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ORIGINAL ARTICLE

Effect of a chitosan additive to a Sn^{2+}-containing toothpaste on its anti-erosive/anti-abrasive efficacy—a controlled randomised in situ trial

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Abstract
Objectives It is well known that Sn^{2+} is a notable anti-erosive agent. There are indications that biopolymers such as chitosan can enhance the effect of Sn^{2+}, at least in vitro. However, little information exists about their anti-erosive/anti-abrasive in situ effects. In the present in situ study, the efficacy of Sn^{2+}-containing toothpastes in the presence or absence of chitosan was tested.
Methods Ten subjects participated in the randomised cross-over study, wearing mandibular appliances with human enamel specimens. Specimens were extraorally demineralised (7 days, 0.5 % citric acid, pH2.6; 6×2 min/day) and intraorally exposed to toothpaste suspensions (2× 2 min/day). Within the suspension immersion time, one half of the specimens were additionally brushed intraorally with a powered toothbrush (5 s, 2.5 N). Tested preparations were a placebo toothpaste (negative control), two experimental toothpastes (F/Sn = 1,400 ppmF^-, 3,500 ppm Sn^{2+}; F/Sn/chitosan = 1,400 ppmF^-, 3,500 ppm Sn^{2+}, 0.5 % chitosan) and an SnF_2-containing gel (positive control, GelKam = 3,000 ppm Sn^{2+}, 1,000 ppmF^-). Substance loss was quantified profilometrically (μm).
Results In the placebo group, tissue loss was 11.2±4.6 (immersion in suspension) and 17.7±4.7 (immersion in suspension+brushing). Immersion in each Sn^{2+}-containing suspension significantly reduced tissue loss ($p \leq 0.01$); after immersion in suspension+brushing, only the treatments with GelKam (5.4±5.5) and with F/Sn/chitosan (9.6±5.6) significantly reduced loss [both $p \leq 0.05$ compared to placebo; F/Sn 12.8±6.4 (not significant)]
Conclusion Chitosan enhanced the efficacy of the Sn^{2+}-containing toothpaste as an anti-erosive/anti-abrasive agent.
Clinical relevance The use of Sn^{2+}- and chitosan-containing toothpaste is a good option for symptomatic therapy in patients with regular acid impacts.

Keywords Abrasion · Erosion · Chitosan · Enamel · In situ · Tin

Introduction

Several studies have been published in the last 5 years that investigate the efficacy of various compounds as anti-erosive agents. Different fluoride compounds exhibit differences in their ability to inhibit enamel erosion [1–3]. Polyvalent metal cations [4], particularly the stannous ion in combination with fluoride, are much more effective than the conventional fluorides, including sodium fluoride (NaF) and amine fluoride (AmF), under both in vitro [2, 5] and in situ conditions [6, 7].

The impact of acids, however, leads not only to the erosive loss of dental substance [8] but also to a loss of surface microhardness, resulting in a higher susceptibility of the acid-altered surface to mechanical tissue losses, at least in enamel [9]. Whether anti-erosive agents are also effective against the combined effect of acids and mechanical impact is not clear since the efficacy of Sn^{2+} as an agent that can prevent abrasion after erosive challenges has not been fully investigated. One in vitro study showed that brushing with a fluoride-free toothpaste after both an erosive challenge and immersion of enamel specimens in an Sn^{2+}- and fluoride-containing rinse results in significantly less substance loss

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than that observed after post-erosion brushing with a conventional NaF toothpaste [10]. Two different Sn^{2+}-containing toothpastes have been tested under in situ conditions. The effect was limited, only offering a degree of protection between 26 % and 34 % compared to a group that was brushed with water only (control). However, relative to an NaF toothpaste, which revealed only a 7 % reduction compared to the control group, this effect was significantly better [11]. Another in situ study investigated the effect of a complex fluoridation procedure on erosive–abrasive tissue loss. It consisted of the use of a fluoridated toothpaste twice daily, directly after erosion, followed by the use of an Sn^{2+}- and fluoride-containing rinse and a highly concentrated fluoride gel every third day. This fluoridation procedure was quite successful, reducing an additional substance loss induced by brushing after an acid impact to a level comparable to those of only eroded specimens [12]. However, due to the complexity of the fluoridation procedure, one cannot draw conclusions regarding the in situ efficacy of the Sn^{2+}-containing preparation alone.

In addition to the application of polyvalent metal cations, the application of biopolymers is a promising approach for the reduction of erosion progression. The supplementation of acids with food-approved polymers, such as polyphosphates, ovalbumin, xanthan or carboxymethyl-cellulose, can reduce their erosivity [13, 14]. Chitosan is a biopolymer, derived from chitin, which is positively charged at low pH [15]. It tends to bind to surfaces with a negative zeta potential, such as the dental hard tissue [16, 17] and the pellicle [18]. Chitosan can reduce carious demineralisation both in vitro [19] and in situ [20]. Therefore, it is quite conceivable that the addition of chitosan to anti-erosive agents might also increase their anti-erosive and their anti-erosive/anti-abrasive effects. The first indications of this impact can be found in two in vitro studies. A chitosan-containing, fluoride-free toothpaste reduced erosive–abrasive loss by approximately 25 %, exhibiting an efficacy that was in the range of the effects of conventional NaF-containing toothpastes [10]. The impact of chitosan addition to an experimental Sn^{2+}- and fluoride-containing toothpaste has also been investigated under in vitro conditions. This study revealed a significant increase in the efficacy of this toothpaste as an anti-erosive/anti-abrasive agent [21]. However, no study has investigated the effect of chitosan on erosion and erosion/abrasion progression under in situ conditions.

Based on the promising effects of Sn^{2+} and chitosan that were observed under in vitro conditions, the anti-erosive/anti-abrasive in situ effects of both an Sn^{2+}- and fluoride-containing toothpaste (F/Sn) and an Sn^{2+}-, fluoride- and chitosan-containing (F/Sn/chitosan) toothpaste were investigated in the present study. Their efficacy was compared to those of an active agent-free placebo toothpaste (negative control) and an Sn^{2+}- and fluoride-containing gel (GelKam, positive control). The null hypothesis was that there is no difference between the preparations under investigation.

Materials and methods

The study was planned as a double-blinded, prospective, single-centre, four-cell in situ trial with a crossover design. The study was performed at the Department for Conservative and Preventive Dentistry at the Dental Clinic in Giessen. The study was approved by the local ethics committee (Ethik-Kommission des Fachbereiches Medizin der Justus-Liebig-Universität Giessen, No. 46/10), following the guidelines of Good Clinical Practice and conforming to the declaration of Helsinki. The report of the study followed the CONSORT statement.

Participants

A total of 10 volunteers were screened and included. All participants volunteered and gave written informed consent. Inclusion criteria for participants consisted of the following: an age at or above the age of consent; a lack of serious diseases, particularly those that interfere with saliva flow rate; the willingness and ability to give written informed consent; no removable dentures or orthodontic devices; healthy or sufficiently restored dentition; no clearly visible plaque; and the absence of signs of salivary hypofunction (clinical examination; Working Group 10 of the Commission on Oral Health, Research and Epidemiology 1992). Exclusion criteria consisted of the following: any known allergy to previously used oral hygiene products and/or oral therapeutic agents and/or dental materials, which were used in the oral cavity or in the throat; the use of medication that interferes with saliva flow rate; and pregnancy or breastfeeding.

Specimen preparation, mouth appliances and tested products

Two hundred and forty enamel specimens were prepared from freshly extracted, previously impacted human third molars. All donors lived in an area with ≤0.03 mg/L fluoride present in the drinking water. The natural surfaces of enamel specimens were ground flat and polished under sufficient water flow (≥50 mL/min, Abrasive Cutting System and Exakt Mikrogrinder, Exakt-Apparatebau, Norderstedt, Germany; P800 and P1200 silicon carbide abrasive paper, Leco, St. Joseph, USA). The preparation resulted in an experimental area of at least 3 mm × 3 mm. Specimens were stored in 100 % humidity until use.

A total of six enamel specimens were recessed in the buccal aspects of mandibular mouth appliances, which were made of cold-cured acrylic and were retained by braces. One half of the experimental area was covered with a light-

curing resin material (Technovit 7230 VLC; Kulzer-Exakt, Wehrheim, Germany) and served as the reference area for profilometry. After covering, specimens were scrutinised under a microscope (magnification ×10, SMZ-1, Zoom Stereomicroscope; Nikon GmbH, Düsseldorf, Germany) to ensure that there was no contamination by the acrylic on the experimental area. For disinfection, the specimens were stored in saturated aqueous thymol solution [22, 23] for at least 2 weeks. Before insertion into the mouth, appliances with the specimens were immersed in 70 % ethanol for 30 min [24].

The products investigated in this study included one experimental Sn^{2+}- and F^--containing toothpaste (F/Sn, 1,400 ppmF^-, 4,223 ppm Sn^{2+}); one experimental Sn^{2+}-, F^-- and chitosan-containing toothpaste (F/Sn/chitosan, 1,400 ppmF^-, 4,223 ppm Sn^{2+}, 0.5 % chitosan); one Sn^{2+}- and F^--containing gel [positive control, GelKam (Colgate Oral Pharmaceuticals, New York, USA), 970 ppmF^-, 3,030 ppm Sn^{2+}]; and one placebo paste (negative control) (Table 1). All toothpastes had an RDA of 70; the RDA of GelKam is 60. RDA measurement was performed according to the method of Grabenstetter et al. [25] (Missouri Analytical Laboratories, Inc., St. Louis, MO, USA).

Intervention

A flowchart of the study is given in Fig. 1. Volunteers were informed about all study procedures and additionally received written information. After giving written informed consent, volunteers were included (hereafter referred to as participants). Impressions were taken from both jaws, and individual appliances were made.

The study was performed in a crossover, split-mouth design. Split mouth refers to the following treatment: On one side, specimens were only treated with toothpaste/gel (immersion in suspension), and on the other side, specimens were additionally brushed with the respective toothpaste/gel (immersion in suspension+brushing). Except for the provided study products, the use of other oral hygiene products was not allowed for the duration of the study.

The total observation time was 4×7 days. Prior to each observation period, a 5-day wash-out phase was included. In each period, a different preparation was used. On each day, appliances with specimens were extraorally demineralised six times per day at 1.5-h intervals (starting at 8.30 A.M.) for 2 min each in 200 mL of 0.5 % citric acid (pH2.6, citric acid monohydrate; Merck, Darmstadt, Germany). Demineralisation was performed under standardised agitation (30 min). After demineralisation, the specimens and appliances were rinsed with tap water for 1 min and reinserted into the mouth.

Treatment with toothpastes/gel was performed intraorally. Brushing was performed with a powered toothbrush equipped with a pressure alert, which is activated at 2.5 N (Oral-B Professional Care 3000; Oral-B, Schwalbach am Taunus, Germany). A pea-sized amount of toothpaste/gel was placed on the head of the toothbrush for treatment. The participants started brushing the occlusal surfaces of their own lower teeth for 15 s to produce a toothpaste–/gel–saliva suspension. Afterwards, they moved from the occlusal surfaces to one buccal aspect of the appliance (to the left side for right handed individuals and to the right side for left handed individuals). The participants were asked to place the head of the toothbrush adjacent to the specimens and to push the brush until the alert was just activated. They then moved the brush over the specimens without changing the pressure and brushed the specimens without any further manual movements for 5 s. The whole brushing procedure was performed under visual control. After the brushing period, the toothbrush was deactivated and removed from the mouth. The suspension, however, was held in the mouth for a total time of 2 min. After this period, the suspension was spit out; the mouth was rinsed with tap water for 3 s, followed by the removal of the appliance. The appliance was then rinsed under tap water for 1 min to remove all toothpaste remnants, reinserted into the mouth and worn until the next demineralisation period.

Preferably, the appliances were worn for 24 h, except during meals and periods of oral hygiene. The patients were only allowed to drink water with the appliances in situ. After meals, a period of at least 15 min elapsed prior to reinsertion

Table 1 Active ingredients and type of abrasives in the toothpastes used

Paste	Active ingredient	Type of abrasives
Placebo (pH6.4) (toothpaste, negative control)	–	Silica
F/Sn (pH4.4) (toothpaste)	1400 ppmF^- (0.935 % AmF; 0.155 % NaF) 4223 ppm Sn^{2+} (0.675 % $SnCl_2$)	Silica
F/Sn/chitosan (pH4.4; toothpaste)	1,400 ppmF^- (0.935 % AmF; 0.155 % NaF) 4223 ppm Sn^{2+} (0.675 % $SnCl_2$) 0.5 % Chitosan (shrimp origin, degree of de-acetylation approx. 80 %, MW approx. 350 kDa)	Silica
GelKam (pH4.0) (gel, positive control)	970 ppmF^-, 3,030 ppm Sn^{2+} (0.4 % SnF_2)	–

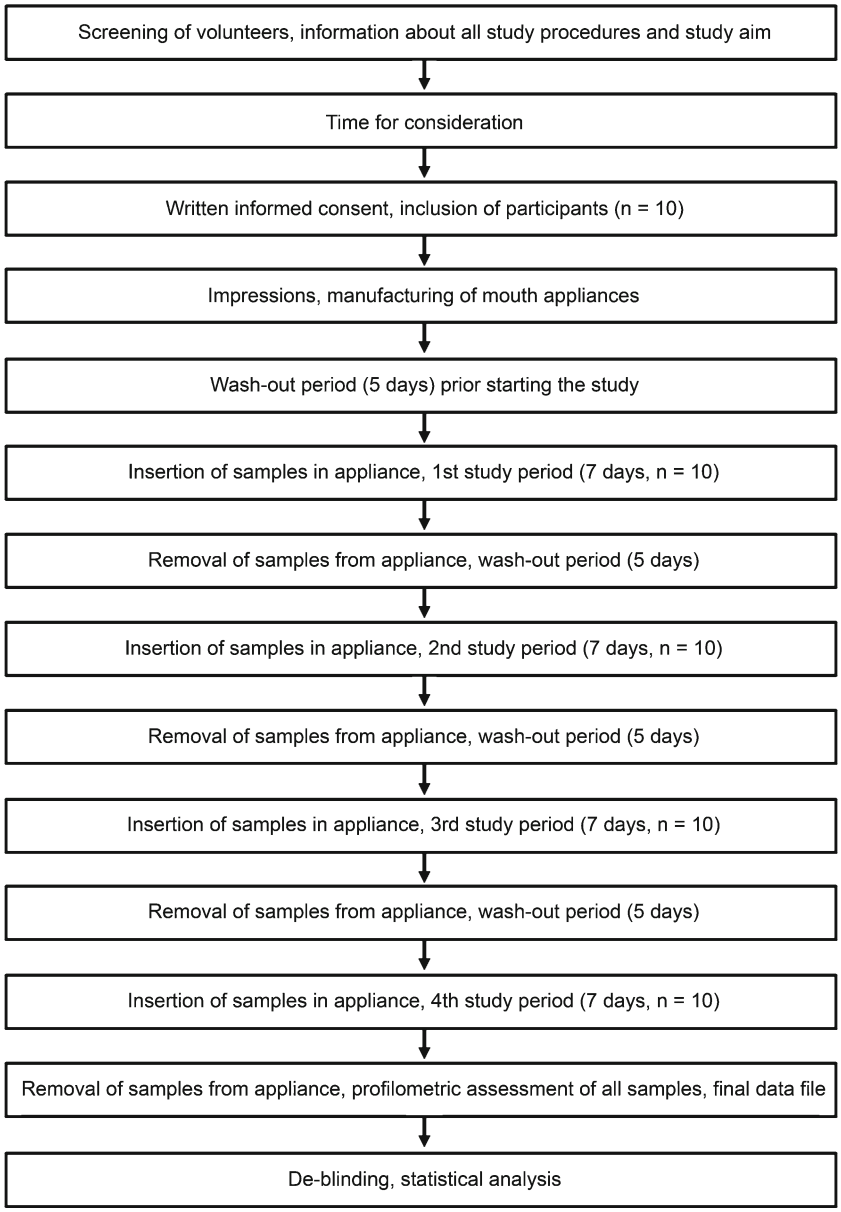

Fig. 1 Flow chart of study procedures

of the appliance. In the evening, the appliances, but not the specimens, were cleaned with a toothbrush without toothpaste. Afterwards, the appliances were immersed for 1 min in chlorhexidine digluconate solution (Chlorhexamed Fluid 0.1 %; GlaxoSmithKline Consumer Healthcare GmbH & Co. KG, Buehl, Germany) to avoid plaque formation. Daily oral hygiene was performed with the placebo toothpaste without appliances in situ.

All participants were extensively trained in all procedures, particularly the brushing procedure, to achieve the best level of standardisation; the participants also received written instructions, a schedule and a checklist on which each treatment was marked. All treatment times were controlled with stop watches. At the beginning of each period, the participants were provided with the respective toothpaste/gel, a new head for the powered toothbrush, the placebo toothpaste for their own oral hygiene, 1.5 L of citric acid, the chlorhexidine solution and the respective containers for these solutions. After each period, the checklist, toothpaste/gel and citric acid were retrieved. To verify compliance, the use of the toothpaste/gel was controlled by reweighing.

Tissue loss measurement

After each period, the specimens were carefully removed from the appliances and fixed on glass slides. Prior to measurement, the acrylic resin was carefully removed from the reference area, and the specimens were scrutinised under a microscope (10-fold magnification; SMZ-1, Zoom Stereomicroscope, Nikon GmbH). Tissue loss was measured profilometrically with an

optical device (MicroProf; Fries Research & Technology GmbH, Bergisch-Gladbach, Germany). On each specimen, three traces, 2 mm in length, were recorded perpendicular to the border between the reference area and the experimental area at 200-μm intervals. Analysis of traces was standardised with the system software (Mark III; Fries Research & Technology GmbH). For both the experimental and the reference areas, parallel regression lines were constructed on the outer 200 μm of the trace. The length of the orthogonal line between both regression lines was defined as tissue loss (μm); the value per specimen was calculated as the mean of the three traces. The used measuring device 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.

Evaluation criteria

The evaluation criterion was tissue loss measured in micrometers. The observation unit was the participant; the mean of three specimens per participants was used.

Responsibilities

The study director was responsible for the proper realisation of the study. Two investigators performed the study. One was responsible for the inclusion of participants and the clinical procedures; the other performed all technical procedures, such as specimen preparation and analysis. Both investigators were carefully trained and calibrated.

Sample size calculation

Sample size was determined based on previous study data [10, 21]. Sample size calculation was performed with Cademo version 3.25 (BioMath, Rostock, Germany). In a previous study, a 67 % reduction of the amount of tissue loss produced by erosion only was achieved by brushing with the positive control (GelKam) [10]. A reduction by 35 % (approximately 50 % of the reduction of the positive control) was assumed to be a clinically relevant difference. An in vitro study with comparable conditions showed a substance loss of 14.4 μm after erosion only [21]. A clinically relevant difference of 35 % would result in a reduction of 5.1 μm of tissue loss. Based on the assumption that the standard deviation is 4 μm [21], α=0.05 and β=0.2, a reduction of 35 % (5.1 μm) by the test products would be detectable with a group size of 10 participants.

Randomisation and blinding

Study products were provided by GABA International in neutral containers that were labelled with the participants' numbers and study periods. Randomisation of the study products was performed by the provider of the study products. The order of treatments differed between participants. All individuals involved in the study (e.g. participants and investigators) were blind to the study products (except for the gel). De-blinding was performed after finalising all procedures.

Statistical analysis

Statistical analyses were performed at the end of the study. No interim analysis was planned or performed. The primary outcome measure was the profilometrically measured tissue loss at the end of the experimental period of 7 days (μm). All statistical procedures were performed with IBM SPSS 20 for Windows (Armonk, NY, USA). The Kolmogorov–Smirnov test was used to check for deviations from the normal distribution. No significant deviation was found. Homogeneity of variance was checked with Levene's test. For the suspension groups, Levene's test revealed a significant deviation from the homogeneity of variances. An analysis of variance with Tamhane's (suspension) or Tukey's post hoc test (suspension+brushing) was performed for the comparison of groups. The level of significance was set at 0.05.

Results

All participants satisfactorily finished the study; no test product-related adverse or serious adverse events occurred. The mean use of pastes was 6.8±2.5 g for placebo, 7.3±1.7 g for F/Sn, 7.3±1.6 g for F/Sn/chitosan and 8.6±2.6 g for GelKam. No significant difference was found between the four preparations.

Eight of 240 specimens (3.3 %) were not analysed: four due to the destruction of the specimens during removal from the appliance and two due to a loss of coverage on the reference area during the test period. Another two specimens could not be analysed.

Individual response data and mean values are presented in Table 2. Only small differences between individuals were found.

The mean tissue loss was highest in the placebo group, both after suspension immersion only (11.2±4.6 μm) and after suspension immersion+brushing (17.7±4.7 μm).

After immersion in suspension only, tissue loss was significantly reduced by all Sn^{2+}-containing preparations relative to placebo. The F/Sn toothpaste reduced tissue loss by 68 % ($p \leq 0.01$), the F/Sn/chitosan toothpaste by 76 % ($p \leq 0.01$) and GelKam by 82 % ($p \leq 0.001$). Among the three Sn^{2+}-containing preparations, no significant differences were found.

Table 2 Individual response data of all participants

Participant	Suspension immersion only				Suspension immersion+brushing			
	Placebo	F/Sn	F/Sn/chitosan	GelKam	Placebo	F/Sn	F/Sn/chitosan	GelKam
1	7.9	2.1 (73 %)	2.4 (70 %)	2.0 (75 %)	13.6	5.2 (62 %)	3.4 (75 %)	1.7 (88 %)
2	15.8	3.4 (79 %)	5.6 (65 %)	0.3 (98 %)	18.4	10.6 (43 %)	9.4 (49 %)	9.0 (51 %)
3	19.4	5.3 (73 %)	3.4 (82 %)	3.8 (80 %)	27.1	24.5 (10 %)	22.0 (19 %)	18.8 (31 %)
4	8.8	4.0 (54 %)	4.0 (54 %)	0.5 (94 %)	20.0	15.8 (21 %)	8.7 (57 %)	2.3 (89 %)
5	16.3	7.3 (55 %)	0.6 (96 %)	0.9 (95 %)	17.0	13.6 (20 %)	10.9 (36 %)	2.1 (88 %)
6	9.8	2.1 (79 %)	−2.2 (122 %)	3.3 (67 %)	18.8	12.4 (34 %)	5.3 (72 %)	7.7 (59 %)
7	7.3	1.5 (80 %)	1.9 (74 %)	2.2 (69 %)	18.0	21.7 (−20 %)	7.0 (61 %)	4.3 (76 %)
8	8.8	1.5 (83 %)	0.5 (95 %)	1.1 (87 %)	19.5	8.9 (55 %)	6.0 (69 %)	1.5 (93 %)
9	12.5	4.4 (65 %)	7.6 (39 %)	2.2 (82 %)	15.3	10.8 (50 %)	16.3 (−6 %)	5.6 (64 %)
10	5.4	4.5 (15 %)	3.7 (32 %)	3.8 (29 %)	8.9	4.8 (46 %)	7.2 (19 %)	1.2 (87 %)
Mean	11.2a	3.6 (68 %)b	2.7 (76 %)b	2.0 (82 %)b	17.7A	12.8 (28 %)A,B	9.6 (46 %)B,C	5.4 (69 %)C
SD	4.6	1.9	2.8	1.3	4.7	6.4	5.6	5.5

Displayed are measured substance losses per participant (micrometers, mean of three specimens), mean values of individual data and standard deviations of individual data. The values in braces are the percentage reduction of substance loss compared to placebo group. Statistically significant differences between groups are marked by different lowercase letters (suspension immersion only) or uppercase letters (suspension immersion+brushing)

After immersion in suspension+brushing, the Sn^{2+}-containing preparations exhibited different efficacies. With a 28 % reduction compared to placebo, the F/Sn toothpaste was the least effective, and no significant difference was found between this product and the placebo. The F/Sn/chitosan toothpaste and GelKam were able to reduce tissue loss significantly compared to the placebo (GelKam—reduction by 69 %, $p \leq 0.001$; F/Sn/chitosan paste—reduction by 46 %, $p \leq 0.05$). No significant difference was found between F/Sn and F/Sn/chitosan or between GelKam and F/Sn/chitosan. The difference between F/Sn and GelKam, however, was significant ($p \leq 0.05$).

Discussion

The use of conventional fluoridated toothpastes, which contain NaF or AmF, regularly used for the prevention of caries, seems to be sufficient for the prevention of erosive substance loss in cases of ordinary acid consumption. In cases of high acid consumption, chronic reflux or eating disorders, however, their efficacy is evidently too low. The present study design imitated frequent acid impacts occurring in patients consuming soft drinks with high frequency spread throughout the day. The pH and the concentration of citric acid employed in this study correspond to the characteristics of commonly consumed soft drinks [26]. A recent study that addressed the brushing habits of adults has shown a mean brushing duration of 96.6 s [27], meaning that each quadrant was brushed for a period of approximately 24 s and each surface of one tooth was brushed for only a few seconds. Therefore, the brushing duration used in this study (5 s per specimen) reflects the clinical situation.

There are few studies that investigate the effects of different toothpastes that contain various active agents on the abrasion of eroded enamel under in situ conditions. A PubMed literature search with the terms "erosion AND abrasion AND in situ AND enamel" revealed only 25 hits; five of these publications were reviews, and one was an in vitro study. From the 19 remaining studies, only seven investigated the effect of toothpastes on the progression of erosive–abrasive tissue loss. Among these located studies, the designs varied considerably in the choice of erosive agent (pH and type of acid), the duration of erosive (40 s to 20 min) or abrasive challenge (10 to 40 strokes, 5 to 60 s) and the erosion and abrasion frequency. Therefore, it is quite difficult to draw conclusions about the effect of toothpastes under in situ conditions. Furthermore, none of these studies investigated the in situ effect of toothpaste using an intraoral brushing model [28]; thus, there is a lack of a standardised intraoral brushing study model for the investigation of the effect of toothpastes as anti-erosive/anti-abrasive agents. With the present study, an intraoral brushing model with standardised brushing procedure was introduced, displaying results that were comparable to those of in vitro studies in terms of the dimension of tissue loss and the variation observed for the results [10].

The process of choosing a positive control is often discussed, and it is not easy to find one control that fulfils all requirements. On the one hand, the comparison to a current benchmark appears to be meaningful. On the other hand, determining the benchmark of toothpastes is not possible, particularly if one considers the lack of available information

regarding the efficacy of toothpastes against erosion–abrasion progression. As toothpastes are complex mixtures of excipients, such as stabilisers, thickeners, preservatives, detergents and abrasives, the variabilities of the composition, abrasivity and active agents of different products are very high. Therefore, the use of any conventional, commercially available, sodium fluoride-containing toothpaste as a positive control appeared not meaningful. Therefore, we decided to use a preparation that contained the same active agent as the experimental toothpastes under investigation. The Sn^{2+}-containing gel (GelKam) was chosen, as previous studies have shown that this product has notable efficacy as an anti-erosive agent. Furthermore, it has been used as positive control in previously performed in vitro studies [10, 21]. The preparation is not a toothpaste in the proper sense, and it is not indicated for the daily use for oral hygiene. However, due to the fact that the gel has a relatively simple composition and contains no abrasives, an investigation of the impact of Sn^{2+} in the tested study model, with only limited interactions between the stannous ion and other ingredients of the basic formulation of the gel, was possible. Furthermore, it was possible to get insights into the impact of the brushing procedure itself without the effect of the abrasives. The lack of possibilities for blinding this product during the study was accepted, as the advantages in obtaining information about the effect of the stannous ion on its own outweighed any risks.

The highest tissue loss was found, as expected, in the placebo group, both after immersion in suspension only and after immersion in suspension with brushing abrasion within the immersion time. The order of magnitude of tissue loss values in the placebo group was comparable to values obtained from an in vitro study with a similar study design [21], indicating that the in vitro and in situ results were comparable. Data of individuals were relatively constant within one group, which was also found for tin-containing mouthrinses under in situ conditions [24].

Immersion in the toothpaste or gel suspensions without brushing led to a very promising reduction of erosive tissue loss of 68–82 %. Interestingly, no significant differences between the three Sn^{2+}-containing preparations were found, even if the Sn^{2+} content in the gel was, in terms of declared Sn^{2+} values, lower than in both toothpastes. An analysis of the available tin content in these preparations, however, revealed that the amount of tin, measurable in the supernatant of centrifuged toothpaste/gel suspensions, was more than twice as high in the suspension of GelKam than in either of the experimental toothpastes [21]. This result is most likely due to the more simple composition of the gel. On one hand, the gel contains no abrasives. This lack of abrasives most likely leads to a higher availability of the stannous ions in this preparation because no interaction between the abrasives (predominantly silica types) and the stannous ions can occur. Silica is a compound with negative zeta potential; therefore, the stannous ion can easily adsorb to it due to electrostatic attraction. Furthermore, the gel formulation is free of water. Stannous ions can react with the oxygen in the water, possibly inducing the formation of stable tin oxide or tin hydroxide [29]. Such a reaction would result in a reduced availability of the stannous ion in the toothpastes. However, whether such reactions actually occur in the toothpastes can only be speculated.

The encouraging effects observed with the suspensions were partially counteracted by the brushing procedure. Brushing with the F/Sn toothpaste without chitosan increased tissue loss compared to immersion only in placebo by 14 %. In comparison with brushing with placebo, this toothpaste only achieved a reduction of the erosive–abrasive tissue of 28 %. This result is in good accordance with an in situ study that investigated the effect of two different Sn^{2+}-containing, commercially available toothpastes. That study used a milder study design (3×5 min demineralisation per day; 4 days; 0.5 % citric acid; no agitation) with an extraoral brushing model (2×2 min toothpaste suspension immersion per day; 10 strokes brushing within immersion time using a brushing machine; load 150 g). The tested Sn^{2+} toothpastes produced a 26–34 % reduction, which is in the same order as observed in the present study.

Brushing with the Sn^{2+}-containing gel, however, offered satisfactory protection (52 % reduction in comparison to immersion only in placebo suspension; 69 % reduction in comparison to brushing with placebo). This result is in good concordance with an in vitro study that was previously performed under more severe erosive conditions (6×2 min demineralisation per day in 1 % citric acid), which showed a reduction of erosive/abrasive tissue loss of 67 % after brushing with GelKam; these results were relative to a control group that only experienced erosion [10].

The difference between the F/Sn toothpaste without chitosan and the gel is most likely related to the presence of abrasive in the toothpaste, which usually produces more pronounced substance losses both in vitro [30, 31] and in situ [32]. In addition, it is quite possible that the amount of Sn^{2+} and abrasives, as well as the presence of glycerine in the gel, played a role. Glycerine is a short-chain sugar alcohol, which is widely used in the medicine and the cosmetic industries as a humectant and a lubricant. It is known that glycerine is able to reduce the abrasivity of silica [33]. The contribution of the toothbrush itself to abrasive or abrasive/erosive tooth wear is still under debate. The toothbrush itself has a nearly nonexistent impact on the wear of sound enamel [34]. Likewise, brushing after a single demineralisation of enamel specimens with citric acid (0.65 %, pH 3.6) with a soft toothbrush (150 g load) or with a toothbrush in combination with toothpaste suspension (RDA 30–40) revealed that the toothbrush on its own resulted in negligible levels of additional abrasive tissue loss [35]. After brushing

with the toothbrush in combination with toothpaste suspension, however, a substance loss of approximately 300 nm (after 150 strokes) was measured [35]. In the present study, however, more severe erosive conditions in a cyclic model were used. Therefore, it is quite conceivable that the lubricating properties of the glycerine may also impact the abrasive potential of the toothbrush itself, possibly resulting in an additional reduction of the abrasive-induced substance loss.

Likewise, under in vitro conditions [21], a significant increase in the anti-erosive/anti-abrasive efficacy was found after the use of chitosan-supplemented toothpaste (F/Sn/chitosan). In contrast to the formulation without chitosan (F/Sn), the modified toothpaste (F/Sn/chitosan) was not only able to reduce the loss compared to the specimens brushed with placebo by 46 % but also by 15 % relative to the specimens that were only immersed in placebo suspension without brushing. Chitosan is the only known cationic polysaccharide, and it abundantly occurs in nature. Most polysaccharides are either neutral or negatively charged. The positively charged molecule [15] can electrostatically bind to surfaces with a negative zeta potential [16, 17]. The dental hard tissue, the pellicle and the abrasives show such negative potential, and in all cases, binding between both components could impact the toothpastes' effect. The interaction of chitosan with negatively charged surfaces is complex. It is assumed that the molecule forms multilayers [36], which are notably resistant to changes in pH and are even stable at an acidic pH [15]. This effect would directly protect the dental hard tissue from acids. Chitosan also has lubricating effects [37]. This property might reduce the abrasivity of silica if chitosan is directly bound to the silica. If it is bound to the dental hard tissue, chitosan might also reduce the abrasive effect of the silica on the dental hard tissue or of the toothbrush itself, as considered for glycerine. Further detailed studies that elucidate the mode of action of chitosan and of Sn^{2+}- and chitosan-containing toothpastes, under both in vitro and in situ conditions, are necessary.

Conclusion

This in situ study conclusively showed that the active ingredients in the tested products are effective in reducing erosion from dietary acids. This positive effect, however, was partially counteracted by brushing with the toothpastes, particularly in the case of the F/Sn toothpaste. The F/Sn/chitosan toothpaste reduced the erosive/abrasive tissue loss significantly compared to placebo and showed an efficacy in the order of the positive control. Therefore, the Sn/F toothpaste containing chitosan is a good option for an anti-erosive/anti-abrasive therapeutic agent in particular for patients with regular acid exposure.

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Conflict of interest The authors declare that they have no conflicts of interest.

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