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# **Conventional and Anti-Erosion Fluoride Toothpastes: Effect on Enamel Erosion and Erosion-Abrasion**

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## **Key Words** Enamel • Erosion • Fluoride • Toothpaste

### Abstract

New toothpastes with anti-erosion claims are marketed, but little is known about their effectiveness. This study investigates these products in comparison with various conventional NaF toothpastes and tin-containing products with respect to their erosion protection/abrasion prevention properties. In experiment 1, samples were demineralised (10 days,  $6 \times 2$  min/day; citric acid, pH 2.4), exposed to toothpaste slurries (2  $\times$  2 min/day) and intermittently stored in a mineral salt solution. In experiment 2, samples were additionally brushed for 15 s during the slurry immersion time. Study products were 8 conventional NaF toothpastes (1,400-1,490 ppm F), 4 formulations with anti-erosion claims (2 F toothpastes: NaF + KNO<sub>3</sub> and NaF + hydroxyapatite; and 2 F-free toothpastes: zinc-carbonate-hydroxyapatite, and chitosan) and 2 Sn-containing products (toothpaste: 3,436 ppm Sn, 1,450 ppm F as SnF<sub>2</sub>/NaF; gel: 970 ppm F, 3,030 ppm Sn as SnF<sub>2</sub>). A mouth rinse (500 ppm F as AmF/NaF, 800 ppm Sn as SnCl<sub>2</sub>) was the positive control. Tissue loss was quantified profilometrically. In experiment 1, most NaF toothpastes and 1 F-free formulation reduced tissue loss significantly (be-

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Accessible online at: www.karger.com/cre tween 19 and 42%); the Sn-containing formulations were the most effective (toothpaste and gel 55 and 78% reduction, respectively). In experiment 2, only 4 NaF toothpastes revealed significant effects compared to the F-free control (reduction between 29 and 37%); the F-free special preparations and the Sn toothpaste had no significant effect. The Sn gel (reduction 75%) revealed the best result. Conventional NaF toothpastes reduced the erosive tissue loss, but had limited efficacy regarding the prevention of brushing abrasion. The special formulations were not superior, or were even less effective. Copyright © 2011 S. Karger AG, Basel

Toothpastes undoubtedly play an important role in maintaining general oral health, and the daily fluoride exposure via toothpastes provides at least a basic protection against erosive demineralisation from everyday exposure to acidic food and drink. However, there is only limited knowledge of the effects that can be expected of this, and the results that have been published range from no protection up to almost complete inhibition [Davis and Winter, 1977; Bartlett et al., 1994; Hooper et al., 2007; Magalhaes et al., 2007; Rios et al., 2008b; Hara et al., 2009a; Kato et al., 2010; Moretto et al., 2010]. Meanwhile,

Carolina Ganss Dental Clinic, Department of Conservative and Preventive Dentistry Schlangenzahl 14 DE-35392 Giessen (Germany) Tel. +49 641 994 6173, E-Mail carolina.ganss@dentist.med.uni-giessen.de new toothpastes have been marketed claiming to be specifically effective against erosion. The new toothpastes contain ingredients promised to exhibit particular efficacy against acid challenges (i.e. SnF<sub>2</sub>, nanohydroxyapatite or biopolymers) or claim to be formulated to provide the best availability of the fluoride ion. So far, however, there is very limited information on the efficacy of these products, particularly in relation to conventional fluoride toothpastes. Analogously to the previously shown antierosive properties of Sn/F-containing solutions [Ganss et al., 2008], there is some evidence that Sn-containing toothpastes might be more effective than conventional NaF toothpastes [Young et al., 2006; Hooper et al., 2007]. For a toothpaste with anti-erosion claim containing NaF and 5% KNO<sub>3</sub>, a significant potential to reharden eroded enamel surfaces has been shown [Zero et al., 2006; Hara et al., 2009b; Maggio et al., 2010], but studies quantifying erosive enamel loss have revealed conflicting results [Rees et al., 2007; Kato et al., 2010].

The aim of the study was to investigate the effects of toothpastes with anti-erosion claims in comparison to conventional fluoride toothpastes in terms of slurry effects (experiment 1) and the additional effects of abrasion (experiment 2). In both experiments, a cyclic procedure with demineralisation in acid, exposure to toothpastes and storage in a mineral solution was performed. The target criterion was loss of enamel, determined profilometrically.

#### **Material and Methods**

#### Preparation of Enamel Specimens

Previously impacted human third molars stored in saturated thymol solution were used. Donors lived in an area with  $\leq 0.03$ mg/l fluoride in the drinking water. Approximately 1-mm-thick longitudinal slices were prepared (Exakt Abrasive Cutting System and Exakt Microgrinder, Exakt-Apparatebau, Norderstedt, Germany), and the natural surfaces were ground flat by removing about 300  $\mu$ m (P800 and P1200 silicon carbide abrasive paper, Leco, St. Joseph, Mo., USA) until an experimental area of approximately 3  $\times$  3 mm was achieved.

Specimens were mounted on microscope slides (R. Langenbrinck, Teningen, Germany; experiment 1) or on sample holders suitable for the automated brushing machine (experiment 2) with light-curing acrylic (Technovit 7230 VLC, Kulzer-Exakt, Wehrheim, Germany). One half of the experimental area was covered with the acrylic and served as a reference area for profilometry. The uncovered area was carefully checked for any contamination. Specimens were stored in 100% humidity until use. 594 specimens were prepared and randomly allocated to 33 groups (n = 18 each).

#### Solutions and Products Used

Erosive demineralisation was performed with 0.05 M citric acid (citric acid monohydrate, pH 2.4; Carl Roth GmbH & Co. KG,

Karlsruhe, Germany). Otherwise, samples were stored in a mineral salt solution containing 4.08 mmol  $H_3PO_4$ , 11.90 mmol NaHCO<sub>3</sub>, 20.10 mmol KCl, and 1.98 mmol CaCl<sub>2</sub> [Gerrard and Winter, 1986], pH 6.7 (all chemicals from Merck, Darmstadt, Germany). The degree of saturation (pK-pI) with respect to hydroxy-apatite was calculated from the pH and the concentrations of calcium and phosphate using a computer program [Larsen, 1986] assuming a solubility product for hydroxyapatite of  $10^{-58.5}$  [Mc-Dowell et al., 1977]. The storage solution was supersaturated with respect to hydroxyapatite and had a pK-pI of 10.37.

All products (toothpaste, gel and mouth rinse) were commercially available and are described in table 1. From the toothpastes and the gel, slurries were prepared by mixing with the mineral salt solution (1 part toothpaste or gel to 3 parts mineral solution, by weight). The slurries were freshly prepared at the beginning of each experimental day. The mouth rinse was used undiluted. The pH values of all solutions were monitored with a pH-sensitive electrode. The relative dentin abrasivity (RDA) of the batches used was determined (Missouri Analytical Laboratories Inc., St. Louis, Mo., USA) after the test method described by Grabenstetter et al. [1958].

#### Determination of Fluoride in the Slurries

For the fluoride-containing toothpastes and the gel, fluoride concentrations in the slurries prepared as described above were determined directly and 6 h after preparation. The slurries were centrifuged at 10,000 g for 10 min and 1 ml of the supernatant was added to 1 ml TISAB II (Thermo Fisher Scientific, Beverly, Mass., USA). The fluoride concentration was determined with a fluoride-sensitive electrode. Analyses were made in triplicate.

#### Experimental Procedure

The study consists of 2 separate experiments. Both experiments were cyclic procedures over 10 days including erosion, application of test or control products and storage in the mineral salt solution. The time between cycles was 1.5 h.

*Experiment 1.* Erosion was performed  $6 \times 2 \min/\text{day} (250 \text{ ml}, 25^{\circ}\text{C})$  under agitation in a shaking bath (Model 1083, GFL mbH, Burgwedel, Germany) with horizontal movements ( $35 \times / \min$ ). After erosion, the samples were rinsed with tap water for 30 s, and then immersed in 200 ml slurry for 2 min after the first and the last erosive demineralisation each day. The samples were rinsed using tap water for 1 min and stored in the remineralisation solution (shaking bath, horizontal movements,  $35 \times / \min$ ;  $25^{\circ}$ C) until the next intervention. The negative control was erosion only and the positive control was erosion and immersion in the mouth rinse for 2 min.

*Experiment 2.* Erosion and immersion in the slurries or in the mouth rinse were performed as in experiment 1. In addition, samples were brushed with an automated brushing machine (SD Mechatronik GmbH, Feldkirchen-Westerham, Germany) for 15 s during the 2-min slurry immersion. The brush moved in a 'zigzag' pattern (150 oscillations/min, linear travel path 6 mm, travel velocity 60 mm/s). American Dental Association reference brushes (soft) were used and the brushing load was 200 g. There were 2 negative control groups: one was erosion only and the other was erosion and brushing with F-free toothpaste. The positive control was erosion and brushing with F-free toothpaste. Afterwards, the samples were immersed in the mouth rinse for 2 min.

#### Table 1. Specification of the products used

	рН	Free fluoride ppm	RDA <sup>1</sup>	RDA <sup>2</sup>	Active ingredients
Conventional toothpastes					
Theramed Natural White	7.1	$317.0 \pm 7.8$	$75 \pm 16$	95-105	1,450 ppm F as NaF
(Schwarzkopf & Henkel)		$(323.7 \pm 2.1)$	(HS)		
Perlodent Kraeuter	8.1	$341.0 \pm 1.7$	$93 \pm 10$	n.a.	1,450 ppm F as NaF
(Rossmann)		$(340.7 \pm 7.6)$	(HS)		
Theramed 2in1 Original	7.3	$350.0 \pm 2.7$	$74 \pm 17$	40-50	1,450 ppm F as NaF
(Schwarzkopf & Henkel)		$(357.0 \pm 3.0)$	(HS)		
Odol Med 3 Pro Clean	7.1	$330.3 \pm 2.9$	$109 \pm 10$	150	1,400 ppm as NaF
(GlaxoSmithKline)		$(311.0 \pm 3.0)$	(HS)		
Blend-A-Med Classic	6.3	$173.7 \pm 4.7$	$69 \pm 10$	n.a.	1,450 ppm F as NaF
(Procter & Gamble)		$(229.7 \pm 41.3)$	(HS)		
Sensodyne MultiCare	6.0	$319.7 \pm 5.1$	$115 \pm 30$	30-40	1,400 ppm F as NaF
(GlaxoSmithKline)		$(287.0 \pm 3.0)$	(HS)		
GUM Original White	7.2	$324.0 \pm 2.7$	$101 \pm 8$	135	1,490 ppm F as NaF
(Sunstar)		$(331.3 \pm 0.6)$	(HS)		
Dentagard Original	7.0	$331.0 \pm 3.5$	$80 \pm 15$	41	1,450 ppm F as NaF
(Colgate Palmolive)		$(337.3 \pm 2.3)$	(HS)		
Toothpastes with anti-erosion claim					
Pronamel	7.0	$295.0 \pm 3.5$	$115 \pm 32$	30-40	KNO <sub>3</sub> , 1,450 ppm F as NaF
(GlaxoSmithCline)		$(280.0 \pm 22.3)$	(HS)		
ApaCare	6.7	$49.8 \pm 0.5$	$77 \pm 17$	50	1% hydroxyapatite nanoparticles.
(Cumdente)		$(55.0 \pm 0.6)$	(DCPD, S)		1,450 ppm F as NaF
BioRepair	7.8	_	$110 \pm 13$	n.a.	Zinc-carbonate-hvdroxyapatite
(Dr. Kurt Wolff)			(HS)		nanoparticles, no fluoride
Chitodent	6.3	_	$83 \pm 11$	n.a.	Chitosan, no fluoride
(B & F)			(HS)		
Sn-containing products					
ProExpert Gum Protection	6.0	$280.0 \pm 2.7$	$119 \pm 16$	n.a.	1.450 ppm F: 1.100 ppm F as $SnF_2$ ,
(Procter & Gamble)		$(281.0 \pm 1.0)$	(HS)		$350 \text{ ppm F as NaF: } 3.436 \text{ ppm Sn as SnF}_2$
Gel-Kam	43	2883 + 22	60 + 22	na	970 ppm F as $SnF_2$ , 3,030 ppm Sn as $SnF_2$
(Colgate Palmolive)	110	$(272.7 \pm 1.5)$	(none)		
		(2/20/2/10)	(110110)		
Controls	4.5				
Elmex Erosionsschutz (mouth rinse)	4.5		-		500 ppm F: 125 ppm F as amine fluoride,
(GABA)	7 5		$77 \pm 21$		$350 \text{ ppm F}$ as NaF; $800 \text{ ppm Sn}$ as $\text{SnCl}_2$
Aronal fluoride-free brand (Japan) (GABA)	7.5		(DCPD)	n.a.	-

German brands are listed with their manufacturer. Free fluoride values are those determined in the slurries directly after preparation, with values determined 6 h after preparation in parentheses. n.a. = Not available.

<sup>1</sup> Value as determined, type of abrasives as declared: DCPD = dicalcium phosphate dihydrate; HS = hydrated silica; S = silica.

<sup>2</sup> Value from *Das Dentalvademekum* [2009]; active ingredients as claimed and declared.

#### Tissue Loss Measurement

Measurements were performed with an optical device (Micro-Prof, Fries Research & Technology GmbH, Bergisch Gladbach, Germany). On each sample, 3 traces were made at intervals of 0.2 mm, each 2 mm in length (200 pixels, 32 Hz, sensor HO). The traces were interpreted with special software (Mark III, Fries Research & Technology GmbH). Two regression lines were constructed on each trace, one in the reference area and one in the experimental area, both 0.5 mm in length. The vertical distance between the regression lines was then defined as tissue loss (micrometres). Ten repeated analyses with the removal and reposition of a randomly selected sample in the system yielded a standard deviation of 0.35  $\mu$ m (mean tissue loss of 16.1  $\mu$ m).

#### **Statistics**

Statistical procedures were performed with PASW Statistics 18 (SPSS GmbH, Munich, Germany). The Kolmogorov-Smirnov test revealed no significant deviation from the gaussian distribution.

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Differences between the groups were analysed with one-way ANOVA (for experiments 1 and 2). Tukey's post hoc test was used for experiment 1 and Tamhane's post hoc test was used for experiment 2 because there was a significant deviation from homogeneity of variance (Levene's test). Univariate ANOVA was used to analyse the interactions between fluoride content and RDA with respect to tissue loss (experiment 2). The significance level was set at 0.05.

## Results

Data of the RDA and fluoride measurements are given in table 1, the results for both experiments are presented in table 2 and figure 1.

### Experiment 1 (Effects of Slurries)

Tissue loss was highest in the group with erosion only (negative control), and all products (except BioRepair and Theramed Natural White) reduced tissue loss significantly in the order of 19–78% ( $p \le 0.05$  when compared to the negative control). However, when compared to the positive control, all products (except Gel-Kam) were significantly less effective ( $p \le 0.05$ ). Comparisons between the NaF toothpastes revealed only minor differences. Of the F-free products, BioRepair showed no significant effect compared to the negative control, and Chitodent reduced tissue loss in the order of conventional NaF products. Among the Sn-containing formulations, ProExpert was significantly more effective than all other toothpastes ( $p \le 0.05$  each, except for ApaCare n.s.), as was Gel-Kam ( $p \le 0.01$ ).

# *Experiment 2 (Effects of Slurries and Brushing Abrasion)*

Compared to erosion only, brushing with the F-free control toothpaste increased tissue loss by 29% ( $p \le 0.001$ ). The positive control reduced tissue loss by 62% compared to the F-free control toothpaste ( $p \le 0.001$ ) and by 51% compared to erosion only ( $p \le 0.001$ ). In addition, it was significantly more effective than all other test products except Gel-Kam ( $p \le 0.01$ ). None of the toothpastes reduced tissue loss when compared to erosion only.

Compared to brushing with the F-free control toothpaste, 3 out of 8 conventional toothpastes revealed a significant reduction in tissue loss ( $p \le 0.01$  each). The special formulation Pronamel led to the lowest tissue loss, while ApaCare was significantly less effective than Pronamel ( $p \le 0.001$ ). The effects of both products were in the order of conventional NaF products. **Table 2.** Tissue loss values ( $\mu$ m; means  $\pm$  standard deviation) after 10 experimental days with 6  $\times$  2 min erosion in 0.05 M citric acid (pH 2.4), and immersion in slurries for 2  $\times$  2 min either without (experiment 1) or with brushing for 15 s (experiment 2)

	Experi- ment 1	Experi- ment 2
Conventional toothpastes		
Theramed Natural White	$32.7 \pm 4.4$	$25.5 \pm 3.2$
Perlodent Kraeuter	$30.2 \pm 3.7$	$17.5 \pm 4.7$
Theramed 2in1 Original	$29.3 \pm 3.6$	$17.2 \pm 2.8$
Odol Med 3 Pro Clean	$28.5 \pm 4.0$	$20.6 \pm 4.7$
Blend-A-Med Classic	$28.4 \pm 4.2$	$20.1 \pm 4.1$
Sensodyne MultiCare	$26.5 \pm 4.6$	$22.1 \pm 4.3$
GUM Original White	$24.9 \pm 4.6$	$17.6 \pm 5.0$
Dentagard Original	$24.2 \pm 5.9$	$22.3\pm5.8$
Toothpastes with anti-erosion claims		
Pronamel	$30.3 \pm 5.8$	$15.7 \pm 3.2$
ApaCare	$21.5 \pm 5.8$	$22.1 \pm 3.6$
BioRepair	$34.2 \pm 4.6$	$28.9 \pm 5.2$
Chitodent	$26.3 \pm 6.5$	$20.7\pm4.3$
Sn-containing products		
ProExpert Gum Protection	$16.8 \pm 6.7$	$19.2 \pm 9.2$
Gel-Kam	$8.3 \pm 3.3$	$6.3\pm2.1$
Control groups		
Negative		
Erosion only	$37.1 \pm 5.5$	$19.3 \pm 3.0$
Erosion and abrasion (Aronal without F)	_	$24.9 \pm 3.4$
Positive		
Elmex Erosionsschutz mouth rinse	$8.2 \pm 3.5$	_
Elmex Erosionsschutz mouth rinse and abrasion with Aronal without F	-	$9.5 \pm 4.3$

The F-free product BioRepair exhibited the overall highest tissue loss values, and was significantly less effective than most of the NaF toothpastes. Chitodent revealed effects similar to that of the conventional NaF toothpastes.

Gel-Kam was significantly better than all the other test products ( $p \le 0.01$  each) and reduced tissue loss in the order of the positive control (n.s.). The effect of Pro-Expert was in a similar order to the conventional NaF toothpastes. Due to the high standard deviation, however, there was no significance to the other tested products.

As to overall effects there was no significant impact of fluoride concentration or RDA on enamel loss, and no interaction between these factors.



**Fig. 1.** Percent reduction of tissue loss compared to erosion only. Light grey columns = immersion in slurries (experiment 1); black columns = immersion in slurries and brushing abrasion (experiment 2). Columns sharing the same letter were not significantly different (lower case letters, experiment 1; upper case letters, experiment 2).

# Discussion

Though acid exposures from various sources are common, for instance from normal dietary intake, most people do not exhibit clinically relevant erosive lesions. Thus, individuals with manifest defects either must be particularly susceptible or must have more than average acid exposure. The present study sought to represent such conditions by frequent acid exposures with citric acid mimicking, for example, the overconsumption of soft drinks. The abrasion protocol approximated clinical conditions. The immersion in slurry was 2 min and during this time brushing was performed for 15 s. A previous tooth brushing study [Ganss et al., 2009] demonstrated that adults had an average brushing time of 96 s, which would equal a brushing time of 24 s per quadrant. The brushing time per sample used here was somewhat longer, but reflects more thorough habits for oral hygiene. Overall, the experimental design followed recent recommendations [Shellis et al., 2011; Wiegand and Attin, 2011].

The fluoride toothpastes included were selected according to the fluoride compound (all NaF, except for 1 toothpaste additionally containing  $SnF_2$ ) and fluoride content (all around 1,450 ppm F) as declared on the containers. Furthermore, it was intended to cover a range of low, medium and high abrasivity. The products were selected based on RDA values published by *Das Dentalvademekum* [2009], which is an extensive compilation of information on products used in dentistry issued by the German Dental Association (Bundeszahnärztekammer)

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and the National Association of Statutory Health Insurance Dentists (Kassenzahnärztliche Bundesvereinigung). The toothpastes were regarded as special products against erosion when this was claimed by the manufacturer.

The RDA of brands might vary depending on the country of origin and was not available for all products included. Therefore, the values for all batches used in the present experiment were determined. Even though differences were expected, it was striking how far the values diverged, indicating that RDA obtained from sources should be used with caution. It should be noted that RDA is not necessarily related to relative enamel abrasivity (REA) [Barbakow et al., 1989]. However, the REA is valid only for sound enamel and very little is known about its relevance to acid-softened enamel surfaces. For example, a study comparing the effects of toothpastes with and without abrasives revealed that the presence of abrasives increased tissue loss, but the degrees were similar for the toothpastes with medium and high REA [Wiegand et al., 2008]. Similar results were obtained for F-free toothpastes in an experiment comparing tissue loss after brushing with experimental F and non-F formulations of different abrasivity [Hara et al., 2009a]. Systematic studies comparing REA and RDA on eroded enamel, however, are lacking. From these considerations and because the REA is generally rarely available, the RDA value is given here. The mouth rinse was used as a positive control because its efficacy has been confirmed in vitro [Schlueter et al., 2010] and in situ [Ganss et al., 2010].

Regarding the degree of tissue loss, it was striking that the order of values obtained from experiments 1 and 2 differed despite all procedures (temperature, shaking frequency, shaking bath, rinsing procedures and transposition of the samples in the fluids) being standardised. The only difference was the position of the samples in the erosion solution: in experiment 1 the samples were on glass slides positioned vertically and in experiment 2 they were on holders for the brushing machine positioned horizontally. A possible explanation for the diverse set of results is that the clearance of dissolution products, which is essential for the erosion process [Shellis et al., 2005], varied depending on the orientation of the samples. Due to this methodological difference, the results of the 2 experiments cannot be directly compared. This finding emphasises the need for the meticulous standardisation and control of experimental conditions [Shellis et al., 2011].

Except for ApaCare and Blend-A-Med Classic, the amount of free fluoride detected in the slurry superna-

tants was in the expected order. ApaCare contains hydroxyapatite which could lower the amount of available F ions, but Blend-A-Med Classic is a standard NaF toothpaste formulation, and from the ingredients/excipients declared there is no explanation for the low available fluoride measured. In contrast to all other products, however, the supernatant from the slurry was very turbid, the cause of which is not clear. The fluoride content increased with time, and it can be speculated that fluoride was bound or adsorbed in the freshly prepared slurry and released again when the mixture was allowed to age. This effect was not found for ApaCare indicating a much more stable binding of the fluoride ions.

In experiment 1 the effect of the toothpaste slurries containing NaF was in the order of 19-42% and was not related to the amount of available fluoride. This was particularly evident for ApaCare for which from the 1,450 ppm fluoride declared only roughly 15% was available. It is not clear how the effects of toothpaste slurries can be explained, but in the case of enamel erosion, it can only be assumed that its mode of action is surface precipitation. After short-term immersion of low-concentrated fluoride solutions on sound enamel, only limited, if any, precipitation of CaF<sub>2</sub>-like material could be expected in vitro [Bruun and Givskov, 1993; Petzold, 2001]. However, providing Ca by pretreatment with CaCl<sub>2</sub> [Saxegaard and Rølla, 1988] or with saliva [Larsen and Richards, 2001] or altering the enamel surface structure by etching [Saxegaard and Rølla, 1988, 1989] can increase the amount of fluoride adsorbed to the surface. Besides these factors, the presence of calcium and phosphate in the slurries could contribute to establishing surface precipitates also in the presence of very low amounts of F, and it can be assumed that protecting effects depend on the amount, type and acid solubility of such deposits. However, the results indicate that further research is necessary to elucidate the mode of action of fluoride toothpastes in the frame of dental erosion.

The product with nano-zinc carbonate-hydroxypatite but without fluoride (BioRepair) did not reduce erosive loss. There is some evidence from an experiment with pre-eroded enamel and application times of the toothpaste during 36 h [Poggio et al., 2010] that precipitation can occur. Zinc-carbonate-hydroxyapatite, however, is easily dissolvable in acids and there is currently no evidence that its nanocrystalline form contributes significantly to solubility.

An interesting finding was that the product containing chitosan but no fluoride reduced tissue loss by about 30%. Chitosan is a natural polysaccharide produced by deacetylation of chitin. It is a cationic polyelectrolyte with a pK<sub>a</sub> of 6–7, it has a strong positive charge at low pH and adsorbs readily to structures with negative zeta potential [Claesson and Ninham, 1992], like enamel [Young et al., 1997]. Thus, the effects observed from Chitodent may be attributed to the build-up of a protective organic layer. It could be speculated that this effect is even more pronounced in vivo because chitosan can also bind to salivary pellicles [van der Mei et al., 2007] and can form multilayers in the presence of mucin [Svensson et al., 2006].

The best effects were found with the Sn-containing products, which complements experimental findings and theoretical considerations on the anti-erosive potential of the tin ion [Ganss et al., 2008; Schlueter et al., 2009a, b]. The tin concentration in the slurries was similar to the mouth rinse used in the positive control, which explains the comparable effect of the gel slurry and the positive control. The toothpaste was somewhat less effective than the gel, which might be due to the reduction of available tin ions from adsorption to the negatively charged silica.

When physical forces were included (experiment 2), none of the toothpastes had protective properties against erosion when erosion protection is defined as the potential to lower substance loss below the level of tissue loss after erosion only, and only a few had abrasion prevention properties by reducing substance loss compared with brushing with the F-free control.

The effects of combined physicochemical impacts on enamel are difficult to interpret, the more so since the composition of toothpastes is very complex. In a first approximation one could assume that a good efficacy of active ingredients would lead to less loss of surface hardness, thus making the eroded surface less susceptible to abrasion. This assumption is supported by the results from the positive control. With the toothpastes used here, however, this relation was not so obvious and there was no clear impact of the RDA. In most of the products with good anti-erosive effects from slurries, the benefit from active ingredients was counteracted by the effects of abrasion. This was particularly evident for the fluoride product with hydroxyapatite (ApaCare) and the Sn-containing toothpaste (ProExpert). Conversely, the products with limited effects of active ingredients revealed good effects in the abrasion experiment.

There is little knowledge of the effects of abrasives and abrasion on eroded enamel independently from the effects of active ingredients. It is well known that sound enamel is relatively resistant to physical impacts [Attin et al., 1997; Eisenburger et al., 2003], whereas significant tissue loss can occur after exposure to acids [Vieira et al., 2006; Voronets and Lussi, 2010]. Similar to sound enamel, the main effect on tissue loss appears to be due to the action of abrasives rather than to the impact of the toothbrush itself [Voronets and Lussi, 2010], at least in early stages. However, conflicting information exists on how the tissue loss due to physical impacts is related to the surface properties of eroded enamel. In an experiment focusing on the initial stages, tissue loss due to tooth brushing remained constant even when tissue and microhardness loss was increasing [Lippert et al., 2005]. In contrast, an experiment focusing on more advanced stages [Attin et al., 1997] clearly demonstrated that the amount of loss depended on how softened the enamel surface had become. It is important to note that this relationship was not linear. Therefore, the relevance of rehardening effects observed from toothpaste applications [Lussi et al., 2008] is difficult to estimate.

Softened enamel has been assumed to be removable even by weak forces, like the friction of oral soft tissues [Gregg et al., 2004; Vieira et al., 2006], but even more so after stronger impacts [Eisenburger et al., 2000]. Scanning electron microscopy studies, however, have revealed that distinct signs of erosive demineralisation persist even after ultrasonication [Eisenburger et al., 2004] or tooth brushing [Rios et al., 2008a]. This is corroborated by hardness measurements demonstrating that the hardness of acid-softened surfaces increases after brushing, but does not reach the order of that of sound tissue by far [Lippert et al., 2005]. From these considerations, it can be assumed that the effects of physical impacts occur within the small demineralised band at the enamel surface. If this assumption is correct, the degree of tissue loss from abrasion would depend not only on the erosion-inhibiting potential of active ingredients, but also on their modes of action. In the case that it is merely surface precipitation, tissue loss would depend on the amount and physical properties of the deposited material. If it is the incorporation of foreign ions in the softened enamel, the question arises as to how stable the resulting enamel structure is against physical forces. This is particularly important for Sn- and F-containing preparations because it has been shown that a broad Sn-containing surface band with a less dense histological feature than sound enamel, developed when applied in a cyclic erosion model [Schlueter et al., 2009b], which might not be very resistant against abrasion. From this background, it was an encouraging finding that the Sn/F mouth rinse used here was not only effective in preventing erosive loss, but was also very effective in the abra-

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sion experiment. However, considerably more research is needed to understand the combined physicochemical interactions occurring in erosion models in particular with respect to the role of abrasives.

In conclusion, conventional NaF toothpastes reduced the erosive tissue loss even under relatively severe erosive conditions, but had limited efficacy regarding brushing abrasion. The formulations with anti-erosion claims were not superior, and some were even less effective compared to conventional products. The tin-containing toothpaste had promising anti-erosion potential, which was, however, counteracted by the effects of abrasion.

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