride (PVC) membranes designed to detect specific ions. They are used to measure free ion concentration in solution. Ion activity is measured by converting it into an electrical potential. The ISE measures the ion activity with the aid of a pH meter or a voltmeter. The voltage is theoretically dependent on the logarithm of the ionic activity according to the Nernst Equation.

The ISE system comprises -ISE electrodes, the ELITE head system, references electrode, an ion/pH analyzer, electrochemical software for ISE/pH, and a two-channel interphase 2.1.22 (Chemputrix Ltd, London UK 1998).

The calcium ISE contains a PVC membrane saturated with organic molecules capable of binding and transporting calcium ions. Inside the electrode, an internal solution with a predetermined calcium chloride concentration is combined with potassium chloride or silver chloride as part of the internal reference system. In modern solid-state ISEs, this reference system is present in a solid form.

There are three types of reference electrodes, classified according to their filling solution composition. In this study, potassium chlorides were used in combination with barium, calcium, fluoride and nitrous oxide.

In this study, the ISE was employed to measure the rate of mineral loss from HAP discs under simulated cariogenic challenges. For each data set, the amount of calcium dissolved as a result of HAP dissolution was plotted as a function of time and calcium dissolution concentration.

Calcium and reference electrode calibration

Calcium and reference electrodes were immersed in 60ml of one of the calibration solutions (Table 1). After the reading stabilized, recording was initiated. Then, 1ml of acid or water was added to dilute the solution, and a new reading was taken. This procedure was repeated 20 times. The readings obtained from the ISE, expressed in millivolts, reflected the free calcium concentration in the solution, resulting from the potential difference between the calcium

Table 1. Calibration of the solutions concentration

Calibration solution concentration (mM)	Medium	Amount of CaCl2. H2O added (grams)
1 (1 litre)	Deionised water	0.1443
1 (1 litre)	Acetic acid	0.1443
1 (60 ml)	Deionised water	0.008658
1 (60 ml)	Acetic acid	0.008658

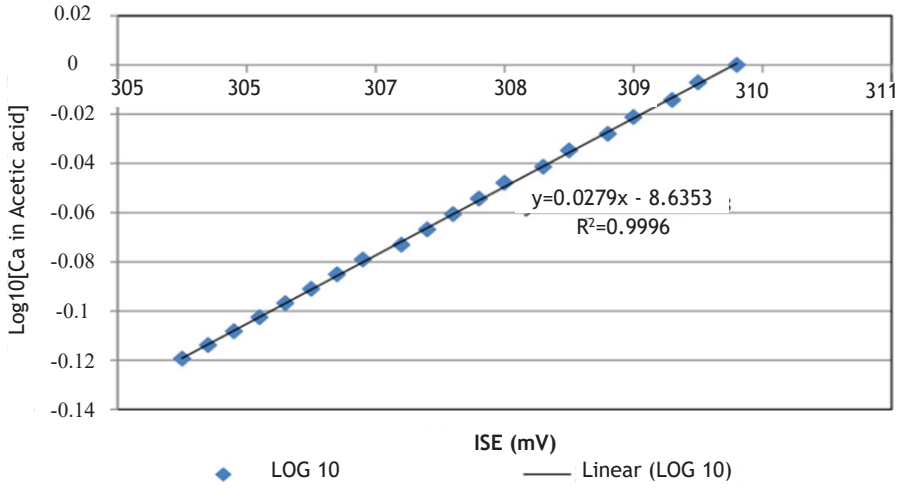

Figure 1. Calibration curve

and reference electrode measurements. These data were used to plot a calibration graph, from which a calibration equation was derived to calculate calcium ion concentration in subsequent experiments. The calibration graph was linear, producing an equation suitable for converting ISE readings into concentration (Figure 1).

Varnish selection

Four dental varnishes were selected for this study (Table 1): (1) MI Varnish with CPP-ACP containing 5% sodium fluoride [2.26% (w/w) fluoride, 22.6mg/ml fluoride or 22600mg/L Fluoride]; (2) ClinPro White with fTCP containing 5% sodium fluoride [2.26% (w/w) fluoride, 22.6mg/ml fluoride or 22600mg/L fluoride]; (3) Fluor Protector S containing 1.5% ammonium fluoride [0.77% (w/w) fluoride, 0.77mg/ml fluoride or 7700mg/L fluoride]; (4) Duraphat containing 5% sodium fluoride [2.26% (w/w) fluoride, 22.6mg/ml fluoride or 22600mg/L Fluoride].

HAP discs

The specimens used to simulate enamel were commercially available, compressed, sintered HAP discs (Plasma Biotal, UK), measuring 13mm in diameter, 2mm in thickness, and with 20% nominal porosity. These were prepared as windowed-type discs for demineralization experiments. Nail polish was applied to varnish the disc surfaces, leaving a 6 mm x 6 mm window exposed.

Demineralising solutions

Acetic acid (0.1 M/L) was used as the acidic medium to simulate demineralization. A solution was prepared by adding 6.05g of pure (100%) acetic acid to 1.0 L of deionised water, yielding a pH of 2.8. The solution was then gradually neutralized with sodium hydroxide via syringe, adjusting to a final pH value of 4.0.

Data collection procedures

Four compressed, sintered HAP discs (Plasma Biotal, UK.) were prepared in windowed-type discs. Nail polish was applied to each disc, leaving a 6 mm x 6 mm window exposed.

All four HAP discs were then coated with a single layer of one of the different fluoride varnishes, as shown in Figure 2. The amount of varnish painted on each HAP disc was determined before and after application. The weight of varnish covering the standardized area (6 mm x 6 mm window) of the HAP disc was MI Varnish (0.086 g); Clinpro White (0.082 g); Fluor protector S (0.088 g); Duraphat (0.089 g). A 60 ml volume of acetic acid solution (pH 4.0) was poured into a beaker.

The calcium and reference electrodes were then immersed into the solution while being stirred continuously using a magnetic stirrer. A few minutes were allowed for the reading to stabilize before HAP discs were added to the solution. The HAP discs were immersed into the solution for 4 hours before ISE reading was stopped.

The HAP discs were then removed from the demineralization solution, air-dried for 2 minutes, and washed with distilled water for another 2 minutes. A single, thin film of each varnish was then applied to the exposed window of each

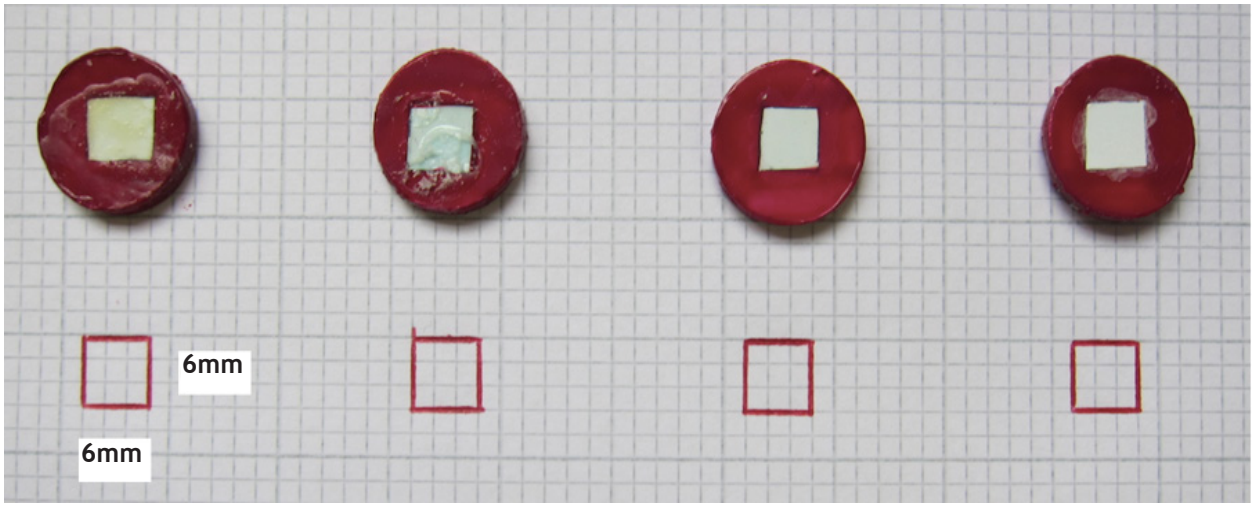

Figure 2. From left, HAP disc after treated with Duraphat, MI Varnish, Fluor protector S and Clinpro White

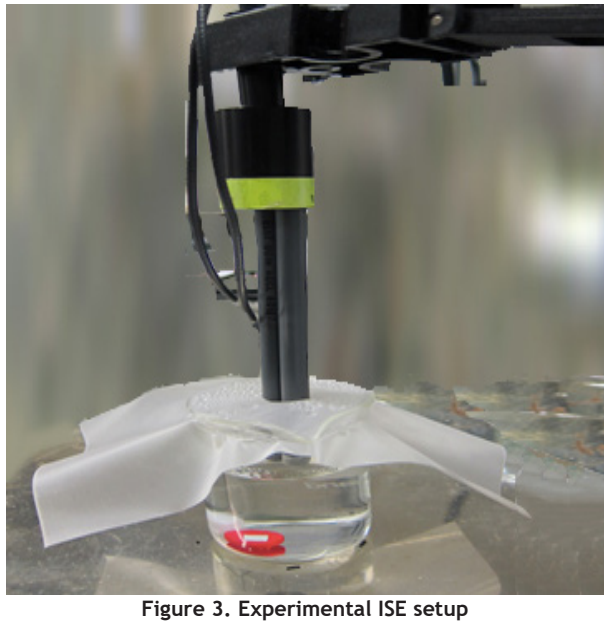

Figure 3. Experimental ISE setup

HAP disc using the supplied or recommended applicator.

The exposed surfaces treated with topical varnishes were maintained at 37°C for 1 minute, air dried, and the amount of varnish applied was determined by reweighing the HAP disc. An attempt was made to equalize the varnish amount across discs within about 10% by adding varnish to those with a lower weight. However, varnish was not removed from discs with higher weight once applied. All HAP discs were then re-immersed in the 60ml acetic acid solution (pH 4.0). The rate of calcium dissolution of HAP discs was determined using the calcium electrode for another 4 hours. Experiments were repeated with each topical fluoride varnish (Clinpro White, Fluor protector S, MI Varnish Duraphat), as shown in Figure 3.

RESULTS

For all cases, prior to the intervention, the mineral mass loss from the hydroxapatite discs was linear with time as previously observed.[11] However, as shown in Figure 4 (A-D), the horizontal tendency indicated that the calcium release nearly ceased after the intervention.

Figure 5 shows a continued drop in slope after 4 hours of ClinPro White treatment, reflecting a reduced calcium dissolution rate.

For each experiment, the percentage change in HAP discs was calculated based on calcium dissolution before and after intervention expressed in mmol/L (Table 2)

Percentage of inhibition of calcium dissolution after intervention with fluoride

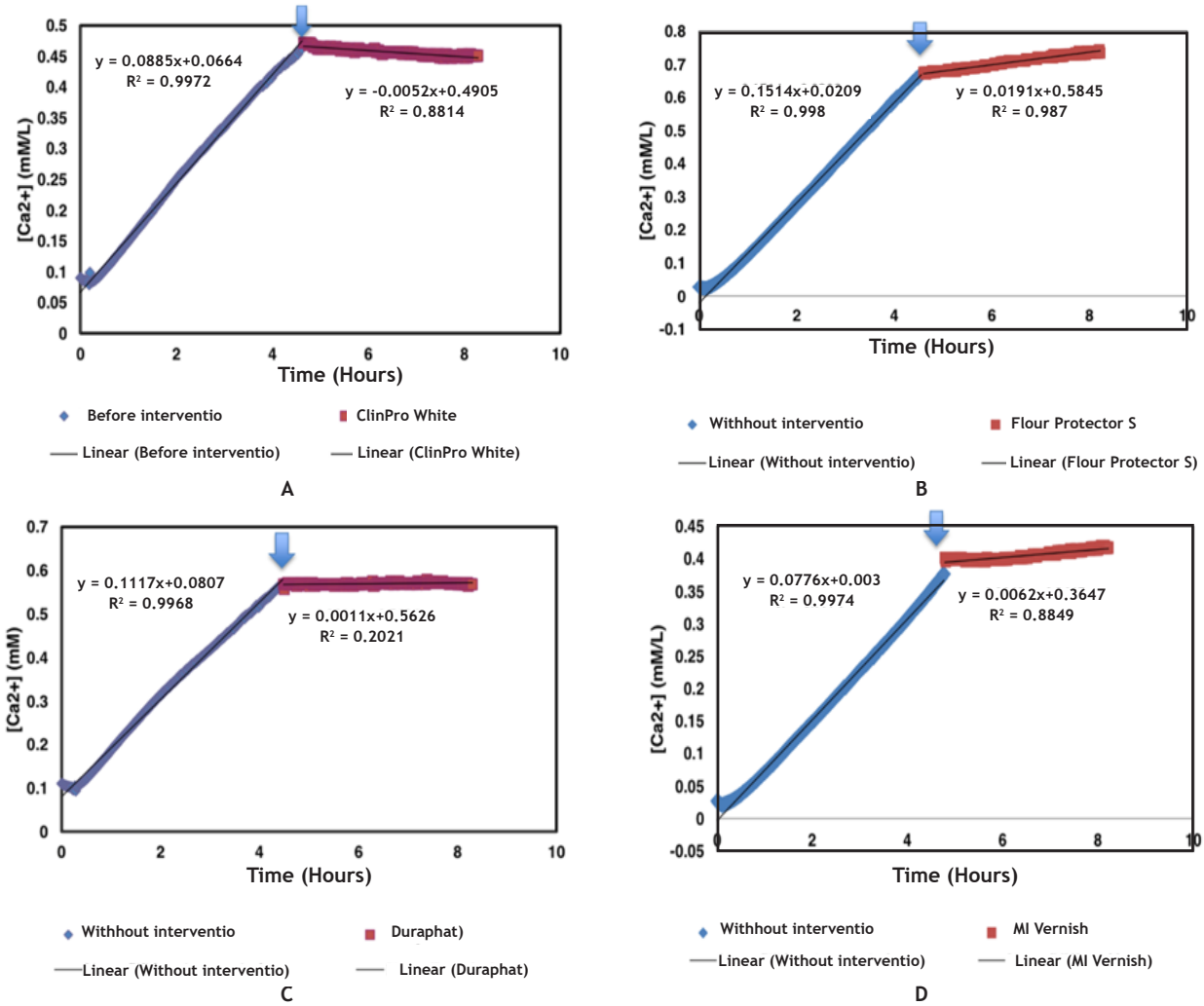

Figure 4. A) Windowed-type disc treated with ClinPro White varnish after 4 hours demineralisation in 60 ml acetic acid pH 4.0.; B) Windowed-type disc treated with Fluor Protector S varnish after 4 hours demineralisation in 60 ml acetic acid pH 4.0.; C) Windowed-type disc treated with MI Varnish after 4 hours demineralisation in 60 ml acetic acid pH 4.0.; D) Windowed-type disc treated with Duraphat varnish after 4 hours demineralisation in 60 ml acetic acid pH 4.0.

Table 2. Amount of calcium dissolution rate before and after intervention with fluoride varnishes

Fluoride varnishes	Before treatment calcium concentration (mmol/L)	After treatment calcium concentration (mmol/L)	Percentage inhibition (%)
Clinpro White	0.0885	-0.0052	0 (100)
Duraphat	0.1117	0.0011	0.98 (99.02)
Fluor protector S	0.1514	0.0191	12.61 (87.39)
MI Varnish	0.0776	0.0062	7.9 (92.1)

varnishes is shown in Figure 6. ClinPro White showed the highest percentage of inhibition of calcium dissolution on HAP disc within 4 hours after the intervention. The hydroxyapatite discs treated with Fluor protector S had the lowest ability in inhibiting calcium dissolution under demineralizing conditions.

The calcium dissolution rate was plotted against the log of fluoride content. Log fluoride values were derived from the sodium fluoride concentrations in each varnish, as provided by the manufacturer (ppm) (Figure 7).

There was an initial and rapid decrease from the control until HAP discs were treated with a range of fluoride varnishes. Fluor Protector S had the highest calcium dissolution rate compared to other fluoride varnishes. ClinPro White showed the lowest calcium dissolution after treatment.

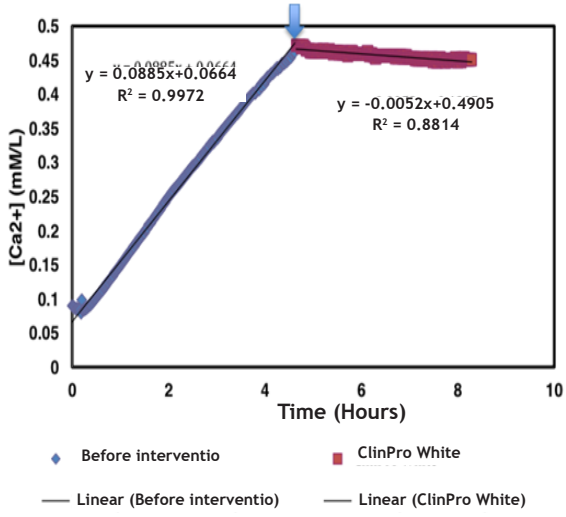

Figure 5. Windowed-type disc treated with ClinPro White varnish after 4 hours demineralisation in 60 ml acetic acid with the pH value of 4.0.

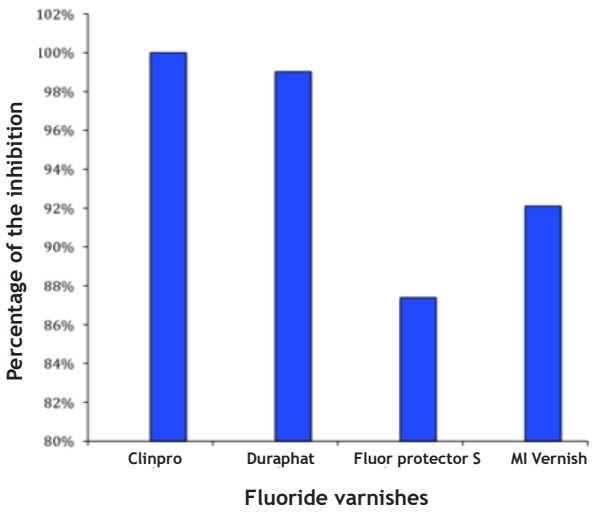

Figure 6. Percentage of the inhibition of calcium dissolution against different fluoride varnishes

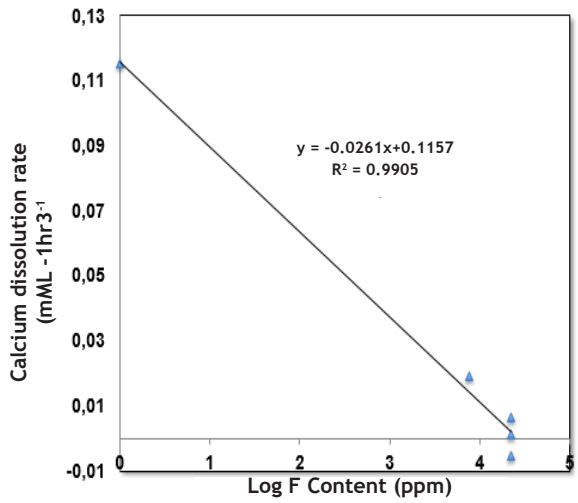

Figure 7. Calcium dissolution rate against Log of the fluoride content

DISCUSSION

The use of professional topical fluoride to the exposed surface of teeth at elevated concentrations for a localized protective effect has increased over recent decades. Recently, various formulations of fluoride varnishes have evolved, each with its own recommended concentration, potentially active ingredients, and flavour, leading to claims of additional preventive benefits. Differences in fluoride release patterns can potentially enhance or reduce the efficacy of fluoride varnishes.[12,13] Numerous clinical trials have demonstrated their ability to prevent and arrest dental caries. The main focus of this study was to evaluate the efficacy of four different fluoride varnishes in inhibiting the demineralization process of the apatite discs and to determine whether one varnish was more effective than the others.[14]

Artificial HAP discs have previously beenused as a model system analogues for natural enamel because of their thickness, homogeneity and composition.[10,15] Furthermore, numerous studies have used acetic acid (pH = 4.0) as a demineralizing solution to mimic caries-like conditions in vitro.[11,15,16]

All the slope and rate values of the experiment were gathered in a table to present the effects of all fluoride varnishes. Minor discrepancies observed during the 4-hour control demineralization may be attributable to manufacturing variation, as individual discs were not identical. New HAP discs were used for each experiment, which may differ slightly in chemical structure. Furthermore, new batches of demineralizing solution were used for each series of experiments. The amount of calcium released over time differed slightly between ClinPro White, Fluor protector S, Duraphat and MI Varnish. However, as shown in the results, the

horizontal tendency indicates that the calcium release nearly ceased after the intervention. These findings identified that continuous ISE time-dependent [Ca2+] release studies demonstrate that dissolution inhibition by different fluoride varnishes was rapid. The findings also support the theory of a 'ceiling effect' whereby high fluoride concentrations

strongly inhibit demineralization. This may be attributed to the formation of CaF2 covering the HAP disc surfaces, which acts as a reservoir of fluoride for fluorapatite establishment later on.6 The thick CaF2 barrier may further inhibit calcium dissolution. However, as the ISE system detects only dissolved ions in the aqueous phase, deposition products such as CaF2 could not be documented. On the other hand, the result was in line with the previous studies which reported that fluoride can also change the rate of dissolution without changing the hydroxyapatite mineral solubility.

Interestingly, in HAP discs treated with ClinPro White, there appear to be signs of remineralization. Additional calcium and inorganic phosphate into fluoride varnish may lead to inhibiting demineralization and further remineralization on HAP disc. There was a continuing drop in slope after 4 hours of treatment with ClinPro White, and the calcium dissolution rate after treatment was (-0.0052). This finding supports the hypothesis that, apart from inhibiting demineralization, fluoride varnishes (ClinPro White) might simultaneously demonstrate signs of remineralization within four hours. Fluoride varnish has a short lifespan in the oral environment as it is removed by the action of the cheeks and tongue, salivary flow, mastication and oral hygiene procedures. Therefore, varnishes should release their ions in a relatively short period before the varnish is lost. It has been estimated that varnishes only remain in situ for up to 24 hours. The combined use of calcium and fluoride, particularly ClinPro White, has shown signs of remineralization within 4 hours under caries-like demineralizing conditions, so this new varnish may have the potential to improve caries prevention further.

In terms of the ability in inhibiting calcium dissolution in demineralizing conditions, ClinPro White has the best outcome (100%) followed by Duraphat (99%), MI Varnish (92%) and Fluor Protector S (87%). These findings are consistent with previous research indicating that some of these secondary ingredients may affect the fluoride ion release of the product.17,18 Fluoride release and subsequent formation of calcium fluoride are thought to be an essential part of the mechanism of action of fluoride varnishes to prevent demineralization.19,20 Moreover, differences in fluoride release patterns can potentially enhance or diminish the efficacy of fluoride varnishes.[21] This finding also agrees with the observation of Seppa22 who reported that the higher the fluoride concentration, the greater the fluoride uptake by enamel. Conversely, Fluor Protector S has the lowest concentration of fluoride (1.5% ammonium fluoride) compared to other dental varnishes and is likely to have the lowest rate of dissolution inhibition. More research is therefore needed to better understand that it is essential to ensure that the addition of calcium and phosphate ions does not reduce the availability of fluoride ions, as fluoride has been shown in clinical trials to provide the caries preventive efficacy of the varnish.23-25 For future studies, the fluoride and phosphate concentration in the demineralizing solution after the application of different fluoride varnishes should be determined. However, these findings should be interpreted with caution, as this study does not fully replicate in vivo oral conditions.

CONCLUSION

Fluoride varnish treatments were found to be effective in inhibiting the demineralization of apatite, regardless of differences in fluoride concentration or additional active ingredients incorporated in some of the fluoride varnishes. Among the four fluoride varnishes in this study, ClinPro White showed possible signs of remineralization under caries-like demineralizing conditions within 4 hours. Sodium fluoride and ammonium fluoride decreased the rate of HAP disc dissolution under caries-like demineralizing conditions. Continuous ISE time-dependent [Ca2+] release studies demonstrate that dissolution inhibition by different fluoride varnishes was rapid.

REFERENCES

1. Walsh T, Worthington HV, Glenny AM, Marinho VC, Jeroncic A. Fluoride toothpastes of different concentrations for preventing dental caries. Cochrane Database Syst Rev. 2019 Mar 4;3(3):CD007868. DOI: 10.1002/14651858.CD007868

2. Hellwig E, Lennon AM. Systemic versus topical fluoride. Caries Res. 2004;38(3):258-62. DOI: 10.1159/000077764
3. Featherstone JDB. Caries prevention and reversal based on the caries balance. Pediatr Dent. 2006;28(2):128-32; discussion 192-8.
4. Ten Cate JM, Buzalaf MAR. Fluoride mode of action: once there was an observant dentist J Dent Res. 2019;98(7):725-30. DOI: 10.1177/0022034519831604.
5. Duggal MS, Toumba KJ, Amaechi BT, Kowash MB, Higham SM. Enamel demineralization in situ with various frequencies of carbohydrate consumption with and without fluoride toothpaste. J Dent Res. 2001;80(8):1721-4. DOI: 10.1177/00220345010800080801
6. Mohammed NR, Kent NW, Lynch RJM, Karpukhina N, Hill R, Anderson P. Effects of fluoride on in vitro enamel demineralization analyzed by (1)(9)F MAS-NMR. Caries Res. 2013;47(5):421-8. DOI: 10.1159/000350171
7. Issa AI, Preston KP, Preston AJ, Toumba KJ, Duggal MS. A study investigating the formation of artificial sub-surface enamel caries-like lesions in deciduous and permanent teeth in the presence and absence of fluoride. Arch Oral Biol. 2003;48(8):567-71. DOI: 10.1016/s0003-9969(03)00095-5
8. Jablonowski BL, Bartoloni JA, Hensley DM, Vandewalle KS. Fluoride release from newly marketed fluoride varnishes. Quintessence Int. 2012;43(3):221-8.
9. Ritwik P, Aubel JD, Xu X, Fan Y, Hagan J. Evaluation of short-term fluoride release from fluoride varnishes. J Clin Pediatr Dent. 2012;36(3):275-8. DOI: 10.17796/jcpd.36.3.q304488478w52334
10. Kosoric J, Hector MP, Anderson P. The influence of proteins on demineralization kinetics of hydroxyapatite aggregates. J Biomed Mater Res A. 2010;94(3):972-7. DOI: 10.1002/jbm.a.32759
11. Bollet-Quivogne FR, Anderson P, Dowker SEP, Elliott JC. Demineralisation of permeable hydroxyapatite with alternating water and acidic buffer: scanning microradiographic study of effect of switching period. Caries Res. 2007;41(2):152-60. DOI: 10.1159/000098050
12. Rirattanapong P, Vongsavan K, Saengsirinavin C, Pornmahala T. Effect of fluoride varnishes containing tri-calcium phosphate sources on remineralization of initial primary enamel lesions. Southeast Asian J Trop Med Public Health. 2014;45(2):499-504.
13. Rirattanapong P, Vongsavan K, Saengsirinavin C, Pornmahala T. Effect of fluoride varnishes containing different calcium phosphate sources on mineralization of initial primary enamel lesions. Southeast Asian J Trop Med Public Health. 2014;45(6):1503-10.
14. Cochrane NJ, Cai F, Huq NL, Burrow MF, Reynolds EC. New approaches to enhanced remineralization of tooth enamel. J Dent Res. 2010;89(11):1187-97. DOI: 10.1177/0022034510376046
15. Elliott JC, Bollet-Quivogne FRG, Anderson P, Dowker SEP, Wilson RM, Davis GR. Acidic demineralization of apatites studied by scanning X-ray microradiography and microtomography. Mineral Mag. 2005;69(5):643-52. DOI: 10.1180/0026461056950276
16. Anderson P, Bollet-Quivogne FRG, Dowker SEP, Elliott JC. Demineralization in enamel and hydroxyapatite aggregates at increasing ionic strengths. Arch Oral Biol. 2004;49(3):199-207. DOI: 10.1016/j.archoralbio.2003.10.001
17. Retief DH, Sorvas PG, Bradley EL, Taylor RE, Walker AR. In vitro fluoride uptake, distribution and retention by human enamel after 1- and 24-hour application of various topical fluoride agents. J Dent Res. 1980;59(3):573-82. DOI: 10.1177/00220345800590030401
18. Lippert F, Hara AT, Martinez-Mier EA, Zero DT. In vitro caries lesion rehardening and enamel fluoride uptake from fluoride varnishes as a function of application mode. Am J Dent. 2013;26(2):81-5. PMID: 24073530.
19. Nalbantgil D, Oztoprak MO, Cakan DG, Bozkurt K, Arun T. Prevention of demineralization around orthodontic brackets using two different fluoride varnishes. Eur J Dent. 2013 Jan;7(1):41-7. PMID: 23408742; PMCID: PMC3571508.
20. Weyant RJ, Tracy SL, Anselmo T, Beltrán-Aguilar ED, Donly KJ, Frese WA, et al. Topical fluoride for caries prevention: executive summary of the updated clinical recommendations and supporting systematic review. J Am Dent Assoc. 2013;144(11):1279-91. DOI:10.14219/jada.archive.2013.0057

21. Lippert F. Fluoride release from fluoride varnishes under acidic conditions. J Clin Pediatr Dent. 2014;39(1):35-9. DOI: 10.17796/jcpd.39.1.b45805v0v17407gl
22. Fluoride Formed on Enamel by Fluoride Varnish or Gel Application: A Randomized Controlled Clinical Trial" Caries Res. 2022;56(1):73-80
23. Shen P,Manton DJ, Cochrane NJ, Walker GD, Yuan Y, Reynolds C, Reynolds EC. Effect of added calcium phosphate on enamel remineralization by fluoride in a randomized controlled in situ trial. J Dent. 2011;39(7):518-25. DOI: 10.1016/jdent.2011.05.002
24. Shahmoradi M, Hunter N, Swain M. Efficacy of fluoride varnishes with added calcium phosphate in the protection of the structural and mechanical properties of enamel. Biomed Res Int. 2017;1(7834905):1-7. DOI: 10.1155/2017/7834905
25. Schemehorn BR, Wood GD, McHale W, Winston AE. Comparison of fluoride uptake into tooth enamel from two fluoride varnishes containing different calcium phosphate sources. J Clin Dent. 2011;22(2):51-4.