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ORIGINAL RESEARCH

Influence exerted by a xylitol and fluoride based mouthwash on the *in vitro* enamel remineralization of primary teeth

Influencia de un enjuague a base de fluoruro y xilitol en la remineralización in vitro *del esmalte en dientes temporales*

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ABSTRACT

The purpose of the present study was to assess the effectiveness on primary teeth of a fluoride and xylitol based mouthwash. 40 caries-free teeth were used. 35% phosphoric acid was applied during 20 seconds. Teeth were then immersed in the mouthwash for 0, 15, 30, 45 and 60 days. 150-250µm longitudinal slices were taken of each sample. Re-mineralization was assessed according to bi-refringence observed after applying Thoulet solution (1.47 IR). Assessment was conducted under polarized light in a photo-microscope. At 15 days, a mean of 0.444 (± 0.527) was observed. After 30 days the observed mean was 0.778 (± 0.441). At 45 days, observed mean was 1.444 (± 0.527), and at 60 days, observed mean was 1.47 (± 0.483). Variance analysis established statistically significant differences among groups ($p < 0.001$) as well as when comparisons among groups were established ($p < 0.05$). After conducting the aforementioned tests it could be concluded that the employed mouthwash exerted a slight re-mineralizing effect upon the enamel of treated teeth.

RESUMEN

El propósito del estudio fue evaluar la eficacia de un enjuague bucal con fluoruro de sodio y xilitol, en la remineralización del esmalte de dientes temporales. Se utilizaron 40 dientes sin caries, aplicando ácido fosfórico al 35% durante 20 segundos, y fueron inmersos en el enjuague por 0, 15, 30, 45 y 60 días. Se observaron cortes longitudinales de 150-250 µm de cada muestra y se valoró la remineralización, de acuerdo a la birrefringencia observada después de aplicarles solución de Thoulet (1.47 IR) bajo luz polarizada en un fotomicroscopio. A los 15 días tuvo una media de 0.444 (± 0.527), a los 30 días de 0.778 (± 0.441), a los 45 de 1.444 (± 0.527) y a los 60 días de 1.47 (± 0.483). El análisis de varianza permitió establecer diferencias estadísticamente significativas entre los grupos ($p < 0.001$), y comparándolos entre sí ($p < 0.05$). Puede concluirse que el enjuague empleado tuvo un ligero efecto remineralizante en el esmalte.

Key words: Fluoride, xylitol, enamel, re-mineralization, primary teeth, polarized light, bi-refringence.
Palabras clave: Fluoruro, xilitol, esmalte, remineralización, dientes temporales, luz polarizada, birrefringencia.

INTRODUCTION

In general terms, products used for oral hygiene contain anti-microbial effect substances which can decrease caries incidence through a mechanism of plaque build-up control, suppressing thus cariogenic species or through the inhibition of the bacterial mechanism.[1]

Since 1970 changes in the mean index of caries have been spectacular. In this field, fluoride plays a main role, through the action exerted by three mechanisms: de-mineralization inhibition (when present in liquid phase), re-mineralization enhancement as well as inhibition of bacteria found in the dental plaque.[2]

Fluoride exerts its main anti-caries effect on the enamel of teeth; it can also exert an antimicrobial effect, which, although subtle, can be extremely important. Although fluoride cannot directly alter microflora composition it can act by preserving plaque's microbial homeostasis, exerting a stabilizing effect upon sugar concentration and pH variation during oscillatory conditions.[1]

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One of the mechanisms through which oral environment homeostasis can be interrupted is through exposition of plaque to a low pH, which can be caused by the frequent intake of fermentable carbohydrates. A suitable prevention mode could be related to a decrease of intake of foods with high contents of fermentable carbohydrates. Another prevention mode would be the use of sugar substitutes, since they cannot be metabolized by dental plaque's micro-organisms.[1]

Sugar substitutes possess the ability of reducing at least one of the four essential etiological factors (diet, micro-flora, susceptibility and time) for dental caries,[1,2] this is the presence of fermentable carbohydrates in the diet in order to break interaction of susceptible teeth with cariogenic bacteria of the plaque and sugar, all of which are causing agents for the disorder.[1,3-5]

Xylitol is one of the most suitable and promising sugar substitutes tested for caries prevention purposes. It is as sweet as saccharose (common sugar) and cannot be metabolized by most of oral bacteriae.[3,5-9]

In the process of caries prevention, dental brushing with a fluoride toothpaste has become a public measure of oral health. Since fluoride concentration found in toothpastes does not provide comprehensive protection for all subjects, and must observe certain limits due to legal regulations, there is doubt upon whether to effectively use additional products with fluoride content as well as incorporation of other ingredients.[10]

Xylitol or pentinol is a natural, 5 carbon sugary alchohol which has proven to be an effective agent in caries prevention in animals and humans. It is naturally found in some fruits and vegetables.[1,3-5] Many countries have approved its consumption in the daily diet. Presently, it is incorporated as sweetener into various products such as sweets, chewing gums, confectioneries, as well as into oral hygiene products, cosmetics and medical drugs.[3,6-8]

The mechanism and re-mineralizing effect of xylitol are triggered into action whenever chewing gum of food containing materials which stimulate salivation cannot be fermented. In those cases, plaque and dental surface found underneath plaque result exposed to an environment exhibiting a pH very similar to the salivary pH, this favors the process of tooth remineralization. When these events occur repeatedly, after de-mineralization episodes, it is probable that potential clinically important re-mineralization episodes will occur.[6]

Therefore, caries clinical studies have reported what was called reversions of early caries lesions (white spots) with unusually high frequencies amongst users of xylitol containing chewing gum.[6]

Former research on xylitol mode of action revealed that fermentation was nil due to most microotganisms of dental plaque. The following was equally observed: absence of significant degradation of dental plaque to acid terminal products, stimulation of salivary flow, increase in buffering capacity inhibition of cariogenic bacteria and plaque accumulation, re-mineralization of de-calcified areas as well as inhibition of healthy enamel de-mineralization.[3,6,8,11,12]

Xylitol has recently been incorporated into fluoride tooth pastes and mouthwashes. *In vitro* studies suggest the fact that the aforementioned ingredients exert additional inhibiting effect upon growth and/or production of cariogenic microorganism acids.[5,9]

Many studies conducted with pure bacterial cultures, dental plaque suspension and *in situ* pH measurements have established the fact that xylitol meets all criteria to be used as dental caries preventive agent.[3,12,13]

MATERIALS AND METHODS

The sample was composed of 40 caries-free primary teeth, randomly selected and about to exfoliate. They were divided into five groups of eight teeth each. Once the teeth were extracted, infection control procedures were undertaken so as to guarantee bio-security in the handling of biological specimens. Therefore, after teeth were extracted, they were immersed in a 6% NaOCl solution in order to neutralize any bacterial or biological component found in the sample. Samples were then rinsed with abundant de-ionized water until total removal of any organic remain on the surface. Once cleansed, specimens were stored in de-ionized water. Crowns were varnished leaving a free 3 x 3 mm surface. The surface was later treated with a 35% phosphoric acid gel during 20 seconds. Teeth were then rinsed with tap water; a control group was set aside and left in de-ionized water, the other four groups were immersed in a rinse of 0.5 g sodium fluoride and 1% xylitol (Fluoxytil®).

Groups were structured so as to have one control group and four experimental groups (according to exposition and observation times)(0 to 60 days), as shown in *table 1*. The control sample as well as samples immersed in xylitol and fluoride rinse were stored in sealed glass jars, and were taken to a Hanau oven to achieve a constant 37 °C temperature, with absolute humidity. The rinse was changed on alternate days.

Once the oral rinse exposition term was completed, specimens were mounted on plastic supports and

fixated with self-curing acrylic. At a later point they were placed on a diamond disk trimmer so as to undertake 150-250 µm longitudinal cuts *(Figure 1)*. These cuts were observed, and re-mineralization was assessed in function of refraction exhibited by the cuts after application of Thoulet 1.47 IR solution.

Samples were observed under polarized light in a photomicroscope (Axiophot, Zeiss, Germany) with 5X and 10 X work objectives *(Figure 2)* so as to determine existing bi-refringence. The following criteria were applied *(Table II)*.

In order to determine surface re-mineralization, photographic records were taken of all specimens at different experimental periods (15, 30, 45 and 60 days) respectively, the records were compared to the control group.

RESULTS

Groups were observed according to their corresponding period. In all sessions, photographic records were taken and a previously determined measurement scale was applied. Data were gathered into statistical collection cards and Anova variance analysis was applied. This procedure was conducted in order to establish the existence of statistically significant differences among groups *(Figure 3)*.

Control surface was treated with 35% phosphoric acid. These surfaces were not rinsed *(Figure 4)*.

In the 15-day group, superficial changes were observed. A 0.444 mean was established (± 0.27 DE). In that group, the surface treated with phosphoric acid did not show significant changes when compared with the control group. Control group also underwent superficial treatment but was not exposed to rinsing *(Figure 5)*.

In the 30-day group, changes observed under polarized light were more evident than in the 15-day group. Nevertheless, these changes did not reach beyond enamel surface. In this group the mean of changes observed in enamel was 0.778 (± 0.441), which was almost double the change observed in the former group *(Figure 6)*.

In the 45-day group a 1.44 mean (± 0.527 DE) was determined. This meant that in all cases there was at least one change in enamel surface, and this change was not only limited to the surface, a sub-surface characteristic modification was observed as well *(Figure 7)*.

Table I. Classification of treated teeth.

Group	Solution	Exposition time
0	de-ionized Water	None
1	Mouthwash	15 days
2	Mouthwash	30 days
3	Mouthwash	45 days
4	Mouthwash	60 days

Figure 1. 150-250 µm longitudinal cut of a tooth exposed to xylitol for a 15 day period.

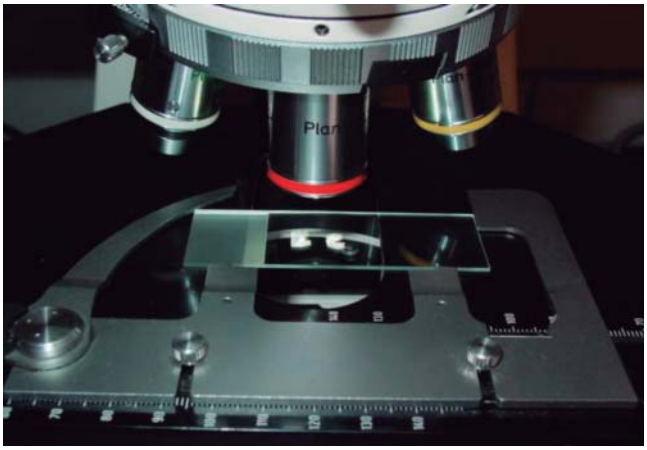

Figure 2. Photomicroscope (Axiophot, Zeiss, Germany).

Table II. Valuation categories of Fluoxytil® re-mineralizing effect.

Scale	Effect
0	No change
1	Superficial change
2	Sub-surface change
3	Profound change

The 60-day group exhibited a 1.47 mean (± 0.483). This showed the fact that changes were found underneath the enamel surface *(Figure 8)*.

Upon application of statistical analysis, it was determined that there was statistically significant difference among groups when compared with control group ($H = 21.992$, $p \leq 0.001$). Differences were found even among different groups. In the same manner, an attempt was made to determine which were the associations when comparisons among groups were established. For this reason, Dunnet multiple comparison method was applied. With it it was found that there were statistically significant differences ($p < 0.05$). This could be interpreted as changes in enamel caused by the effect of the rinse in all studied periods.

DISCUSSION

Re-mineralization and de-mineralization processes which take place in dental tissues are dynamic mechanisms which are carried out during the time in which the tooth remains in the mouth.[3,4] For example, an unbalanced environment generated by pH and saliva as endogenous factors, together with diet as exogenous factor, allow bacteria to not only be the causing agents for a de-mineralization phenomenon, but also to contribute to it. On a contrary basis, a balanced oral environment favors the re-mineralization process.[3,4,6]

Fluoride use is an undisputed viable and cost-effective method. This is probably still the situation

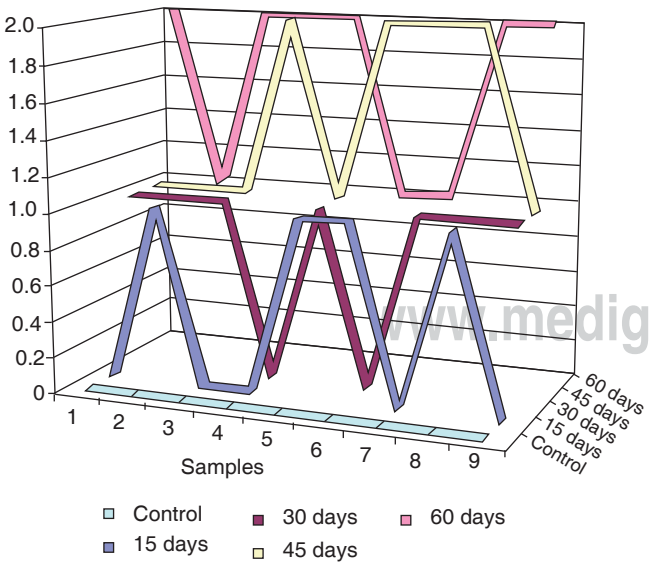

Figure 3. Bi-refrigence in enamel samples immersed in xylitol solution (Fluoxytil®) observed under polarized light.

Figure 4. Sample corresponding to control group (Axiophot 5x and Tholuet 1.47 IR solution).

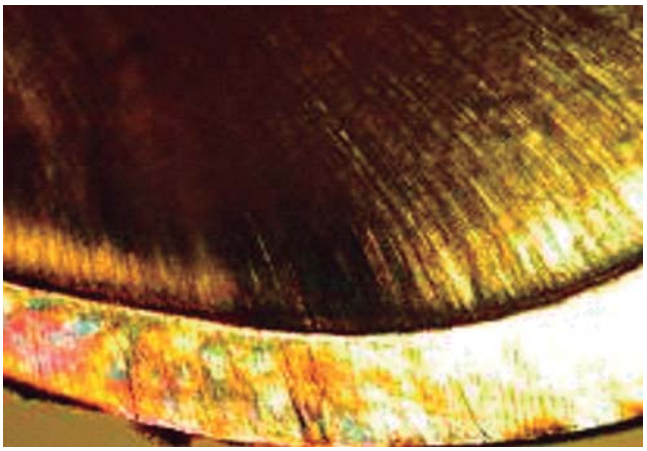

Figure 5. Sample corresponding to group 1 (15 days) exposed to mouthwash (Axiophot 5X and Tholuet 1.47IR solution).

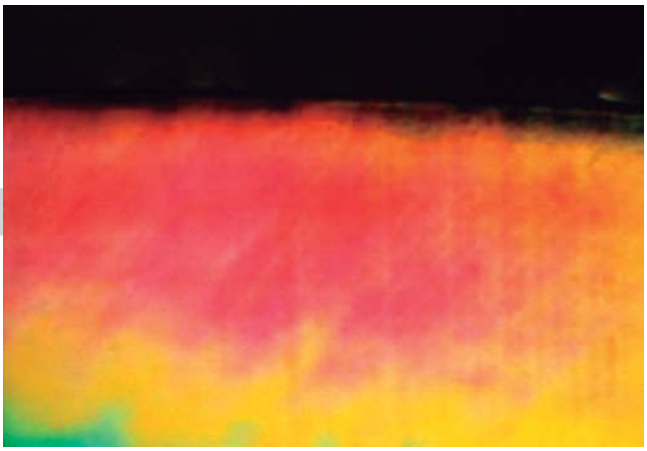

Figure 6. Sample corresponding to group 2 (30 days 9 exposed to mouthwash (Axiophot 10X and Tholuet 1.47 IR solution).

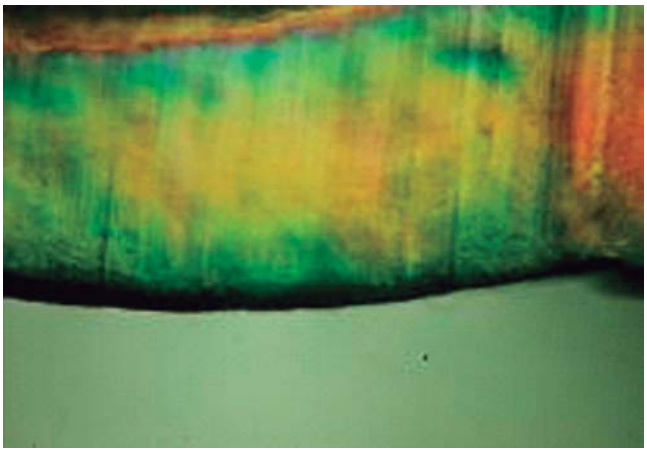

Figure 7. Sample corresponding to group 3 (45 days) exposed to mouthwash (Axiophot 10X and Tholuet 1.47 IR solutions).

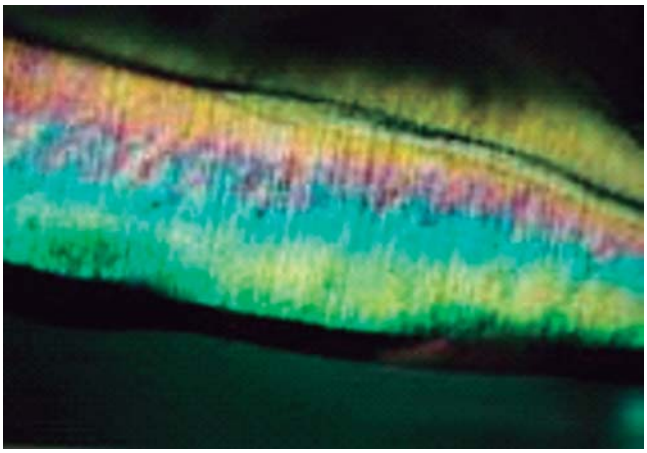

Figure 8. Sample corresponding to group 7 (60 days) exposed to mouthwash (Axiophot 5X and Tholuet 1.47 IR solution.

in countries where there are not nationally-minded preventive systems, or those countries with relatively high caries incidence.[2]

Apparently, when fluoride oral rinse is used as an adjuvant of unsupervised oral hygiene, with a fluoride toothpaste, caries inhibition is approximately 10 to 20%. In this context, it is important to observe that effectiveness of any caries prevention measure not only depends on the isolated measure, it also depends on other factors such a caries risk, awareness of dental health, and presence of organized dental health care. Therefore, one fluoride rinse program could result effective in one type of population, while remaining ineffective in others.[13]

Reports on xylitol used in toothpaste upon saliva and caries incidence have been contradictory. Effect of xylitol in mouthwash upon saliva and plaque have been scarcely studied.[5]

In scientific literature, there are reports which suggest a re-mineralization effect based upon different compounds. One example would be glass-ionomer cements, which can be directly or indirectly applied on enamel surfaces.[3,4,6] Purportedly they exert a re-mineralizing effect due to the interaction and concentration of the fluoride ion from the fluoride-aluminum-silicate which forms part of the matrix of these cements.[6] The last decade has seen the development of alternative treatments to reduce carious lesions incidence, as well as resources to obtain re-mineralization areas through procedures which although simple, are very valuable for the prevention of this disease.[3,4,6] In fact, enamel surface re-mineralization function has been attributed to xylitol based compounds.[3,4-6,9,12]

As was the case with Amaechi,[6] in the present research, an experimental model was applied with the aim of assessing the re-mineralizing ability exhibited by the aforementioned compound, with the difference that our study employed primary human teeth and not bovine teeth. Results of the present study concur with other results reported in literature inasmuch as they suggest lesser mineral loss as well as reduced cariogenic potential due to its re-mineralizing activity.[1,3,4-6,9]

Wennerholm & al[13] reported a study where, after having used chewing gum with xylitol for a 21-25 days period, they discovered decrease of salivary pH. That study was conducted *in vivo* in adult patients.

In a similar manner, Gertsen & al[5] could not prove the effect of xylitol and fluoride mouthwashes (by themselves or combined) upon microflora or salivary secretion indexes, on the accumulation of dental plaque, on the development of gingivitis or upon plaque's acidogenic potential . A possible explanation for the finding of contradictory results in several research projects could be the fact that the xylitol content in the vehicle varies greatly, another fact to consider could be the diversity of presentations and differences in the frequency and duration of use.[5,9]

Besides the already mentioned variations, in the different studies there are other variants tailored to intended objectives. Very few studies purported the same materials and methods. For this reason, the present study could hardly totally coincide with other consulted research. In order to better assess the product effectiveness, it would probably be necessary to allow teeth to remain longer time in contact with the fluoride and xylitol solution.

The determination of changes elicited by polarized light upon mineral components is a technique used to assess re-mineralizing effects. Results showed the aforementioned re-mineralizing effect on at least the enamel surface in the 45 day period. Even though mechanisms involved in homeostasis of enamel re-mineralization processes are complex, the role played by xylitol when assessed under polarized light cannot be discarded, at least that was the case in the present study.

To be more knowledgeable on these variables, evaluation time must be longer. It is also necessary for experimental conditions to consider variables such as presence of certain electrolytes, and, whenever possible, simulation of dental vitality environments. Moreover, application of techniques for the characterization of enamel surface must be considered, so as to allow changes in the composition. It would then be reasonable to hope achievement of these phenomena through use of chemical and optical characterization instruments so as to determine changes in the enamel structure.

CONCLUSIONS

In the present study, mouthwash with sodium fluoride and xylitol exerted a slight re-mineralizing effect upon surface and sub-surface of enamel of primary teeth.

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