

Impact of Different Toothpastes on the Prevention of Erosion

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Key Words

Erosive tooth wear · Fluoride · Prevention of erosion · Toothpaste · Saliva

Abstract

The aim of the present study was to test the impact of different toothpastes on the prevention of erosion. Enamel demineralization and remineralization were monitored using surface microhardness (SMH) measurements. Human enamel specimens were treated following two different procedures: (1) incubation in toothpaste slurry followed by acid softening and artificial saliva exposure; (2) acid softening followed by incubation in toothpaste slurry and artificial saliva exposure. For the control procedure, toothpaste treatment was excluded. The following toothpastes were tested: Zendium, Sensodyne Proschmelz (Pronamel), Prodent Rocket Power, Meridol and Signal active. Normalized SMH values compared to the baseline (= 1.00) after 1-hour artificial saliva exposure for procedure 1 (respectively for procedure 2) were as follows (mean: 95% CI): Sensodyne Proschmelz 0.97: 0.93, 1.00 (0.92: 0.90, 0.94), Zendium 0.97: 0.94, 1.00 (0.89: 0.83, 0.95), Meridol 0.97: 0.94, 1.00 (0.94: 0.92, 0.96), Signal active 0.94: 0.91, 0.97 (0.95: 0.91, 0.99), Prodent Rocket Power 0.92: 0.90, 0.94 (0.93: 0.89, 0.97) and control 0.91: 0.88, 0.94. Further exposure to artificial saliva for up to 4 h showed no significant improvement of SMH. Regression analyses revealed a significant impact of the applied procedure. Incubation in toothpaste slurries before the acid challenge seems to be

favorable to prevent erosion. None of the tested toothpastes showed statistically significant better protection than another against an erosive attack.

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There is some evidence that the prevalence of dental erosion is on the increase. Therefore it is important to diagnose this condition as early as possible and to initiate preventive measures to diminish further progression [Jaeggi and Lussi, 2006].

In recent years, research groups have investigated the preventive effect of several fluoride regimes on dental erosion and found different efficacy [Attin et al., 1999; Ganss et al., 2001, 2004; Jones et al., 2002; van Rijkom et al., 2003; Hughes et al., 2004; Lussi et al., 2004; Schlueter et al., 2007]. Larsen and Richards [2002] showed in vitro that fluoride treatment was unlikely to provide a preventive effect against erosion because an acidic drink will rapidly dissolve accessible calcium fluoride and remove the remaining traces of a previous topical fluoride treatment. Further saturation of the drinks with calcium fluoride did not significantly reduce the erosive potential of the drinks unless fluoride concentrations in a harmful range were used [Larsen and Richards, 2002]. However, it has to be kept in mind that the calculations and measurements of the latter study did not take into account the coating of the CaF₂ layer by phosphates and proteins, which occurs in the mouth. These stabilized particles are

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more resistant to an acidic attack [Øgaard et al., 1994; Ganss et al., 2007].

Obviously, substance loss of acid-softened tooth surfaces by mechanical load is greater than loss of sound surfaces under the same conditions [Attin et al., 1997; Jaeggi and Lussi, 1999; Hemingway et al., 2006]. In one study fluoride dentifrice had some protective effect on eroded enamel to brushing abrasion when immersed in vitro in a cola drink for 5 min, 4 times a day [Magalhaes et al., 2007]. Bartlett et al. [1994], using a cycling model of erosion (citric acid, 5 min, pH 3.5) and abrasion, showed less wear in the presence of fluoride toothpaste than in the presence of a non-fluoride toothpaste.

It seems reasonable to develop new toothpastes with good protection against erosion. Currently, conventional (fluoride-containing) toothpastes do not appear to be able to protect efficiently against erosion as this condition seems to be still on the increase. Nevertheless, it seems that a growing market for devices preventing erosion is emerging.

The aim of the present study was to compare the impact of five different toothpastes on the prevention of erosion. Three of the toothpastes studied claim to prevent dental erosion.

Material and Methods

Preparation of Enamel Specimens

Ninety-nine caries-free human premolars with no cracks evident on the facial surface when viewed under a stereomicroscope (magnification $\times 25$) were selected from a pool of extracted teeth. All teeth had been extracted by dental practitioners in Switzerland (no water fluoridation, 250 ppm F in table salt). Prior to the extraction, the patients were informed about the use of their teeth for research purposes and consent was obtained. After brushing thoroughly under running tap water the crowns were separated from the roots. Then the facial sides were cut out under water cooling and ground flat on a rotating polishing machine (Knuth-Rotor, Struers, Copenhagen, Denmark) as follows. Each slab was embedded in resin (Paladur, Heraeus Kulzer GmbH, Hanau, Germany) in two planar parallel molds. The thinner mold (200 μm thick) was removed, and the thicker mold (7 mm thick) was serially abraded under constant tap water cooling on the Knuth Rotor polishing machine with silicon carbide paper discs of grain size 18, 8 and 5 μm for 30 s each. Then the embedded enamel blocks were taken out of the molds before being polished for 60 s with 3- μm diamond abrasive on Struers polishing cloth under constant cooling (LaboPol-6, DP-Mol Polishing, DP-Stick HQ, Struers). Between the polishing steps and after the final polishing, all slabs were sonicated for 3 min in a detergent solution and rinsed under running tap water. This way, specimens were produced, with a flat ground area from which 200 μm of enamel had been removed in the center of the window. Polished enamel specimens

were selected and six indentations were made for standardization. The procedure for preexperimental stratified random sampling was described earlier [Lussi et al., 1993]. After the randomization procedure and before the experiments all specimens were stored in a supersaturated mineral solution (1.5 mmol/l CaCl_2 , 1.0 mmol/l KH_2PO_4 , 50 mmol/l NaCl, pH 7.0) [Zero et al., 1990]. Prior to the experimental procedures the specimens were further polished with a 1- μm diamond abrasive for 60 s (LaboPol-6, DP-Mol Polishing, DP-Stick HQ, Struers).

Surface Microhardness Measurement

Surface microhardness (SMH) measurements were performed with a Knoop diamond under a force of 0.49 N applied for 20 s (MHT-10 Microhardness tester, Anton Paar, Paar Physica, Austria). Indentations were made with the long axis parallel to the coronal axis of the enamel at intervals of 50 μm in the center of the exposed area within an area of 500 μm (mesiodistal) and 100 μm (coronapical). Each indentation was neighbored by the corresponding next indentation following the experimental procedure. Indentation lengths were measured with an optical analysis system and transferred to a computer (DMR Microscope, Leica, Wetzlar, Germany). The apparatus was calibrated before each use.

Toothpaste Slurry

Each specimen was immersed in 6 ml toothpaste slurry (30 wt%, toothpaste/deionized water) for 3 min at 30°C under constant agitation on an orbital shaker (Salvis, Reussbühl, Switzerland) [ten Cate et al., 2006].

The following toothpastes were tested:

- 1 Zendium Syreforsvar (batch 125222, 166601), Sara Lee/DE, Den Haag, The Netherlands, 1,450 ppm NaF, pH of the slurry 5.21;
- 2 Sensodyne Proschmelz (Pronamel) (batch 6060 P1), Glaxo-SmithKline, Bühl, Germany, 1,450 ppm NaF, 5% KNO_3 , pH of the slurry 6.81;
- 3 Prodent Rocket Power (batch 220111, 243503), Sara Lee H&BC, Veenendaal, The Netherlands, 1,100 ppm NaF, pH of the slurry 9.37;
- 4 Meridol (batch 545202, 05-2008), Gaba AG, Therwil, Switzerland, 1,400 ppm Olafur/Sn(II)F, pH of the slurry 4.74 (positive control), and
- 5 Signal active (batch 60513IWC), Unilever Schweiz, Zug, Switzerland, 1,450 ppm NaF, pH of the slurry 6.67 (positive control).

After incubation, the specimens were carefully rinsed with deionized water and dried for 5 s with oil-free air.

Surface Softening

Each specimen was immersed in 20 ml of 1% citric acid, pH 4, for 3 min, at 30°C under constant agitation on an orbital shaker (Salvis, Reussbühl, Switzerland). The specimens were then carefully rinsed in deionized water and dried for 5 s with oil-free air.

Artificial Saliva

The specimens were incubated in 20 ml artificial saliva at 37°C under constant agitation for 1, 2 and 4 h on an orbital shaker (Salvis). One liter of artificial saliva contained: 0.002 g ascorbic acid, 0.58 g NaCl, 0.17 g CaCl_2 , 0.16 g NH_4Cl , 1.27 g KCl, 0.16 g NaSCN, 0.33 g KH_2PO_4 , and 0.34 g Na_2HPO_4 [Lennon et al.,

2006]. The pH was adjusted to 6.4. Then, specimens were carefully rinsed in deionized water and dried for 5 s with oil-free air before SMH measurements.

Study Design

Three (preexperimental) indentations were done in the center of the enamel window of each prepared specimen and the average SMH was calculated. Based on these data the specimens were assigned to nine groups according to the distribution of all specimens. Statistically determined outliers were discarded. Then, one specimen from each of the nine groups was randomly selected and assigned to a test group in a randomized block design [Jaeggi and Lussi, 1999]. Thereafter, enamel specimens were treated following two different procedures and a control.

Procedure 1. Six baseline indentations were made and the average SMH was calculated. Then, the specimens were incubated in the toothpaste slurry followed by additional hardness measurements (six indentations). Thereafter surface softening was executed and surface hardness was again measured (six indentations). To check the effect of saliva exposure after toothpaste treatment and softening on SMH, further measurements were made after exposure to artificial saliva for 1, 2 and 4 h (six indentations each). During experimental procedures specimens were stored in a chamber with 100% humidity.

Procedure 2. In contrast to procedure 1 the erosive challenge took place prior to incubation in the toothpaste slurries. Except for this difference in experimental procedure the measurements were as described in procedure 1.

Negative Control. These specimens were tested in the same manner without incubation in a toothpaste slurry.

For each experimental run 9 specimens were tested. A total of 99 specimens were used for experimental procedures (5 toothpastes \times 2 procedures \times 9 specimens + 9 specimens for the control group).

SMH Analysis

The change in normalized SMH was calculated and expressed as fractions relative to the baseline. In procedures 1 and 2, the initial baseline hardness was set to 1.0 (SMH_B). The measured hardness values after each step (SMH_x) downstream were normalized relative to the baseline, according to the formula $SMH_n = (SMH_x/SMH_B)$ where SMH_n is the normalized hardness after a certain step.

Statistics

Descriptive analyses with box- and quantile-quantile plots showed a normal distribution of the data (box plot/QQ plot, SPSS 11.0, SPSS Inc., Chicago, Ill., USA). Comparisons of two groups were performed using the t test. When the groups were dependent, paired t test was performed. Values between procedures 1 and 2 were compared with the independent t test. For multiple comparisons the p values were corrected by the Bonferroni adjustment procedure. Furthermore, the data were subjected to analyses of variance using the GLM procedure (GLM, SAS Institute Inc., Cary, N.C., USA). The relationship between the change in SMH from baseline after artificial saliva exposure (dependent variable) and toothpaste, pH of toothpaste slurry, procedure and baseline SMH (independent variables) were investigated. Thereby, the variables toothpaste and procedure were set as categorical variables. The significance level for all statistical tests was set at 0.05.

Results

Table 1 gives an overview of SMH values and the 95% confidence interval (CI) measured at each step of procedures 1 and 2 and the negative control as well as the corresponding normalized SMH. In both procedures, there was no statistically significant difference in SMH after 1-, 2- and 4-hour incubation in artificial saliva ($p > 0.05$). Therefore, the statistical comparisons were made with the values after 1-hour incubation.

Procedure 1. After 3-min incubation in toothpaste slurry, all test groups showed an increase in Knoop SMH. The greatest SMH increase was found for Zendium (20.9, $p < 0.001$). The SMH increases of Prodent Rocket Power (11.2, $p > 0.05$) and Signal active (6.8, $p > 0.05$) were not statistically significant. After softening, all groups showed a statistically significant decrease in SMH compared to baseline. The SMH values after incubation in artificial saliva for 1, 2 and 4 h showed greater values in all groups compared to SMH, directly measured after softening but decreased values compared to baseline. Significant decreases in SMH after 1-hour immersion in artificial saliva compared to baseline were found for Prodent Rocket Power ($p < 0.001$), the control ($p < 0.001$), Signal active ($p = 0.004$) and Meridol ($p = 0.041$).

Procedure 2. Softening showed statistically significant decreasing values in all groups compared to baseline. The immersion of erosively altered enamel specimens in toothpaste slurry gave increasing and decreasing values with no statistically significant differences ($p > 0.05$). In all groups the SMH measurements after immersion in artificial saliva for 1, 2 and 4 h showed increased values compared to SMH measurements after softening. Thereby significant increases were found for Prodent Rocket Power, Meridol and Signal active ($p < 0.05$). Compared to baseline hardness all products showed decreased values after artificial saliva exposure. With the exception of Signal active ($p = 0.068$) all SMH decreases after 1-hour exposure to artificial saliva were statistically significantly different ($p < 0.05$).

Compared to procedure 1, procedure 2 showed significantly lower hardness after artificial saliva exposure of 1 h ($p = 0.031$), 2 h ($p = 0.049$) and 4 h ($p = 0.041$). The baseline hardness showed no significant differences ($p > 0.05$).

The change in hardness after artificial saliva exposure compared to the baseline was significantly influenced by the two procedures. The respective p values were 0.013 after 1-hour, 0.027 after 2-hour and 0.01 after 4-hour exposure. Between 14 and 18% of all variations could be explained by this parameter.

Table 1. Mean (95% confidence interval = CI) Knoop surface microhardness = SMH values and the corresponding normalized SMH compared to the baseline measured at each step of procedures 1 and 2 for all tested toothpastes and the negative control

Procedure 1	Baseline		After toothpaste		After softening		After saliva 1 h		After saliva 2 h		After saliva 4 h							
	SMH	CI	SMH	CI	SMH	CI	SMH	CI	SMH	CI	SMH	CI						
													lower	upper	lower	upper	lower	upper
Zendium	339	327	351	349	369	314	300	328	330	311	349	324	309	339	327	313	341	
Sensodyne Proschmelz	340	334	346	360	354	317	305	329	330	317	343	322	313	331	328	315	341	
Prodent Rocket Power	345	341	349	356	339	292	272	312	316	307	325	312	300	324	316	304	328	
Meridol	334	322	346	346	338	315	307	323	322	314	330	324	316	332	322	313	331	
Signal active	338	332	346	345	333	300	283	317	319	307	331	321	306	336	321	311	331	
	Normalized SMH																	
Zendium	1.0		1.06	1.04	1.08	0.93	0.91	0.95	0.97	0.94	1.00	0.96	0.93	0.99	0.97	0.94	1.00	
Sensodyne Proschmelz	1.0		1.06	1.04	1.08	0.93	0.90	0.96	0.97	0.93	1.01	0.95	0.93	0.97	0.96	0.93	0.99	
Prodent Rocket Power	1.0		1.03	0.99	1.07	0.85	0.79	0.91	0.92	0.90	0.94	0.90	0.87	0.93	0.91	0.88	0.94	
Meridol	1.0		1.04	1.02	1.06	0.95	0.92	0.98	0.97	0.94	1.00	0.97	0.94	1.00	0.97	0.95	0.99	
Signal active	1.0		1.02	1.00	1.04	0.89	0.84	0.94	0.94	0.91	0.97	0.95	0.91	0.99	0.95	0.92	0.98	
	Procedure 2																	
Procedure 2	Baseline		After softening		After toothpaste		After saliva 1 h		After saliva 2 h		After saliva 4 h							
	SMH	CI	SMH	CI	SMH	CI	SMH	CI	SMH	CI	SMH	CI						
													lower	upper	lower	upper	lower	upper
Zendium	335	325	345	291	274	308	297	277	317	297	277	317	300	286	314	298	276	320
Sensodyne Proschmelz	345	338	352	305	296	314	301	292	310	316	311	321	311	301	321	310	304	316
Prodent Rocket Power	343	336	350	299	285	313	311	295	327	318	301	335	319	301	337	319	306	332
Meridol	343	334	352	312	305	319	312	303	321	321	313	329	322	314	330	325	314	336
Signal active	332	323	341	303	293	313	307	296	318	316	300	332	310	297	323	317	302	332
	Normalized SMH																	
Zendium	1.0		0.87	0.83	0.91	0.88	0.84	0.92	0.89	0.83	0.95	0.90	0.86	0.94	0.89	0.84	0.94	
Sensodyne Proschmelz	1.0		0.89	0.88	0.90	0.87	0.85	0.89	0.92	0.90	0.94	0.90	0.87	0.93	0.90	0.89	0.91	
Prodent Rocket Power	1.0		0.87	0.83	0.91	0.91	0.87	0.95	0.93	0.89	0.97	0.93	0.88	0.98	0.93	0.89	0.97	
Meridol	1.0		0.91	0.89	0.93	0.91	0.89	0.93	0.94	0.92	0.96	0.94	0.92	0.96	0.95	0.93	0.97	
Signal active	1.0		0.92	0.89	0.95	0.93	0.89	0.97	0.95	0.91	0.99	0.93	0.89	0.97	0.96	0.92	1.00	
	Negative control																	
Negative control	Baseline		After softening		After saliva 1 h		After saliva 2 h		After saliva 4 h									
	SMH	CI	SMH	CI	SMH	CI	SMH	CI	SMH	CI								
											lower	upper	lower	upper	lower	upper		
338	334	342	291	279	303	307	296	318	305	292	318	303	291	315				
	Normalized SMH																	
1.0		0.86	0.83	0.89	0.91	0.88	0.94	0.90	0.87	0.93	0.93	0.90	0.86	0.94				

Discussion

In this study, three toothpastes that claimed to prevent erosion were tested. They were compared with two conventional toothpastes (positive control) and a negative control. The tested toothpastes showed comparable but different fluoride content ranging from 1,100 to 1,450 ppm. The range of free-fluoride availability measured as described earlier [Newby et al., 2006] was between 1,090 and 1,320 ppm. This rather small difference could be one reason why no overall significant differences between products were found. However, the pH of toothpaste slurries showed distinctive differences (pH range: 4.74–9.37). Zendium slurry (pH 5.21) and the Meridol slurry (pH 4.74) were slightly acidic and would be expected to provide higher F intensity than the neutral ones. Brighenti et al. [2006] found a smaller decrease in percent SMH in a pH cycling model for 7 days when using acidified toothpastes compared to neutral ones. In our study no overall better performance could be shown of the slightly acidic toothpaste slurries compared to the neutral ones. The most basic pH (9.37) was found with Prodent Rocket Power toothpaste slurry. When this slurry was used after softening, slightly better rehardening could be measured. Although the teeth were rinsed with water after softening this slurry probably increased the pH at a faster rate at the tooth surface than the other formulations. However, this may be interpreted with caution as no statistically significant effect of this parameter was found.

In an *in situ* study Zero et al. [2006] found a beneficial effect of an experimental toothpaste containing 1,100 ppm F and 5% KNO₃ compared to a toothpaste available on the market with 1,100 ppm F. In addition Bartlett et al. [1994] and Magalhaes et al. [2007] showed a beneficial effect of fluoride dentifrice. In contrast to the surface softening during 3 min (pH 4) in our experiment these researchers used a more severe and longer attack (e.g. 25 min with grapefruit juice with its low pH and high buffering capacity).

Regression analyses revealed a significant impact of the applied procedure on SMH. SMH values after 1, 2 and 4 h in saliva for procedure 1 were significantly higher than the corresponding values for procedure 2. Therefore, the incubation of enamel specimens in toothpaste slurry prior to softening seems to be more favorable than postexposure incubation, which is in accordance with other studies [Hughes et al., 2004; Ponduri et al., 2005]. This could be due to some incorporation of material into and/or deposition of material onto the enamel surface, most probably as a CaF₂-like material, which will lead to

less softening than in the absence of this layer [Ganss et al., 2001; Lussi and Hellwig, 2006]. Independent of the applied procedure and dentifrice, all groups showed increased SMH values after immersion in artificial saliva compared to SMH values directly measured after softening. But there were no significant changes in SMH between 1-, 2- and 4-hour immersion in saliva. The remineralization/rehardening effect is controversial. There is evidence that acid-softened enamel can reharder after exposure to saliva or to a remineralization solution and that dietary products and fluoride can enhance the rehardening process [Feagin et al., 1969; Gedalia et al., 1991a; Zero et al., 1994; Amaechi and Higham, 2001; Kim et al., 2001]. Other investigations did not find a significant rehardening effect of saliva [Garberoglio and Cozzani, 1979; Gedalia et al., 1991b; Collys et al., 1993; Lippert et al., 2004a, b].

In the presence of saliva pellicle is formed, which might act as a diffusion barrier, therefore diminishing acid exposure and ion dissolution of the tooth surface. Thus, salivary pellicle may protect against further erosion. We did not intend to simulate the acquired pellicle as this barrier for acid diffusion could masque the potential of a toothpaste to protect from erosion. Further, the protection of a very thin pellicle is small [Amaechi et al., 1999].

Toothpaste application prior to an erosive challenge seems to be favorable compared to postexposure tooth cleaning. However, in practice it would be difficult to motivate patients prone to erosive tooth wear to execute a fluoride regime prior to an acid attack such as vomiting. On the other hand each fluoride application after an erosive challenge has the benefit of being a fluoride input before the next erosive challenge (e.g. toothpastes applied after dinner would be beneficial for reflux during the night).

In summary, neither the use of the toothpastes under study nor immersion for up to 4 h in artificial saliva led to complete recovery of microhardness. More research is needed to evaluate the influence of possible preventive measures against erosion.

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