The Erosive Effect Evaluation of Chlorinated Pool Waters by Using the Molecular Absorption Spectroscopy Method

Effective methods of enamel protection

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In the light of multiple research on dental erosion, by using the spectrophotometry absorption method, we have conducted a in vitro study with the aim to highlight the erosive effect of chlorinated pool water, as well as the protective role of a lacquer and of two different toothpastes with remineralizant effect. Followed targets: the erosive capacity evaluation of three chlorinated waters used for swimming pools, the quantitative determination of the calcium and phosphates concentration absorbed by these waters, the quantitative determination of the remineralization and protection capacity of some dental materials on the enamel exposed to the action of chlorinated waters. After exposing the enamel to the action of these waters, we determined that the swimming pool waters improperly chlorinated represent a risk factor for the performant and casual swimmers. These waters have the capacity to demineralize the dental enamel in vitro, the severity of the demineralization being influenced by the pH and the capacity of extracting different quantities of calcium and phosphates. The three materials used for protection are able to remineralize the dental enamel, the best effect being given by the application of fluorinated lacquers. Optimal maintenance strategies of the swimming pools and specific prophylactic methods for the performant swimmers are necessary.

Keywords: dental erosion, tooth wear, gas chlorinated water

The dental erosion is defined as being the loss of rough dental tissue due to intrinsic and extrinsic factors [1]. The loss of hard dental tissues provoked by dental erosion constitutes a major problem both for teenagers and adults [2]. Despite numerous in vitro and in vivo studies, the ethiopatogenic mechanisms are not completely understood [3-9]. The performant swimmers may be affected by dental erosions thanks to the effect of the chlorinated water in the swimming pools [10, 11]. The swimming pools are chlorinated in order to reduce the contamination with bacteria or algae through chlorine addition, with a concentration of 2-3 ppm. In water, chlorine produces hypochlorous acid and the hipochlor ion.

The *p*H is being adjusted to 7.5 by adding of acids and alkaline solutions. The tablets used for the disinfection of the swimming pools contain salts of the cyanuric acid to reduce the decomposition of the hypochlorous acid in the sun. If the acid is not neutralized by the sodium carbonate from the water, the water pH can drop to 3 [12]. The 5.5 *p*H is considered to be a critical verge for the dental erosion. If the solution *p*H is lower than the critical value, the solution is unsaturated, though the mineral substance inclines to dissolve itself until the solution will become saturated. The saliva and the liquid from the dental plaque are normally oversaturated, the minerals not getting dissolved in them [13]. The dental enamel is made of hydroxyapatite and impurities such as the carbonates or the fluorine which can influence its solubility. When getting in contact with water, the hydroxyapatite dissolves itself, freeing calcium, phosphates and the hydroxyl ion. The process continues until the water is saturated without the change of the hydroxyapatite. From this point of view the speed of demineralization will be equal to the one of mineral precipitation. The higher the calcium and phosphates concentration is, that much smaller will be the critical value of the *p*H [14]. When a solution/liquid comes in contact with the enamel surface it has to disseminate through the film formed by the proteins and glycoproteins of the saliva and just afterwards it will be able to interact with the mineral phase of the tooth. The young film is a barrier against erosive agents. When the film is mature and weaker, the diffusion process of the acid solution may begin. In contact with the enamel, through the hydrogen ions or through the chelation capacity, the acids are able to dissolve the mineral crystals. The non-ionised stage of the acids will disseminate in the interprismatic spaces of the enamel, dissolving the minerals and leading to the release of calcium or phosphorus ions and to the growth of the *p*H [15]. The process stops when a new acid or chelating agent is present. The *p*H value, the calcium and phosphates, as well as the fluorine content in liquids and food have an influence on the erosive attack [15]. The application of a sodium fluoride gel which interacts with the enamel forming the fluorapatite, less soluble, can reduce the critical value of the *p*H [16]. The molecular absorption spectrophotometry is a high precision method for the determination of small mineral quantities extracted from the dental tissues [16-18].

Experimental part

We conducted a in vitro study in order to highlight the erosive effect of chlorinated pool waters, as well as the prophylactic effect of some dental materials after the protocol designed by Rirattanapong, 2011[19]. By sectioning extracted teeth from patients with different ages, we obtained 96 enamel fragments, with dimensions of 5mm/4mm/2mm, without caries or tartar deposits. These were stored in containers with artificial saliva, at a constant temperature of 37^0C, in thermostat for 12 h. The

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Mineralizing agent	Composition
Sensodyne Repair and Protect GlaxoSmithKline, SmithKline Beecham Ltd, EUCHCQ	Glycerin, PEG-8, sodium phosphosilicate and calcium (NOVAMIN), sodium lauryl sulfate, sodium monofluorophosphate, tastes, titanium dioxide, carbomer, acetate potassium sulfate. sodium monofluorophosphate 1,08% (1450 ppm fluorine)
Remin Pro, VOCO GmbH, Germany	Hydroxyapatite (calcium and phosphates), fluorine, tastes.
Bifluoride 10, VOCO GmbH, Germany	Ethyl acetate, sodium fluoride, calcium fluoride, cellulose ester, eugenol. 1g Bifluoride 10 contains 50mg sodium fluoride (equal with 23mg fluorine) and 50mg calcium fluoride (equal with 24mg fluorine)

Table 1
THE CHEMICAL COMPOSITION OF THE PRODUCTS USED FOR MINERALIZATION

artificial saliva has been prepared in the laboratory using the following formula (1):

Sodium chloride (*NaCl*), 0.400g; *potassium chloride (KCl)*, 0.400g; *calcium chloride* ($CaCl_2H_2O$), 0.795g; *sodium phosphate acid* (NaH_2PO_4), 0.69g; *sodium sulfite* ($Na_2S\cdot 9H_2O$), 0.005g; *urea*, 1.0g; *distilled water*, 1000mL

By adding hydrochloric acid, the *p*H has been adjusted to 7. A water sample has been harvested from a Olympic swimming pool. Two samples have been prepared in the laboratory with the help of pills according to the recipe on the label, adding 1 mg of chloride from the pill in 1L tap water. One prepared water sample has been maintained at the recommended pH value of 7.4-7.6. The *p*H of the other sample has been adjusted to 5.06 by adding 0.1 M hydrochloric acid to 1L water. A digital portable Hanna Combo model H198129 pH-meter has been used for the measurements. The calibration has been made in one-two points, with standard buffer values, after each determination. Each determination has been realized twice, taking into account the average value of the records. We determined the tamponage capacity of the artificial saliva and of the chlorinated waters by using the titration method with phenolphthalein as indicator, phosphate buffer solution with *p*H=7 and sodium hydroxide solution N/10. From each water sample we have titrated 10 mL and we have then registered the values.

The calcium concentration in artificial saliva and waters has been determined before and after the immersion of the enamel fragments with the spectrophotometer Spekol UV/VIS, programmable with scanning. This uses the molecular absorption specrtophotometry technique. For the calcium dosage from the solution, we used the Calcium Arsenazo III reagent, which contains 100mmol/L MES, at a *p*H=6.5 and 200μmol/L Arsenazo III. The principle of the method consists in the formation, at a neutral *p*H, of a complex between the calcium ion (Ca^{2+}) and the reagent Arsenazo III, the color intensity being directly proportional with the calcium concentration of the analyzed sample. The reagent has been prepared with a absorbance of 1.148, at a wavelength of 600nm and a temperature of 37^{0}C. We prepared a blanc and a standard sample out of 10ml reagent and 10μL bidistilled water. The extinction has been read a minute after the mixing and the incubation. The calibration, with the Arsenazo method, has been made at a temperature of 37^{0}C with bidistilled water and a calcium calibrator Calcium Standard Cat. No.:52001. The calculation of the calcium concentration has been made using the following formula (2):

Sample absorbance/ standard absorbance x standard concentration= sample concentration, (standard concentration = 10.79mg/dL)

The analyzed solutions have been introduced into the machine in identical cuvettes with the standard and blanc solutions. The calculation of the absorbed calcium quantity by the three waters has been made through the difference between the values measured before and after the immersion of the tested samples.

The phosphorus absorption has been determined the same way, in the presence of the following reagents: ammonium molybdate solution from the acidic medium, hydroquinone solution 1% and sodium sulfite solution 20%. The principle of the method consists in the reaction between the inorganic phosphate from the chlorinated water and the ammonium molybdate, which form the ammonium phosphomolybdate (a yellow colored complex). This is reduced to molybdate blue by the hydroquinone and then with sodium sulfite. The intensity of the blue color is proportional with the quantity of inorganic phosphate from the sample, the color produced by the molybdate oxides having the ability to be subjected under photometry. The calibration has been done after a standard sample in the presence of the trichloroacetic acid 20% and the prepared reagents, the extinction being read at a wavelength of λ=600nm. The phosphate determination has been made reporting the extinction of the read sample towards the blanc to the calibration curve.

The mineralization of the enamel fragments has been realized using toothpaste P1, cream P2 and fluorine varnish P3, applied on the surface of the enamel in a 0.5 mm layer, for 5 min, according to the indications of use in the prospectus (table 1).

The demineralization has been realised by introducing the enamel fragments into the three chlorinated waters, 24 hours, at room temperature. The 96 enamel specimens have been divided into three groups: A1, A2, A3 and each group into 4 other subgroups in accordance with the conducted treatment: 0-without treatment, P1-toothpaste, P2-remineralizant cream, P3-protective fluorine varnish.

The statistical analysis has been made with the help of the GraphPadPrism programme, by applying the T student tests Wilcoxon and Kruskal-Wallis [20]. The significance verge has been established to be <0.05.

Results and discussions

The measured values of the *p*H, of the tamponage capacity and of the calcium and phosphorus concentration in the chlorinated waters before the enamel fragments immersion are presented in table 2.

Table 2
THE CALCIUM AND PHOSPHATES CONCENTRATION CONTAINED BY THE CHLORINATED WATERS BEFORE THE INTRODUCTION OF THE ENAMEL FRAGMENTS, THE *p*H AND THEIR TAMPONAGE CAPACITY.

Water	pH	Tamponage capacity (mmol/L NaOH)	Calcium concentration (mg/dl)	Phosphorus concentration (mg/dl)
Pool water	7.11	0.04	10.459	0.043
Prepared water with adjusted pH	5.06	0.4	12.645	0.0062
Prepared water with unadjusted pH	7.46	0.4	11.853	0.0109

The calcium concentration values of the water samples are shown in figures 1- 3.

The phosphorus concentration values are illustrated in figures 4-6.

The performant swimmers make exercises that require maintaining their head under water for a long period of time in which their teeth are constantly exposed to the water in the swimming pool. For maintaining hygienic conditions in the swimming pools there are some strict recommendations to follow. It is known that the added substances for disinfection have a limited effect or that they are even inoffensive for the swimmers. However, there are studies that sustain swimming is a risk factor for the appearance of dental erosions [21, 22]. The *p*H we have measured for the three chlorinated waters finds itself within the recommended limits, 7.46, for the water prepared in the laboratory, under these limits, 7.11, for the swimming pool water and 5.06 for the one prepared in the laboratory by adding chlorine. The small values of the *p*H are not exclusively responsible in the dissolution process of the hydroxyapatite within the dental structures, but also the concentration of the calcium and phosphorus ions which have as an effect its saturation. Although the water has a neutral *p*H, the dissolution of the hydroxyapatite can occur due to the fact that the pool waters do not get saturated [22]. The long time exposure to this water type or their continuous renewal may be one of the main factors of these modifications. We must also take into consideration the fact that both the performant and the occasional swimmers often consume acid beverages and foods, known as responsible for the dental erosion [17, 23]. The pool water with low tamponage capacity has extracted the biggest calcium quantity, but a smaller phosphates quantity, while the water with *p*H = 5.06 has extracted the biggest quantity of phosphates. The comparison of the obtained data can be made just with information from studies on the erosive effect of different acid beverages on the dental enamel [18, 23, 24].

The dental enamel is the tissue with the highest mineral concentration in the organism. During the erosion, after the reaction between the hydroxyapatite crystals and the acids, phosphates and calcium ions are released from the enamel [25]. The determination of the enamel demineralization by measuring the calcium and phosphates absorption from the hydroxyapatite crystals represents a quantification method of the dental erosion.

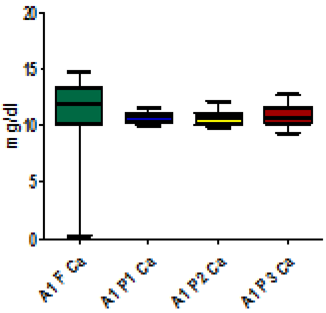

Fig. 1 The comparative graphic representation of the calcium absorption capacity by the pool water, according to the applied treatments.

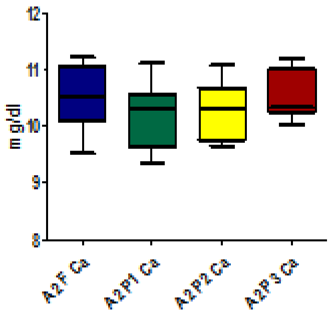

Fig. 2 The comparative graphic representation of the calcium absorption capacity by the prepared chlorinated water, with adjusted *p*H, according to the applied treatments.

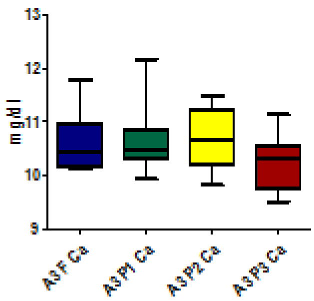

Fig. 3 The comparative graphic representation of the calcium absorption capacity by the prepared chlorinated water, with unadjusted *p*H, according to the applied treatments

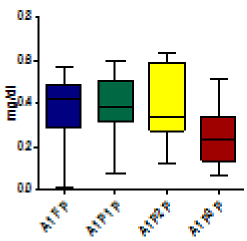

Fig. 4 The comparative graphic representation of the phosphorum absorption capacity by the pool water, according to the applied treatments.

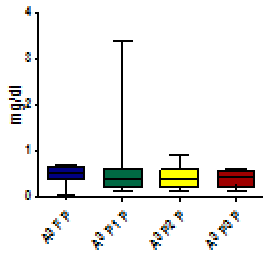

Fig. 5 The comparative graphic representation of the phosphorum absorption capacity by the prepared chlorinated water, with unadjusted *p*H, according to the applied treatments.

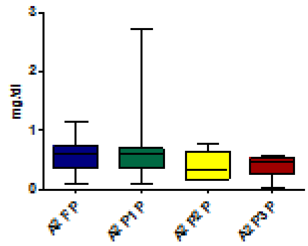

Fig. 6 The comparative graphic representation of the phosphorum absorption capacity by the prepared chlorinated water, with adjusted *p*H, according to the applied treatments.

This study is among the few ones that analyzed the erosive effect of chlorinated waters on the dental enamel, using the molecular absorption spectrophotometry.

The erosive capacity of some chlorinated waters has also been observed through profilometry. [26] In vitro or in situ studies have shown the protective effect of these products, while others sustain that a high fluorine concentration does not bring any benefits to the protection against dental erosions [27-29].

Conventional toothpastes cannot efficiently protect the enamel against erosions [30]. The Sensodyne Repair and Protect toothpaste contains sodium monofluorophosphate (1450 ppm fluorine), sodium phosphosilicate and calcium, with preventive effect for the dental erosions and reduction of the dentinal sensitivity effects. The ReminPro cream with fluorine and hydroxyapatite nanocrystals content has excellent biologic effects, a low toxicity, is anti-inflammatory and has a immunologic response. The hydroxyapatite microcrystals are identical to those in the dentine and enamel structure. Their activity inside the enamel consists in the ability to bind themselves to the natural tissue, closing the microporosities at this level.

By the spectrophotometrical comparison of the phosphates and calcium concentration extracted from the enamel fragments immersed in the three waters with demineralizant effect, treated with the two toothpastes, the differences between the values found were very small. The results we obtained are similar to those shown by other authors [31-34]. There are few studies in the specialty literature referring to the protection methods against demineralization produced by chlorinated waters and these are more related to the protective effect of some ordinary toothpastes or mouthwashes [19, 35, 36].

This study is a experimental one, disregarding the conditions in the oral cavity. The demineralization produced by the studied chlorinated waters varies according to the enamel fragments subjected to erosion in different conditions and to the applied treatments. These variations cannot be detailed explained because they come from different donors and the dimensions of the fragments were not identical. The qualities of the saliva, the *p*H, the tamponage capacity, as well as the composition and the thickness of the salivary film, recognized as having a remineralizant effect, should be also taken into consideration.

Conclusions

The waters from the swimming pools improperly chlorinated represent a risk factor for both performant and occasional swimmers.

The tested waters have the capacity to demineralize the dental enamel in vitro, the severity of the demineralization being influenced by the pH. The three types of water have the capacity to extract different quantities of calcium and phosphates out of the enamel structure, the highest quantity being extracted from the fragments treated with fluorine varnish.

The molecular absorbtion spectroscopy is a efficient quantitative method of demineralization testing and through chemical measurement of the demineralization which appears after erosive attack, we can detect small quantities of extracted minerals.

Strategies regarding optimal conditions maintenance of the swimming pools are necessary, taking in consideration the increasing preoccupation of the population, no matter their age, for sport.

The performant swimmers must benefit of specific prophylactic methods in order to avoid the erosive effect of waters from the swimming pools they train in.

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