Abstract: This study aimed to determine the effect of xylitol on the development and remineralization of caries in vitro, and to compare this effect with that of fluoride alone and in combination. Two experiments were devised. In experiment 1, bovine incisors were each sectioned into 4 portions which were randomly assigned to 4 demineralizing agents: A) acidic buffer (x), B) x + 0.5 ppm fluoride, C) x + 20% xylitol, and D) x + 20% xylitol + 0.5 ppm fluoride. Caries-like lesions were produced in specimens. In experiment 2, carious lesions were produced in teeth. Five lesion-bearing slabs were cut from each tooth. While one was reserved as control (UN), others were randomly assigned to 4 remineralizing agents: 1) artificial saliva (y), 2) y + 0.05 ppm fluoride, (3) y + 20% xylitol, and 4) y + 20% xylitol + 0.05 ppm fluoride. Mineral loss (ΔZ) and lesion depth (ld) were quantified after 4-week remineralization. In experiment 1, numerical values of ΔZ and ld observed can be ranked as A>C>B>D. These differences were significant only in B and D when compared with A for ΔZ, but not between any group for ld. In experiment 2, the numerical values of ΔZ and ld for control UN (unmineralized) and remineralized groups (1-4) ranked as UN>3>4>1=2. Compared with UN, this difference was significant in all groups with ld, but not in any group with ΔZ. We concluded that tolerable levels of xylitol alone may not show a significant caries inhibiting and remineralizing effect, but may act as a caries inhibitor additively with fluoride. (J. Oral Sci. 41, 71-76, 1999)

Key words: xylitol; fluoride; demineralization; remineralization; inhibition; artificial saliva.

Introduction
A falling prevalence of dental caries has long been reported (1, 2). This was achieved not only by dental health education, but also through the implementation of preventive programmes involving the incorporation of agents (e.g. fluoride), which can inhibit demineralization and/or enhance remineralization of caries, into drinking water, food and commonly used dental products (e.g. toothpastes, mouthrinses). Another promising approach is the replacement of cariogenic dietary components (e.g. glucose, sucrose, etc) with one with either non-cariogenic (nonfermentable by oral microorganisms) or anticariogenic (impairs the metabolism and growth of bacteria/dental plaque) properties. Though cariogenic, these sugars are energy providers, and are also used as sweeteners in various foods and drinks, hence the substitute should possess these good qualities without the adverse cariogenic effect.

Xylitol, a pentitol, which occurs naturally in many fruits, berries and vegetables (3), has been used as an artificial sweetener for many years (4), and has been found to meet these requirements (5, 6). It has the same calorific value and sweetness as sucrose (3), and has been shown to be non-cariogenic as the sweetener in gum (5). The caries inhibiting effect (anticariogenicity) of xylitol has, so far, been demonstrated by its ability to inhibit the growth and metabolism of the mutans group of streptococci and dental plaque (6). Xylitol gum has also been reported to reharden and remineralize natural and artificial caries (5), and to arrest the progress of caries (7); and these were attributed to the salivation-stimulating property of xylitol-sweetened gum. However, xylitol has been shown to possess the ability to form complexes with Ca²⁺ and phosphate ions (8), and to penetrate into demineralized enamel (9), and hence could participate in caries prevention by acting as a Ca²⁺ carrier and an agent that can concentrate calcium (9). This has drawn attention to the possibility that xylitol, in addition to its effect on oral microorganisms and salivation-stimulation, could influence the process of de- and remineralization by altering the diffusion coefficient of calcium and phosphate ions to and from the lesion into the de- or remineralization solutions.

This study, therefore, aimed to determine the effect of xylitol on the development and remineralization of artificial caries in vitro, and to assess this effect relative to that of fluoride, whose ability to inhibit demineralization and enhance remineralization has long been established (10, 11), and with combined xylitol/fluoride. It was envisaged that this might provide information necessary to develop xylitol-based preventive programmes such as the incorporation of xylitol in dental products (such as toothpastes and mouthrinses), and the substitution of cariogenic sweeteners by xylitol in acidic

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drinks/beverages and other food items.

Materials and Methods
Demineralization and remineralization agents
The demineralization solution used was similar to the acidic buffer solution described by ten Cate and Duijsters (10), and contained, 2.2 mM KH₂PO₄; 50 mM acetic acid; 2.2 mM of 1M CaCl₂; 0.5 ppm fluoride, and the pH adjusted to 4.5 using concentrated KOH. This was modified in the following way to produce four different demineralization solutions: A) acidic buffer without 0.5 ppm fluoride, B) normal acidic buffer with 0.5 ppm fluoride, C) acidic buffer without 0.5 ppm fluoride but supplemented with 20 % w/v xylitol (by dissolving 200 g of xylitol in 1 litre of the buffer solution), and D) acidic buffer with 0.5 ppm fluoride and 20 % w/v xylitol.

The remineralization solution used was an artificial saliva similar to that described by McKnight-Hanes and Whitford (12) but without the sorbitol, and contained Methyl-p-hydroxybenzoate (2.0g/l), sodium carboxymethyl cellulose (10.0 g/l), KCl (8.38 mM), MgCl₂.6H₂O (0.29 mM), CaCl₂.2H₂O (1.13 mM), K₂HPO₄ (4.62 mM), KH₂PO₄ (2.40 mM), and the pH adjusted to 6.7 using KOH. This was also modified by supplementation with either 0.05 ppm fluoride or 20 % w/v xylitol or both, to produce four different types of remineralization solutions: 1) artificial saliva only, 2) artificial saliva plus 0.05 ppm fluoride, 3) artificial saliva plus 20 % w/v xylitol, and 4) artificial saliva plus 0.05 ppm fluoride and 20 % w/v xylitol. The pH of either the demineralization solution (4.5) or remineralization solution (6.7) was not altered following supplementation with 20 % w/v xylitol.

Experimental Procedure
Forty freshly extracted bovine incisors were cleaned, and polished with wet pumice to remove organic contaminants. A carbon pencil was used to map out a horizontal rectangular window (8 × 2mm) on the middle region of the labial surface of the enamel of each tooth. Two experiments were devised and carried out as follows:

Experiment 1: Demineralization. Twenty of the above-prepared teeth were used in this section. Each tooth was cut buccolingually into four portions using a water-cooled diamond wafering blade (Buehler, Warwick, U.K). Each portion was air dried and coated with two layers of acid-resistant nail varnish (Max Factor®) except for an exposed window (2 × 2 mm) corresponding to the rectangular window. The hollow cavities (pulp chamber) in the specimens were filled with blue in-lay wax. Portions from each tooth were assigned randomly to one of four experimental groups (A-D) devised with respect to the four demineralization solutions described above. Caries-like lesions were produced in specimens in each group following three-day demineralization in their respective solutions (20 ml/specimen). Following this, each specimen was washed with de-ionized distilled water, air-dried, and the nail varnish removed with acetone.

Experiment 2: Remineralization. The remaining twenty teeth were coated with two layers of acid resistant nail varnish as above except for the rectangular window. Carious lesions

Fig. 1a Effect of xylitol and fluoride on the development of artificial carious lesions as measured by mineral loss. The mean (n = 15) values of mineral loss following three-day exposure of enamel samples in different groups to their respective demineralization solutions. A) acidic buffer solution with neither fluoride nor xylitol added, B) acidic buffer with 0.5 ppm fluoride added, C) acidic buffer with 20 % w/v xylitol added, and D) acidic buffer with 0.5 ppm fluoride/20 % w/v xylitol added. *significantly lower than A (p <0.001), a significantly higher than B and D (p < 0.01).

Fig. 1b Effect of xylitol and fluoride on the development of artificial carious lesions as measured by lesion depth. The mean (n = 15) values of lesion depth following three-day exposure of the enamel samples in different groups to their respective demineralization solutions. A) acidic buffer solution with neither fluoride nor xylitol added, B) acidic buffer with 0.5 ppm fluoride added, C) acidic buffer with 20 % w/v xylitol added, and D) acidic buffer with 0.5 ppm fluoride/20 % w/v xylitol added.
were produced in each tooth using an acidified gel system based on that described by Edgar (13) made by mixing 0.1 M lactic acid and 0.1 M sodium hydroxide in proportion to give a pH of 4.5, and gelled with 6 % hydroxethylcellulose (HEC) (Aldrich, Dorset, UK). Caries-like lesions were created by five-day demineralization in the above gel (20 ml/ specimen) until clearly observable "white spot" lesions were obtained. Following this, four enamel sections (taken from different parts of the lesions) and four enamel slabs bearing the lesion were produced from each tooth using a water-cooled diamond saw (Well, Walter Ebner, Germany). The four slabs were assigned randomly to one of four experimental groups (1-4) devised in accordance with the four varieties of artificial saliva described above, while the sections were used as controls. Prior to the test, the control sections were processed and examined as follows: the sections were mounted on brass anvils with nail varnish and polished to give planoparallel specimens of 80 μm thickness using a diamond disc. Each section was imbibed with water and examined under polarized light using a Nikon Optiphov® light microscope with rotating stage, polarizer and analyzer at a magnification of x450 (Nikon, Tokyo, Japan). This was carried out as an initial check on the production of a regular subsurface enamel lesion. Having confirm the production of a suitable lesion in each tooth, sections in each group were subjected to remineralization in their respective agents (20 ml/ slab) in a universal container on a roller mixer at room temperature (approximately 20 °C) for 28 days. The artificial saliva was changed daily.

Sectioning and Microradiography

Four sections were cut from each specimen in both experiments, and these were processed to 80 μm thickness as described above. The sections were mounted on a microradiographic plate-holder bearing an aluminium stepwedge (25 μm steps). The microradiographs were taken with a 20-minute exposure on Kodak high-resolution plates (Type 1A) using a Cu(Kα) X-ray source (Philips B.V, Eindhoven, The Netherlands) operating at 25 Kv and 10 mA at a focus-specimen distance of 30 cm. The plates were developed using standard techniques.

Image analysis

The microradiographs of the sections were examined, using a Leica DMRB optical light microscope (Leica, Wetzlar, Germany). The image was captured at a magnification of x20/0.40 via a CCD video camera (Sony, Tokyo, Japan) connected to a computer (Viglen PC, London, U.K). The lesion parameters (integrated mineral loss and lesion depth) were quantified using a software package (TMRW v.1.22, Inspektor Research System BV, Amsterdam, The Netherlands) based on the work described by de Josselin de Jong et al. (14).

Statistical analysis

Statistical processing of the data was performed using the Biosoft Stat-100 package, with a level of significance pre-chosen at 0.05. The data from the image analysis were analysed statistically using paired Student t-tests and Duncan’s multiple

Fig. 2a Effect of xylitol and fluoride on the remineralization of artificial carious lesions as measured by mineral loss. The mean (n = 15) values of mineral loss of the control (UN) and the remineralized groups (1-4) after 4 weeks of exposure to different variety of artificial saliva. (UN) control samples, 1) artificial saliva only, 2) artificial saliva supplemented with 0.05 ppm fluoride, 3) artificial saliva supplemented with 20 % w/v xylitol, and 4) artificial saliva supplemented with 0.05 ppm fluoride and 20 % w/v xylitol.

Fig. 2b Effect of xylitol and fluoride on the remineralization of artificial carious lesions as measured by lesion depth. The mean (n = 15) values of lesion depth of the control (UN) and the remineralized groups (1-4) after 4 weeks of exposure to different variety of artificial saliva. (UN) control samples, 1) artificial saliva only, 2) artificial saliva supplemented with 0.05 ppm fluoride, 3) artificial saliva supplemented with 20 % w/v xylitol, and 4) artificial saliva supplemented with 0.05 ppm fluoride and 20 % w/v xylitol. *significantly lower than UN (p < 0.05).
comparison test.

**Results**

**Demineralization**

The ability of the individual demineralization agents to inhibit the formation of carious lesions was determined by the level of mineral loss and lesion depth (Fig. 1a,b). It was observed under both the polarized light and optical light microscopes that instead of caries-like lesions (with sound surface layer and subsurface lesion) eroded enamel lesions were produced in five specimens in group A with neither fluoride nor xylitol and four specimens in group C with only xylitol. This affected the values of the mineral loss and lesion depth observed in these lesions in that the values were enormously greater compared with those of caries-like lesions. In order to compare lesions of a similar nature, only data from caries-like lesions were analysed, and for an equal number of samples to be used in all groups only 15 samples were used for comparison (hence n = 15). Using the acidic buffer solution with neither fluoride nor xylitol (group A) as the control, a highly significantly lower mineral loss (vol%μm) was observed in group B (2377.5 ± 533.6; p < 0.001) with only fluoride added and also in group D (2275.2 ± 469.4; p < 0.001) with both fluoride and xylitol added, when compared with group A (3518.5 ± 749.3). However, this effect was more pronounced in group D than in group B indicating an additive effect from fluoride and xylitol (Fig. 1a). Though the mineral loss in group C (3148.4 ± 556.6) with only xylitol added, was lower compared with group A, this was not statistically significant at the 5% level of confidence. Duncan’s multiple comparison test showed no significant difference in mineral loss between groups B and D, but when these groups were compared with group C, the mineral loss was significantly lower in groups B (p < 0.01) and D (p < 0.01). This demonstrates that the effect of only fluoride in group B was comparable to the effect of combined fluoride and xylitol in group D. A different trend was observed with the lesion depth (μm) as shown in figure 1b. When compared with the control A (105.9 ± 17.1), lower numerical values of lesion depth were observed with the other groups (B-D), and can be ranked as D (98.3 ± 12.4) < B (97.7 ± 16.1) < C (104.3 ± 7.1), but these differences were not statistically significant using t-tests. Also Duncan’s multiple comparison test showed no significant difference in lesion depth among the groups.

**Remineralization**

The degree of remineralization obtained with each variety of artificial saliva was estimated from the difference in mineral loss and lesion depth between the control (UN) and the remineralized groups (1-4) which is equivalent to the mineral gain. When the amount of mineral loss (vol%μm) was compared between the control (UN = 2790.7 ± 437.2) and the remineralized groups (1 = 2446.6 ± 502.9; 2 = 2421.1 ± 423.4; 3 = 2458.1 ± 519.8; 4 = 2450.6 ± 392.8), there was no significant difference between either the control and the remineralized groups or between the remineralized groups (Fig. 2a). This shows that supplementation of the artificial saliva with either fluoride (0.05 ppm), xylitol (20 %/w/v) or combined xylitol/fluoride in the same concentrations did not have a significant influence in remineralization of the caries lesions. With the lesion depth (Fig. 2b), significant (p < 0.05) decreases were observed when the control (UN = 103.9 ± 9.0) was compared with the remineralized groups (1 = 92.0 ± 9.5; 2 = 90.9 ± 9.2; 3 = 93.8 ± 10.0; 4 = 92.1 ± 14.4). There were no significant differences in lesion depth between the groups.

**Discussion**

**Demineralization**

The composition of an acidic buffer solution partially saturated with respect to enamel hydroxyapatite (10) was altered using xylitol in order to assess the effect of this substance on the demineralization of enamel with regard to caries development. The degree of mineral loss and lesion depth following the production of caries-like lesions in enamel samples were quantified. A greater protective effect against demineralization was obtained when the solution was supplemented with both xylitol and fluoride (Fig. 1a,b; group D) than with fluoride alone (group B). This additive effect was not surprising considering the chemical properties of these two elements. Xylitol in high concentration is known to form complexes with calcium ions (8), and to penetrate into demineralised enamel (9), where it can interfere with the transport of dissolved ions from the lesions to the demineralizing solution by lowering the diffusion coefficient of calcium and phosphate ions from the lesion into the solution (9). The transport of dissolved materials from the inner part of the lesion to the bulk solution has been shown to control the rate of progression of demineralization (15). The continuous presence of fluoride ions in solution, on the other hand, has long been established to inhibit enamel demineralization (10, 11, 15), and this was demonstrated in the present study in which a significant protective effect was observed with the presence of 0.5 ppm fluoride in the buffer solution (Fig. 1a,b; group B). The transported enamel lesions observed only in demineralization solutions devoid of fluoride (groups A and C) was a demonstration of the importance of fluoride in induction of a sound surface layer in a typical caries-like lesion (16, 17). The limitation on the protection offered by xylitol alone as observed in group C (Fig. 1a,b), may be argued to be due to the low level (20 %/w/v) used in this study. However, the low level of xylitol supplementation was selected in order to be sufficient to facilitate significant complex formation (8), while still avoiding the gastrointestinal upset caused by high xylitol concentrations (18). It is speculated that substitution of microorganism-fermentable cariogenic dietary components with xylitol, especially in combination with a low level of fluoride, may have a beneficial influence with respect to tooth demineralization. It is also envisaged that if xylitol is incorporated into fluoridated caries preventive agents such as toothpastes or mouthrinses, it may produce a substantial inhibitory effect on demineralization.
Remineralization

Saliva substitutes has been shown to possess the potential of remineralizing artificial caries (19-23) even in the absence of fluoride (22, 23). This was re-examined in the present study in which caries-like lesions produced on different groups of enamel samples were remineralized using an artificial saliva supplemented with either fluoride, xylitol or both substances combined. The degree of remineralization was determined by the difference in mineral loss and lesion depth before and after the remineralization procedure. Though a significant difference was observed only in lesion depth following remineralization, both parameters exhibited similar trends graphically (Fig. 2a,b). The limited degree of remineralization observed with all varieties of the artificial saliva, may be attributed to the presence of carboxymethylcellulose (CMC), a major component of the artificial saliva, which has been shown to reduce the rehardening potential of saliva substitutes (20, 21). This effect of CMC was attributed to its properties of 1) forming complexes with calcium and/or phosphate ions (21), thereby making these elements unavailable for lesion remineralization, 2) increasing the viscosity of the saliva substitutes thereby decreasing the diffusion rate within the solution (24). Carboxymethylcellulose represents the mucin content of natural saliva, but porcine gastric mucin has an even greater effect (21). As previously reported by other workers (19), this adverse effect of CMC may have been reduced by the presence of fluoride in group 2 (Fig. 2a,b) where the greatest degree of remineralization was observed. The lowest level of remineralization recorded when the artificial saliva was supplemented with xylitol alone (Fig. 2a,b; group 3) may be attributed to the complex formation with calcium and phosphate ions by xylitol (8, 23) which would further deplete the solution of these elements necessary for remineralization of the caries lesion in addition to a similar effect from CMC. An additive effect from xylitol and fluoride was sought in group 4 by supplementation of the saliva with both fluoride and xylitol, but the beneficial effect of fluoride obtained in group 2 was rather reduced. This must be, as stated above, the result of depletion of the solution of the calcium and phosphate ions which would have enhanced the action of the fluoride. Hence the remineralization demonstrated in the two groups (3 and 4) in which the saliva was supplemented with xylitol can be ascribed to the presence of calcium and phosphate ions in the solution as can be seen from the effect in group 1 in which the saliva was supplemented with neither fluoride nor xylitol, thus demonstrating an inability of xylitol alone to influence the remineralization of a caries-like lesion. Thus the reported rehardening and remineralization of natural and artificial caries lesions (9, 18, 21, 25), and caries arrest (7) by xylitol in vivo must be the result of a combination of two factors. Firstly, its sweetness, contributing to a food's salivation-stimulating effect (26) would foster remineralization of the caries (27, 28). Secondly, its ability to inhibit the growth and metabolism of Streptococcus mutans and dental plaque (6), thereby suppressing the demineralization part of the de- and remineralization cycle occurring in vivo, and consequently enhancing remineralization.

It can be concluded from the present study that xylitol alone may not show a significant effect as a caries inhibitor and as a remineralizing agent, but can have a significant inhibiting effect on caries in the presence of fluoride and other inorganic elements such as calcium and phosphate. Hence, it may be useful in fluoridated caries preventive and dietary products.

References


