Protective & Corrective efficacy of Xylitol Versus Fluoride on Primary Teeth Enamel subjected to in Vitro Demineralization by Chlorinated Pool Water

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Abstract

Background: Although chlorine is used as an important disinfectant for pool water yet its effect on the teeth could be a cause of concern. This study aimed to assess the protective and corrective effect of Xylitol versus standard use of fluoride on surface morphology and ionic profile of primary enamel exposed to chlorinated water in vitro, to tailor a feasible regimen for young swimmers.

Materials and Methods: Thirty recently exfoliated, deciduous central incisors, (Negative Control,n=30) were examined by scanning electron microscope and energy dispersive X-ray analysis (EDAX). Teeth were divided into Two groups, (n=15). I. Corrective groups: Pool water group (P): teeth were subjected to chlorinated water, then half the tooth was coated with fluoride (P&F): and the other half xylitol (P&X):, II. Preventive groups: The other 15 specimens, were half coated with fluoride: subgroup (F), and half with xylitol Subgroup(X) (positive controls): teeth were then subjected to chlorinated water. (F+P) & (X+P). A blinded operator to the tooth condition examined all groups between all steps by scanning electron microscope to detect surface morphological changes and energy dispersive X-ray analysis to evaluate Ca, P, C, Cl ions.

Results: scanning electron microscope revealed areas of erosion on enamel subjected to chlorinated water, which improved by both agents but xylitol showed a smoother surface which was supported by statistical analysis of ionic EDXA results. Fluoride demonstrated a better protective effect as par ionic measuring results.

Conclusion: young swimmers should use a fluoride agent prior to pool, and a xylitol agent after training to minimize detrimental effects of chlorinated water. Xylitol showed a higher significant effect whether as a protective or corrective agent.

Key words: Chlorinated Pool Water, Young Swimmers Remineralization, Xylitol, Fluoride, Primary Teeth Enamel.

Introduction

The understanding of the properties of dental tissue as well as the mechanisms involved in their interaction with their surrounding media improves quality and progress of dental care 1. Enamel has the highest degree of mineralization almost 96-98%, mainly in the form of hydroxyapatite crystals which allows the exchange of ions through the hydroxyl ion (OH-) 2. Although it is considered the hardest human tissue surface, dental enamel can still be attacked by acids generating mineral loss at the microscopic and macroscopic levels 3.

Dental caries and dental erosion are the two main causes of hard dental tissue loss(mainly surface enamel) 4. While caries requires the presence of microorganisms, erosion is caused by chemical chelation due to intrinsic factors as recurrent vomiting or the regurgitation of the gastric contents 5 or extrinsic factors as diet acidic foods, carbonated beverages, citrus fruits, low pH medications or environmental exposure to acidic agents 6. Most clinical research has focused on the impact of diet and lifestyle 7.

Another external factor would be exposure to chlorinated pool water, the low pH as well as the under saturation of pool...
water with hydroxypatite components increases the risk of dental erosion on enamel surface but still both public as well as “backyard” swimming pools must be chlorinated to reduce bacterial and algal contamination 8.

Chlorine could be added by several ways, with a preferable concentration of 2–3 ppm (minimum concentration one ppm). Chlorine gas, is used mainly in large public swimming pools but the most commonly used method is “stabilized” chlorine, which is created by combining chlorine into a tablet or granular form which is dissolved first in a bucket and then thrown daily in pool with the salts of cyanuric acid which retards the rate of breaking down of hypochlorous by sunlight 9. However, inadequately acid buffering with sodium carbonate (soda ash (Na2CO3), could lead to a rapid decrease in pH to decalcifying levels as low as 3 allowing tooth dissolution 10.

It was suggested that a low pH pool water (acidic) can cause very rapid and extensive dental erosion 8. Clinically, intensive swimming should be considered on diagnosing general dental erosion 16 which is a common lesion in competitive swimmers caused by a low pH of the pool water 11.

Both fluoride and more recently xylitol play a protective role against caries as well as erosion and are considered as anti-caries & anti-erodive agents. Especially when used as varnishes. Fluoride which is a naturally occurring mineral has been the number one protector against caries and demineralization for the past decades, fluoride ions also enhance remineralization 12. Xylitol, a five-carbon polyalcohol sugar substitute which has the same sweetening power of sucrose 5,6 has gained attention in the last years as it cannot be fermented by plaque bacteria and has been proven to have a caries inhibiting effect.

Although research has proven that primary enamel is more susceptible to demineralization than permanent 2, it would not be logic to prevent young children enjoying neither recreational nor competitive swimming & as most studies were performed using permanent teeth. Therefore this in vitro study was designed to evaluate the preventive and/or corrective performance of fluoride versus xylitol varnishes on primary enamel subjected to demineralization from chlorinated pool water. This was done through analysis of the enamel by scanning electron microscopy (SEM) & ionic enamel profile.

**Material and Methods**

**Specimens & Solutions**

Teeth: Thirty recently exfoliated and caries-free deciduous incisors, were selected from the outpatient clinic of Pediatric Dentistry Department, Ain Shams University. All teeth were examined with stereomicroscope and those presenting visible pigmentation, enamel cracks and/or fractures were excluded. Teeth were washed thoroughly with inert detergent followed by rinsing with double deionized water.

Artificial saliva: All teeth specimens were stored in artificial saliva till samples examination and at intervals between applications which was constituted of:

0.65 grams per liter potassium chloride British Pharmacopoeia (BP), 0.058 g/l magnesium chloride BP, 0.165 g/l calcium chloride BP, 0.804 g/l dipotassium hydrogen phosphate US Pharmacopeia, 0.365 g/l potassium dihydrogen phosphate, 2 g/l sodium carboxymethyl cellulose BP and deionized water to make 1 liter as modified from 13. The solutions were stored 4°C and pH of artificial saliva was optimal, 7.0.

Pool water: Gas chlorinated water was freshly collected from a public pool known to have high frequency of swimmers per day of which those who regularly trained were reported to have teeth erosions and higher teeth sensitivity. The pH was randomly measured and was found to be 4.4-5.5 (Acidic).

Fluoride* Flor-opal Varnish White with 5% sodium fluoride in a resin carrier was used

* Ultradent USA

Xylitol: Xylitol was prepared from 100% pure xylitol powder * at a concentration of 35%100ml of ethyl alcohol for * Swanson SIGMA-ALDRICH Germany

**Experimental design:**

Negative Control (C)

All Specimens (n=30) were labeled and coated with an acid-resistant varnish except for the middle one third of the tooth cervico-incisalay, which was examined by scanning electron microscope and the mean ionic weight percent profile (IW%) was recorded and considered as the negative control group readings. Any extremes in measurements were excluded. The middle third of all specimens was then symmetrically divided into two halves using pink wax. Teeth specimens were then stored in artificial saliva at room temperature till further applications.

Teeth were numbered then randomly allocated by a sequence program into two major groups, (n=15) each and assigned for the following:

Pool water group (P): The teeth of this group were subjected to chlorinated water for two hours each day for two successive days (total of four hours) with exchanging of water each half an hour. In between the two applications teeth were washed with deionized water, blotted and immersed in artificial saliva. (These teeth were considered as Positive Control and were then examined after 48 hours).

Corrective groups or remineralization groups (P+F) & (P+X): In order to test the corrective (remineralizing) effect of xylitol & fluoride against enamel erosion, specimens of the positive control were subjected to the following:

Pool & Fluoride group (P&F): Half the middle third of teeth specimens (n=15) was coated with fluoride varnish for 4min, then rinsed thoroughly with distilled water for another 4 min.

Pool and Xylitol group (P&X): The other half of the middle third of specimens was coated with xylitol varnish for 4 min., then rinsed thoroughly with distilled water for another four min. All Specimens were kept in artificial saliva for 48 hours, then examined.

As regards to the other 15 specimens, they were subjected to the
following:

**Fluoride and xylitol groups (F) & (X):**

**Fluoride subgroup (F):** Half the middle third of specimens was labeled and coated with fluoride varnish for 4 min then rinsed thoroughly with distilled water for four min.

**Xylitol Subgroup (X):** The other half was labeled and coated with xylitol varnish for four min., then were rinsed thoroughly with distilled water for 4 mins both groups F & X can be considered as positive controls. Teeth were kept in artificial saliva for 48 hours then examined.

**Preventive or protective groups (F+P) & (X+P)**

In order to test the protective or preventive effect of xylitol and fluoride against enamel erosion, F & X specimens were subjected to chlorinated water as the previous regimen, blotted and immersed in artificial saliva for 48 hours till examination hence testing the protective capacity of both varnishes & creating the protective groups (F+P) & (X+P)

**SEM-ADEXA examination:**

In order to observe microstructural features of the enamel after the treatments, middle thirds of specimens of different groups were examined at 30KV using secondary electron LFD detector under magnification (X1500) and (X4000) with a spot size (4.3-5.3 nm) in each magnification after each step in each group.it must be noted that the specimens were numbered and coded so that the examiner was blinded as to which group the examined surface belonged to.

**EDAX analysis:**

For each tooth the middle third of labial surface was adjusted to be examined in three different areas to measure the Calcium (Ca), Phosphorus (P), Carbon (C), Chloride (Cl) and other elements like Oxygen(O) in weight % using energy dispersive X-ray analysis (EDAX) with a S-UTW detector (EDXA INC, Mahwah, NJ,USA). The count rate of the detector was between 1800 and 2000 counts per second. Measuring time was 71 seconds with a resolution of 132.8 eV an amplification time of 100 us after each step in all groups.

**Statistical Analysis:**

The mean and standard deviations of the ionic values of Ca, P, Cl, O and C in weight % of each EDAX analysis in each of the negative and positive controls as well as the experimental groups were calculated & tabulated.

The difference in the mean of each element in the ionic profile of enamel was calculated in all groups using the independent T test. All statistical analysis were conducted with PASW statistics 17.0 (SPSS, Chicago, IL, USA). The significance level was set at 0.05.

**Results:**

Variation of Mineral values in different situations

The mean & standard deviation of the ionic weight percentage (IW%) of the five tested minerals calcium, phosphorous, oxygen, chloride & carbon and their levels in each of the 8 groups are represented in Table 1 & there relation to each other demonstrated in Table 1 Fig 1

<table>
<thead>
<tr>
<th>Tooth</th>
<th>C</th>
<th>O</th>
<th>P</th>
<th>Cl</th>
<th>Ca</th>
</tr>
</thead>
<tbody>
<tr>
<td>N Control</td>
<td>32.18±4.21</td>
<td>23.97±1.32</td>
<td>11.03±2.44</td>
<td>0.6±0.032</td>
<td>32.22±3.04</td>
</tr>
<tr>
<td>Pool</td>
<td>51.78±5.31</td>
<td>36.99±1.28</td>
<td>1.89±3.01</td>
<td>1.27±0.04</td>
<td>8.07±2.97</td>
</tr>
<tr>
<td>Fluoride</td>
<td>30.9±5.11</td>
<td>20.91±1.18</td>
<td>12.32±3.51</td>
<td>0.58±0.053</td>
<td>35.29±3.17</td>
</tr>
<tr>
<td>Xylitol</td>
<td>20.68±4.41</td>
<td>19.61±1.25</td>
<td>16.16±3.21</td>
<td>0.63±0.038</td>
<td>42.92±4.17</td>
</tr>
<tr>
<td>Pool+F</td>
<td>25.7±4.31</td>
<td>22.21±1.25</td>
<td>13.12±3.11</td>
<td>1.28±0.05</td>
<td>37.69±3.77</td>
</tr>
<tr>
<td>Pool+X</td>
<td>0.01±0.67</td>
<td>34.01±1.29</td>
<td>21.35±3.3</td>
<td>0.73±0.044</td>
<td>43.9±3.68</td>
</tr>
<tr>
<td>F+Pool</td>
<td>35.26±4.81</td>
<td>21.1±1.37</td>
<td>13.24±3.01</td>
<td>0.1±0.052</td>
<td>30.3±3.97</td>
</tr>
<tr>
<td>X+Pool</td>
<td>33.72±5.01</td>
<td>10.49±1.28</td>
<td>9.47±2.51</td>
<td>0.67±0.04</td>
<td>45.65±4.97</td>
</tr>
</tbody>
</table>

n=30

**Table (1) Mean of Ionic weight percent of enamel Minerals following different applications**
Statistically significant differences were found in each group as regards to IW% of the different elements (p<0.05). Table 2 demonstrates that specimens after pool water had significantly decreased the calcium & phosphorous IW%, while it had significantly increased the carbon & chloride IW%. The opposite effect took place after the application of fluoride & xylitol with the latter causing a more positive significant difference. Oxygen was omitted from the rest of the tables due to its irrelevance.
Discussion

The comparison of the results obtained from different groups in the present study allowed the evaluation of the protective and corrective potential of fluoride versus xylitol on primary dental enamel subjected to chlorinated pool water, which was the erosive cracks.

Specimens assigned for examining the protective influence of the varnishes against demineralization attacks were exposed to xylitol (X+P) and fluoride (F+P) application then chlorinated pool water. (X+P) showed smooth surface with areas of minerals deposits and no signs of erosions or cracks (Fig. 3c) while group (F+P) presented irregular enamel surface and minute cracking (Fig. 3d).

It should be mentioned that the results from SEM analysis were matched with those from IW%.

Fig. 3: SEM pictures of enamel specimens showing the corrective influence of:

- **a**: Xylitol. The teeth in this group (P + X) were first exposed to chlorinated pool water then xylitol application.
- **b**: Fluoride. The teeth in this group (P + F) were first exposed to chlorinated pool water then fluoride application for remineralization.
- **c**: Xylitol. The teeth in this group (X + P) were first exposed to xylitol application then chlorinated pool water.
- **d**: Fluoride. The teeth in this group (F + P) were first exposed to fluoride application then chlorinated pool water.

SEM Analysis Results

Areas of irregular enamel surface with defined deepened enamel cracks (Fig. 2a) and localized areas of enamel erosion were observed in chlorinated pool water treated teeth group (P) (Fig. 2b).

Minimal surface changes with almost non-observable cracking was seen in both positive control groups subjected to Xylitol (X) (Fig. 2c) and Fluoride (F) which showed area of surface mineral deposits. (Fig. 2d).

To evaluate the corrective influence of the varnishes on the specimens that were earlier subjected to pool water, the specimens were examined after xylitol (P+X) and fluoride (P+F) application, and they presented no signs of enamel erosions. The specimens of group (P+F) Fig 3b demonstrated surface irregularities in comparison (P+X) Fig 3a group which represented a smoother surface with minimal minerals deposits sites and very shallow cracks.
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demineralization agent of choice in an attempt to find the best regimen that could be applied by young swimmers to protect themselves from the demineralization that occurs from continuous training 8,14. Competitive swimmers are highly susceptible to acid erosive cavities of surface enamel which clinically varied in the degree of its sensation. 14. The accepted pH range for swimming pools is between 7.2 and 8.0. However, inadequately buffering of the acids, which is usually done with sodium carbonate named soda ash (Na2CO3), could lead to rapid decrease to the pH to decalcifying levels as low as 3 allowing tooth dissolution. Though swimmers may not sense the low pH, yet it may cause eye irritation. However, it was suggested that a low pH pool water (acidic water) can cause very rapid and extensive dental erosion 8,10,11. It must be mentioned that the chlorinated water used in this study was taken from a pool where young swimmers suffered from erosive cavities and teeth sensitivity and a random measuring of the pH of the pool found it to be 5.5. 16.

The period of exposure of teeth to the pool water was determined in view of the daily average training time which competitive swimmers teeth would be subjected to it, which was 120 minutes 15. In order to stimulate normal oral conditions of swimmers, specimens were immersed in artificial saliva between application periods to allow remineralization from the salivary components 17.

Although the enamel of deciduous teeth is more susceptible to acid digestion than permanent teeth as the former contained significantly less fluoride in every layer in comparison with the later, the number of studies found in the literature monitoring the surface enamel alteration and ionic profile of surface enamel of primary teeth subjected to pool water was limited 2 and therefore targeted in the present study.

Both varnishes whether applied before immersion in pool water as protective agents or after the tooth was subjected to pool water as corrective agents were washed off the tooth after four minutes as recommended 18 with artificial saliva then immersed in it, this was to simulate the clinical conditions in the child’s mouth where the varnish wouldn’t be left in dry conditions due to normal oral activity, and also to focus on the chemical rather than the mechanical effect of the varnish.

The use of ADEXA-analysis conducted on the SEM was used in this study as a tool for detecting the presence and relative quantity of elements IW % in the teeth, also the blinded detection of the morphological observations allowed the researchers to monitor and analyze the changes that occurred to the primary enamel through the experiment without any chance of bias. The methods used appeared to be the most suitable technique for examining elements composition within localized small areas of mineralized enamel structure. Its main advantage is its capacity to analyze in situ weight percentages on the order of cubic microns in non-destructive way and to relate the destruction of various elements to histological structure of the tissue 19.

After subjection to the chlorinated water with the low pH, the primary teeth specimens showed surface irregularities as well as areas of enamel cracks and erosion. These results come in parallel to a study done where teeth specimens soaked in chlorinated water (pH of 5.0), at room temperature for 24 hours reduced the mean microhardness of enamel to 9.76% below baseline (was 343.87±14.14 VHN). Also surface mineral loss, together with appearance of surface erosive lesions was reported with administration of acidic drinks by several investigators 20.

The detected erosive lesions and the ionic profile that showed a significant decrease in the Ca & P ionic weight % versus a significant increase in that C & Cl could be related to the dissociation of hydroxyapatite and impaired mineralization of enamel due to the under saturation of hydroxyapatite minerals in low pH swimming pool water 18. The increased carbon ions wt % in specimens of this group compared to control group could confirm the demineralization and enamel loss in erosive sites. It is known that the carbonate may substitute OH or P in the apatite lattice. As the P content decreased, it is supposed that phosphorus had been replaced by carbonate. The increase in carbon content reflects the decrease in the enamel hardness 21 and logically increases the enamel solubility resulting in an erosive effect.

Exchange of ions is an important property of hydroxyapatite with the hydroxyl ion (OH-) exchanging particularly easily for the fluoride ion (F-). F- ions are capable of stabilizing the structure of hydroxyapatite by reducing its solubility and in consequence increasing enamel resistance to acid attacks. Statistically, application of fluoride before subjection to pool water was accompanied by a decline of Carbon wt % and a significant increase in both Calcium and Phosphorous wt %. This was in accordance with the data presented by 21 about the individual capacity of fluoride in remineralization and reflected in increase in enamel surface hardness and decrease in its solubility.

SEM results in this study showed that fluoride application was found to be more successful before pool water application demonstrating minimal cracking. This was in accordance with 22 who reported capability of fluoride toothpaste application to prevent dental erosion and reduce it before being subjected to acidic drinks. The Ionic weight % results as well supported fluoride remineralization potential (protective effect). This could be related to the CaF ions reservoir formed on the tooth surface inducing reprecipitation of released minerals ions in the form of fluorhydroxyapatite preventing loss of mineral ions 23. Also these reservoirs might have gradually released fluoride into artificial saliva or apatite structure of the tooth 24. This remineralization process might also be enhanced by keeping specimens in artificial saliva in between and after test periods since it has been reported to have remineralization potential as well 15,20. A 40% concentration of xylitol was used as recommended by Da Silva et al. 25 who concluded in their study that only the 40% xylitol promoted the protection and correction of the teeth erosion.
caused by exposure to lemon juice (acid drink) versus lower concentrations (10,20& 30%).

Statistically, application of xylitol before subjection to pool water was accompanied by a decline of Carbon wt % and a significant increase in both Calcium and Phosphorous wt %. This was in accordance with the data presented by Smits and Arends 26, about the individual capacity of xylitol in remineralization.

Interestingly, xylitol was also reported to have a capacity in inhibition of demineralization which was elaborated by its a protective effect against pool water as it can maintain a higher pH value in saliva and simultaneously maintain a supersaturation of Ca level in saliva 27. It could also be related to the capacity of xylitol to form complexes with Ca ions 23 and prevent decalcification by inhibiting translocation of dissolved Ca and Phosphorus ions from lesions by lowering their diffusion coefficients 28, which did not occur with fluoride application. This might explain why fluoride - in comparison to xylitol - couldn’t help reduce enamel erosion after acidic pool water application.

The results of the present study agree with Souza et al 29 who concluded that xylitol had a significant remineralization potential after acid attacks as revealed by SEM results while it disagreed with the results of the study by Chunmuang et al.30 that conclude that various protective agents including 40% xylitol was unable to reduce tooth surface and mineral loss due to acid attacks (orange juice). This might be due to the difference in the erosive protocol as they used successive erosive challenges which might have limited the protective and remineralizing effects of the agents used.

Despite the limitations of this in vitro study regarding the reproduction of the natural conditions of the mouth, it enabled the examination of the microstructural changes & ionic weight profile of primary enamel and how to limit the detrimental effect of chlorinated pool water. Further studies should be performed to test the maintenance effect of the agents used following successive erosive challenges.

**Conclusions**

The present work clarified that(500ppm) fluoride had a better performance when used as a protective agent against demineralization while xylitol was superior as a remineralizing agent, therefore for clinical applications of this in vitro study, we can strongly recommend that young swimmers use a fluoride mouthwash or toothpaste before plunging into the pool ,and then use a xylitol containing vehicle such as chewing gum or lozenges after their training to minimize detrimental effects of chlorinated pool water .Xylitol would be preferable if only one agent is used as it showed a higher significant effect whether as a protective or corrective agent.

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