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Randomised in situ Study on the Efficacy of a Tin/Chitosan Toothpaste on Erosive-Abrasive Enamel Loss

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Key Words
Abrasion · Chitosan · Enamel · Erosion · Tin

Abstract
Tin is a notable anti-erosive agent, and the biopolymer chitosan has also shown demineralisation-inhibiting properties. Therefore, the anti-erosive/anti-abrasive efficacy of the combination of both compounds was tested under in situ conditions. Twenty-seven volunteers were included in a randomised, double-blind, three-cell crossover in situ trial. Enamel specimens were recessed on the buccal aspects of mandibular appliances, extraorally demineralised (6 × 2 min/day) and intraorally treated with toothpaste slurries (2 × 2 min/day). Within the slurry treatment time, one-half of the specimens received additional intraoral brushing (5 s, 2.5 N). The tested toothpastes included a placebo toothpaste, an experimental NaF toothpaste (1,400 ppm F^-) and an experimental F/Sn/chitosan toothpaste (1,400 ppm F^-, 3,500 ppm Sn^{2+}, 0.5% chitosan). The percentage reduction of tissue loss (slurry exposure/slurry exposure + brushing) compared to placebo was 19.0 ± 47.3/21.3 ± 22.4 after use of NaF and 52.5 ± 30.9/50.2 ± 34.3 after use of F/Sn/chitosan. F/Sn/chitosan was significantly more effective than NaF ($p \leq 0.001$) and showed good efficacy against erosive and erosive-abrasive tissue loss. This study suggests that the F/Sn/chitosan toothpaste could provide good protection for patients who frequently consume acidic foodstuffs.

Stannous ion-containing solutions are notably effective in preventing erosion progression under severe erosive in vitro [Hove et al., 2007; Schlueter et al., 2009a, 2010a] and in situ conditions [Hove et al., 2008; Ganss et al., 2010], occurring in patients with high risk of acid-induced dental substance loss. For persons with less severe erosive challenges, preparations that can be used as part of the daily oral hygiene regime, such as toothpastes, would be a good option for erosion prevention. It can be deduced from the data in the literature that toothpastes containing sodium fluoride, which are the most commonly used toothpastes, offer only a protection of 10–30% against tissue loss [Hooper et al., 2007; Moretto et al., 2010; Ganss et al., 2011] and that erosive lesions can develop in spite of their use. Sn^{2+}- and F^--containing toothpaste slurries are notably more effective, showing reductions of up to 80% [Hooper et al., 2007; Ganss et al., 2011]. This beneficial effect of the active agent tin in combination with fluoride, however, is partially lost if an additional abrasive challenge occurs [Ganss et al., 2012], resulting in an efficacy comparable to NaF-containing toothpastes [Ganss et al., 2011, 2012]. Therefore, enhancement of the anti-erosive/anti-abrasive efficacy would be desirable. It is known that the addition of polymers to acids or acidic foodstuff such as polyphosphates [Barbour et al., 2005], xanthan [West et al., 2004; Barbour et al., 2005] or casein [Barbour et al., 2008] and ovalbumin [Hemingway et al., 2008] can reduce their erosivity. Polymers in oral hygiene products, such as

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casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) [Ranjitkar et al., 2009] or chitosan [Ganss et al., 2011], have also been investigated as anti-erosive agents. A fluoride-free, chitosan-containing preparation showed promising results, as it is able to reduce erosive tissue loss in a dimension comparable to sodium- or stannous fluoride-containing toothpastes [Ganss et al., 2011]. Altogether, the single compounds sodium fluoride, tin with fluoride and chitosan are able to reduce erosive-abrasive tissue loss in comparable dimensions. The effect of the combination of these single compounds has been investigated under in vitro conditions [Ganss et al., 2012]. This study clearly demonstrated that the addition of chitosan to a stannous chloride-, amine- and sodium fluoride-containing toothpaste significantly enhanced its anti-erosive/anti-abrasive efficacy. Whether this also holds true under in situ conditions in the presence of saliva and pellicle has not been investigated yet.

The aim of this study was, therefore, to investigate the in situ effect of an experimental chitosan- and Sn^{2+}-containing amine and sodium fluoride toothpaste on erosive and erosive-abrasive tissue loss in enamel and to compare its effect with the effects of experimental sodium fluoride and placebo toothpastes.

Materials and Methods

The single-centre, prospective, double-blind, three-cell study was conducted at the Dental Clinic of Justus Liebig University, Giessen according to the Good Clinical Practice Guidelines. This study conformed to the Declaration of Helsinki, was approved by the local ethics committee (Ethik-Kommission, Fachbereich Medizin, Justus-Liebig-Universität Giessen, No. 05/11) and was externally monitored (date of initiation: February 14, 2011; date of completion: June 6, 2011). The study was performed by a principal investigator, who was responsible for the observance of the protocol, and two investigators, who performed the clinical and technical procedures.

Participants

Twenty-seven participants were included. Inclusion criteria were age of consent, absence of serious diseases (particularly diseases interfering with saliva flow rate), written informed consent, no removable dentures or orthodontic devices, healthy or sufficiently restored dentition, no clearly visible plaque, and absence of clinical signs of salivary hypofunction [Ship, 2002]. Exclusion criteria were any known allergy to oral hygiene products and/or oral therapeutic agents and/or dental materials, medication interfering with saliva flow rate, and pregnancy or breastfeeding.

Specimens

Freshly extracted, previously impacted human third molars were disinfected in saturated, aqueous thymol solution for at least 2 weeks, from which 486 enamel specimens were prepared by grinding and polishing the natural smooth surfaces (Abrasive Cutting System and Microgrinder, EXAKT Apparatebau, Norderstedt, Germany; P1200 silicon carbide abrasive paper, LECO, St. Joseph, Mich., USA; P4000 diamond abrasive paper, Bühler GmbH, Düsseldorf, Germany). The experimental area was at least 3 × 3 mm. The specimens were stored at 100% humidity until use. Specimens prepared as described showed a maximum deviation from flatness of 0.5 μm.

Procedures

After screening and informing the participants, written informed consent was obtained. Afterwards, impressions were taken and individual mandibular mouth appliances were made. Six specimens (3 on each side) were fixed on the buccal aspects of each appliance at the beginning of each study period. One-half of the experimental area served as reference and was covered with light-curing resin (Technovit 7230 VLC, Kulzer-Exakt, Wehrheim, Germany). As described previously [Schlueter et al., 2009c], the appliances with the specimens were disinfected for 30 min in 70% ethanol prior to incorporation into the oral cavity [Ingram et al., 1997; Amaechi et al., 1998]. The study procedures are displayed in a flowchart (fig. 1). The study was performed in a cross-over, split-mouth design with a total observation time of 3 × 7 days. The term split-mouth refers to specimens' treatment. The specimens on one side of the appliance were demineralised and exposed to toothpaste slurries, while the specimens of the other side were additionally brushed with a powered toothbrush during the toothpaste slurry exposure. Right-handed participants performed brushing on the left side and left-handed participants on the right side. The toothbrush was equipped with a pressure light alert system, which was activated at 2.5 N.

The participants were intensively trained in all procedures and received written instructions and a schedule of procedures, on which they had to mark the study procedure steps. Each period began with a 5-day wash-out phase in which the participants used only fluoride-free oral hygiene products and were asked to consume no food containing high amounts of fluoride such as sea-fish, fluoridated salt, mineral water with high content of fluoride or black and green tea.

After the wash-out period, participants were asked to wear the appliance for 24 h per day, except for meals and while performing their own oral hygiene. The specimens were demineralised extraorally for 6 × 2 min with a 0.5% citric acid solution (200 ml, pH 2.6, citric acid monohydrate, Merck, Darmstadt, Germany) under standardised agitation (30/min). After demineralisation, the specimens were rinsed with tap water for 1 min and reinserted into the mouth. The first demineralisation began at 08:30 in the morning; the following demineralisations were performed at 1.5-hour intervals. After the first and last demineralisation of each day, participants used the test products intraorally. They placed a pea-sized amount of toothpaste on the head of the toothbrush. A toothpaste slurry was produced by placing the toothbrush on the occlusal surfaces of the participant's own lower molars and activating the toothbrush's electric head for 15 s. Then, the head was moved to the appropriate buccal aspects of the appliance adjacent to the specimens and pressure was applied until the alert was just activated. The toothbrush was then moved onto the specimens while the alert was activated, and the specimens were brushed for 5 s without any additional manual movement.

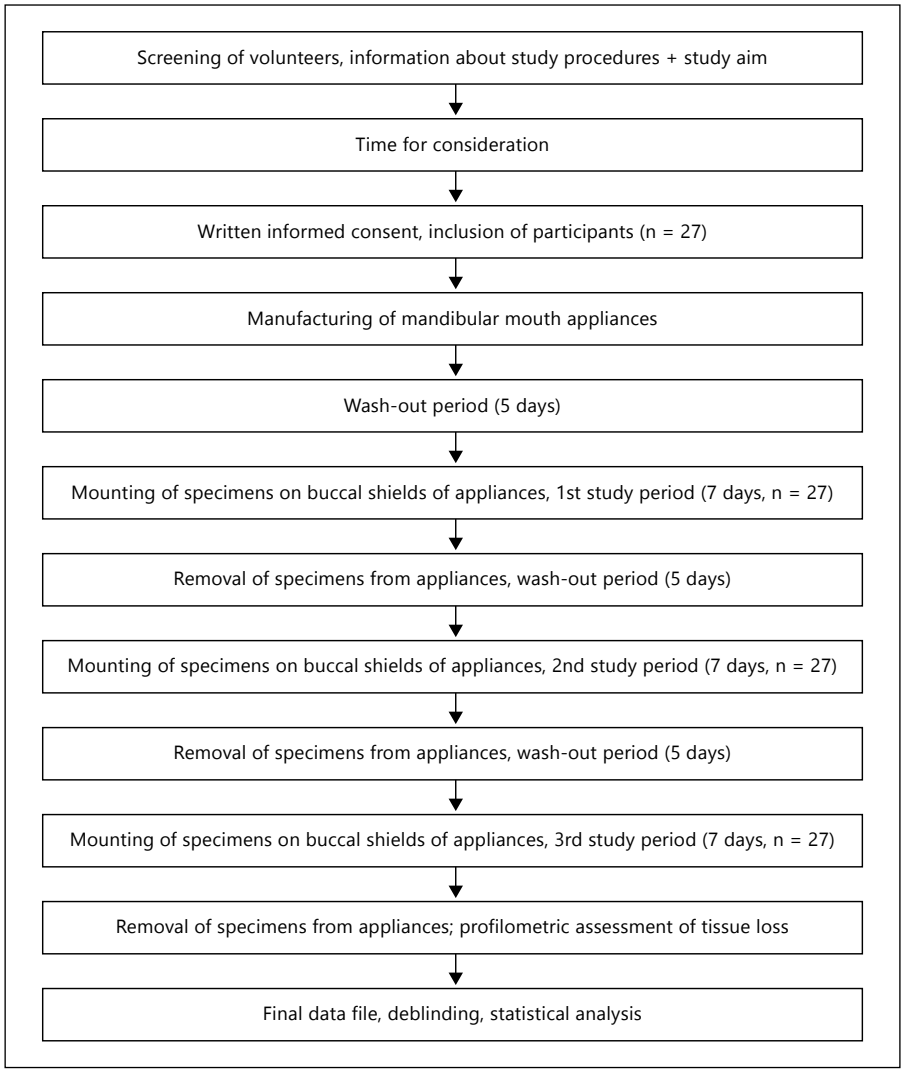

Fig. 1. Flowchart of the study procedures.

Table 1. Numbers of volunteers classified by percentile range with regard to the reduction in tissue loss compared to placebo

	<10%	≥10%	≥20%	≥30%	≥40%	≥50%	≥60%	≥70%	≥80%	≥90%
NaF										
Slurry + brushing	7	5	3	8	3	1	0	0	0	0
Slurry	11	2	2	3	3	3	1	0	0	2
F/Sn/chitosan										
Slurry + brushing	2	1	0	5	3	4	4	6	1	1
Slurry	4	0	1	1	4	6	3	3	3	2

Afterwards, the toothbrush was deactivated and removed from the oral cavity. The slurry was held in the mouth for up to 2 min. After 2 min, the slurry was spit out and the oral cavity was rinsed for 3 s with tap water. Then the appliance was removed from the oral cavity and rinsed under tap water for 1 min.

On the evening of every experimental day, the participants cleaned the appliances, but not the specimens, with a toothbrush without toothpaste. To avoid plaque formation on the specimens, the appliances were immersed for 1 min in chlorhexidine digluconate solution (Chlorhexamed Fluid 0.1%, GlaxoSmithKline Con-

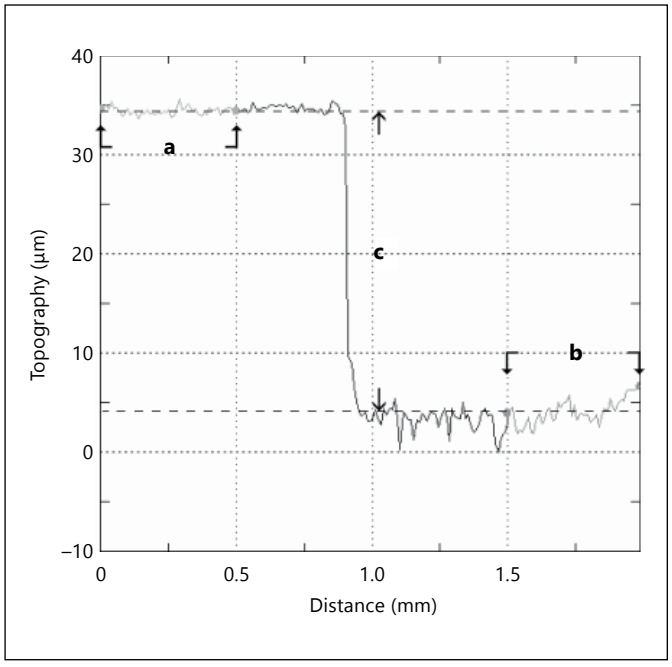

Fig. 2. Example of an analysed trace. The dashed lines (**a**, **b**) are the regression lines constructed by the software on the reference area (**a**) and on the experimental area (**b**). The vertical distance (**c**) between the regression lines is defined as the tissue loss.

sumer Healthcare GmbH & Co. KG, Bühl, Germany). All applications were documented in the schedule, and the durations were controlled using stop watches. The participants' own oral hygiene was performed with placebo toothpaste; no other oral hygiene products were allowed.

Study Products

The study products under investigation were a placebo toothpaste (pH 6.4), an NaF toothpaste (0.31% NaF, 1,400 ppm F^-, pH 4.7) and an F/Sn/chitosan toothpaste (0.935% amine fluoride, 0.155% NaF, 1,400 ppm F^-, 0.675% $SnCl_2$, 3,500 ppm Sn^{2+}, 0.5% chitosan, pH 4.4). The placebo toothpaste had the same composition as the F/Sn/chitosan toothpaste except for the active ingredients and the pH.

Tissue Loss Measurement and Outcome

Tissue loss was quantified profilometrically with an optical measuring device (MicroProf, Fries Research & Technology GmbH, Bergisch-Gladbach, Germany). The specimens were removed from the appliances at the end of each period, fixed on glass slides, and the covers were removed from the references areas. Three traces were performed on each specimen; the traces were each 2 mm in length at 200-µm intervals and were interpreted with the system software (fig. 2; Mark III, Fries Research & Technology GmbH). Parallel regression lines (500 µm in length) were constructed at the ends of the reference and experimental areas. The vertical distance between the regression lines was defined as the tissue loss. The mean of the three traces was defined as the specimen's tissue loss. The measuring device used has a vertical resolution of 10 nm and a measuring accuracy of 100 nm. The repeated measurement (n = 10) of one specimen with a mean step height of 2.3 µm revealed a standard deviation of 0.507 µm and with a mean step height of 8.9 µm a standard deviation of 0.736 µm.

The primary outcome was defined as the percentage reduction of tissue loss by the test toothpastes compared to tissue loss after use of the placebo toothpaste based on individual reduction data. The observation unit was the participant; the means (µm) of both the three brushed and the three unbrushed specimens per participant were used.

Sample Size Calculation, Randomisation, Blinding and Statistical Analysis

Samples size calculation was performed with CADEMO version 3.25 (BioMath, Rostock, Germany) and was determined based on a previously performed pilot study with the same design. In this pilot study, the percentage reduction of tissue loss by the toothpaste compared to that by the placebo was 45.1 ± 27.3. Under the assumptions that the toothpaste in the present study would show an effect of the same order and that a difference of 15% relative to the placebo would be clinically relevant, a group size of 24 would substantiate a detectable difference of at least 14.2% (standard deviation 27, $\alpha = 0.05$, $\beta = 0.2$). Considering drop-outs, 27 volunteers were included.

The study products were provided by GABA International AG (Therwil, Switzerland) in neutral containers labelled with the participants' numbers and study periods. Randomisation was performed with the software for creating randomisation schedules ('randommethod.htm') as described in McLeod [1985] and Wichmann and Hill [1982]. All individuals involved in the study (i.e. participants and investigators) were blind to the study products. De-blinding was performed after finalising all procedures.

Statistical analysis was performed with SPSS 20 for Windows (IBM Corporation, Armonk, N.Y., USA). The percentage reduction on an individual base and the mean ± standard deviation thereof were calculated. The data were checked for normal distribution (Kolmogorov-Smirnov test). Comparison of the groups was performed with paired sample t tests. Additionally, the mean tissue loss values (in µm) per group were calculated. Comparison of the groups was performed with analysis of variance (ANOVA). Levene's test showed a significant deviation in the homogeneity of variances for the slurry but not for the brushing groups. Therefore, Tamhane's post hoc test was used for the slurry groups, and Tukey's post hoc test was used for the brushing groups. The level of significance was 0.05.

Results

The study was completed by all participants with no occurrence of product-related adverse or serious adverse events. With the exception of 10 specimens (2%), all specimens were analysable.

The mean of percentage reduction compared to use of the placebo toothpaste based on individual reduction data showed significantly higher values after the use of F/

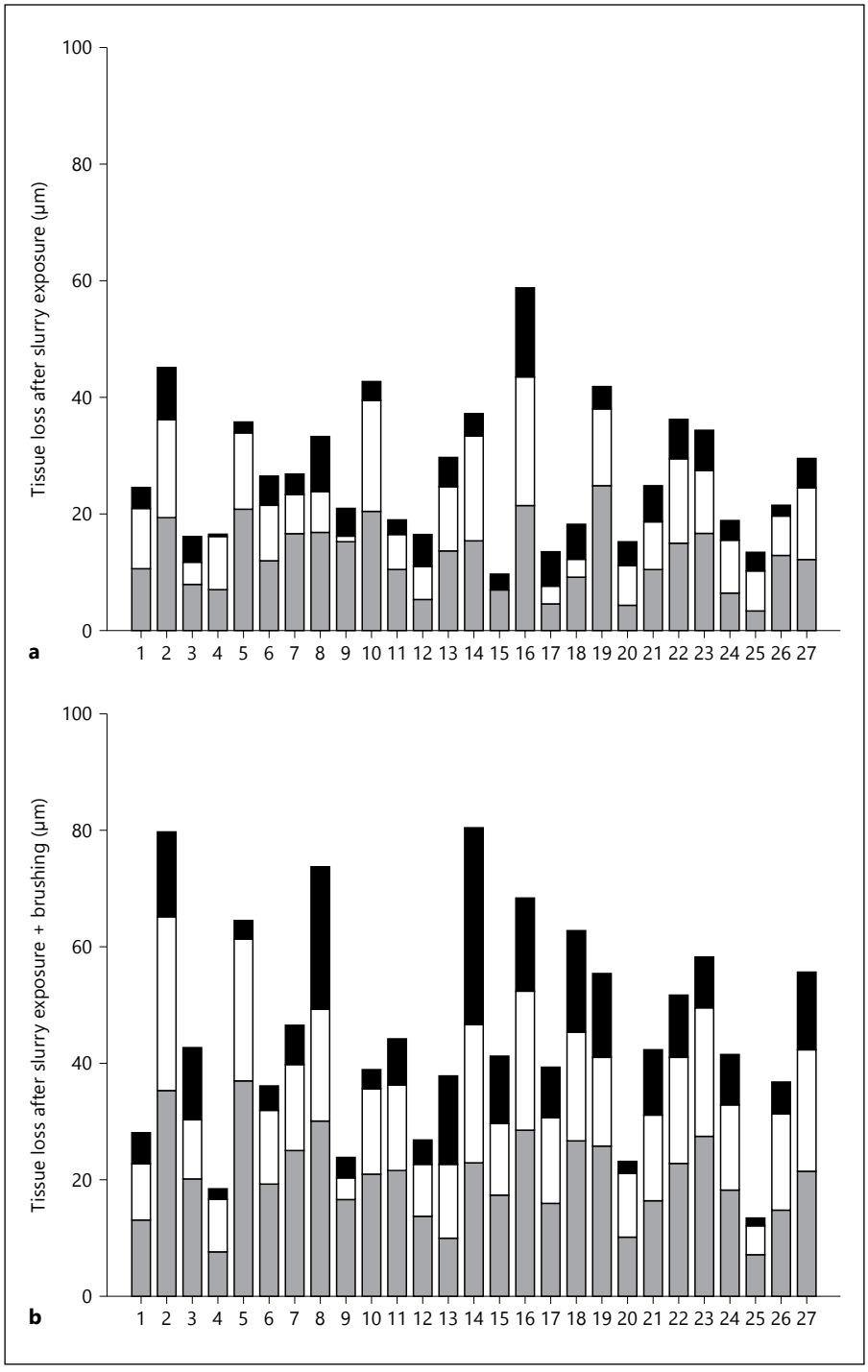

Fig. 3. Individual response data (µm; grey: placebo toothpaste; white: NaF toothpaste; black: F/Sn/chitosan toothpaste) after intraoral exposure of the specimens to toothpaste slurries (**a**) or after intraoral exposure to and intraoral brushing of specimens with toothpaste slurries (**b**). Note: In participant #15, after exposure to NaF toothpaste slurry, the tissue loss was –1.6 µm and is therefore not visible in the bar chart.

Sn/chitosan than after the use of NaF for both the slurry exposure data (NaF vs. placebo: 19.0 ± 47.3; F/Sn/chitosan vs. placebo: 52.5 ± 30.9; $p \leq 0.001$) and the slurry exposure + brushing data (NaF vs. placebo: 21.3 ± 22.4; F/Sn/chitosan vs. placebo: 50.2 ± 34.3; $p \leq 0.001$). The numbers of volunteers classified by percentile range of tissue loss reduction relative to placebo are presented in table 1.

The mean tissue loss (in µm) was highest after the use of the placebo, with 12.5 ± 5.9 after slurry exposure and 20.2 ± 7.8 after slurry exposure + brushing. NaF reduced

tissue loss to 9.3 ± 5.6 (slurry; nonsignificant compared to placebo) and 15.4 ± 6.1 (brushing; $p \leq 0.05$ compared to placebo); after the use of F/Sn/chitosan, the mean tissue loss was 4.9 ± 2.9 (slurry; $p \leq 0.001$ compared to placebo) and 10.0 ± 7.4 (brushing; $p \leq 0.001$ compared to placebo). The F/Sn/chitosan toothpaste was significantly better than the NaF toothpaste both after slurry exposure only ($p \leq 0.01$) and after slurry exposure + brushing ($p \leq 0.05$). The individual response data are displayed in figure 3.

Discussion

No standard for in situ erosion-abrasion studies exists, resulting in a high variation of study designs in the literature [Wiegand and Attin, 2011]. A main goal in designing study models is that they should reflect the clinical situation as near as possible with emphasis on standardisation of all procedures, since changes in parameters can have a distinct impact on study outcome [Schlueter et al., 2010b]. In in situ studies, particularly the consideration of the impact of saliva is of major importance. On the one hand, the saliva forms the pellicle, having erosion- [Hannig et al., 2003] and abrasion-protective properties [Joiner et al., 2008]. On the other hand, the nature of the preparation of the toothpaste slurry might have an impact on the toothpaste's efficacy. The efficacy of NaF preparations is based on the formation of CaF_2-like layers on the surface, which is formed on dental hard tissue in the presence of calcium ions either from the dental hard tissue itself in case of acidic preparations [Rolla et al., 1993] or from the medium with which the slurry is prepared. It has been shown that in presence of saliva, the application of fluoride preparations can lead to higher amounts of calcium fluoride-like material on the dental hard tissue [Larsen and Richards, 2001], making an intraoral application of the toothpaste meaningful. Therefore, in the present study, all procedures except for demineralisation were performed intraorally and, to the knowledge of the authors, this is the first study using an intraoral brushing regime. The 1-minute rinsing procedure of specimens after toothpaste treatment was performed in order to remove all toothpaste remnants from the specimens' surfaces to achieve best possible standardisation. All parameters were chosen according to current recommendations for the design of erosion-abrasion studies [Wiegand and Attin, 2011]. The brushing time of 5 s and the load of 2.5 N correspond to brushing parameters found in an observational study (duration: 97 s for the whole mouth; mean pressure used: 2.3 N) [Ganss et al., 2009]. The demineralisation was performed extraorally to protect the participants' own teeth against erosive demineralisation; however, demineralisation was in general performed after wearing the specimens intraorally and after formation of a 1-hour pellicle [Hannig et al., 2004]. The demineralisation period corresponded to the time required to neutralise the salivary pH after the consumption of acidic foodstuffs [Imfeld, 1983]. The pH and concentration of the acid used corresponds to those found in soft drinks. All in all, the present study design represented the situation of patients with regular exogenous acid impacts.

The choice of a comparison preparation is in general difficult; however, the comparison to a commonly used compound appears meaningful. Thus, NaF was chosen for comparison since it is the most commonly used compound in oral hygiene products and has been used in various published studies. In several studies a commercially available formulation was used; however, the efficacy of those products substantially varies between different brands, with a range of 10–30% reduction of erosive-abrasive tissue loss [Hooper et al., 2007; Moretto et al., 2010; Ganss et al., 2011]. Due to this high variation in efficacy, it is not readily possible to define a single preparation as a suitable comparison toothpaste. Therefore, an experimental NaF formulation was used to hold, except for the active agents, all factors constant, including pH and abrasives.

The highest tissue loss was found for the placebo toothpaste; this loss was comparable to the values obtained from an in vitro study with a similar study design. In that study, tissue loss was 14.4 ± 4.5 μm after erosion only and 20.2 ± 3.8 μm after additional brushing with placebo toothpaste [Ganss et al., 2012] compared to 12.5 ± 5.9 μm and 20.2 ± 7.8 μm in the present study. The use of the NaF toothpaste led in the present study to a reduction of 21% after brushing with and exposure to toothpaste slurry, values also comparable to the above-mentioned study [Ganss et al., 2012]. These results clearly indicate that the mentioned in vitro study supplies a good predictive value for the in situ study. Another in situ study showed only a reduction in erosive-abrasive tissue loss by 7% after the use of an NaF toothpaste [Huysmans et al., 2011]. That study had, except for the intraoral application of the test toothpastes, a comparable study design. However, this difference might explain the notable difference in efficacy between the NaF toothpastes of both studies. In the cited study, the application of toothpastes was performed extraorally with a slurry mixed with distilled wa-

ter, perhaps reducing the preventive effect of the NaF toothpaste.

The F/Sn/chitosan toothpaste resulted in an approximately 50–60% reduction of tissue loss and showed the best efficacy against both erosive and erosive-abrasive tissue loss. This is a result comparable to those from the above-mentioned in vitro study [Ganss et al., 2012], showing again its good predictive value. In that in vitro study a significant enhancement of efficacy of an F/Sn toothpaste was achieved by the addition of chitosan to this toothpaste, resulting in an increased reduction in erosive-abrasive tissue loss from 39% after use of the F/Sn toothpaste to 67% after the use of the chitosan-modified preparation [Ganss et al., 2012]. The comparable efficacy indicates that the combination of chitosan, Sn^{2+} and F^- is also effective in the presence of saliva and pellicle. Chitosan is a cationic polysaccharide with the ability to bind electrostatically to surfaces with a negative zeta potential [Guo and Gemeinhart, 2008], such as dental hard tissue and abrasives. The zeta potential of pellicle-covered enamel is lower than that of uncovered enamel [Weerkamp et al., 1988]; therefore, the chitosan molecule has a strong tendency to bind to the pellicle coverage [van der Mei et al., 2007]. The interaction of chitosan with such negatively charged surfaces is complex. It is assumed that the molecule forms multilayers stable at acidic pH [Claesson and Ninham, 1992], resulting in a lower susceptibility of the underlying substrate to erosive demineralisation [Lee et al., 2012]. It can also bind to mucin, occurring in the pellicle structure, with the possibility to form multilayers with the mucin molecule [Dedinaite et al., 2005; Svensson et al., 2006]. Chitosan has also lubricating effects [Guo and Gemeinhart, 2008], which might result in a lower abrasiveness of the toothpaste and, thus, a lower tissue loss after the combined erosive-abrasive challenge.

After the slurry application of the NaF toothpaste, a somewhat higher inter-individual variability in reduction of tissue loss was found. A similar effect has been shown in previous studies investigating the anti-erosive effect of mouthrinses [Schlueter et al., 2009c; Ganss et al., 2010]. It is reasonable to assume that the anti-erosion efficacy of NaF mainly depends on the formation of CaF_2-like layers. The amount of KOH-soluble fluoride deposited in situ [Laheij et al., 2010] as well as the total amount of fluoride on and in enamel after the intra-oral use of an NaF toothpaste [Mobley, 1981] vary considerably between individuals, which might be an explanatory factor for the high proportion of non-responders after NaF treatment. The efficacy of Sn^{2+}- and F^--containing preparations, however, is due to tin-rich precipitates on the tooth surfaces [Ganss et al., 2008; Schlueter et al., 2009b] rather than on establishing CaF_2-like material. The formation of such tin-rich salts is apparently more independent from individual biological factors, explaining the overall good response to the F/Sn/chitosan-containing toothpaste. Chitosan may also play a role here. The brushing procedure seems to level off these differences between NaF and the Sn^{2+}-containing preparations to some extent, with currently unknown reasons.

The individual response to the toothpaste used was notably constant for the F/Sn/chitosan preparation. If a non-responder to a toothpaste is defined as an individual who shows a reduction below 10% compared to placebo, 11 participants showed non-response to the NaF toothpaste after slurry exposure and 7 participants after slurry exposure + brushing. The number of non-responders was markedly lower after the use of the F/Sn/chitosan toothpaste, with 4 (slurry) and 2 (slurry exposure + brushing) non-responsive participants.

Considering all these data from the present study and from the literature, there is much speaking for an increase in efficacy related to the addition of chitosan to an F/Sn-containing toothpaste. However, detailed studies about the mode of action of such toothpaste are necessary.

In summary, the study showed that F/Sn/chitosan-containing toothpaste is an effective anti-erosive/anti-abrasive agent and that this toothpaste is a good option for erosion prevention in the case of regular acid impacts occurring in patients who frequently consume acidic foodstuffs.

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Author Contributions

N.S. and C.G. conceived and designed the experiments, supervised the clinical examination and analysed the data; N.S., C.G. and J.K. wrote the paper.

Disclosure Statement

The authors declare that there is no conflict of interest.

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