Fluoride dentifrice containing xylitol: In vitro root caries formation

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ABSTRACT: Purpose: To evaluate the effects of experimental xylitol dentifrices with and without fluoride on in vitro root caries formation. **Methods:** Root surfaces from caries-free human permanent teeth (n=10) underwent debridement and a fluoride-free prophylaxis. The tooth roots were sectioned into quarters, and acid-resistant varnish was placed with two sound root surface windows exposed on each tooth quarter. Each quarter from a single tooth was assigned to a treatment group: (1) No treatment control; (2) Aquafresh Advanced (0.15% F = 1,150 ppm F); (3) Experimental xylitol dentifrice without fluoride (0.45% xylitol); and (4) Diamynt fluoride dentifrice with xylitol (0.83% sodium monofluorophosphate = 1,100 ppm F and 0.20% xylitol). Tooth root quarters were treated with fresh dentifrice twice daily (3 minutes) followed by fresh synthetic saliva rinsing over a 7-day period. Controls were exposed twice daily to fresh synthetic saliva rinsing daily over a 7-day period. In vitro root caries were created using an acidified gel (pH 4.25, 21 days). Longitudinal sections (three sections/tooth quarter, 60/group) were evaluated for mean lesion depths (water inhibition, polarized light, ANOVA, DMR). **Results:** Mean lesion depths were 359 ± 37 μm for the control Group; 280 ± 28 μm for Aquafresh Advanced; 342 ± 41 μm for the experimental xylitol dentifrice without fluoride; and 261 ± 34 μm for Diamynt. Aquafresh Advanced and Diamynt had mean lesion depths significantly less than those for the no treatment control and the experimental xylitol without fluoride dentifrice ($P< 0.05$). There were minimal non-significant differences in mean lesion depths between Aquafresh Advanced and Diamynt ($P> 0.05$). (*Am J Dent* 2013;26:56-60).

CLINICAL SIGNIFICANCE: Fluoride dentifrices provided significant reductions in in vitro root caries lesion depths compared with root surfaces not exposed to dentifrice treatment (no treatment control) or exposed to the experimental xylitol without fluoride dentifrice ($P< 0.05$), considering the limitations of the in vitro artificial caries system. Diamynt fluoride dentifrice with xylitol reduced lesion depth to a similar extent as Aquafresh Advanced fluoride dentifrice.

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Introduction

A current review of the available epidemiological data from many countries clearly indicates that there is a marked increase in the prevalence of dental caries.[1]

Although dental caries is primarily observed in children and adolescents, this oral disease continues throughout adulthood.[2-4] Furthermore, it is estimated that the aging population and the tendency of the elderly to retain their teeth will increase the risk for development of root caries.[5-7]

Dentifrice delivers anticaries ingredients, such as fluoride, which has proven to prevent or at least reduce enamel and root surface demineralization. Some dentifrices have been introduced with multiple ingredients to target specific issues, such as plaque, gingivitis, hypersensitivity, and malodor.[8-13]

Xylitol is a sugar alcohol (polyol) used as a non-caloric sweetener in the food industry. From a dental standpoint, xylitol is an interesting natural product that is not fermented by dental plaque bacteria.[14] Studies[15,16] have shown anticaries properties of fluoride dentifrices containing xylitol, as well as lower *mutans Streptococci* levels in plaque and saliva after 6 months.[17] A xylitol and fluoride containing dentifrice has shown lower glucose retention in the oral cavity compared to a non-xylitol containing dentifrice.[18] Additionally, xylitol has been shown to reduce plaque and saliva levels of *mutans streptococci*.[19] Furthermore, xylitol can induce remineralization of the deeper layers of demineralized enamel by facilitating calcium ion movement and accessibility.[20]

This study evaluated the effects of xylitol dentifrices with and without fluoride on in vitro root caries using a well-tested artificial caries system based on an acidified gelatin gel.[21-25]

The hypothesis of this laboratory study was that no statistically significant differences existed among commercially available dentifrices, with or without xylitol, containing different amounts of fluoride on in vitro root surface caries formation.

Materials and Methods

Root surfaces from caries-free human permanent teeth (n=10) underwent debridement and a fluoride-free prophylaxis. The tooth roots were sectioned into quarters, and acid-resistant varnish was placed with two sound root surface windows exposed on each tooth quarter. Each quarter from a single tooth was assigned to a treatment group:

1. No treatment control (synthetic saliva[21] exposure only);
2. Aquafresh Advanced[a] dentifrice (0.15% w/v = 1,150 ppm sodium fluoride);
3. Experimental xylitol dentifrice without fluoride (45% xylitol);
4. Diamynt[b] xylitol dentifrice with fluoride (0.83% w/v = 1,100 ppm sodium monofluorophosphate, 0.20% xylitol, and herbal extracts (chamomile flower extract, sage leaf extract, peppermint leaf extract).

Tooth root quarters were treated with fresh dentifrice twice daily (3 minutes) followed by fresh synthetic saliva[21] rinsing over a 7-day period. Dentifrice treatment was performed by submerging each tooth portion into 0.5 mL of dentifrice. Following the dentifrice exposure period, the dentifrice was removed from the root portions by using a 15-second gentle air-water rinse until all visible dentifrice was removed. Controls were exposed twice daily to fresh synthetic saliva, rinsing daily over a 7-day period. In vitro root caries were created using an acidified gel (pH 4.25, 21 days)[22-25] developed by using a 10%

Table. Mean lesion depth of the different groups.

Groups	Mean lesion depth	Lesion depth reduction
• No treatment control (n=60 caries-risk sites)	359 ± 37 μm	
• Fluoridated dentifrice (Aquafresh Advance) (n=60 caries-risk sites)	280 ± 28 μm*	22%*
• Xylitol experimental dentifrice (n=60 caries-risk sites)	342 ± 41 μm	5%
• Xylitol with fluoride dentifrice (Diamynt) (n=60 caries-risk sites)	261 ± 34 μm*	27%*

* Mean lesion depths for fluoridated dentifrice and xylitol with fluoride experimental dentifrice significantly different compared with no treatment control and xylitol only experimental dentifrice (P< 0.05, ANOVA, DMR).

gelatin powder (G8 Gelatin Laboratory Grade Type A[c]) dissolved into deionized distilled water. Addition of lactic acid to the gelatin to a terminal pH of 4.25 was performed. The tooth specimens with exposed enamel windows were placed into the gelatin and in vitro root surface caries were created during a 21-day period. This method of artificial caries formation creates lesions within root surfaces and enamel that mimic the initial phase of natural caries formation.[22-25]

Following artificial root surface caries creation, longitudinal sections (three sections/tooth quarter, 60 lesions/group) were evaluated for mean lesion depths (water imbibition, polarized light microscopy).

The results were statistically analyzed using ANOVA and Duncan's multiple range test (P< 0.05).

Results

The Table shows the mean lesion depths (in μm ± SD) for all groups and Figs. 1-4 illustrate the polarized light microscopic results (water imbibition). Aquafresh Advanced and Diamynt had mean lesion depths significantly less than those for either the control group or the experimental xylitol without fluoride dentifrice group (P< 0.05).

There were non-significant differences in mean lesion depths between the Aquafresh Advanced and Diamynt groups (P> 0.05).

Discussion

Dental caries, whether clinically detectable or invisible, is the outcome of numerous episodes of de- and remineralization, rather than a demineralization process without intervening periods of remineralization.[26-28]

Fluorides have been extensively used in the prevention of dental caries. Fluoride acts as a catalyst and influences reaction rates with reversal of dissolution and transformation of more soluble, less stable calcium-phosphate mineral phases into those that are less soluble and more stable within the tooth structure and mineral phases that reside within plaque adjacent to tooth surfaces. The incorporation of minimal amounts of fluoride into hydroxyapatite yields fluoride-substituted hydroxyapatite that resists demineralization to a significantly greater degree than hydroxyapatite without fluoride.[5]

The effect of fluoride has been mainly studied in enamel surfaces. However, there is a considerable difference in mineral composition and solubility between enamel and root surfaces

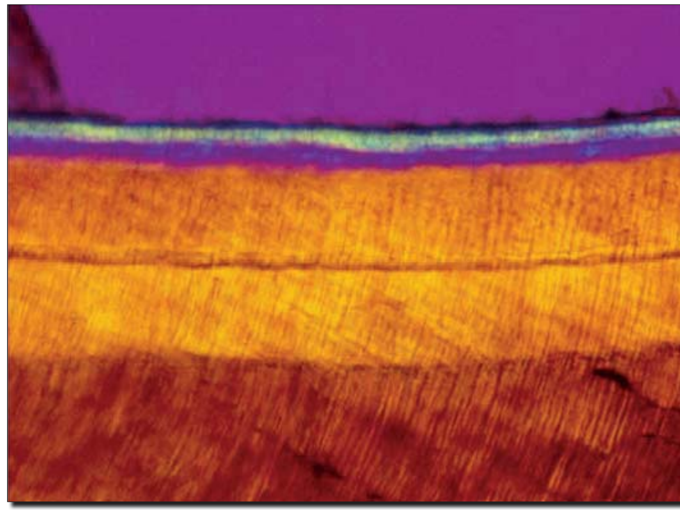

Fig. 1. Representative in vitro root surface caries lesion from no treatment control group that received only synthetic saliva exposure (polarized light microscopy, water imbibition, original magnification ×200).

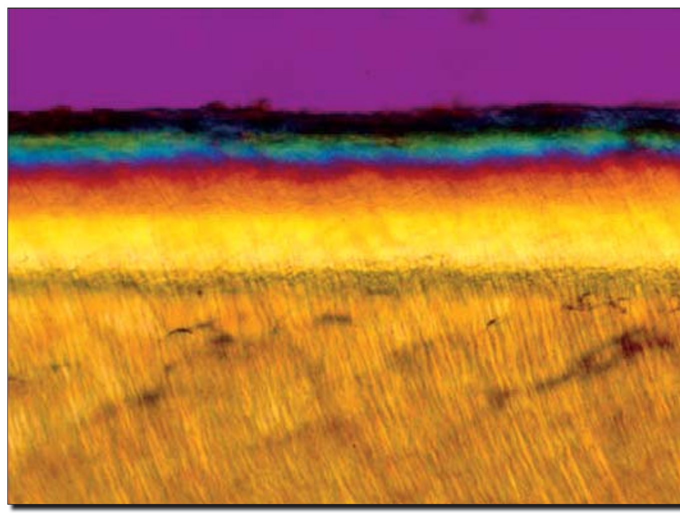

Fig. 2. Representative in vitro root surface caries lesion from the fluoride dentifrice (Aquafresh Advance; polarized light microscopy, water imbibition, original magnification ×200).

(cementum overlying dentin), which results in a greater degree of caries susceptibility for root surfaces and dentin.[29] With root surface lesions, the anticaries effect of different fluoride combinations has been suggested to be due to the intrinsic nature of the substrate, and the fact that root surfaces have greater uptake of fluoride than enamel.[30] The role of fluoride in preventing root-surface lesions has been shown in both in vivo and in vitro studies.[24,31-34]

While root surfaces have a higher affinity for fluoride uptake than enamel, root surfaces also have a higher susceptibility to dissolution in an acidic environment than enamel surfaces.[24,31,35-40] The critical pH at which enamel dissolves is approximately pH 5.[41,42] The critical pH for dissolution of cementum and dentin is slightly above pH 6.0,[31,34,37-39] indicating that even a mild to moderate decrease in the resting pH leads to a critical pH (derived from cariogenic bacteria) that may initiate the caries process in root surfaces.

Fluoride concentrations within dental plaque have a critical effect on the virulence factors of *S. mutans* in vitro, such as acid production and glucan synthesis, but the clinical

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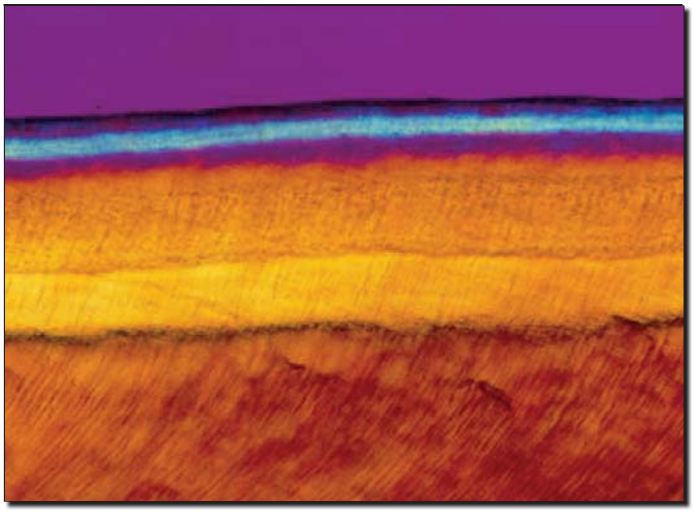

Fig. 3. Representative in vitro root surface caries lesion from xylitol with no fluoride dentifrice group (Experimental dentifrice; polarized light microscopy, water imbibition, original magnification ×200).

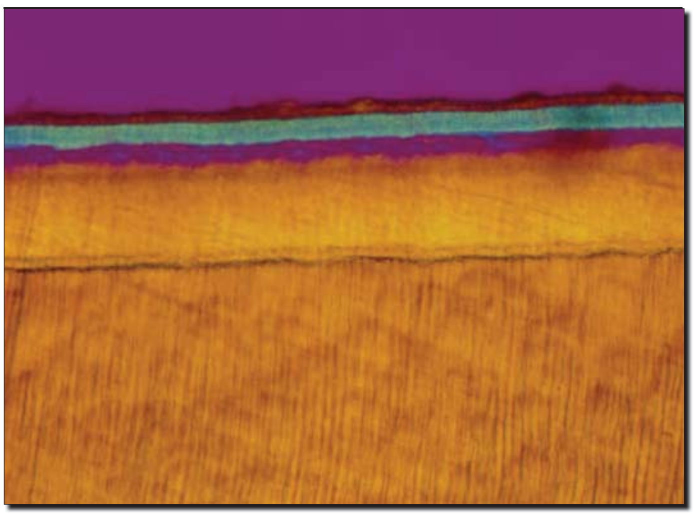

Fig. 4. Representative in vitro root surface caries lesion from fluoride with xylitol dentifrice group (Diamynt; polarized light microscopy, water imbibition, original magnification ×200).

implications of this are still not clear.[43] Studies assessing plaque exposed to fluoride showed that fluoride inhibited the metabolism of bacteria, as supragingival plaque metabolized carbohydrates derived from saliva and bacteria-stored polysaccharides more slowly and at a lower constant rate.[44] Fluoride is preserved in supragingival plaque[45] and inhibits bacterial glycolysis which may interrupt bacterial growth over time.[45] This metabolic inhibitory effect by fluoride on bacterial glycolysis may repress bacterial growth over the long-term.[44] Additionally, the levels of fluoride found to inhibit streptococcal enolases were much lower than previously reported, and these levels of fluoride are likely to be present in plaque, especially during acidogenesis, thereby exerting an antiglycolytic effect.[46] Overall, the anticaries actions of fluoride have an effect on both the plaque bacteria and the root surface mineral structure. The antibacterial actions of fluoride are complex, but seem to be dominated by weak-acid effects.[47] Fluoride reduces the acid tolerance of the bacteria, and is most effective at acid pH values. In the acidic conditions of cariogenic plaque, fluoride at levels as low as 0.1 mM can cause complete arrest of glycolysis by *Streptococcus mutans*.[47]

Adding fluoride to a cell suspension of *S. mutans*, *S. sobrinus*, and *S. sanguinis* resulted in intracellular accumulation of 3-phosphoglycerate and 2-phosphoglycerate (enolase substrate) and a decrease in phosphoenolpyruvate (enolase product) in the EMP pathway,[48,49] confirming the inhibitory effect of fluoride on enolase. However, this inhibitory mechanism of fluoride has not been confirmed in dental biofilm, comprised of multiple bacterial species, which may behave differently in vivo when compared with in vitro behavior.

Xylitol, a polyol, has been approved by the US Food and Drug Administration for its non-cariogenic properties and reduction in risk for dental caries.[50] The European Union has also approved a health claim regarding xylitol as a 'tooth friendly' component in chewing gums.[51]

The effect of xylitol in prevention and reduction of enamel demineralization was suggested by Arends et al[52] in 1984. Later, Arends et al[53] reported the beneficial combined effects of xylitol and fluoride in laboratory studies on enamel demineralization.

To our knowledge, no study has been published evaluating the effect of xylitol containing dentifrices (with or without fluoride) on in vitro root caries formation. This laboratory study did not show a significant difference in lesion depth formation between the fluoride dentifrice and the fluoride dentifrice containing xylitol. This could be due to the fact that laboratory studies cannot fully replicate the multifactorial nature of dental caries in the oral environment, and this study involved a physicochemical model of artificial root surface caries. It would be expected that in a model using a bacterial biofilm the effects of xylitol alone on bacterial glycolytic metabolism could result in improved caries resistance.

Xylitol primarily acts on bacteria in the oral environment. Xylitol is known to repress acid production from glucose by *S. mutans*.[54-56] It is not an inhibitor of plaque acid production, but it is a non-fermentative sugar alcohol.[44] When xylitol is taken up by oral bacteria, it is incorporated as xylitol 5-phosphate which inhibits bacterial enzymes involved in metabolism.[44,54-56] The role of xylitol in caries prevention seems to be as a non-fermentative sugar substitute, because there is no lactate production from xylitol. Also, there is no effect of xylitol on the metabolome profile.[44] One study[57] showed that xylitol did not reduce mutans streptococci in plaque. However, xylitol inhibited the glycolysis and growth of *Streptococcus mutans* to different degrees among bacterial strains and with different xylitol concentrations.[58] Reduction in glycolysis was suggested to be caused by the accumulation of intracellular xylitol-5-phosphate in some plaque bacteria during xylitol exposure.[59,60] Therefore, the exact role of xylitol in oral bacteria is not clear and needs further research.

Xylitol's main effect on root caries formation will not be seen in an experimental physicochemical model, such as the one used in the present study because it lacks a bacterial biofilm. However, in a clinical study, Sintes et al[16] showed that a toothpaste containing 0.243% sodium fluoride and 10% xylitol significantly reduced caries during a 3-year period. In a 30-month clinical study using a 0.836% (1,100 ppm) sodium monofluorophosphate and 10% xylitol dentifrice, caries was significantly reduced when compared to a similar fluoride dentifrice without xylitol.[15] In another clinical study, Sano et al[14] showed that the combination of 500 ppm sodium fluoride and 5% xylitol significantly enhanced remineralization.

It appears that xylitol use is clinically relevant, as a 12-year longitudinal clinical study[60] showed that root caries was present in 62% of patients within the first 4 years after treatment for advanced periodontal disease. By the end of the 12-year study, root surface caries had developed in 90% of these patients.[60,61] The addition of xylitol to fluoridated dentifrices may provide an additional benefit with respect to inhibition of glycolysis by bacteria and a reduction in acid production with resultant increase in plaque pH, hopefully above the critical pH for root surfaces (cementum overlying, dentin) and enamel alike. This present study showed that the addition of xylitol and herbal ingredients to a fluoridated dentifrice (Diamynt) had a similar effect on artificial root caries when compared to the conventional fluoride dentifrice.

It is recognized that the artificial caries model (acidified gelatin gel) utilized in this study had certain limitations regarding release of fluoride from sodium monofluorophosphate within the experimental xylitol dentifrice with fluoride. Release of fluoride from monofluorophosphate requires either enzymatic or acid hydrolysis. The uptake of fluoride or retention of dentifrice within imperfections and hypomineralized mineral within the root surface may have allowed for fluoride uptake and penetration into tooth substance in this particular model that repeatedly exposed the root surface to the dentifrices followed by synthetic saliva rinsing. The retained fluoride and/or dentifrice in root surface imperfections and hypomineralized tissue may have undergone hydrolysis with diffusion controlled acid release from the acidified gel (pH 4.25). It would be expected that in the presence of intraoral conditions with acid and enzymatic hydrolysis, the effects of fluoride release from monofluorophosphate and the bacterial effects of xylitol would be more pronounced than in this in vitro caries system.

In conclusion, the fluoridated dentifrices tested provided significant reductions in lesion depths with in vitro root surface caries compared with root surfaces that were not exposed to dentifrice treatment (no treatment control) or exposed to xylitol without fluoride dentifrice ($P< 0.05$), considering the limitations of any artificial caries system. Diamynt (sodium monofluorophosphate dentifrice containing xylitol and herbal extracts) reduced lesion depth to a similar extent ($P> 0.05$) as Aquafresh Advanced (sodium fluoride dentifrice).

Further studies including metabolome analyses and analysis of plaque glycolysis need to be conducted to elucidate the role of dentifrices with and without xylitol on the dynamic demineralization and remineralization process in caries development and reversal.

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b. LMKao, LLC, New York, NY, USA.
c. Fisher Scientific, Fair Lawn NJ, USA.

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