Comparison of Three Topical Fluorides Using Computer Imaging

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The purpose of this in vitro study was to evaluate the short and long-term effectiveness of fluoride varnish and compare it with the two most commonly used topical fluorides, i.e., fluoride gel and foam. A second purpose of the study was to compare the effectiveness of these preparations on primary and permanent teeth. Enamel slabs with a thickness of 500 microns were obtained from caries free primary molars and premolars. They were divided into four groups: control, foam (F), gel (G) and varnish (V). Fluorides were applied to the enamel slabs according to the manufacturer's instructions and were placed in a 5.1 pH acidic gel for one week. The amount of demineralization from the enamel was measured by calculating the amount of light reflected from these surfaces. This was done by using a Charged Couple Device attached to a high resolution microscope with image processing software, Optima 5.22. The enamel slabs were placed in a freshly prepared acidic gel for a second week without application of fluorides. This was done to mimic a situation where fluoride is eventually brushed away from teeth. Reflective images were recorded under the previously described conditions. A two-way analysis of covariance was used to compare the treatments. The results showed no statistically significant difference (with Bonferroni correction) in the effectiveness of different fluoride preparations over the short-term (Week I comparison; p-values: F vs. G 0.079, F vs. V 0.030, G vs. V 0.44). However, the long-term protection provided by fluoride varnish was far more than fluoride gel and foam (Week II comparison; p-values: F vs. G 9X10-5 , F vs. V 7X10-8, G vs. V 1X10-4). Fluorides were equally effective for both primary and permanent enamels (p-value 0.24). The results of this study suggest that fluoride varnish is beneficial for use with white spot lesions, newly erupted permanent teeth and early decalcification in primary dentition.

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Introduction

Fluoride varnish is increasingly being used by pediatric dentists in prevention programs as well as in the interception of demineralizations associated with early childhood caries.

Earlier studies,[4,6,7,9,10,11,18-20,23,26,28,31] mainly from Scandinavia, report that fluoride varnishes and gels are equally effective when applied topically. However, it has been suggested that varnishes may be a more effective method of delivering "longer term" protection to teeth and that it may be especially beneficial for primary teeth. The rationale given is that the fluoride varnish maintains better contact with the enamel in the hours and days after application resulting in better uptake and creating more resistance to acid demineralization.

Topical fluorides have been applied to teeth as aqueous solutions or gels and have been incorporated into prophylactic pastes and varnishes. The results of numerous clinical studies[1,2,7,15-18,20] indicate that the vehicle in which fluoride is incorporated can influence the level of clinical effectiveness. The time consuming procedure of applying aqueous solutions led to the development of fluoride-containing gels in the belief that their viscosity would make them easier to work with.[1] These products have essentially the same formulations as the aqueous solutions, with addition of alkyl cellulose as the gelling agent.[2] Fluoride solutions and gels leach out absorbed fluoride from the surface enamel. To prevent this immediate loss, fluoride was incorpo-

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rated into varnishes that have the ability to adhere to enamel for long periods and it was hypothesized, to slowly release the fluoride to the teeth. These retentive and slow release effects increase the exposure time of the teeth to the fluoride by several days, without increasing chair time, and presumably, allow fluoride to be more permanently bound to the teeth.[1]

In 1994, fluoride varnish was cleared by the U.S. Food and Drug Administration (under class II regulations, as listed in the Code of Federal Regulations, Title 21, Parts 800 to 895) as medical devices to be used as cavity liners and for the treatment of hypersensitive teeth. Laboratory evidence suggests that fluoride varnishes have properties equivalent to other dentinal tubule sealants, but because caries prevention is considered a drug claim, manufacturers would have to submit appropriate clinical trial evidence for review by the FDA before they could be accepted as anticaries agents.[4]

The cariostatic effect of fluoride on primary teeth has been well documented in water fluoridation studies.[5] However, the cariostatic effectiveness seems to be slightly lower for primary teeth than for permanent teeth.

Grodzka *et al.*[6] evaluated caries increments in primary teeth after application of topical fluoride in high-risk children. The fluoride was applied bi-annually in preschoolers and followed for two years. They found that topical fluoride provided little benefit in this population. Koch *et al.*[7-11] reported an insignificant effect of topical fluoride application in primary dentition in a group of individuals with high caries activity. It may, however, be more effective when combined with other methods, e.g. fluoride tablets, tooth pastes and low fluoride concentration gels.[7-11] Edenholm *et al.*[12] evaluated fluoride concentrations in primary enamel after application *in vitro* of five fluoride varnishes at different concentrations. The teeth were analyzed for their fluoride content by using a stepwise acid etch technique followed by a fluoride determination. Their results indicated a high fluoride uptake after a single application of the varnishes. However, a considerable loss of fluoride took place when the teeth were stored in synthetic saliva for one week after application. The authors concluded that while it was possible to greatly increase fluoride concentration in primary enamel, the high rate of leakage seems to influence the cariostatic effects. Koch *et al.*[8] followed the kinetics of fluoride in primary enamel after application of topical fluoride. The fluoride concentration was determined using a micro-acid-drop technique from 24 hours up to six months after treatment. Their results showed a marked increase of fluoride in surface as well as subsurface enamel 24 hours after treatment. Thereafter, release of fluoride from enamel seems to reduce this concentration. They concluded that the caries inhibiting effect of fluoride in primary enamel is based more upon the kinetics of fluoride than a permanent uptake.

Previous *in vitro* studies, comparing the effectiveness of different fluoride vehicles, were all of short duration. In the acid-etch technique, the teeth were immersed in synthetic saliva at pH-7 for a short duration, less than 24 hours in most cases. There was no attempt to test the efficacy of the absorbed fluoride by subjecting the teeth to a caries challenge. Further study is needed to evaluate the effectiveness of fluorides by subjecting enamel to an acidic challenge. The majority of the studies included enamel from permanent teeth only. Due to the known differences in the mineral content, thickness and possible kinetics of fluorides in the enamel of primary teeth, it is important to evaluate the effectiveness of fluorides in the primary teeth also.

The purpose of this *in vitro* study was to evaluate the short and long term effectiveness of three topically applied fluorides. These included a fluoride varnish (5% NaF in colophonium base) and two other commonly used topically applied fluorides, an acidulated phosphate fluoride (APF) gel (1.23% F^- from NaF + HF and 0.1 M Orthophosphoric acid, pH = 3) and foam (1.23% F^- from NaF + HF with pH = 3.5). A second purpose of the study was to compare the effectiveness of these preparations in preventing demineralization of primary and permanent enamel after being exposed to an artificial caries challenge.

MATERIALS AND METHOD:

Twelve sound premolars and 12 primary molars (caries and restoration free) were used. The teeth were cleaned with pumice powder and stored in Hanks Balance Salt Solution at 3ºC. Twenty four flat and highly polished enamel slabs were prepared from these teeth using a three step grinding and polishing procedure. The gross grinding was carried out with a 600 grit silicon carbide (SiC) paper. The area was carefully evaluated with magnifying lenses for the presence of exposed dentin, cracks or different planes. The polishing was carried out with 1 and 0.5 micrometer Aluminum oxide (Al2O3) suspensions respectively. The final luster of the flattened enamel was the same as uncut enamel. A section incorporating the flattened surface area was cut from the tooth. The underlying surface of this sample was ground and polished in the same manner described. The thickness of these slabs ranged from 395 to 755 microns. The under surface of the samples were covered with a thin layer of transparent nail polish and dried in a desicator for 24 hours.

The enamel slabs were divided into primary and permanent categories. They were further subdivided in a control group (n=3), a fluoride foam group (n=3), a fluoride gel group (n=3) and a fluoride varnish group (n=3). The amount of demineralization from enamel was calculated by comparing the reflectance of light from the enamel slabs before and after an artificial caries challenge. This was done by using a charged couple device (CCD) and the computer program, Optima version 5.22.® The CCD was attached to a high-resolution microscope that captured images after light was reflected from the observed surfaces. The amount of light hitting each surface was standardized and the relative lightness and darkness of each sample was compared to standard white and black backgrounds. This is described as "gray scale value." The images were taken three times: at baseline, one week after artificial caries challenge and two weeks after the caries challenge. Since light is reflected more from a white surface, the artificially created white spot lesions in the enamel reflected more light. The difference in the amount of light reflected was a measure of the demineralization for the control and fluoride groups. The same standardized imaging technique was repeated for each of the permanent and primary enamel slabs.

After taking images at baseline (week 0 images), the three topical fluorides were applied according to the manufacturer's recommendations. Fluoride foam (Oral-B Minute-Foam®) and Fluoride gel (Nupro APF 1-Minute Treatment®) were applied to dry enamel slabs for one minute. Fluoride varnish (Duraflor® sodium fluoride varnish) was applied to a wet enamel slab. No fluoride was applied to the enamel slabs in the control group. All enamel slabs were immersed in an acidic gel at 22.5°C for one week. The gel consisted of 6% by weight of hydroxyethyl cellulose in a 0.1 mol/L lactic acid solution adjusted to pH-5.1 with 1.0 mol/L sodium hydroxide (NaOH). After removal from the acid gel, the enamel slabs were washed with de-ionized water. This was followed by immersion in acetone for one minute to remove all residues of the acid gel and varnish. The samples were washed again in de-ionized water and then dried in desicator for 24 hours. Reflective images were taken for a second time in the previously described standardized manner (week 1 images). The difference in the reflectance between these images and the base line images measured the amount of demineralization and represented the short term effects of the three topical fluorides on both the primary and permanent enamel slabs. The enamel slabs were then re-immersed in a freshly prepared 5.1-pH gel at 22.5ºC for a second week. This time no fluoride was applied. Any protection against the acidic challenge was provided by the amount of fluoride absorbed from the earlier application. At the end of the second week, the enamel slabs were washed and dried in the same fashion and images were taken for a third time (week 2 images). The difference in the reflectance between week one and week two images represented the long term effects of the topical fluoride applications.

A two-way analysis of covariance (ANCOVA) was performed for the treatment groups (control vs. fluoride foam, gel and varnish) and the tooth type (primary vs. permanent enamel slabs). Bonferroni correction was applied for the six pairs to be compared among the four treatments. These six pairs included: control group vs. fluoride foam, control vs. fluoride gel, control vs. fluoride varnish, fluoride foam vs. fluoride gel, fluoride foam vs. fluoride varnish and lastly fluoride gel vs. fluoride varnish.

RESULTS

Effects of Short-term Fluoride Application (Week 1 – week 0)

The control group showed the highest amount of demineralization, significantly higher than the three fluoride groups. The enamel slabs that were protected by fluoride varnish showed the least demineralization. There was no statistically significant difference between the three fluoride groups (Foam vs. Gel, $p = 0.079$; Foam vs. Varnish, $p = 0.030$; Gel vs. Varnish, $p = 0.44$). (Figure 1 & Table 1)

Table 1. Comparison of Effects of Different Fluoride Preparations on Enamel Demineralization (p values)

COMPONENTS	SHORT-TERM EFFECTS	LONG-TERM EFFECTS
Control vs. Foam	0.0163*	0.0068**
Control vs. Gel	0.0005**	8x10-7**
Control vs. Varnish	0.0011**	3x10-8**
Foam vs. Gel	0.079	9x10-5**
Foam vs. Varnish	0.030*	7x10-8**
Gel vs. Varnish	0.44	1x10-4**

*Significant without Bonferroni correction
** Significant with Bonferroni correction (0.05/5= 0.0083).

Effects of Long-term Fluoride Application (Week 2 – week 1)

The control group showed the highest amount of demineralization, significantly more than the three treatment groups. The fluoride varnish group showed the least amount of demineralization, and differed significantly from the fluoride foam and gel group (Foam vs. Varnish, $p = 7x10^{-8}$; Gel vs. Varnish, $p = 1x10^{-4}$). There was also a significant difference between the fluoride foam and gel group while the foam group showed the

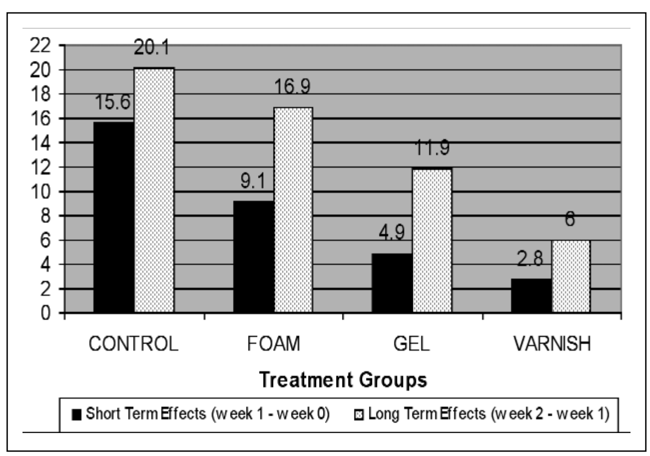

Figure 1: Comparison of Effects of Different Fluoride Preparations on Enamel Demineralization

highest amount of demineralization among the treatment groups (Foam vs. Gel, $p = 9x10^{-5}$). (Figure 1 & Table 1)

The primary and permanent enamel slabs in the fluoride group did not differ significantly in the amount of demineralization observed for week 0 to week 2 ($p = 0.24$). (Figure 2) However, the permanent enamel slabs in the control group showed significantly higher demineralization than the primary enamel slabs for the same period ($p = 0.013$). (Figure 2)

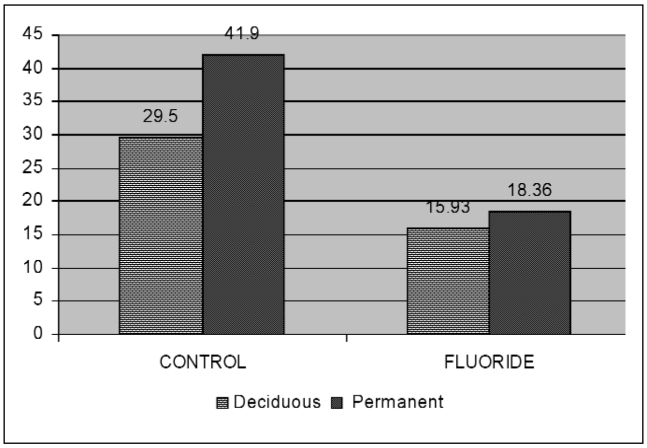

Figure 2. Comparison of Fluoride Effects on Primary and Permanent Enamel

DISCUSSION

The results of this study cannot be directly compared with the previous *in vitro* studies[7,10,14,15-18,20,28] as the methodologies were completely different. The majority of the earlier comparative studies of topical fluorides employed the micro-acid etch technique. This study evaluated the quantitative effects of topical fluoride agents by comparing the amount of light reflected from the enamel surface before and after demineralization as previously described by Ko *et al.*[13] They compared the results of this method with a micro-hardness technique and found them to be highly correlated. They concluded that light scattering properties could reliably predict the severity of white spot lesions, thus indirectly measuring demineralization. This procedure has the advantage of being non-destructive and allows continuous evaluation of the same sample material. Unlike the earlier *in vitro* studies, the enamel in this study was subjected to an acid challenge by immersion in a 5.1 pH gel. The acidic medium was used in the gel form because the diffusing calcium ions are maintained close to the surface of the enamel in the viscous gel. This aids remineralization in the presence of fluoride. In an acidic solution, calcium ions diffuse out and spread through the medium. This results in irregular surface erosion rather then the required sub-surface demineralization for the creation of white spot lesions.

This study evaluated the short and long term effects of two commonly used topical agents (APF foam and gel) and the increasingly popular, fluoride varnish. The long-term effects were studied in order to determine whether longer contact time of these fluoride agents with the tooth enamel had an additive benefit in prevention of caries. Any fluoride vehicle applied in clinical settings is eventually lost due to mastication or brushed off. Lastly, the effects of fluorides on primary and permanent enamel were compared.

The result of this study showed that there was no difference between fluoride foam, gel or varnish in preventing demineralization after an immediate acidic challenge. However, when the fluoride agents were no longer in physical contact with the teeth, the fluoride varnish worked best followed by APF gel and foam. This is likely due to the longer initial contact time of fluoride varnish with the enamel as compared to the other agents. Retief *et al.*[14,15,16] reports that the fluoride acquired by tooth enamel from topical fluoride solutions is not permanently incorporated and coating materials applied to enamel surfaces immediately after topical fluoride treatment markedly increase the amount of fluoride retained in the enamel. They showed that the suspension of teeth in synthetic saliva with fluoride results in leaching out of fluoride from the tooth enamel. However, this leaching out was far less for fluoride varnishes than for APF gel. Fluoride is released slowly from the natural resin base of the varnish and the relatively low F^- concentrations at the enamel-varnish interfaces will enhance fluoroapatite formation. The protective effect of varnishes allows sufficient time for a second-order reaction in which CaF_2 formed during the induction stage is converted into fluoroapatite. Topical application of APF will result in formation of CaF_2 as the main reaction product, which is unfixed and less well retained. This result differed from the study of Whitford *et al.*[17] Using a less sophisticated methodology, they measured the amount of fluoride retained in the enamel and saliva at 15 minutes and 16 days after application of APF foam and gel. They concluded that the two products were equivalent with respect to enamel fluoride uptake over the long-term although the difference in uptake was higher for APF gel immediately after application.

The results of this study conform with earlier clinical findings in which subjects receiving different treatment modalities with fluorides were followed from six months to five years. Tewari *et al.*[18] studying 6-12 year old children found the highest caries reduction in a fluoride varnish group when compared to NaF solution and APF gel. Similar conclusions were drawn from the studies of Seppa *et al.*[19,27] Shobha *et al.*,[28] Pettersson *et al.*[29-31]

The second purpose of this study was to evaluate the effectiveness of fluorides in preventing demineralization in both primary and permanent tooth enamel. When the effects of all fluoride agents were considered together, there was no statistically significant difference in the amount of demineralization between primary and permanent enamels after two weeks. Retief *et al.*[14,16] reported that fluoride acquired by enamel at three acid etch depths, from topical fluoride agents, was inversely related to the enamel fluoride concentration prior to topical application. The caries free premolars used in this study were obtained from teenagers undergoing orthodontic therapy and hence they were immature in terms of enamel fluoride content. Therefore, these slabs must have acquired proportionately greater amounts of fluoride than the primary enamel slabs (obtained after physiological exfoliation) having a mature mineral content. Our results do not support the idea of high kinetic activity of fluoride in primary enamels as reported by Koch *et al.*[8] An additional finding of this study was the significantly higher amount of demineralization of the premolar enamel when compared to the primary molar enamel in the control group. It has been reported that 97% of new enamel lesions in primary teeth reached the dentin within one-year.[32] An explanation for the findings of this study may be the relative immaturity of the premolar enamel as compared to the primary enamel. This might have decreased the resistance of the premolar enamel to demineralization when subjected to acidic challenge.

Most studies showing a higher efficacy of fluoride varnish attribute it to its increased contact time. However the deposition of fluoride into surface enamel is influenced by many factors. These include pH, concentration and nature of fluoride salts, the transporting media and the duration and mode of application.[33] The fluoride varnish used in this study was twice as concentrated as the APF foam or gel. Although the fluoride concentration of the APF foam and gel were equal, a significantly lesser amount of fluoride foam is applied to the enamel due to its foamy nature. The pH of APF foam and gel ranged between 3 and 3.5 as compared to the neutral pH of NaF varnish. Although the low pH helps with the absorption of fluoride into the enamel, this fluoride is not permanently bound and is easily washed away when compared to the slow releasing fluoride from varnishes. This forms fluoroapatite, which is much more efficient in controlling demineralization.

Although clinical trial data is still needed by the Food and Drug Administration (FDA) for clearance of fluoride varnishes as caries-preventive agents, some US dental professionals are using fluoride varnish in an off-label manner. The lack of FDA clearance of fluoride varnish as a caries-preventive agent and US dental professional's limited familiarity with the technique and its efficacy, explain why fluoride varnishes have not been more widely used despite their endorsement by international dental professionals. Based on the effectiveness, safety and practicality, one might anticipate increased interest in these agents in the future as the scientific evidence continues to document their usefulness in caries prevention.

CONCLUSIONS

1. Fluoride foam and gel and varnishes were equally efficacious in the *short-term* at preventing enamel demineralization.
2. In the *longer term*, fluoride varnish provided more protection against demineralization.
3. There was no difference in the effectiveness of fluorides in preventing demineralization of either primary or permanent enamel.

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REFERENCES:

1. Mellberg JR, Ripa LW. Fluorides in preventive dentistry. Chap 7, Professionally applied topical fluoride. Quintessence Pub Co., Inc. pp 186–192, 1983
2. Stamm JW: New fluoride agents and modalities of delivery. In: Wei SHY. Clinical uses of fluorides. Lea & Febiger. pp177–180, 1985.
3. Horowitz HS, Heifetz SB. Topically applied fluorides. In: Newbrun E. Fluorides and dental caries. 3rd Ed. Charles Thomas Ltdpp 71–87, 1986.
4. Eugenio D, Aguilay B, Goldstein JW, Lockwood SA. Fluoride varnishes. J Amer Dent Assoc., 131:589–596, 2000.
5. Allmark C, Green HP, Linney AD, Wills DJ, Picton DCA. A community study of fluoride tablets for school children in Portsmouth. Br Dent J., 153:426–30, 1982.
6. Grodzka K, Augustyniak J, Budny K, Czarnocka J, Janicha K, Mlosek B, Moszczenska M, Szpringre M, Wacinska L, Petterson L, Frostell G. Caries increment in primary teeth after application of Duraphat fluoride varnish. Community Dent Oral Epidemiol., 10:55–59, 1982.
7. Koch G, Petterson L.G, Ryden H. Effect of fluoride varnish (Duraphat) treatment every six months compared with weekly mouth rinses with 0.2 percent NaF solution on dental caries. Swed Dent J., 3:39–44, 1979.
8. Koch G, Petterson LG, Gleerup A, Lowstedt E. Kinetics of fluorine in deciduous enamel after application of fluoride-containing varnish (Duraphat). Scand Dent J., 6:39–44, 1982.
9. Koch G, Petterson LG. Caries preventive effect of a fluoride containing varnish (Duraphat) after 1 year's study. Community Dent. Oral Epidemiol., 3:262–266, 1975.
10. Koch G, Pettersson LG, Ryden H. Effect of fluoride varnish (Duraphat) treatment every six months compared with weekly mouthrinses with 0.2 per cent NaF solution on dental caries. Scand Dent J., 3:39–44, 1979.
11. Koch G, Pettersson LG. Fluoride content of enamel surface treated with a varnish containing sodium fluoride. Odont. Revy. 23:437–446, 1972.
12. Edenholm H, Johnson G, Koch G, Petterson L.G. Fluoride uptake and release in deciduous enamel after application of fluoride varnishes. Swed Dent J., 1:59–64, 1977.
13. Ko CC, Tantbirojn D, Wang T, Douglas WH. Optical scattering power for characterization of mineral loss. J Dent Res., 79(8): 1584–1589, 2000.
14. Retief DH, Harris BE, Bradley EL. Enamel fluoride uptake, distribution and retention from topical fluoride agents. Caries Res., 17:44–51, 1983.
15. Retief DH, Harris BE, Bradley EL. In vitro enamel fluoride uptake from topical fluoride agents. Dental Materials, 1:93–97, 1985.

16. 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., 59(3):573–582, 1980.
17. Whitford GM, Adair SM, Hanes CM, Perdue EC, Russel CM. Enamel uptake and patient exposure to fluoride: Comparison of APF gel and foam. Ped Dent., 17(3):199–203, 1995.
18. Tewari A, Chawla HS, Utreja A. Comparative evaluation of NaF, APF and Duraphat topical fluoride applications in the prevention of dental caries—a 2 1/2 years study. J Ind Soc Pedo Preven Dent.,8(1):28–35, 1991.
19. Seppa L, Heikki Tuutti, Luoma H. A 2-year report on caries prevention by fluoride varnishes in a community with fluoridated water. Scand J Dent Res., 89:143–148, 1981.
20. Seppa L, Leppanen T, Hausen H. Fluoride varnish versus acidulated phosphate fluoride gel: A 3-year clinical trial. Caries Res., 29:327–330, 1995.
21. Seppa L, Pollanen L, Hausen H. Caries-preventive effect of fluoride varnish with different fluoride concentrations. Caries Res., 28(1): 64–7, 1994.
22. Seppa L, Pollanen L. Caries preventive effect of two fluoride varnishes and a fluoride mouthrinse. Caries Res., 21:375–379, 1987.
23. Seppa L, Tolonen T. Caries preventive effect of fluoride varnish applications performed two or four times a year. Scand J Dent Res., 98:102–105, 1990.
24. Seppa L. Effect of dental plaque on fluoride uptake by enamel from a sodium fluoride varnish in vivo. Caries Res., 17:71–75, 1983.
25. Seppa L. Luoma H, Hausen H. Fluoride content in enamel after repeated application of fluoride varnishes in a community with fluoridated water. Caries Res., 16:7–11, 1982.
26. Seppa L. Studies of fluoride varnishes in Finland. Proc Finn Dent Soc., 87(4):541–7, 1991.
27. Seppa L. Topical fluorides. Proc Finn Dent Soc., 85:445-456, 1989.
28. Shobha T. Nandhal B, Prabhakar A.R. Sudha P. Fluoride varnish versus acidulated phosphate fluoride for school children in Manipal. Community Dent. Oral Epidemiol., 59(6):157–160, 1987.
29. Pettersson LG, Arthursson C, Ostberg C, Jonsson G, Gleerup A. Caries-inhibiting effect of different modes of Duraphat varnish reapplication. A 3-year radiographic study. Caries Res., 25:70–73, 1991.
30. Pettersson LG, Magnusson K, Andersson H, Deierborg G, Twetman S. Effect of semi-annual application of a chlorhexidine/fluoride varnish mixture on approximal caries incidence in schoolchildren. A three year radiographic study. Eur J Oral Sci., 106:623–627, 1998.
31. Pettersson LG. Fluoride mouthrinses and fluoride varnishes. Caries Res., 27(Suppl 1):35–42, 1993.
32. Peyron M, Matsson L, Birkhed D. Progression of proximal caries in primary molars and the effect of Duraphat treatment. Scand J Dent Res., 100:314–8, 1992.
33. Bang S, Kim YJ. Electron microbe analysis of human tooth enamel coated in vivo with fluoride-varnish. Helv. Odont., 17:84–88, 1973.