Markus J. Altenburger[1]
Jasmin Bernhart[2]
Thurid D. Schicha[1]
Karl-Thomas Wrbas[1]
Elmar Hellwig[1]

[1] University Dental School, Department of Operative Dentistry and Periodontology, University of Freiburg
[2] University Dental School, Department of Prosthodontics, University of Freiburg

Corresponding author
Dr. M. J. Altenburger
Universitätsklinik für Zahn-, Mund- und Kieferheilkunde, Abteilung für Zahnerhaltungskunde und Parodontologie
Hugstetter Straße 55,
79095 Freiburg, Germany
Tel. +49 761 270 4957
Fax +49 761 270 4762
E-mail:
markus.altenburger@uniklinik-freiburg.de

Comparison of *in vitro* fluoride uptake from whitening toothpastes and a conventional toothpaste in demineralised enamel

Key words: Fluoride uptake, enamel, abrasives, whitening toothpastes

Summary   Studies on the compatibility of abrasives and fluoride compounds deal exclusively with fluoride uptake and remineralisation after storing the enamel specimens in a toothpaste-saliva mixture. The influence of brushing on the fluoride uptake when highly abrasive toothpastes are used has hardly been investigated so far. The aim of the present study was to investigate fluoride uptake in initially demineralised dental enamel after storage in, or brushing with, whitening toothpaste slurries, compared to a conventional toothpaste. For this purpose two widely available whitening toothpastes with ionically-bound fluoride (sodium fluoride NaF), two with covalently-bound fluoride toothpastes (sodium monofluorophosphate, NaMFP) and a conventional amine fluoride toothpaste (AmF) were compared. The fluoride uptake after use of the AmF toothpaste was shown to be statistically significantly higher than that after application of the NaF toothpastes, which in turn was statistically significantly higher than the uptake resulting from NaMFP application. The fluoride uptake was slightly higher when the enamel samples were brushed with NaF toothpaste, rather than just stored in the respective toothpaste slurry. Brushing with highly abrasive toothpastes did not negatively influence fluoride uptake in demineralised dental enamel.

The ionic form of the fluoride in toothpastes appears to be critical for increased fluoride uptake. The acidic components of the AmF toothpaste improved fluoride uptake compared to alkaline NaF toothpastes.

Introduction

The importance of tooth whitening products for patients has risen along with an increased interest in health, personal hygiene and beauty. Extrinsic stains are removed using partly abrasive toothpastes and the natural tooth colour is restored (Zantner 2006). Nevertheless, apart from the cosmetic effect, a caries protective effect should also be achieved with these toothpastes.

This effect is based on the inhibition of demineralisation and enhancement of remineralisation of dental hard tissue by different fluoride compounds (Thylstrup et al. 1990, Rølla et al. 1993). In toothpastes, ionically-bound fluoride (sodium fluoride (NaF), stannous fluoride (SnF$_2$), amine fluoride (AmF) and covalently-bound fluoride (NaMFP) are used (Mellberg, 1991). The different forms of fluoride differ in the mechanisms by which they protect against caries. It is thought that monofluorophosphate, exchanges with orthophosphate in enamel and afterwards an intracristalline transposition of F$^-$ and OH$^-$ takes place, whereby fluorapatite is formed (Gron et al. 1971). Therefore, the caries protective effect is limited by the number of the reactive molecules in the crystal lattice (Ingram 1972). Ericsson (1967) described another reaction channel. The MFP-molecule is hydrolysed by phosphatases from the oral bacterial flora and saliva (Eanes 1976, Klimek et al. 1997). The fluoride, which has been released, reacts with dental hard tissue like ionic fluoride compounds. Ionically-bound fluoride is deposited primarily as a CaF$_2$-layer on the dental hard tissue during

brushing. With time, this reservoir is used up and the fluoride concentration of the enamel and the saliva rises (BUCHALLA ET AL. 2002, BUCHALLA ET AL. 2007).

Covalently-bound fluoride is often used in whitening toothpastes (MELLBERG 1991), due to good compatibility with different cleaning particles such as $CaCO_3$ or $Ca_2P_2O_7$ or Al_2O_3. When combined with ionically-bound fluoride these cleaning particles quickly decrease the freely available fluoride and are therefore not suitable. For this reason, cleaning particles derived from SiO_2 for example silica gel, are predominantly used in toothpastes containing ionically-bound fluoride. Up until now, studies addressing the subject of the caries prophylactic effect of whitening toothpastes have only looked at the freely available fluoride content of toothpaste/saliva slurries or the enamel fluoride content or remineralisation following placement in these slurries (WHITE & FALLER 1986, CURY ET AL. 2001, PICKLES ET AL. 2005, WATANABE ET AL. 2005, JOINER ET AL. 2008). It remains largely unclear whether brushing enamel with abrasive toothpastes changes the fluoride uptake and to what extent this differs from brushing with conventional toothpastes. This may be particularly important for demineralised enamel, which has a pseudo-intact surface with increased pore volume due to acid attack and is, therefore, mechanically instable.

The increased mechanical wear caused by whitening toothpastes may result in enlargement of the enamel surface area and thereby increase the opportunity for applied fluoride to react with the calcium in enamel. On the other hand, brushing may also destroy the developing CaF_2-surface layer. Therefore, the aim of this in-vitro investigation was to measure the fluoride uptake of demineralised enamel samples after placement in and after brushing with different whitening toothpaste/saliva slurries and to compare these with fluoride uptake after brushing or placement in a conventional amine fluoride containing toothpaste slurry.

Materials and Methods

60 freshly-extracted bovine mandibular incisors were used for the study. Three enamel-dentin samples were prepared from each crown using a trephine mill (custom made, Gebr. Brasseler, Lemgo, Germany). These were placed in a special mould (Multiform Einbettform, Struers, Copenhagen, Denmark) and embedded in acrylic (Technovit 4071, Heraeus Kulzer GmbH, Hanau, Germany). The sample blocks were then ground parallel and polished using a water-cooled bench grinding machine (EXAKT-Mikroschleifsystem, Nordstedt, Germany) and 1000, 1200, 2400 and 4000 grit sandpaper. Samples were marked

according to the teeth they were taken from (1a–c to 60a–c) and randomly divided into 5 treatment groups with 12 teeth each. The samples were stored in moist conditions until they were used. The teeth were placed in 10 litres of demineralisation solution for 5 days (BUSKES ET AL. 1985). The pH-value was checked daily (target: pH 5) and buffered if necessary using KOH. Samples were not actively vibrated or rinsed in order to avoid mechanical damage to the enamel surface. The monofluorophosphate toothpastes chosen for the high abrasion cosmetic toothpaste groups were Dr Best Zahnweiss (GlaxoSmithKline Consumer Healthcare GmbH & Co. KG, Bühl, Germany) and Settima (GlaxoSmithKline). The test toothpastes with ionically-bound fluoride were Colgate Sensation White (Colgate Palmolive GmbH, Hamburg, Germany) and Odol-med3 samtweiss (GlaxoSmithKline) and the conventional toothpaste elmex (GABA GmbH, Lörrach, Germany).

Relevant ingredients and physical properties are given in table I.

The (a) sample of each sample-triplet was placed in a slurry of test toothpaste and artificial saliva for 60 minutes. The mixture was made using 1 g toothpaste per 5 ml artificial saliva. 20 ml toothpaste–saliva mixture was used for each sample. The artificial saliva was freshly made for the study according to KLIMEK ET AL. (1982), with a total calcium fraction of 1.53 mmol and a total phosphate fraction of (anorganic) 4.82 mmol (ATTIN ET AL. 2001). The pH-value of the saliva was between 6.2 und 6.4. The samples for the brushing test (c) were mounted in a specially-designed brushing machine (VDD Elektronik, Freiburg, Germany) and the respective slurry added. The toothbrushes were mounted so that the brushing field lay parallel to and on the sample surface. The contact pressure of the toothbrush was 2.75 N. The samples were brushed with 3600 brushing strokes in 60 min. The (b) samples were left untreated. Then all samples were gently rinsed with double distilled water (Fresenius Kabi, Bad Homburg, Germany), carefully dabbed with gauze and allowed to air dry until needed for analysis.

A specially-fabricated apparatus was used to measure the fluoride content in enamel (Technischer Betrieb, Universitätsklinikum Freiburg, Freiburg, Germany, Figure 1) (WEATHERELL ET AL. 1985, BUCHALLA ET AL. 2002). In order to remove a defined volume of enamel, the samples were fixed in a special holder planoparallel to the grinder. Strips of 0.8×10 cm were cut from 1200 grit sandpaper and then agitated in perchloric acid for 8 hours at 25 °C. One sample and one sandpaper strip each were fixed in the apparatus and 100 μm enamel was ground away according to the integrated gauge. The resulting enamel grit and the paper strip were placed in 0.5 ml 0.5 M perchloric

Tab. I	Composition and relevant properties of the toothpastes tested.				
Product	**Abrasive**	**Fluoride content [ppm]**	**Fluoride form**	**pH-value**	**RDA-value**
Settima	Calciumcarbonate (Chalk)	1350	Sodiummono-fluorophosphate	8	180–200
Dr. Best Zahnweiss	Calciumcarbonate (Chalk)	1350	Sodiummono-fluorophosphate	8	180–200
Colgate Sensation White	Hydrated Silica (silica gel)	1500	Sodium fluoride	7.8	180
Odol-med3 samtweiss	Hydrated Silica (silica gel)	1100	Sodium fluoride	9	90–100
elmex	Hydrated Silica (silica gel)	1250	Amine fluoride	4.6	77

Fig. 1   Large image: custom made apparatus for controlled dental hard substance abrasion testing. Small image: Abrasion slide with a sample attached in the holder as well as a sandpaper strip with abraded enamel powder.

acid and 2.5 ml TISAB II and buffered to a pH of 5.5. The solution was mixed using a magnetic stirrer and then the fluoride content of the solution was determined at a constant 25°C using a fluoride sensitive electrode (Modell 96-09, Thermo Orion, Beverly, Ohio, USA). The fluoride content for a particular sample was calculable using the fluoride concentration measured in the solution since the volume of the solution and of the ground enamel were known.

The statistical analyses were carried out by the institute for medical biometrics of the University of Freiburg. The statistical significance of different results was tested using analyses of variance (ANOVA) followed by Tukey's Studentised Range Test. The level of significance was set at $p \leqslant 0.05$.

Results

None of the enamel samples included in the study were lost, therefore, all were evaluated (Table II). The fluoride content of

the reference samples (b samples), which were only demineralised, was between 250 und 380 $\mu g/cm^3$. No statistically significant differences were found between the samples of the 5 toothpaste groups. No statistically significant difference could be found between the two monofluorophosphate toothpastes, Dr. Best Zahnweiss and Settima, and the non-fluoridated control group, either after storage in toothpaste slurry or after brushing abrasion. The mean fluoride concentration was numerically higher in both toothpaste groups after storage in slurry but this difference was not statistically significant. There was a significant increase in enamel fluoride concentration ($p < 0.001$) after storage in and brushing with the sodium fluoride containing toothpaste slurries, Colgate Sensation White and Odol-med3 samtweiss compared to the non-fluoridated control group. No significant difference could be shown between the two treatment methods. The highest fluoride uptake was found for the amine fluoride toothpastes after storage in the slurry (2061 $\mu g/cm^3$) and also after brushing the samples (2809 $\mu g/cm^3$). The fluoride concentrations were statistically significantly different from the non-fluoridated control. There was no significant difference between the treatment methods.

A comparison of the toothpastes (Table II) showed, after storage in the slurries, that use of elmex resulted in a significantly higher fluoride uptake in enamel than NaF and NaMFP toothpastes. While the NaF toothpastes did not differ significantly from each other, they did result in significantly better fluoride uptake than the NaMFP toothpastes, which in turn did not differ significantly from the non-treated controls. The same order was observed after brushing with the respective slurries. Fluoride uptake was, however, significantly higher after using Colgate Sensation White compared to Odol-med3 samtweiss.

Discussion

Fluorides play a decisive role in caries prevention today as ingredients in oral hygiene products. Freely-available fluoride on the enamel surface in the form of a CaF_2-like layer has a greater caries protective effect than structurally-bound fluoride in enamel (FEATHERSTONE 1999). Several studies have shown that plaque fluoride plays an important role (AXELSSON 2004). However, plaque is removed by toothbrushing, and if some plaque remains, even after careful oral hygiene, it has been shown to have a relatively low fluoride content (DUCKWORTH ET AL. 1994). Moreover, newer studies show that the usual fluoride treatments do not cause a significant increase in plaque fluoride concentration (DUCKWORTH ET AL. 1994, HEIJNSBROEK ET AL. 2006). Therefore, the structurally-bound and surface-layer fluoride is thought to have the most important function in caries prevention.

Fluoride uptake in demineralised enamel was determined in the present study using a special abrasion procedure, which had been used in previous studies (WEATHERELL ET AL. 1985, BUCHALLA ET AL. 2002). Hereby, no distinction was made between the calcium fluoride layer on the surface of the enamel (KOH-soluble fluoride) and the structurally-bound fluoride. This fluoride test was chosen because two of the toothpastes used contained sodiummonofluorophosphate. This covalently-bound fluoride does not form a calcium fluoride layer. It has been proposed that the reaction of monofluorophosphate with demineralised enamel may be an exchange reaction (GRON ET AL. 1971). The approximately 100 μm enamel layer removed in the study should allow a complete assessment of precipitated and structurally-bound fluoride in the demineralised enamel,

which, after topical fluoridation, is found primarily in the outer 40 μm (DUSCHNER ET AL. 1997).

The results show, that the fluoride content was significantly higher in samples following storage in, or brushing with, the following test slurries: Colgate Sensation White, Odol-med3 samtweiss and elmex compared to the demineralised reference samples. This effect is mainly due to the formation of a CaF$_2$ layer on the enamel surface, which is formed within minutes with ionically-bound fluoride (PETZOLD 2001, HOLLER ET AL. 2002). Fluoride uptake after brushing with the respective product was only slightly increased and not significantly higher despite the long storage and brushing time. One explanation for this is that the enamel was previously demineralised (IMFELD ET AL. 1993). Demineralised enamel, as such, is more susceptible to mechanical wear (ATTIN ET AL. 2001, KIELBASSA ET AL. 2005). Both the use of abrasive materials and brushing itself may cause dental hard tissue wear (BARBAKOW ET AL. 1989), which removes the intact enamel surface and exposes the superficial portion of the porous body of the lesion. This increases access to the body of the lesion allowing more fluoride to be incorporated. Only RDA values (Radioactive Dentin Abrasion) can be given for the toothpastes tested. Although no linear correlation was found between REA values (Radioactive Enamel Abrasion) and RDA values (BARBAKOW ET AL. 1989, PHILPOTTS ET AL. 2005), they may still be used to characterise the toothpastes examined here. However, increased fluoride uptake after brushing with toothpastes with higher RDA values could not be shown. The fluoride uptake after brushing with Colgate Sensation White was significantly higher than after brushing with Odol-med3 samtweiss. One possible explanation for this is that Colgate Sensation White has a higher fluoride content (DUNIPACE ET AL. 1997). A further factor for the higher fluoride uptake is that Colgate Sensation White has a low pH. This may also explain why the highest fluoride uptake was measured after using elmex toothpaste, which is less abrasive than other toothpastes but has a low pH. In this case, the low pH factor seems to have had an influence on the fluoride uptake, so that after storage of the samples in the elmex-slurry higher fluoride uptake was measured than after brushing with Colgate Sensation White toothpaste. It is well known, that the reaction between enamel and fluoride is faster and that fluoride uptake on and in enamel is higher at lower pH (FRIBERGER 1975, SCHREIBER ET AL. 1988, PETZOLD 2001). This effect may be intensified by mechanically wearing the enamel surface (IMFELD ET AL. 1993). While this study could not exclude the influence of the composition and pH value of the toothpaste on fluoride uptake when ionically-bound fluoride is used, these connections have not yet been confirmed (PETZOLD 2001, HOLLER ET AL. 2002).

When using the two monofluorophosphate toothpastes Dr. Best Zahnweiss and Settima we were able to show a slight but not statistically significant fluoride uptake after brushing and storage in slurry. This was significantly lower than after use of the NaF toothpastes. The fluoride uptake may be explained by the marginal ionically-bound fluoride portion in the NaMFP toothpastes (BRUUN ET AL. 1984, BRUUN & GIVSKOV 1993). A second theory proposes that the NaMFP is structurally changed, thus allowing uptake into the enamel surface (ARENDS ET AL. 1985). However, this effect is negligible and would only make sense if, as mentioned above, brushing resulted in opening of the porous lesion body. The enlarged "reaction surface" resulting from brushing of the enamel would also have to be reflected in increased fluoride uptake. Nevertheless, because fluoride uptake has been proven clinically in dental enamel after application of NaMFP-containing toothpastes (REINTSEMA & ARENDS 1987), it is thought that phosphatases in saliva and pellicle may be responsible. Nevertheless, it must be remembered, that the change from NaMFP to an ionic form of fluoride only takes place after several minutes and that this reaction seems to depend on the salivary composition (KLIMEK ET AL. 1997).

Today, from a caries protective view, whitening toothpastes with a combination of silica gel and ionically-bound fluorides need not be classified as problematic. In this study, this combination achieved the greatest fluoride uptake in enamel compared to NaMFP-containing pastes. A possible negative influence on fluoride uptake, due to brushing with highly abrasive toothpastes in demineralised enamel, was not confirmed. A positive trend was seen; this, however, was not statistically significant.

Résumé

Les recherches sur la compatibilité des abrasifs et des composés de fluorure dans les dentifrices se penchent uniquement sur l'absorption de fluorure et sur la reminéralisation après la déposition des échantillons d'émail dans un mélange de dentifrice et de salive. L'influence du brossage avec des dentifrices hautement abrasifs sur l'absorption de fluorure dans des échantillons d'émail n'a été jusqu'à présent que très peu étudiée. Le but de cette étude in vitro était de déterminer le degré d'absorption de fluorure sur des échantillons d'émail initialement déminéralisés après déposition ou brossage avec mélanges de dentifrice très abrasif et de salive et de comparer celle-ci avec le degré d'absorption d'un dentifrice normal. Deux dentifrices blanchissants avec fluorure composé ionique (fluorure de sodium, NaF), deux dentifrices avec fluorure composé covalent

Tab. II Mean and standard deviation in μg/cm³ of the single groups after demineralisation, storage in and brushing of the enamel samples with the respective toothpastes/toothpaste-slurries. Statistical grouping of the results by means of Tukey's studentised rank test. Groups with statistically significant differences are marked with different letters.

	After demineralisation			After storage			After brushing		
	Mean	Standard deviation	Mean statistical group	Mean	Standard deviation	Mean statistical group	Mean	Standard deviation	Mean statistical group
elmex	247.86	39.18	A	2061.60	702.23	A	2809.84	943.77	A
Colgate Sensation White	378.38	197.20	A	1117.99	560.71	B	1679.37	594.31	B
Odol-med3 samtweiss	314.31	69.91	A	851.57	295.86	B	1083.49	454.41	C
Dr. Best Zahnweiss	336.26	64.27	A	430.46	135.73	C	388.46	73.82	D
Settima	280.17	119.93	A	373.73	157.60	C	323.07	64.57	D

(monofluorophosphate de sodium, NaMFP) et un dentifrice normal (fluorure d'amines), tous usuels, ont été comparés dans cette étude. On a constaté que l'absorption de fluorure était statistiquement plus importante après l'utilisation des dentifrices avec fluorure d'amines qu'après celle des dentifrices avec fluorure de sodium. De même l'absorption de fluorure est plus importante après l'utilisation de dentifrice NaF qu'après celle du dentifrice avec monofluorophosphate de sodium. Comparé à la déposition des échantillons d'émail dans un mélange de dentifrice et de salive, le brossage des échantillons avec du dentifrice avec fluorure de sodium améliore de façon signifi-

cative l'absorption de fluorure. Les dentifrices blanchissants contenant du fluorure composé ionique (NaF) ont un degré d'absorption de fluorure sur de l'email déminéralisée supérieur aux dentifrices avec monofluorophosphate de sodium (NaMFP). Le brossage avec des dentifrices hautement abrasifs n'amoindrit pas l'absorption de fluorure de l'émail déminéralisé. Il paraît que l'absorption de fluorure est influée positivement par le fluorure composé ionique. Entre ces, le fluorure d'amines acidulé dans le dentifrice normal augmentait l'absorption de fluorure plus que le fluorure de sodium basique dans les dentifrices blanchissants.

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