FLUORIDE UPTAKE OF ENAMEL TREATED WITH DIFFERENT FLUORIDE AGENT

Hasić Branković Lajla*[1], Tahmiščija Irmina[1], Korać Samra[1], Džanković Aida[1], Konjhodžić Alma[1], Selimović Dragaš Mediha[2]

[1] Department of Restorative Dentistry with Endodontics, Faculty of Dentistry with Clinics, University of Sarajevo, Bosnia and Herzegovina

[2] Department for Preventive Dentistry and Pedodontics, Faculty of Dentistry with Clinics, University of Sarajevo, Bosnia and Herzegovina

*Corresponding author

Lajla Hasić Branković, PhD
University of Sarajevo,
Faculty of Dentistry with Clinics
Department of Restorative Dentistry with Endodontics
Bolnicka 4A, 71 000 Sarajevo,
Bosnia and Herzegovina.
E mail: lajlabrankovic@gmail.com
Phone: +387(33)214249

ABSTRACT

The aim of the paper was to examine the efficacy of individual topical fluoride preparations and CPP-ACP agents and to evaluate the efficacy of their combination on the processes in the initial carious lesion. The effectiveness of these agents was tested by assessing fluoride uptake using a fluoride-selective electrode.

Material and methods: The enamel samples used in this study were prepared with intact premolars extracted for orthodontic reasons. In order to produce an initial caries lesion, the enamel slabs were submitted to a pH cycling regimen. The specimens were distributed in four groups of 15 samples receiving the following treatment, respectively: Group I-1% NaF solution, Group II-1% TiF4 solution, Group III- 1% NaF+CPP-ACP, Group IV- 1% TiF4+CPP-ACP. Fluoride uptake was determined using a fluoride-selective electrode by measuring the concentration of fluoride ions in solution before and after immersion of the sample.

Results: The results showed that fluoride uptake was the largest in the IV experimental group (TiF4 +CPP-ACP). The fluoride uptake was evaluated as significantly higher compared to the other experimental groups. A surprising effect of blocking the influx of fluoride ions into the lesion occurred in group III.

Conclusion: The technique of the fluoride uptake measurement from the solution proved to be a relevant and valid method for estimating the initial carious lesion capacity for the fluoride ions absorption.

Keywords: white spot lesion, fluoride, fluoride-selective electrode, fluoride uptake, sodium fluoride, titanium tetrafluoride

Introduction

The current emphasis on new technologies of enamel remineralization suggests changes that occurred in our understanding of caries disease during the last century. Identifying white spots lesions (WSL) as an early symptom of carious disease, which can develop into cavitation, specified the moment when clinicians began to think about possibilities of non-invasive treatment to repair defects or even reverse the process of its occurrence. [1-3]

By their influence to the dynamics of the carious process, fluorides are proven to be very effective assets in slowing down the progression of carious destruction. [4] It is generally known that fluorides have two primary mechanisms by which they interfere with the dynamics of the carious process: the first is prevention of healthy enamel demineralization and the second is incorporation into previously demineralized enamel with the promotion of remineralization. The third mode of fluoride action is interference with bacterial metabolism. [5-9] The anticariogenic effect of fluoride depends on factors such as concentration, pH value, frequency and treatment duration, dosage and the optional addition of antimicrobial agents. [10,11]

Under the action of fluoride, changes have been observed to occur both on the surface and in the sub-surface zone of WSL. It has been shown that the critical pH at which demineralization and remineralization processes occur in maximum speed is between pH 4.3 to 5. A main microscopic feature of such WSL is a well-defined superficial layer. That layer mainly consists of fluorapatite crystals. [1,12] As the pH of oral fluids decreases, saliva and plaque become less saturated relative to hydroxyapatite, until the pH reaches a critical point below which the solution becomes unsaturated with respect to hard dental tissues. [6] Calcium hydroxylapatite then dissolves. As fluorapatite is less soluble compared to hydroxyapatite, the plaque fluid is still supersaturated compared to fluorapatite, and this mineral does not dissolve. Subsurface hydroxyapatite dissociates as resistant fluor hydroxylapatite forms on the surface. Competitive supersaturation in relation to fluor hydroxyl apatite is the main reason for maintaining the integrity of the surface layer. [1] As long as the surface layer of the enamel is intact and has a moderate mineral content, fluorides cannot diffuse into the body of the lesion.

Due to the very slow diffusion of ions in the body of the WSL, fluid supersaturation in the lesion is never achieved, nor is complete remineralization in the body of the lesion. Thus, the superficial well-mineralized layer protects the body of the lesion from further dissolution, but also from complete remineralization. [12] Therefore hypothetically, any procedure that temporarily alters the integrity of the surface well-mineralized layer should allow the diffusion of fluoride ions into the body of the lesion. This should allow complete remineralization of the body of the lesion. Hence, fluoride preparations are used in various formulations, in combination with other agents or methods that should improve the fluoride penetration into the body of the lesion. One of the standard methods is an acidic attack on the surface of well-mineralized layer. Fluoride agents can have very low pH. Such an agent is titanium tetrafluoride (TiF_4) with a pH value of around 2. Numerous studies show TiF_4 has a strong protective effect in enamel, not only due to reaching fluoride content (4 fluoride ions) but that a significant part can also be attributed to the protective reaction of the titanium component, further enhanced by the possibility of oxidative coating on the enamel. [13-16]

However, the fluoride's ability to promote remineralization in the enamel is limited by the presence of calcium in saliva. [17] For every two fluorine ions, ten calcium and six phosphate ions are needed to form one unit of fluorapatite. Thus, despite fluoridation, Ca and P ions deficiency may be a limiting factor for overall remineralization, especially in terms of xerostomia. [18,19]

Reparation of early carious lesions can be accelerated by exogenously introduced ions of calcium and phosphates. These findings led to the development of a remineralization system based on the complexes casein-phosphopeptidestabilized amorphous calcium phosphate (CPP-ACP;

Recaldent®) [8] and casein-phosphopeptide-stabilized amorphous calcium fluorophosphate (CPP-ACFP). [20-25] Casein is a protein derived from milk. It is a so-called "ion-binding" protein. CPP can bind 25 Ca ions, 15 phosphate groups, and 5 fluoride ions per molecule, and stabilize calcium phosphate in the solution. [2] CPP-ACFP (casein-phosphopeptide-amorphous calcium fluoro-phosphate) contains 18% Ca ions and 30% phosphate relative to its molecular weight. It provides all the necessary elements for enamel remineralization (calcium, phosphates, fluoride, and water). [2]

Therefore, the prospect of caries preventing action in the future is in the development of a system that will optimize and control the delayed release of fluoride ions in the oral cavity. Moreover, the concept is fundamentally changing by seeking to provide a simultaneous supply of calcium, phosphates and fluorides to increase the amount of fluorapatite produced. [2,12,17,18] Although plenty of time has passed since the introduction of fluoride ion-exchange systems into dental practice [17], the simultaneous introduction of calcium, phosphate and fluoride through ion-exchange systems is still the subject of research. [17-19]

The main obstacle to remineralization of the deep WSL zones is precisely the superficial well - mineralized layer, which pores are so narrow so that molecules carrying calcium and phosphate groups and fluoride ions cannot penetrate through them. If we apply solely topical fluoride agents, we achieve even better mineralization and additional pores reduction. This study's idea was to use a low pH agent which is able to transiently open diffusion pathways through the superficial well-mineralized enamel zone in order to allow remineralization of deeper layers of the lesion as well. This would, in theory, achieve the "healing" of the carious lesion through its entire depth being the main problem in remineralization challenge.

The aim of the research was to establish fluoride uptake of the enamel with initial caries lesions when treated only with 1% topical fluoride agents, and determine whether it is increased when these agents are used in combination with the CPP-ACP.

Materials and methods:

The study was conducted on enamel slabs origin of 60 intact permanent premolars extracted for orthodontic reasons. The teeth were firstly examined and the following specific inclusion criteria were established: intact teeth without obvious and initial carious lesions or WSL, or enamel cracks (infractions). In the teeth that satisfied the inclusion criteria, the root and crown were separated. Thereafter, each tooth crown was separated into two halves with a high-speed handpiece under water cooling. Enamel blocks were immersed in self-curing transparent acrylate and the enamel slabs surfaces were coated with acid resistant varnish. Self-adhesive tape with a round perforation 2mm in diameter was made by a rubber dam hole punch plier and placed over varnished enamel. The varnish was then removed with acetone through circular tape perforation. The performed procedure produced a uniform experimental surface of 3.14 square millimeters on each enamel slab.

Samples underwent a pH-cycling procedure with a daily regime of 3 hours of demineralization and the remaining time of remineralization. Demineralization consisted of immersing the samples in a sufficient amount of demineralization solution whose pH was set at 4.3. The remineralization solution had practically the same composition as artificial saliva. All the samples received 8 daily cycles. The precise composition of demineralization and remineralization solutions, as well as a description of the pH cycling regime, were given in the previous study [26]. Described pH-cycling regime produced WSL with an average depth of 50 micrometers, which was confirmed by scanning electron microscopy and cross-section microhardness testing.

Samples were randomly divided into four experimental groups, containing 15 samples each. The first group received topical fluoride treatment with 1% sodium fluoride (NaF) solution, group II received treatment with 1% titanium tetrafluoride (TiF_4) solution, and group III received combined treatment with 1%NaF + CPP-ACP (GC "Tooth

mouse" Recaldent®), while group IV received combined 1% TiF_4+CPP-ACP. Topical fluoride solutions were prepared with super clean distilled deionized water.

A topical fluoride treatment with 1% fluoride solutions consisted of the following: a 10 µl of 1% fluoride solution was carefully applied on to a round experimental window on the enamel with a micro-pipette and left to act for 15 minutes. Immediately thereafter, each sample was washed with 10 ml of TISAB solution and added with a burette (TISAB- total ionic strength adjusting buffer containing CDTA, solution for sample preparation for fluoride determination). The fluoride concentration in the solution was measured in the same vessel in which the topical fluoride treatment was performed.

The procedure for the combination of fluoride solutions and "Tooth Mouse" was more complicated, as there was no known reaction between the paste and the fluoride solutions used in the experiment. Theoretically, it was possible for the paste to bind a certain amount of fluoride ions by a chemical reaction and thus "mask" them. If the fluoride ions in the solution are not free but bounded in the form of various compounds, their precise concentration cannot be measured by the flour-selective electrode method. As the aim of this experiment was to determine the precise quantities of fluoride ions that the enamel "absorbed", based on the determined difference in the concentration of fluoride in the solution before and after enamel treatment, the nature of the possible chemical reaction between paste and fluoride solutions had great importance. Especially in the case of the combination of TiF_4 solution and Tooth Mouse paste, since a review of the literature could not establish that this combination has been the subject of the scientific research previously. First, an experiment was performed on a series of solutions, which aimed to determine whether the Tooth Mouse paste binds fluoride ions to itself. The concentration of fluoride ions in a series of fluoride solutions was measured before and after the addition of a precisely determined mass of the paste. The paste was weight with the precise analytical scale. When it was determined that the Tooth Mouse paste does not bind fluoride ions from either NaF solution or TiF_4 solution, portions of the paste weighing about 1-3 mg were applied onto disposed of enamel. At that point, 10 µl of fluoride solution was added by a micropipette. With a chemically pure dentin-adhesive applicator, the resulting mixture was rubbed on the enamel surface for 15 minutes. The applicator was left in the measuring vessel to avoid measurement error. The mixture of paste and fluoride was washed from the enamel surface and the applicator by the addition of 10 ml of TISAB solution. After the treatment, the samples were tested for the amount of fluoride absorbed from the solution (so-called fluoride uptake) by the fluoride selective electrode method.

The fluoride-selective electrode is mostly used nowadays in determining the concentration of fluoride in various samples due to its outstanding performance and ease of application. [27] The fluoride-selective electrode WTW, F 500, Fluorides, DIN CONNECTOR was used to measure the electrode potential in this study (Measuring range:0.02-gesät.mg/L, 10^{-6}-gesät.Mol/L; Bridge electrolyte- ELY/BR/503). First, the whole system was checked with a standard solution (WTW solution NaF 10g /l) used for activation, testing and calibration of the fluoride electrode. Solutions of known concentrations were then made from the standard solution to test the electrode action and construct a calibration curve.

The measurement consists in first making a series of so-called "blank tests" for each individual solution, i.e., the experimental group. "Blank test" was made by placing an identical amount of the active substance in a chemically clean container in which the measurement was performed, which would be applied to the enamel veneer, i.e., 10 µl, and diluted with an identical amount of TISAB solution (10ml). The concentration of ions measured in a solution containing an enamel sample was lower than the concentration of ions in the "blank test" precisely for the value that the enamel "absorbs" from the topical agent. The measurement was performed by immersing both,

the reference and the fluoride-selective electrode, simultaneously in a solution in which the concentration of fluoride ions was measured. The result was considered stable if the value that appears on the LCD of the measuring instrument remains unchanged for 3 seconds.

After each sample, both electrodes were washed with a copious amount of distilled deionized water, dried with super-absorbent paper, and then re-immersed in the new sample. After every twentieth measured sample, the electrodes were calibrated again in standard solutions of known concentrations, and the semipermeable membrane on their top was cleaned with a special polishing paper provided by the electrode manufacturer. Results are given in Table 1.

Results and statistical analysis

Shapiro-Wilk distribution normality test showed that group I has a distribution that deviates from the normal (Table 2).

To test the significance of differences between individual groups, an ANOVA test was performed. The values found by ANOVA analysis are given in Table 3.

Table 1. Fluoride uptake given in µMol of fluoride ions

Sample sequence no	Group I	Group II	Group III	Group IV
1.	0.80	2.85	0.51	2.27
2.	0.80	2.85	1.47	1.41
3.	1.99	2.55	0.51	3.82
4.	1.41	2.85	0.84	3.58
5.	1.41	2.23	0.84	2,54
6.	0.49	1.24	1.16	2.27
7.	1.70	2,85	0.51	4.29
8.	4.52	2.23	2.38	3.58
9.	1.70	2.85	0.17	3.33
10.	1.41	3.15	0.84	2.54
11.	4.29	2.23	1.47	4.29
12.	5.58	0.90*	0.51	6.35
13.	0.16	2.85	0.51	7.22
14.	0.49	1.91	0.84	6.53
15.	1.41	2.55	0.51	6.35

Group I: 1%NaF solution; Group II: 1% TiF4 solution; Group III: 1%NaF solution +Tooth mousse; Group IV: 1% TiF4 solution Tooth Mousse; *Result not included in statistical processing.

Table 2. Shapiro-Wilk distribution normality test

	Shapiro-Wilk		
	Statistic	df	Sig.
NaF F-uptake (I gr)	.782	8	.018
TiF_4 F-uptake (II gr)	.859	8	.117
NaF+Tooth Mousse F-uptake (III gr)	.919	8	.419
TiF_4+Tooth Mousse F –uptake (IV gr)	.920	8	.429

Table 3. ANOVA for the fluoride uptake values

	Sum of Squares	df	Mean Square	f	Sig.
Between Groups	75.345	3	25.115	15.08	.000
Within Groups	89.878	54	1.664	9.00	
Total	165.222	57			

Table 4. Post-hoc "Scheffe" test (Dependent Variable: fluoride uptake values)

(I)Type of product /uptake	(J) Type of product /uptake	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
NaF	TiF_4	-.63624	.47942	.626	-2.0197	.7472
	NaF +Tooth Mousse	.98019	.47942	.255	-.4033	2.3637
	TiF_4+ Tooth Mousse	-2.14733(*)	.47108	.000	-3.5067	-.7879
TiF_4	NaF	.63624	.47942	.626	-.7472	2.0197
	NaF +Tooth Mousse	1.61643(*)	.48762	.018	.2093	3.0236
	TiF_4+Tooth Mousse	-1.51110(*)	.47942	.027	-2.8946	-.1276
NaF +Tooth Mousse	NaF	-.98019	.47942	.255	-2.3637	.4033
	TiF_4	-1.61643(*)	.48762	.018	-3.0236	-.2093
	TiF_4+Tooth mousse	-3.12752(*)	.47942	.000	-4.5110	-1.7441
TiF_4+Tooth Mousse	NaF	2.14733(*)	.47108	.000	.7879.	3.5067
	TiF_4	1.51110(*)	.47942	.027	1276	2.8946
	NaF +Tooth Mousse	3.12752(*)	.47942	.000	1.7441	4.5110

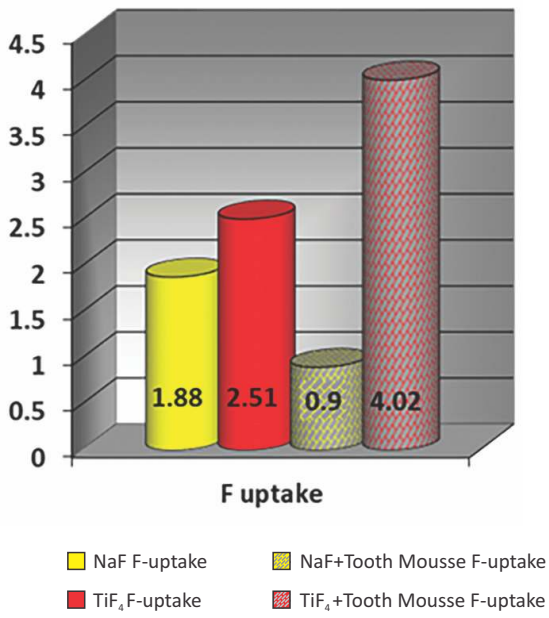
* The mean difference is significant at the .05 level

Table 5. Statistically significant differences overview obtained using the post-hoc "Scheffe" test

Difference between groups	Sig.
Group I↔ Group II	.626
Group I ↔ Group III	.255
Group I ↔Group IV	.000
Group II↔ Group III	.018
Group II ↔ Group IV	.027
Group III ↔ Group IV	.000

In order to determine whether there are statistically significant differences between the groups, a post-hoc "Scheffe" test was performed, the values of which are given in Tables 4 and 5.

Graph 1. Average values of fluoride uptake given in µMol of fluoride ions



Discussion

The fluoride uptake evaluation performed in this study was somewhat specific in methodology. The specifics consist of the following:

- The nature of the chemical bond that fluorides have in/on enamel was not the subject of this study, and therefore, no analysis of fluoride concentration in enamel was performed, but simply the WSLs ability to "absorb" fluoride ions was examined.
- Therefore, the experiment was designed so that the topical fluoride agent and enamel were placed in a closed system in which the loss of active fluorine ions was prevented during the manipulation and measurement. The concentration of fluoride ions was previously determined in the solution to treat the enamel with, and then re-measured after the enamel with an artificial initial carious lesion was added to this closed system. Therefore, any decrease in the concentration of fluoride ions in this closed system is considered to be a direct consequence of their incorporation into the enamel.
- The experiment aimed to determine the effect of a paste with bio-available calcium and phosphates on the chemistry of the "absorption" of fluorine by enamel.

Artificial initial carious lesion produced by pH-cycling has the capacity to "absorb" fluoride ions, depending on the concentration of fluoride in the topical fluoride agent, as well as the potency of the topical agent. The potency of a topical fluoride agent is not only a consequence of the concentration of fluorine ions but also of other physico-chemical characteristics such as the degree of ion reactivity, solubility constants, stability of the agent in aqueous solution, etc.

Founded degree of fluoride uptake was slightly higher by 1% TiF4 solution (2.51 ± 0.5 µMF-) compared to 1% NaF solution (1.88 ± 1.62 µMF-), but the difference found was not significant (Sig. = 0.626).

A particular challenge was to determine the effect of a paste with bio-available calcium and phosphates on the chemistry of "absorption" of fluorine by enamel. Namely, the literature review found that TiF4 has not been tested in combination with CPP-ACP agents. The presence of the CPP-ACP agent reduces fluoride uptake of 1% NaF, but not significantly. On the other hand, the combination of TiF4 and CPP-ACP significantly increased fluoride uptake. The mean value for 1% TiF4 was 2.51 ± 0.5 µMF-, while for 1% TiF4 +Tooth Mousse was 4.02 ± 0.027µMF-. The difference found is significant and amounts to Sig. = 0.027 (Table 5).

As a series of tests have shown that CPP-ACP derivatives do not bind fluoride ions, it can be concluded that "blocking the entry" of fluoride ions into the enamel in experimental group III was caused by some other physicochemical mechanism.

Comparison of the results obtained in this research with the results of other authors must include additional recalculation (Table 6), as well as some necessary adaptations mainly for the following reasons:

- Previous research on fluoride uptake did not refer to the influence of TiF4, and especially not to the combination of TiF4 + CPP-ACP derivatives. For this reason, fluoride uptake can be compared mainly to NaF.
- The researchers examined "uptake" by chemical analysis of the enamel. Chemical analysis of enamel fluorides implies that KOH soluble fluorides release first, the so-called loosely bound fluorides, followed by acid extraction of structurally bound fluorides, which mainly exist in the form of fluorapatite. (28)
- Enamel blocks from different studies were of different origins. Many authors work on bovine enamel slabs, which are similar to human enamel, but the existence of differences in chemical composition should not be overlooked. Other authors have worked on human enamel, but one that already contains a certain amount of fluoride ions in the form of

Table 6. Mean values of fluoride ions amount converted into mass units of fluoride ions per unit area of enamel

Group	The amount of ions F- in µMol	Ion mass F- in µg applied to 0.0314 cm^2	Uptake in µg F^-/cm^2
Group I	1.88	35.72	1137.570
Group II	2.51	47.69	1518.789
Group III	0.90	17.10	544.586
Group IV	4.02	76.38	2432.480

fluorapatite, thanks to the fact that enamel comes from the teeth of people living in areas with fluoridated drinking water. In addition, the enamel blocks went through different models of pH-cycling, with differently long regimes of de- and remineralization, as well as with different concentrations of acid that demineralize.

Most authors express "fluoride uptake" as units of mass per unit area (µg F-/cm2). The results of our research, therefore, require recalculation in order to be comparable. The recalculated values are given in Table 6.

Thus, Casals [8] found that ion incorporation in enamel after treatment with 4 different kinds of toothpaste available on the market ranged from 3.6 to 40.4 µg F^-/cm^2. As can be clearly seen from the results, the range is quite wide, and the amounts of "absorbed" fluoride ions were very low compared to our research. Casals used standard toothpaste available on the market, with very low fluoride concentrations adapted for everyday use, ranging between 500 and 2000 ppm fluoride (parts per million). In this study, a solution for topical fluoridation was used with a very high concentration of fluoride, which is intended only for professional use in the dental office with all precaution measures. Thus, the concentration of fluorine in our 1% NaF solution was around 10.000 ppm of fluorine, as well as in other solutions intended for professional topical fluoride treatment. Therefore, such a big difference in fluoride uptake values should not surprise. Zero [11] found the fluoride uptake is between 16 and 19 µg F^-/cm^2 for 100 ppm NaF solution. Campus [27] found that the uptake was 34.9 µg F^-/cm^2 for 1500 ppm F as NaF +1000ppm F as SMFP (Fluocaril ®Bifluore 250).

In the research of Attin et al. [29], preparations for professional topical fluoride treatment (Mirafluoride and Duraphat) were used. The fluorides were divided into KOH soluble (loosely bound) and structurally bound fluorides (fluorapatite). However, as the concentration of structurally bound fluorides was given as a mass per volume unit of enamel (µg F^- per cm^3 of enamel), unfortunately, these results were not comparable with our research. Moi [28] and co-workers found that fluoride uptake after treatment with 0.05% NaF was 3.5 µg F^-/cm^2 in the form of CaF_2 (loosely bound) + 2.2 µg F^-/cm^2 in the form of FA (tightly bound).

Conclusions

The method of measuring fluoride uptake from the solution proved to be a relevant and valid method for assessing the capacity of the initial carious lesion to "absorb" fluoride ions. A statistically significant difference in the efficiency of 1% TiF_4 and NaF solution could not be determined from the point of view of F-uptake. In other words, both were potent fluoride topical agents that have provided a sufficient amount of fluoride ions for the initial carious lesion. Fluoride uptake turned out to be the largest for the combination of TiF_4 +Tooth Mousse and the

smallest for the combination of NaF +Tooth Mousse. Tooth Mousse does not react with TiF_4 in terms of binding or blocking free fluoride ions. The nature of the chemical interaction between Tooth Mousse and TiF_4 was synergistic in terms of a statistically significant increase in fluoride uptake over pure TiF_4 solution. On the other hand, the nature of the interaction of the paste Tooth Mousse and NaF was the opposite and reduced the F-uptake in relation to NaF itself, although not significantly.

Literature

1. Cury JA, Tenuta LM. Enamel remineralization: controlling the caries disease or treating early caries lesions? Braz Oral Res. 2009;23 Suppl 1:23-30.
2. Llena C, Forner L, Baca P. Anticariogenicity of casein phosphopeptide-amorphous calcium phosphate: a review of the literature. J Contemp Dent Pract. 2009;10(3):1-9.
3. ten Cate JM. Remineralization of deep enamel dentine caries lesions. Aust Dent J. 2008;53(3):281-5.
4. Silva MF, Giniger MS, Zhang YP, DeVizio W. The effect of a triclosan/ copolymer /fluoride liquid dentifrice on interproximal enamel remineralization and fluoride uptake. J Am Dent Assoc. 2004;135(7):1023-9.
5. Cury JA, Tenuta LM. How to maintain a cariostatic fluoride concentration in the oral environment. Adv Dent Res. 2008;20(1):13-6.
6. Featherstone JD. The science and practice of caries prevention. J Am Dent Assoc. 2000;131(7):887-99.
7. Pessan JP, Al-Ibrahim NS, Buzalaf MA, Toumba KJ. Slow-release fluoride devices: a literature review. J Appl Oral Sci. 2008;16(4):238-46.
8. Casals E, Boukpessi T, McQueen CM, Eversole SL, Faller RV. Anticaries potential of commercial dentifrices as determined by fluoridation and remineralization efficiency J Contemp Dent Pract. 2007;8(7):1-10.
9. Arnold WH, Haase A, Hacklaender J, Gintner Z, Bánóczy J, Gaengler P. Effect of pH of amine fluoride containing toothpastes on enamel remineralization in vitro. BMC Oral Health. 2007; 7:14.
10. Villena RS, Tenuta LM, Cury JA. Effect of APF gel application time on enamel demineralization and fluoride uptake in situ. Braz Dent J. 2009;20(1):37-41.
11. Zero DT, Zhang JZ, Harper DS, Wu M, Kelly S, Waskow J, Hoffman M. The remineralizing effect of an essential oil fluoride mouthrinse in an intraoral caries test. J Am Dent Assoc. 2004;135(2):231-7.
12. Cochrane NJ, Cai F, Huq NL, Burrow MF, Reynolds EC. New approaches to enhanced remineralization of tooth enamel. J Dent Res. 2010;89(11):1187-97.
13. Magalhães AC, Rios D, Honório HM, Jorge AM Jr, Delbem AC, Buzalaf MA Effect of 4% titanium tetrafluoride solution on dental erosion by a soft drink: an in situ/ex vivo study. Arch Oral Biol. 2008;53(5):399-404.
14. Magalhães AC, Comar LP, Rios D, Delbem AC, Buzalaf MA. Effect of a 4% titanium tetrafluoride (TiF4) varnish on demineralisation and remineralisation of bovine enamel in vitro. J Dent. 2008;36(2):158-62.
15. Wiegand A, Laabs KA, Gressmann G, Roos M, Magalhães AC, Attin T. Protection of short-time enamel erosion by different tetrafluoride compounds. Arch Oral Biol. 2008;53(6):497-502.
16. Nóbrega CB, Fujiwara FY, Cury JA, Rosalen PL. TiF(4) varnish-A (19)F-NMR stability study and enamel reactivity evaluation. Chem Pharm Bull (Tokyo). 2008;56(1):139-41.
17. Torrado A, Valiente M, Zhang W, Li Y, Muñoz CA. Remineralization potential of a new

toothpaste formulation: an in vitro study. J Contemp Dent Pract. 2004;5(1):18-30.

18. Reynolds EC. Calcium phosphate-based remineralization systems: scientific evidence? Aust Dent J. 2008;53(3):268-73.
19. Kitasako Y, Cochrane NJ, Khairul M, Shida K, Adams GG, Burrow MF, Reynolds EC, Tagami J. The clinical application of surface pH measurements to longitudinally assess white spot enamel lesions. J Dent. 2010;38(7):584-90.
20. Aimutis WR. Bioactive properties of milk proteins with particular focus on anticariogenesis. J Nutr. 2004;134(4):989S-95S.
21. Manton DJ, Walker GD, Cai F, Cochrane NJ, Shen P, Reynolds EC. Remineralization of enamel subsurface lesions in situ by the use of three commercially available sugar-free gums. Int J Paediatr Dent. 2008;18(4):284-90.
22. Reynolds EC, Cai F, Cochrane NJ, Shen P, Walker GD, Morgan MV, Reynolds C. Fluoride and casein phosphopeptide-amorphous calcium phosphate. J Dent Res. 2008; 87(4):344-8.
23. Cochrane NJ, Saranathan S, Cai F, Cross KJ, Reynolds EC. Enamel subsurface lesion remineralisation with casein phosphopeptide stabilized solutions of calcium, phosphate and fluoride. Caries Res. 2008;42(2):88-97.
24. Iijima Y, Cai F, Shen P, Walker G, Reynolds C, Reynolds EC. Acid resistance of enamel subsurface lesions remineralized by a sugar-free chewing gum containing casein phosphopeptide-amorphous calcium phosphate. Caries Res. 2004;38(6):551-6.
25. Cai F, Shen P, Morgan MV, Reynolds EC. Remineralization of enamel subsurface lesions in situ by sugar-free lozenges containing casein phosphopeptide-amorphous calcium phosphate. Aust Dent J. 2003;48(4):240-3.
26. Hasić Branković L, Tahmiščija I,Korać S, Konjhodžić A, Džanković A. Is a pH cycling model a suitable simulator of actual psychochemical processes in initial carious lesions? Stomatological review. 2020;9(2):2-10.
27. Campus G, Lallai MR, Carboni R. Fluoride Concentration in Saliva after Use of Oral Hygiene Products. Caries Res 2003; 37:66-70.
28. Moi GP, Tenuta LM, Cury JA. Anticaries potential of a fluoride mouth rinse evaluated in vitro by validated protocols. Braz Dent J. 2008;19(2):91-6.
29. Attin T, Lennon AM, Yakin M, Becker K, Buchalla W, Attin R, Wiegand A. Deposition of fluoride on enamel surfaces released from varnishes is limited to vicinity of fluoridation site.Clin Oral Investig. 2007;11(1):83-8.

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