In vitro Evaluation of the Erosive Potential of Chlorinated Pool Water on Dental Enamel and the Protective Effect of Three Dental Materials

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This study reports on the in vitro erosive capacity of three different pH chlorinated waters on dental enamel, and the anti-erosive protection conferred by three dental materials (toothpaste, remineralising cream, and fluoride varnish), assessed by scanning electron microscopy. Fluoride varnish provided the best protection, forming a resistant thin film on the enamel's surface. The observed ultrastructural changes of the enamel surface were low when the tooth paste was used, and more pronounced when the remineralising cream was used.

Keywords: dental erosion; tooth enamel; chlorinated water; fluoride; scanning electron microscopy

Dental erosion is a prevalent condition and an essential factor when considering dental health management, and it is important to find materials and methods for its efficient prevention and treatment. Dental erosion is defined as the loss of dental hard tissue due to intrinsic and extrinsic factors. Intrinsic factors include vomiting and gastro-esophageal reflux, while the extrinsic ones include exposure to acidic foods and drinks, certain medication, occupational factors (wine tasters, manufacturing electrolytic/ galvanic batteries, etc.), and lifestyle [1]. Dental erosion might also affect performance swimmers due to the exposure to chlorinated water in swimming pools [2].

The purpose of swimming pool chlorination is to reduce bacteria/ algae contamination; the recommended concentration is of 2-3 ppm, and the accepted *p*H value is 7.2 to 8. Within these recommended limits, there is no erosive effect on dental enamel. A *p*H of 5.5 is considered a critical threshold for dental erosion, and only a very high concentration of chlorine in water can decrease the pH. Although researchers found a relation between enamel erosion and swimming pool water, this is probably due to insufficient monitoring or inadequate buffering. The classic paper of Centerwall et al. showed dental erosion rates of only 3% in non-swimmers, compared to 12% in swimmers and 39% in members of professional swim teams [3]. Some reports have documented extremely severe cases of erosion: total loss of enamel/ generalized erosion after as little as 14 to 27 days of swimming in improperly chlorinated pools [4].

In this *in vitro* study, we used scanning electronic microscopy to evaluate the erosive effect of chlorinated pool water and the protective effects of several dental products. Study objectives were: to assess the erosive capacity of three chlorinated waters used for swimming pools, to qualitatively determine erosion changes in the structure of enamel, and to analyze the dental materials' remineralisation and protection effect on enamel samples exposed to chlorinated waters.

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Experimetal part

We used the method of Rirattanapong et al. [5], modified as follows:

Enamel sampling

Enamel fragments were obtained from 26 teeth extracted from patients of different ages for periodontal or orthodontic reasons. Teeth were cavity-free and without large deposits of tartar. Using a flexible double-sided diamond disc and continuous cooling with distilled water, teeth were sectioned into 104 enamel fragments of 5mm/ 4mm/2mm (4 quadrants each). In order to simulate the natural conditions from the mouth, enamel fragments were kept at a constant temperature of 37 °C in freshly prepared artificial saliva, for 12 h.

The fragments were initially divided into 4 groups: a negative control group (S) of 8 samples kept only in artificial saliva, and 3 experimental groups of 32 samples each (W1, W2, W3), which were immersed in the 3 different waters. The three experimental groups were further divided into 4 sub-groups of 8 samples each, according to the treatment used: 0 – no treatment (positive controls), P – toothpaste, C – remineralizing cream, and V – protective fluoride varnish (table 1).

Preparing the artificial saliva

The chemical composition of the prepared artificial saliva was: 0.4 g NaCl, 0.4 g KCl, 0.795 g $CaCl_2$. H_2O, 0.69 g NaH_2 . PO_4 0.005 g $Na_2S{\cdot}9H_2O$, 1.0 g urea, and distilled water to 1000 mL. Its buffering capacity was determined using N/10 NaOH titration in the presence of Bogen's indicator.

Table 1
CODING OF THE ENAMEL SAMPLES

Negative controls	Positive controls	Treatment with paste	Treatment with cream	Treatment with varnish
S	W10 swimming pool	W1P	W1C	W1V
	W20 with adjusted pH	W2P	W2C	W2V
	W20 with unadjusted pH	W3P	W3C	W3V

Preparing chlorinated waters

One of the water samples was collected from a local Olympic swimming pool (W1, measured *p*H 7.11). The other two samples were prepared in the laboratory from grinded pills, according to the formula of a commercial disinfectant used for pool maintenance: 1 mg of chloride to 1 L of tap water. These samples had a *p*H of 5.06 (W2, obtained with 0.1 M HCl), and 7.46 respectively (W3). A portable digital Hanna Combo H198129 pH meter (Hanna Instruments, Smithfield, USA) was used for all *p*H measurements. The three chlorinated waters' buffering capacity – as determined by titration – were 0.04 mmol/L NaOH for the pool water, and 0.4 mmol/L NaOH for the two prepared samples.

Reproduction of demineralization and mineralization

For demineralisation, enamel samples were placed into the 3 chlorinated waters for 24 h at room temperature (positive control groups W10, W20, W30).

For mineralisation, enamel samples were treated with three dental materials: a toothpaste (groups W1P, W2P, W3P), a cream for remineralisation (groups W1C, W2C, W3C), and a fluoride varnish (groups W1V, W2V, W3V). Treatments were applied for 5 min just before immersing the samples in the different waters. The paste and the cream were applied on the enamel surface in a layer of 0.5 mm, while the varnish was applied by brush on the dry surface according to manufacturer directions. These materials have different fluoride concentrations, and are known for their remineralising effect.

Scanning electron microscopy (SEM)

Treated and untreated enamel samples were compared to the negative control group of normal enamel samples, with all samples preserved in artificial saliva until prepared for SEM analysis.

After mounting on aluminium stubs (Agar Scientific Ltd., UK) using double-sided adhesive carbon tabs and colloidal silver for electric conductibility, enamel samples were dehydrated for 1 h at 0.15 Torr in a Polaron E- 5100 sputter coater (Polaron Equipment Ltd, Watford, UK). They were metallised with gold in the same device at 0.04 Torr for 1 minute at 2400 V and 30 mA and examined at 25 kV and different magnifications in a JEOL JSM - 25S scanning electron microscope (Jeol Ltd., Tokyo, Japan). Images were captured with a Pixie 3000 system (Deben Ltd., Debenham, UK).

Results and discussions

SEM analysis

Control groups – negative control (S): examination of the control samples preserved in artificial saliva revealed the smooth, normal ultrastructural aspect of the dental enamel surface. Although the enamel displayed superficial scratches and bacteria, its aprismatic layer was relatively uniform in all negative control samples (fig. 1A,B).

Control groups – positive controls (W10, W20, W30): all 3 chlorinated waters produced ultrastructural changes of the enamel surface (fig. 2A, 3A, 4A). When comparing enamel fragments immersed in different waters, important changes were only present in the samples kept in the modified *p*H water (group W20) (fig. 3A), with altered micromorphology of both aprismatic and prismatic structures, penetrating into dentin. Samples introduced into pool water (W10) showed generally normal enamel, with a few areas of demineralisation and a heterogeneous aspect, but without destroying the prisms (fig. 2A). The same changes were observed in samples placed in the water with unchanged pH (W30), but on a much larger surface area, and in depth (Fig. 4A).

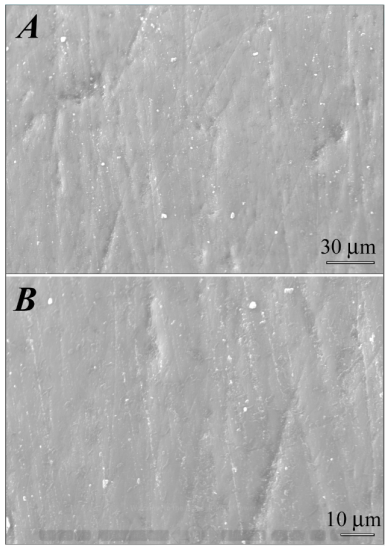

Fig. 1. Scanning electron micrographs of the surface of negative control enamel samples kept in artificial saliva (S), showing normal enamel surface ultrastructure, with uniform aprismatic layer, superficial scratches and bacteria

Experimentally treated groups W1P, W1C, W1V: samples introduced into the pool water after toothpaste treatment (W1P group) showed minimal changes of the enamel surface (fig. 2B) compared to negative controls; the changes only affected the aprismatic layer and were similar to those recorded in the corresponding positive controls (W10 group), but without the same level of heterogeneity. Compared to these, samples treated with the remineralising cream (W1C group) had more changes produced by the chlorinated water (fig. 2C), while samples treated with fluoride varnish (W1V) were not altered by the pool water (fig. 2D). In the latter samples, enamel surface showed no differences compared to the negative controls.

Experimentally treated groups W2P, W2C, W2V: from the enamel samples immersed in the water with modified *p*H, the ones treated with toothpaste (W2P group) showed minimal demineralisation areas, without denudation of the prisms (fig. 3B). Comparable changes were identified in samples treated with remineralising cream (W2C), but with a low degree of prisms denudation (fig. 3C). In the fluoride varnish group (W2V) samples were covered by a continuous layer of varnish, with only small cracks due to dehydration observed in some cases (fig. 3D).

Experimentally treated groups W3P, W3C, W3V: exposing enamel samples to the chlorinated water with unmodified *p*H led to minor surface changes, with limited distribution and only visible at high magnifications – this was observed in samples from W3P and W3C groups (fig. 4B,C). In samples from the varnish group (W3V), dehydration destroyed the varnish surface, revealing an enamel surface with normal appearance (fig. 4D).

As their training programs requires them to spend many hours in swimming pools, mostly with their head immersed under water, the oral cavity and teeth of performance

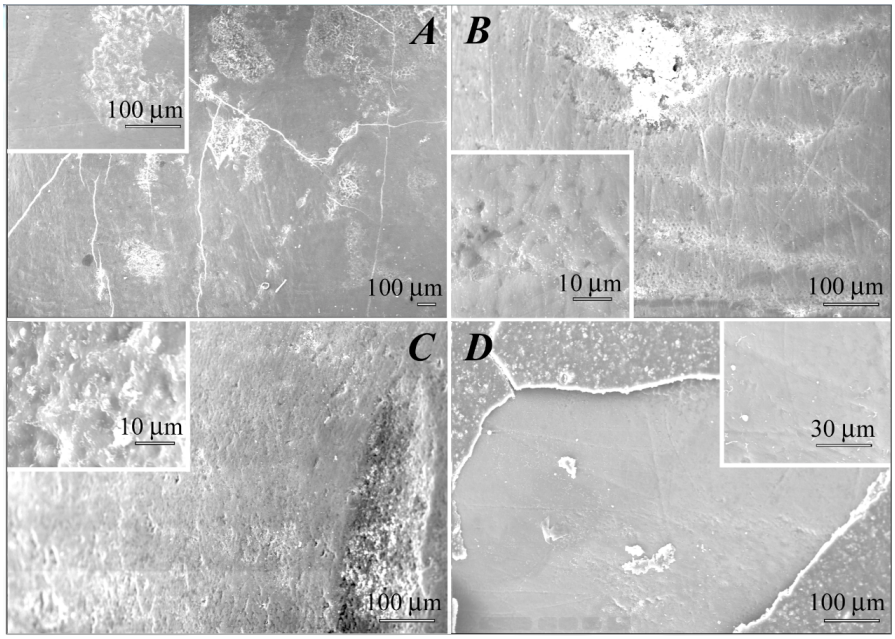

Fig. 2. Scanning electron micrographs of the surface of enamel samples after immersion in swimming pool water (W1): A – positive control sample (no treatment – W10) showing low levels of ultrastructural changes and a heterogeneous aspect, B – sample treated with toothpaste (W1P), C – sample treated with remineralising cream (W1C), and D – sample treated with fluoride varnish (W1D)

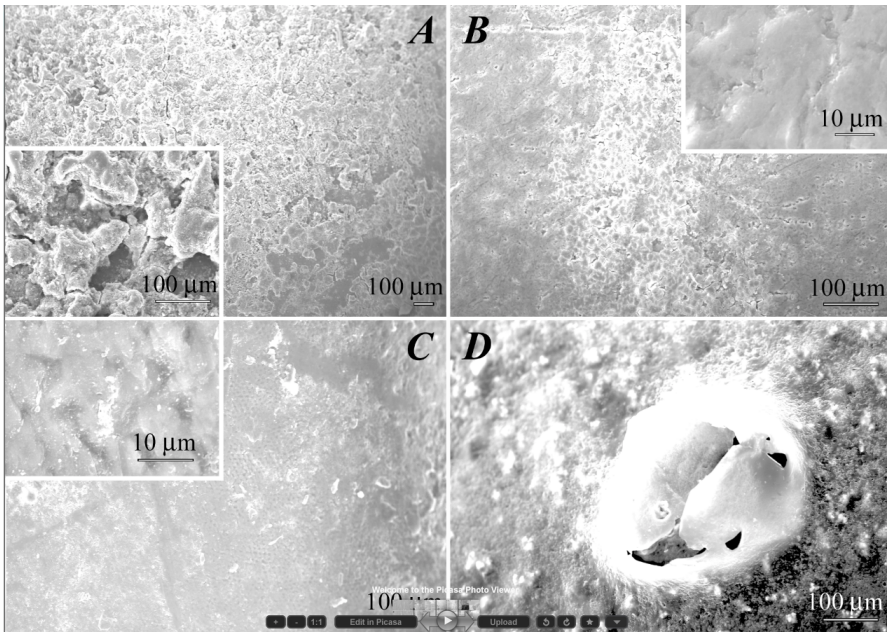

Fig. 3. Scanning electron micrographs of the surface of enamel samples after immersion in the modified pH water (W2): A – positive control sample (no treatment – W20) showing deep ultrastructural changes affecting the aprismatic and prismatic structure of enamel, B – sample treated with toothpaste (W2P), C – sample treated with remineralising cream (W2C), and D – sample treated with fluoride varnish (W2V)

swimmers are exposed to pool water for an extended period of time. Pool water has to ensure high standards of hygienic conditions, following strict recommendations, but with the assumption that substances used for water disinfection have limited effects on, or are safe for swimmers. However, studies have shown that swimming is a risk factor for dental erosion [6]. In addition, performance and occasional swimmers might also consume acidic foods and drinks known to be responsible for dental erosion, enhancing the negative effects of chlorinated water [7,8].

Chlorine is the most commonly used agent to maintain swimming pool water *p*H at a balanced level (7.2-7.8), and to prevent bacterial growth [2]. In our study we compared the demineralising effect of chlorinated water with a pH within the recommended limits (W3, *p*H 7.46), and 2 chlorinated waters with *p*H under these limits (W1 – *p*H 7.11, taken from a swimming pool, and W2 – *p*H 5.06, prepared in the laboratory). The water sample with the lowest *p*H (W2) showed the highest demineralisation, causing major ultrastructural changes of the enamel surface, with prisms destruction and penetration to dentin, while W3 had the lowest demineralising effect, with minimal changes of enamel structure. These results are comparable to those reported by other authors on the corrosive effect of acidic beverages with a *p*H of 2.85 to 5.6, showing the same pattern of dental enamel changes [9,10]. In addition, studies evaluating the erosion produced by chlorinated waters using profilometry have shown that the results depend on *p*H levels and exposure time [5,11].

The mechanism of dental erosion involves the release of calcium and phosphate ions from the enamel due to acids reacting with hydroxyapatite crystals in the structure of teeth. Thus, dental erosion can be quantified by measuring the absorption of calcium and phosphate from hydroxyapatite crystals. Dissolution of hydroxyapatite from the dental structures depends not only on low *p*H values of water, but also on the concentrations of calcium and phosphorus ions. This explains why even in case of a neutral *p*H swimming pool water, hydroxyapatite dissolution can occur due to the water being unsaturated in calcium and phosphorus ions [6]. Therefore, long-term exposure to such waters may be the main factor of dental erosion in swimmers. In our case, the 2 water samples prepared in the laboratory (W2, W3) had the same buffering capacity, 10x higher than that of the water taken from the swimming pool (W1), which explains the demineralisation produced by W2, even at a *p*H of 7.1. In a similar study using spectrophotometric analysis of calcium and phosphate absorption, we have shown that waters with smaller

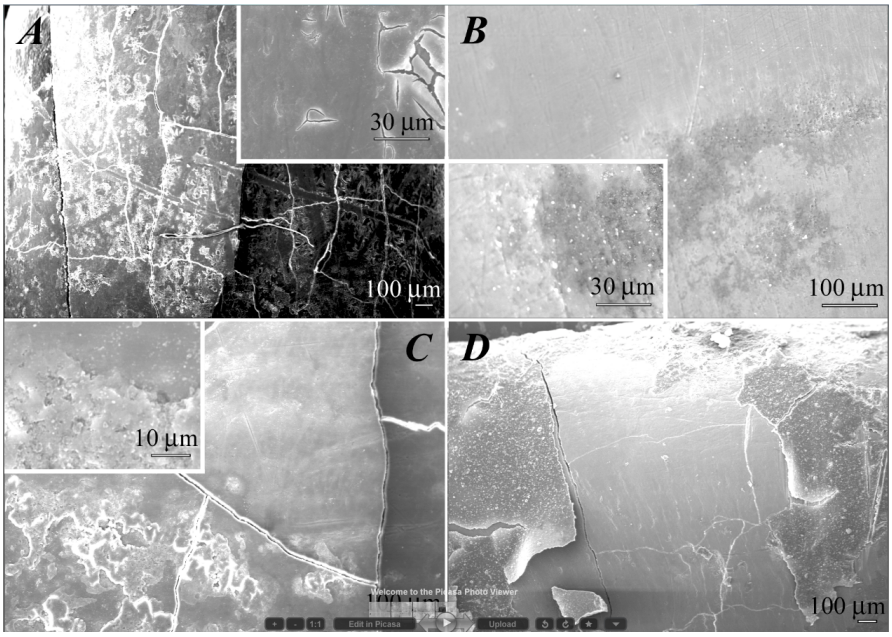

Fig. 4. Scanning electron micrographs of the surface of enamel samples after immersion in the water with unmodified pH (W3): A – positive control sample (no treatment – W30) showing relatively low levels of ultrastructural changes, but on larger areas, B – sample treated with toothpaste (W3P), C – sample treated with remineralising cream (W3C), and D – sample treated with fluoride varnish (W3V)

buffering capacity extract the greatest amount of calcium and small amounts of phosphorus, while waters with lower pH extract the largest amount of phosphates [12]. These results were similar to those obtained in studies on the erosive effect of acidic beverages [7,9].

Our study looked at remineralisation of dental tissues after applying three different protective dental materials: a toothpaste, a remineralising cream, and a fluoride varnish. Sensodyne Repair and Protect (GlaxoSmithKline, UK) toothpaste contains sodium monofluorophosphate (1450 ppm fluorine) and calcium sodium phosphosilicate with preventive effect on dental erosion and reduction of dentinal sensitivity. ReminPro (Voco, GmbH, Germany) is a cream that contains fluorine and hydroxyapatite nanocrystals with low toxicity and excellent biological effects, and anti-inflammatory and immunological response; the contained hydroxyapatite nanocrystals are the same as those found in the structure of dentine and enamel, and can bind to natural tissue and obturate microporosity. Bifluorid 10 (Voco, GmbH, Germany) is a fluoride varnish containing sodium fluoride and calcium fluoride equal to 23 mg and 24 mg fluorine, respectively, per 1 g of product. Compared to the untreated enamel samples, fluoride varnish offered the best protection against demineralisation, while the remineralising cream provided the lowest level of protection, as highlighted by SEM analysis. Enamel samples treated with Sensodyne Repair and Protect revealed large areas of minimal demineralisation caused by chlorinated waters as compared to samples treated with ReminPro, which showed only isolated areas of minimal demineralisation. In both cases, the enamel maintained its inter-prismatic structure, even in the 5.06 *p*H water, results comparable to those reported by other authors [13-16]. The fluoride varnish's protection might be due to the pellicle formed by it on enamel surface, which remained intact in almost all examined samples. In areas where dehydration destroyed this pellicle, we observed a normal structure of enamel surface, thus demonstrating the material's protective effect. Although some authors found similar protective effects in both *in vitro* and *in situ* studies [17-19], others reported that a higher fluoride concentration failed to protect the enamel against dental erosion [20-22].

Although the application of common materials with high concentration of fluoride is considered the method of election in preventing dental erosion [23], recent studies have questioned the usefulness of high concentrations of fluorine in the prevention of dental erosion. These suggest a better evaluation of the methods of prevention instead of those of fluorine therapy in patients with high risk of dental erosion. The mechanism by which fluorine exerts its protective effect against erosion is yet to be fully understood, but it is assumed that calcium fluoride forms a protective layer on the enamel surface. This might act both as a barrier protecting enamel surface against acid attack, and as a reservoir of fluorine ions to form acid-resistant fluoroapatite. Concentration of calcium fluoride consequently stored in the enamel increases in time, depending on fluoride concentration and calcium availability [22,24].

Because of its *in vitro* design, our study could not include the particular conditions of the oral cavity, with the remineralising effect of saliva due to its *p*H and buffering, as well as the salivary pellicle's composition and thickness. There were also limitations due to the working protocol, since enamel fragments came from different donors and were not identical in size. Studies demonstrated that the aspect of enamel surface is age-dependent [25], while their size differences could result in a relative large variation of the results. Additionally, although SEM can be used for qualitative analyses [26], the method is limited by the lack of information regarding the percentage of mineral loss, unless the microscope is equipped for Energy-Dispersive X-Ray Spectroscopy [27]. Future studies must consider these aspects in order to develop an effective preventive therapeutic strategy on dental erosion caused by chlorinated waters.

Conclusions

In this study, we demonstrated that improperly chlorinated swimming pool waters are responsible for changes in the ultrastructure of teeth enamel as evaluated by scanning electron microscopy. The three studied different *p*H chlorinated waters had the ability to demineralise the enamel *in vitro*, in a *p*H-dependent manner – the lowest pH water produced the most important changes in enamel ultrastructure. Among the three dental materials tested, fluoride varnish offered the best protection against demineralisation, by forming a resistant thin film at the enamel surface. Since chlorinated waters represent a risk factor for dental erosion, maintenance strategies are required to ensure optimal conditions of swimming pools. Performance swimmers would be the main beneficiaries

of specific preventive methods in order to avoid the erosive effect of swimming pool water.

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Manuscript received: 23.02.2016