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In situ analysis of usefulness of strontium-fluoride toothpaste for enamel remineralization

Przydatność pasty do zębów z dodatkiem fluoru i strontu do remineralizacji szkliwa – badania *in situ*

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Abstract

Aim. The aim of the study was to investigate the usefulness of an experimental fluoride toothpaste supplemented with Sr^{2+} for enamel remineralization.

Material and methods. Two experimental groups comprised volunteers aged 20–30 years who had all the oral cavity sanation procedures carried out and all the primary and secondary caries lesions filled before undergoing investigations. The material included twenty healthy premolar teeth extracted for orthodontic reasons due to their abnormal position in the arch or to teeth crowding in 12–14 year old children. The teeth were sectioned into blocks and artificially demineralized. The samples were placed in the oral cavity on the buccal surfaces of the first molar teeth of volunteers who used toothpaste supplemented with Ca^{2+}, $P0_4^{3-}$, F^- (group I), toothpaste supplemented with Ca^{2+}, $P0_4^{3-}$, F^-, Sr^{2+} (group II) and control toothpaste supplemented with Ca^{2+}, $P0_4^{3-}$ (Hydroxyapatite-HAP). The content of calcium and phosphorus was analyzed on the lateral walls of the enamel lesions, at preselected depths of 15 μm and 100 μm, by using EDS microanalysis.

Results. After three months, the content of calcium in both studied groups was significantly lower at 15 μm and 100 μm of enamel depth than at the baseline (immediately after enamel demineralization). After six months, the content of calcium was lower only at 15 μm in both groups. At 100 μm of depth the calcium content was significantly higher in group II and the reference group (HAP). After three months the phosphorus content was significantly lower in group II at 15 μm. After six months the content of phosphorous was significantly higher in group II at 15 μm and 100 μm of enamel depth.

Conclusion. The results suggest that supplementation of fluoride toothpaste with strontium improved the effect of enamel remineralization.

Keywords: human teeth, enamel remineralization, strontium-fluoride toothpaste, EDS microanalysis.

Streszczenie

Cel. Określenie użyteczności eksperymentalnej fluorkowej pasty do zębów wzbogaconej jonami strontu do remineralizacji szkliwa

Materiał i metody. Utworzono dwie grupy badawcze złożone z ochotników w wieku 20–30 lat, u których przed rozpoczęciem badań przeprowadzono sanację jamy ustnej, wypełniając wszystkie ubytki próchnicowe. Materiałem badawczym było 20 zdrowych zębów przedtrzonowych usuniętych z powodów ortodontycznych u dzieci w wieku 12–14 lat. Zęby pocięto na bloczki (próbki), w których wywołano sztuczną demineralizację. Próbki umieszczano w jamie ustnej na powierzchniach policzkowych pierwszych zębów trzonowych ochotników, którzy przez okres 3 i 6 miesięcy stosowali pastę wzbogaconą jonami Ca^{2+}, PO_4^{3-} i F^- (grupa I) jonami Ca^{2+}, PO_4^{3-}, F^- i Sr^{2+} (grupa II) oraz pastę bazową z jonami Ca^{2+}, PO_4^{3-} (grupa kontrolna HAP). Zawartość wapnia i fosforu analizowano na bocznych ścianach próbek szkliwa na głębokości 15 i 100 μm za pomocą mikroanalizy EDS.

Wyniki. Po trzech miesiącach obserwacji zawartość wapnia we wszystkich badanych grupach była istotnie niższa na obu badanych poziomach 15 i 100 μm (ściana boczna) niż uzyskana bezpośrednio po demineralizacji; po sześciu miesiącach zawartość wapnia była niższa tylko na poziomie 15 μm we wszystkich badanych grupach. Na poziomie 100 μm natomiast, zawartość wapnia była istotnie wyższa w grupie II i grupie kontrolnej (Hydroksyapatyt). Zawartość fosforu była istotnie niższa w grupie II na poziomie 15 μm po 3 miesiącach badań, natomiast po sześciu miesiącach istotnie wyższa w grupie II na poziomie 15 i 100 μm w stosunku do uzyskanej bezpośrednio po demineralizacji.

Wnioski. Wyniki badań sugerują, że dodatek jonów strontu do fluorkowej pasty do zębów wpływa pozytywnie na remineralizację szkliwa.

Słowa kluczowe: zęby, remineralizacja szkliwa, fluorkowa pasta z jonami strontu, mikroanalizy EDS.

Introduction

The role of trace elements, including fluoride, in the incidence of dental caries has long been investigated. A high content of strontium in drinking water and soil results in low caries incidence [1, 2, 3]. However, the effect of certain strontium compounds on the human`s body should not be ignored. The content of trace elements in drinking water approved by Polish and European Union standards and the level of strontium in drinking water in Poland do not indicate any toxicological activity [4].

Strontium leads to changes in the apatite crystal structure in the surface layer of enamel [5, 6] and formation of a strontium apatite complex [$Ca_6Sr_4(P04_4)_6(0H)_2$]. The incorporated strontium apatite complex makes enamel more resistant to demineralization than calcium apatite does [7]. Recent in vitro studies indicated that strontium can enhance the effect of enamel remineralization [8]. Strontium salts have been also widely used as components of dentifrices and mouth rinses in alleviating the symptoms of uncomplicated dentinal hypersensitivity. Their effectiveness has been well recognized and documented.

Results of clinical and laboratory tests attempting to determine the effects of strontium – supplemented mouth rinses [9, 10], gels [11] and toothpastes [12] on dental hard tissues have been inconclusive, although recent findings suggest that a toothpaste supplemented with strontium- can diffuse into decalcified enamel increasing its resistance to caries [13].

Fluorides still play a predominant role in caries prevention and most of the marketed toothpastes are supplemented with fluoride. Some studies indicate that the reduction in caries incidence may be an effect of a joint action of strontium and fluoride ions on dental enamel which inhibits a dissolution of the apatite [12, 14, 15, 16]. Therefore, the effect of combining strontium and fluoride in treating caries needs to be tested.

Thus, the aim of our study was to investigate remineralization potential after application of an experimental fluoride toothpaste supplemented with strontium.

Material and methods

Experimental toothpastes

Two types of toothpastes were used in the study: calcium, phosphorus and fluoride (1000 ppm) supplemented toothpaste (F toothpaste), and calcium, phosphorus, fluoride (1000 ppm) and strontium (250 ppm) supplemented toothpaste (F+Sr toothpaste). As a base toothpaste – (the control group) was used such, containing calcium and phosphorus (Hydroxyapatite) (HAP). Toothpastes were prepared using a saturated solution of precipitated hydroxyapatite obtained in the following reaction:

$$6Na_2HP0_4 + 10CaCl_2 + 8Na0H \_\_\_\_\_\_ \_\_\_\_\_\_ Ca_{10}(P0_4)_6(0H)_2 + 20NaCl + 6H_20$$

Preparation of the calcifying solution:

Assuring a continuous mixing, equal volumes were mixed of the two principal solutions of

60 mM Na_2HP0_4 and 100 mM $CaCl_2$. During mixing of the two solutions, pH of was controlled so that its value would not drop below 6.95. A decrease in pH was corrected by supplementation of 1 M K0H. The final saturated (and containing a sediment) solution manifested a stable pH of 7.00–7.05.

Depending on the type of the prepared calcifying solution, before mixing of the principal solutions, solution of disodium phosphate was supplemented with sodium fluoride while that of calcium chloride was supplemented with strontium chloride. One or both of the supplements were added in such an amount that in the volume of water equal to the volume of the calcifying solution they would provide concentrations 1000 ppm and 250 ppm of F and Sr, respectively.

For preparation of a calcifying paste, the calcifying solution was used together with the sediment suspended in the solution.

Preparation of the calcifying tooth paste:

100 g paste contained:

- sorbitol, 70% solution 40 g
- silica (grains of 8–19 μm in diameter) 22 g
- other components, i.e. whitening agents, preservatives, polishing, taste and smell correcting agents and a densifier 6.2 g
- calcifying solution (suspension) 30.0 g

Human enamel and the in situ model

Twenty healthy premolar teeth extracted for orthodontic reasons from children aged 12–14 years were used for this study. After extraction the teeth were rinsed in tap water with a detergent. Remnants of the soft tissue were removed, the apex of each tooth root was then amputated and the pulp extirpated. The teeth were finally cleaned in distilled water using ultra sound. The teeth were cut into blocks of 2x2x1.5 mm using of a diamond blade cooled with distilled water (blade thickness = 0.1 mm). These blocks (samples) were then coated with nail polish, except for their enamel surfaces, which were exposed for 5 days to a decalcifying gel of buffered 0.025 M lactic acid at pH 4.5 and 37 °C.

Twenty-four samples were selected for Energy Dispersive Spectrometry (EDS) microanalysis to measure the concentration of Ca and P in the lateral walls of the demineralized enamel at 15 and 100 μm of depth (**Figure 1**). The samples were autoclaved (10 minutes, 134 °C, 0.22 MPA) for the *in situ* experiment. Six healthy volunteers, aged 20–30 years, took part in this experiment. Before

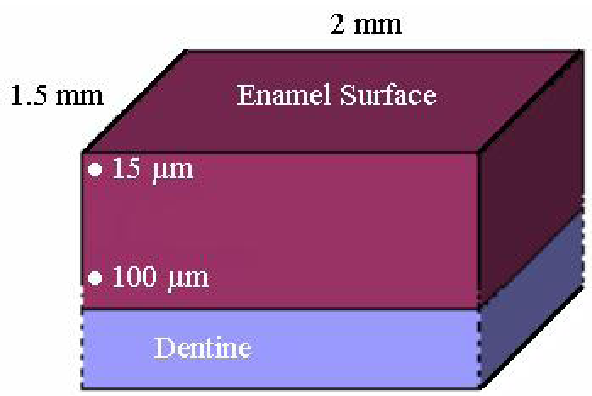

Figure 1. Schematic diagram of enamel sample – enamel-dentine block

Rycina 1. *Schemat próbki szkliwa: blok szkliwno-zębinowy*

undertaking the experiment a thorough prophylaxis was carried out and all the carious lesions in the participants were filled. The enamel blocks were cemented to the buccal surfaces of the first upper molar teeth using 3M Single Bond and Valux composite material. Only the natural enamel surface was directly exposed to the oral cavity (**Figure 2**). All participants were instructed to brush their teeth twice daily for two minutes with the experimental toothpastes and given toothbrushes. They were instructed to discontinue any other oral cavity hygiene procedures and they had their toothbrushes replaced every month. Participants were randomly assigned to three experimental groups (three persons to each group): group I (brushing teeth by using toothpaste supplemented with Ca^{2+}, $P0_4^{3-}$, F^-) and group II (using toothpaste supplemented with Ca^{2+}, $P0_4^{3-}$, F^-, Sr^{2+}) and the reference group using synthetic hydroxyapatite toothpaste (HAP). The study samples were removed from the oral mouth after three and then six months of tooth brushing with the experimental toothpastes. The content

Figure 2. *In situ* model – enamel sample placed in the oral cavity environment on buccal surfaces of the first upper molar teeth

Rycina 2. *Model in situ – próbka szkliwa umieszczona w środowisku jamy ustnej na policzkowej powierzchni pierwszego zęba trzonowego górnego*

of Ca and P was measured at 15 and 100 µm of dental enamel depths by EDS microanalysis using a Jeol Scanning Microscope (JSM) 35 C connected to a X-ray detector. The enamel samples were coated with carbon under vacuum.

The Regional Ethics Committee approved the study and each participant signed the informed consent.

Statistical analysis

The Shapiro-Wilk's test was first performed to check the normality of our results. Since the results have not passed the normality test, the exact nonparametric methods were used for statistical evaluation.

Differences between two independent groups (group I and group II) were evaluated by using the exact Mann-Whitney test. Results obtained at three and six months after applying the experimental toothpastes were compared to those obtained before the experiment, by using the exact Wilcoxon test. Content of Ca and P at 15 µm and 100 µm of enamel depth was also compared by the exact Wilcoxon test. A p-value less than 0.05 indicated a significant difference between results. StatXact statistical software (Cytel Inc., USA) for small-sample statistical evaluation was applied.

Results

After three months, the content of calcium in all studied groups was significantly lower at 15 µm and 100 µm of enamel depth than at baseline (immediately after enamel demineralization). After six months, the content of calcium was lower only at 15 µm in all groups. At 100 µm of depth the calcium content was significantly higher in group II and the reference group (HAP) (**Table 1, Figure 3**). After three months the phosphorus content was significantly lower in group II at 15 µm. After six months the content of phosphorous was significantly higher in group II at 15 µm and 100 µm of enamel depth in comparison to the samples immediately after demineralization (**Table 2, Figure 4**).

Discussion

The *in situ* model [17, 18] consisted of enamel samples placed for three and six months in the oral cavity on the buccal surfaces of the first upper molar teeth. The use of *in situ* models is approved in dental research [9, 12, 17, 18]. Such placement is known to be the most reliable way to protect the samples against mechanical damage and the site of dental plaque formation – the latter being indispensable for the demineralization and subsequent remineralization process.

It was surprising to find out, that after three and six months of using the experimental toothpastes, the content of calcium in the lateral walls of the decalcified enamel samples was significantly lower at 15 µm and 100 µm of enamel depth, than before

Table 1. The effect of HAP, F and Sr+F toothpastes on calcium (Ca) contents (% wet) in lateral enamel walls after three and six months of the experiment compared to its content in decalcified enamel at baseline

Tabela 1. *Wpływ past do zębów: HAP, F i Sr+F na zawartość wapnia (Ca) na bocznych ścianach szkliwa po trzech i sześciu miesiącach badań w porównaniu do szkliwa poddanego demineralizacji*

Enamel depth	Decalcified enamel at baseline	The effect of toothpastes after 3 months			The effect of toothpastes after 6 months		
		HAP	F (Group I)	Sr+F (Group II)	HAP	F (Group I)	Sr+F (Group II)
15 µm	29.48 ± 2.28	28.63 ± 0.37 ****	27.92 ± 1.81 ***	28.23 ± 0.38 ****	28.91 ± 0.25 ***	28.85 ± 0.57 **	28.22 ± 2.49 *
100 µm	29.07 ± 0.52	28.63 ± 0.34 ****	28.57 ± 0.30 *	28.68 ± 0.32 **	28.32 ± 0.69 **	28.62 ± 0.41	29.65 ± 2.09 *

** p < 0.05 *** p < 0.001*
*** p < 0.01 **** p < 0.0001*

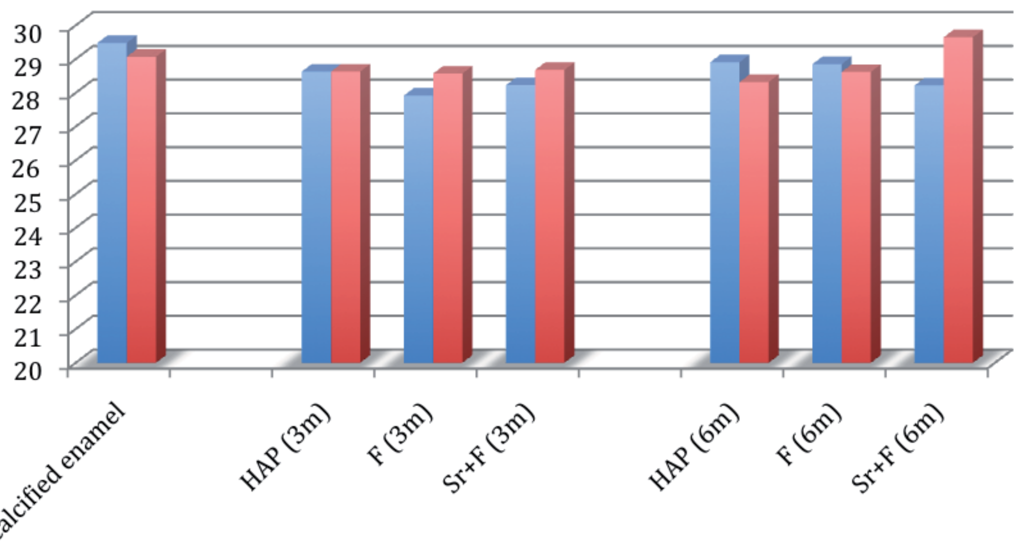

Figure 3. The effect of HAP, F and Sr+F toothpastes on calcium (Ca) contents (% wet) in lateral enamel walls after three and six months of the experiment compared to its content in decalcified enamel at baseline (blue 15 microns, red 100 microns)

Rycina 3. *Wpływ past do zębów: HAP, F i Sr+F na zawartość wapnia (Ca) na bocznych ścianach szkliwa po trzech i sześciu miesiącach badań w porównaniu do szkliwa poddanego demineralizacji (15 µm – niebieski, 100 µm – czerwony)*

Table 2. The effect of HAP, F and Sr+F toothpastes on phosphorus (P) contents (% wet) in lateral enamel walls after three and six months of the experiment compared to its content in decalcified enamel at baseline

Tabela 2. *Wpływ past do zębów: HAP, F I Sr+F na zawartość fosforu (P) na bocznych ścianach szkliwa po trzech i sześciu miesiącach badań w porównaniu do szkliwa poddanego demineralizacji*

Enamel depth	Decalcified enamel at baseline	After 3 months			After 6 months		
		HAP	F (Group I)	Sr+F (Group II)	HAP	F (Group I)	Sr+F (Group II)
15 µm	15.70 ± 0.22	15.69 ± 0.21	15.57 ± 0.27	15.41 ± 0.30 **	15.78 ± 0.13	15.70 ± 0.13	15.86 ± 0.54
100 µm	15.45 ± 0.31	15.60 ± 0.20	15.53 ± 0.20	15.32 ± 0.25	15.43 ± 0.34	15.66 ± 0.37	15.68 ± 0.11 *

** p < 0.05 ** p < 0.005*

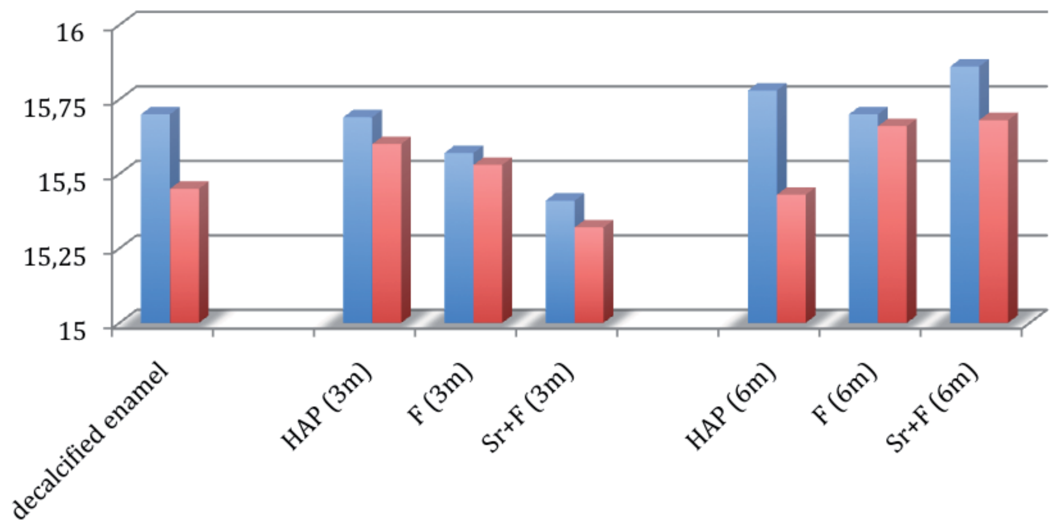

Figure 4. The effect of HAP, F and Sr+F toothpastes on phosphorus (P) contents (% wet) in lateral enamel walls after three and six months of the experiment compared to its content in decalcified enamel at baseline (blue 15 microns, red 100 microns)

Rycina 4. *Wpływ past do zębów: HAP, F I Sr+F na zawartość fosforu (P) na bocznych ścianach szkliwa po trzech i sześciu miesiącach badań w porównaniu do szkliwa poddanego demineralizacji (15 μm – niebieski, 100 μm – czerwony)*

the experiment. A concurrent decrease of the phosphorus content, with the exception of the deepest enamel layers, was also detected. These changes could be explained by further decalcification of demineralized samples due to their exposure to the dental plaque in course of the experiment. Schafer et al. [19] showed that in *in situ* conditions, the initially mild enamel decalcification may progress, although the degree of remineralization is directly proportional to the degree of enamel decalcification only for a certain time. Dijkman et al. [20] also pointed out, that artificially decalcified enamel is in a state of equilibrium with the saliva, however the decalcification time is unknown.

During the first three months of our experiment, the fluoride toothpastes were not so therapeutically effective as we expected, although differences between the content of calcium decreased with depth (i.e. the deeper the layer the smaller the difference). Such changes of calcium content associated with unchanged phosphorus content at 100 μm of dental enamel depth, could indicate a beginning of remineralization process in deeper enamel layers. The use of both experimental toothpastes for 6 months proved more conducive to the remineralization processes. Mean content of Ca and P increased, though not significantly, at 100 μm of depth in the group I (F toothpaste). However, there was significant increase of calcium and phosphorus content at 100 μm in the group II (F+Sr toothpaste). This confirms earlier studies in which the strontium, hydroxyapatite supplemented silica gels induced remineralization of artificially decalcified enamel in *in situ* conditions [21].

Upon chemical analysis, Luoma et al. reported a reduced loss of calcium from decalcified bovine enamel following its exposure to a fluoride and strontium ions supplemented mouth rinse, as compared to mouth rinses that were supplemented either with strontium ions or with fluoride ions only [15]. Similarly, the best therapeutic effect was obtained in our study following the exposure of the demineralized enamel to a toothpaste that contained both fluoride and strontium ions. These observations can suggest that the remineralization effect of fluoride ions is enhanced in the presence of strontium ions.

Various effects of remineralization of enamel lesions at different depths have been reported. In earlier *in vitro* studies, Silverstone [22] showed that the degree of remineralization was dependent of the concentration of Ca^{2+} ions (plus 0.05 mMF) solution. Low concentration of calcium (1 mM) produced a reduction in porosity from the base of the artificially created lesions to the surface, whereas the 3 mM Ca^{2+} solution only remineralized the surface of the lesion. The increased concentration of both Ca^{2+} and P_i at a depth of 100 μm but no corresponding increase in both the Ca^{2+} and P_i at 15 μm after six months application of Sr+F toothpaste (**Table 2**) would suggest that the remineralization was occurring from the base of the lesion towards the surface, thus imitating the effects reported earlier by Silverstone.

Very few studies, which carried out with strontium supplemented toothpastes showed increasing uptake and penetration of fluoride into partially demineralized enamel [12]. This may also explain a higher content of calcium in deeper layers of dental enamel in patients using Sr+F toothpaste when compared to those using the F toothpaste.

The *in vitro* experiment [16] showed that the content of strontium with fluoride (10 ppm Sr^{2+} and 1 ppm, 0.1 ppm or 0.05 ppm F^-) enhanced enamel remineralization and was dependent on the fluoride concentrations. In the present study, fluoride was added at 1000 ppm, and strontium at 250 ppm, similarly to the concentrations used by Spets-Happonen et al [11], who carried out the *in vitro* studies with chlorhexidine-fluoride gel.

Conversely, Shigemi et al [23] investigated a relationship between dental caries and the strontium levels in saliva of school children. They suggested that the saliva strontium level increases because of caries susceptibility and fluoride treatment inhibits strontium dissolution by reducing the solubility of enamel.

A synergy between fluoride and strontium in enhancing remineralization still needs further studies.

During the last twenty years, most of studies on caries prevention focused on the use of fluoride, whereas in the seventies and eighties there the interest in strontium`s cariostatic role was quite significant. In later nineties, a renewed interest in the role of strontium in caries prevention has appeared indicating that the biological monitoring of strontium levels serum, urine, bone, and soft tissues may help to identify its role in caries prevention [24].

Concluding, the results presented in this paper suggest, that a remineralization effect of fluoride in toothpastes is enhanced with Sr^{2+} in course of time.

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Conflict of interest statement
The authors declare that there is no conflict of interest in the authorship or publication of contribution.

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