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Fluoride uptake and retention following combined applications of APF and stannous fluoride in vitro

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Abstract

This study compared the original, more concentrated acidulated phosphate fluoride (APF) and stannous fluoride (SnF_2) regimens and one of the commercial products with respect to the stability of the fluoride deposited from the treatments in vitro. Specimens of human enamel were treated with two-step sequential applications or mixtures of APF and SnF_2 and subsequently were exposed to one of three washing procedures of varying intensity. Data obtained from successive acid-etch biopsies were used to construct fluoride-by-depth profiles for each treatment. The sequential application of the more concentrated 1.23% APF followed by 0.5% SnF_2 was the only treatment which produced elevated enamel fluoride levels compared to untreated controls following the most rigorous wash.

Controlled clinical trails have shown that semi-annual applications of 8-10% stannous fluoride (SnF_2) or 1.23% acidulated phosphate fluoride (APF) can reduce the incidence of caries in children, especially in nonfluoridated areas.[1] As a result, these agents have been used independently as part of caries prevention programs in dental offices for many years. The results of laboratory studies of enamel solubility reduction (ESR) by Shannon et al.[2,3] and artificial caries inhibition by Crall et al.[4,5] have suggested that combined applications of 1.23% APF and 0.5% SnF_2, either by a two-step sequential regimen or as a mixture, might have the potential to provide greater protection against caries than if either agent was used alone.

Commercial firms have developed and promoted treatment packages containing APF and SnF_2 for combined application in the dental office.[a,b] However, the APF and SnF_2 solutions recommended for topical application in these packages have lower fluoride (F) concentrations than those tested in the previously cited studies. The use of the lower F concentrations apparently is based upon the results of a separate in vitro study by Shannon and Edmonds[6] which showed no difference in ESR when the F concentration of the APF was lowered to 0.25%. The manufacturers of these commercial products also suggest that a two-min application period be used for their APF/SnF_2 mixture as opposed to the four-min treatment time employed in the studies by Shannon and Crall.

The objective of this study was to compare two of the commercial APF/SnF_2 topical regimens with the previously tested regimens in terms of their ability to deposit F in enamel in a relatively stable form in vitro.

Methods and Materials

The study was designed to minimize the effect of the variability which exists in baseline enamel fluoride levels between teeth without physically altering the enamel prior to the fluoride application (as is done in some analytical techniques, ie., ESR). To accomplish this, three separate experiments were performed. The same six treatment regimens were used in each experiment, but three different washing procedures of varying duration were employed to assess the stability of the F deposited as a result of each regimen.

The treatment regimens examined in this study are outlined in Table 1. Regimen 1 served as a control; the specimens assigned to this group were exposed to deionized distilled water for 4 min Regimen 2 consisted of a 4-min exposure to a conventional 1.23% APF solution.[c] Regimens 3 and 4 were the sequential and mixture treatments originally tested by Shannon et al. and also by the authors. Regimens 5 and 6 em-

[a] Gel-Kam, Scherer Laboratories, Inc., Dallas, TX.
[b] Omni, Dunhall Pharmaceuticals, Inc., Gravette, AR.
[c] Karidium, Lorvic Corp., St. Louis, MO.

ployed commercially available APF/SnF_2 products[d] using the manufacturer's recommended application times for sequential and mixture treatments. Extracted, macroscopically caries-free human molars were sectioned to provide multiple specimens of buccal enamel from each tooth. A total of 168 specimens were obtained for study. The specimens were assigned to the various treatment groups using a stratified random distribution and a balanced incomplete blocking design. Each specimen was labeled according to tooth, treatment regimen, and washing procedure and subsequently was covered with an acid-resistant varnish except for a circular window of exposed enamel (area = 4.1 mm^2). The specimens then were treated with the appropriate regimens as outlined in Table 1.

Following the topical applications, the specimens from each treatment regimen were divided into three groups corresponding to one of three washing procedures. All specimens were washed in an inorganic solution at 25°C with constant stirring for 18 hr (wash 1) or 24 hr (washes 2 and 3) with solution changes as outlined in Table 2. The inorganic wash solution contained 1 mM Ca, 3 mM P, and 20 mM $NaHCO_3$ at pH 7.0.[7] In addition, the specimens assigned to washing procedure 3 were exposed to a 1 M solution of potassium hydroxide (KOH) following the inorganic wash. The KOH has been shown to remove the more soluble, nonapatitic reaction products such as calcium fluoride.[8]

Successive acid-etch biopsies subsequently were performed on each specimen using 0.5 M perchloric acid. F concentrations were determined with an Orion F-ion specific electrode®[e] and calcium content was determined by atomic absorption spectrophotometry. F-by-depth profiles were constructed for each treatment group for each of the three washing procedures.[4]

TABLE 1. Treatment Regimens

	Agent		*F Concentration (%)*	*Application Time (min)*
1.	dd	H_2O	0	4
2.	APF		1.23	4
3.	APF + SnF_2	(sequential)	1.23	2
			0.12	2
4.	APF + SnF_2	(mixture)	0.92	4
5.	Omni® + Omni®	APF (sequential) SnF_2	0.31	2
			0.40	2
6.	Omni® + Omni	APF (mixture) SnF_2	0.33	2

TABLE 2. Washing Regimens

Solution	*Duration (hrs)*	*Changes (hrs)*
1. Inorganic wash solution	18	½, 1
2. Inorganic wash solution	24	½, 1, 18
3. Inorganic wash solution + 1M KOH	24	½, 1, 18
	24	

Results

The mean enamel F concentrations at various standard depths beneath the enamel surface are shown in Table 3. The data for washes 2 and 3 contain enamel F concentrations for depths beyond those for wash 1 because additional biopsies were performed on those specimens. A General Linear Model Procedure and Duncan's Multiple Range Test were used to test for significant differences between treatment groups at the standard depths for each of the three washes (alpha level = 0.05).

Analysis of the data revealed that no significant differences existed between the values for control specimens for the three washing procedures. Therefore, the data were pooled to allow comparisons between the data for the other treatment groups at similar depths.

Following the 18-hr inorganic wash (wash 1), all topical F regimens resulted in significantly increased enamel F levels at all depths compared to untreated controls with the exception of regimen 6 — the commercial mixture using the recommended 2-min application time. Although enamel F levels for the other treatments (groups 2-5) were significantly greater than for groups 1 and 6, no significant differences were noted between the values for groups 2-5.

Mean enamel F concentrations after the 24-hr inorganic wash tended to be lower than the respective 18-hr values for groups 2-5; however, the differences between the 18-hr and 24-hr values were not significant. Comparisons between treatment groups following the 24-hr wash indicated that no significant differences existed between the values for groups 3 and 6; however, group 3 values were significantly greater than controls (group 1), while no significant differences were noted between group 6 and control values.

Data obtained after the 24-hr inorganic wash followed by the 24-hr exposure to 1 M KOH indicated that enamel F levels for groups 2, 4, and 5 were sig-

[d] Omni, Dunhall Pharmaceutical, Gravette, AR.

[e] Orion Research, Cambridge, MA.

TABLE 3. Mean F Concentrations (ppm) and Pooled Standard Deviations (ppm) at Standard Depths (μm)

	Treatment Groups						
Depth (μm)	1 Control	2 APF	3 Original Sequential APF/SnF_2	4 Original APF/SnF_2 Mixture	5 Sequential Omni® APF/SnF_2	6 Omni® APF/SnF_2 Mixture	Pooled S.D.
Wash 1 (n = 60)							
5	1418	2861	2510	2601	2506	1744	575
10	977	1704	1687	1714	1615	1167	379
15	740	1400	1329	1347	1293	895	343
Wash 2 (n = 60)							
5	1470	2297	1860	2047	2282	1785	246
10	1135	1730	1497	1632	1790	1314	226
15	905	1372	1225	1320	1427	1135	149
25	617	933	895	937	967	782	135
35	475	677	697	697	712	590	121
45	420	558	590	580	577	500	120
Wash 3 (n = 48)							
5	1485	1445	1674	1475	1381	1453	184
10	1118	1057	1223	1098	1088	1180	159
15	890	840	961	875	888	932	123
25	573	568	627	594	592	642	93
35	436	440	470	438	440	473	60
45	345	359	376	348	348	375	51

nificantly less than the respective values following both the 18-hr and 24-hr washes. The only regimen which demonstrated an elevated mean enamel F concentration compared to untreated controls was regimen 3, the original sequential treatment employing the higher concentration of APF followed by 0.5% SnF_2. The enamel F concentration for regimen 3 was also significantly greater than the mean values for the other topical F regimens. This effect was confined to the outer 5 μm of enamel, as no differences were detected among any of the groups at 10 μm and beyond.

Discussion

The results of this in vitro study suggest that although several of the regimens tested were capable of depositing F on or in sound enamel, the reaction products formed as a result of these treatments were relatively labile in nature. The lone exception was the sequential APF/SnF_2 regimen employing 1.23% APF followed by 0.5% SnF_2 which demonstrated a significant increase in enamel F concentration even after the KOH wash. Two of the regimens, the conventional 4-min application of 1.23% APF (regimen 2) and the 4-min sequential application of the commercial APF/SnF_2 products employing the lower 0.31% APF (regimen 5), gave higher enamel F levels after the 24-hr inorganic wash than did the original APF/SnF_2 sequential treatment (regimen 3). However, regimen 3 was the only treatment that effected the formation of a stable reaction product as determined by the results following the KOH wash.

The 18-hr and 24-hr inorganic washes were used to remove the more soluble reaction products. Regimen 6 was the only treatment that did not produce significantly higher enamel F levels following the 18-hr wash, a finding which was not altogether surprising given the lower F concentrations and especially the reduced application time suggested by the manufacturer. This treatment mode would seem to be more appropriate for home use and other situations where frequent applications would be possible than for professional application.

When considering the significance of these findings, several points are noteworthy. First of all, the concept of F reacting with hydroxyapatite to form fluorapatite probably is not a reasonable expectation during the course of a professional topical application of APF or SnF_2. The limited surface area of a tooth and the relatively slow nature of the reaction between a liquid and such a dense solid would necessitate a considerable period of contact between the F and the enamel for ion exchange to occur. Dissolution and reprecipitation processes occur more rapidly, but the products formed (i.e., calcium fluoride) are relatively soluble in the oral environment.[9,10] In vitro studies have shown that little, if any, of the F initially deposited on the tooth surface by conventional APF topical solutions is retained in an apatitic form.[8,10]

The KOH washing procedure used in this study

was modified from the previously cited reports in that the 24-hr KOH exposure was preceded by a 24-hr inorganic wash. This was done in order to remove the more soluble reaction products but still allow for continued reaction between any less soluble F products and the enamel, a condition which would seem to simulate more accurately the in vivo situation.[11] However, even after this additional exposure, the conventional APF regimen failed to demonstrate formation of an apatitic phase as evidenced by the results following the KOH wash. A recent in vitro study[12] has reported that calcium fluoride can be converted to fluorapatite, but the transformation occurs very slowly under the conditions which would be found in the mouth. This observation appears to be borne out by the results of several in vivo studies which have shown that the F deposited as a result of a conventional APF application essentially is lost within seven days.[13-15]

Secondly, several recent studies[16-18] have stressed the effect of low levels of F on the process of remineralization — a phenomenon which may result in the arrest or reversal of the caries process in enamel. If this indeed is a primary mechanism of action of F, professionally applied topical fluorides still could prove beneficial by creating F reservoirs[19] whereby the dissolution of soluble reaction products deposited on or in enamel could release additional F into the environment over a period of time. Since the level of F necessary to enhance remineralization has been shown to be quite low, the goal of professional, topical F therapy might warrant reevaluation. Approaches which form products more resistant to dissolution in the oral environment may prove more beneficial than treatments which deposit F in a more soluble form, given the prolonged interval between applications in the dental office.

Finally, it seems reasonable that a regimen which is capable of depositing F in enamel in significant amounts in a stable form (i.e., the concentrated APF/SnF_2 sequential treatment used in this study) should provide greater protection against caries. However, no clear-cut relationship between enamel F levels and caries resistance has been established. In vitro tests, while possibly providing some indication of the anticaries potential of various agents and regimens, should not be regarded as substitutes for well-controlled clinical caries trials. Until results of clinical caries trials are made available, recommendations for adopting regimens employing new agents or modifications of treatment modalities should be viewed with circumspect.

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