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ORIGINAL ARTICLE

Efficacy of tin-containing solutions on erosive mineral loss in enamel and dentine in situ

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Abstract The addition of tin to mouth rinses is, at least in vitro, a promising strategy for symptomatic therapy of dental erosion. The aim of this study was to evaluate the in situ efficacy of an experimental tin-containing fluoride solution on erosive tissue loss in human enamel and dentine. The study was a three-cell (7 days each) crossover design involving eight healthy participants. Samples were mounted on buccal shields of mandibular mouth appliances, which were worn for 24 h except during meals and drinks. Specimens were demineralised extraorally with 0.05 M citric acid (pH 2.3) for 6×5 min daily and were treated with test solutions intraorally once per day for 30 s after the first demineralisation. Three solutions were used: placebo (negative control), a commercially available tin- and fluoride-containing (SnF_2) mouth rinse (positive control, 409 ppm Sn^{2+}, 250 ppm F^-, pH 4.2) and an experimental solution (pH 4.5) containing 1,900 ppm Sn^{2+} ($SnCl_2$) and 1,000 ppm F^- (AmF/NaF). Tissue loss (micrometre) was determined profilometrically. In enamel, tissue loss was 54.8±8.6 in the placebo, 24.5±14.4 in the positive control and 9.7±4.1 in the experimental solution group. The respective values for dentine were 48.5±13.0 in the placebo, 32.8±9.6 in the positive control and 26.2±6.7 in the experimental solution group. The experimental solution was notably effective for enamel but was less effective for dentine. The positive control solution was less effective than the experimental solution; its effects for enamel and dentine were similar.

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Introduction

The intention of a symptomatic therapy for dental erosion is to modify the tooth surface by enhancing its resistance to acidic impacts. Such acidic impacts can occur in persons who regularly and frequently consume acidic beverages like soft drinks or juices, in those who live on a vegetarian diet and, in particular, in patients with an eating disorder in combination with vomiting (bulimia nervosa) or in those suffering from gastro-oesophageal reflux disease. These patients are at a higher risk of erosion than the general population [1–4].

Several approaches for modifying the surface of the dental hard tissue have been discussed. The use of dentine adhesives has been investigated but has the shortcomings of limited long-term success and reliance of patients on their dentist [5]. Another approach is the use of fluoride preparations, which can form mineral precipitates on the tooth surface. Besides common fluoride preparations such as NaF and AmF, fluoride compounds containing polyvalent metal cations have recently been investigated with respect to their erosion-inhibiting potential. In this context, tin-containing preparations showed, at least for enamel, a promising reduction in erosive mineral loss of 50–90% [6–11]. The in situ efficacy of these solutions in enamel, however, has only rarely been investigated [10, 12–14] but has shown similar promising results. One serious limitation of most of these solutions was that they were not pursuant to the directives for dental hygiene products and cosmetics (high concentrations of active agents or high acidity of

the preparations) and therefore were not suitable as over-the-counter products.

In own in vitro studies, the efficacy of different solutions with various tin and fluoride concentrations was investigated [7, 8]. These solutions all had a pH of 4.5, and the concentrations used were in accordance with the directives for dental hygiene products and cosmetics. It turned out that the combination of stannous chloride (1,400–2,800 ppm Sn^{2+}) and amine and sodium fluoride (1,000–1,500 ppm F^-) was exceptionally effective with respect to erosion-inhibiting potential.

Regarding the efficacy of tin-containing solutions on dentine, to the authors' knowledge, only one study has investigated its efficacy on dentine in vitro [15], and no study has examined the effect of tin-containing preparations on dentine erosion in situ.

The aim of this study was therefore to test the efficacy of an experimental stannous chloride and amine/sodium fluoride-containing solution with respect to its erosion-inhibiting potential in human enamel and dentine under in situ conditions, using a cyclic demineralisation and remineralisation model. This solution was compared to a commercially available stannous/amine fluoride-containing mouth rinse, which was used as a positive control. A fluoride and tin-free placebo solution was used as a negative control. The null hypothesis was that there is no difference between the various solutions.

Materials and methods

The study was planned as a prospective, single-centre, double-blind, three-cell study with a crossover design in accordance with the CONSORT guidelines and an overall observation period of 3×7 days. It was conducted in the Dental Clinic, Department of Conservative and Preventive Dentistry of the University of Giessen. The study conformed to the declaration of Helsinki and was performed according to the guidelines of Good Clinical Practice. The study was approved by the local Ethical Committee (Ethik-Kommission des Fachbereiches Medizin der Justus-Liebig-Universität Giessen, No.: 78/07).

A study director was responsible for the realisation of the study and the randomisation procedure. Clinical procedures and inclusion of participants were carried out by investigator 1. Investigator 2 was responsible for specimen preparation and tissue loss measurements. Investigators were carefully trained.

All subjects volunteered and were given oral and written information about the products and the purpose of the study. All participants gave their informed consent. The inclusion criteria were age of consent, good oral health (no frank cavities, significant gingivitis/periodontitis or visible plaque), physiological saliva parameters (Table 1 [16, 17]) and no removable dentures or orthodontic devices. Exclusion criteria were serious diseases, pregnancy, breast-feeding, medication that influenced salivation and allergies against dental materials or oral hygiene products.

Sample size calculations were based on previously performed in vitro and in situ studies [7, 18]. A tissue loss of 40 µm was estimated in the negative control group, and the aim was to achieve a reduction of 50%. Therefore, a tissue loss of 20 µm was defined as a clinically relevant difference. A mean SD of 10 µm was found averaged over the mentioned studies. A sample size of seven participants was calculated (α=0.05, β=0.80, Cademo Vers. Light 3.25, BioMath, Rostock, Germany), and upon consideration of potential dropouts, a sample with a size of eight was planned.

Specimen preparation and mouth appliances

Seventy-two longitudinal enamel and 72 longitudinal dentine specimens were prepared from previously completely impacted, freshly extracted human third molars without cracks. All donors lived in an area with ≤0.03 ppm fluoride in their drinking water. The natural surfaces of enamel specimens and the surfaces of dentine specimens were ground flat and polished under sufficient water flow (Exakt Abrasive Cutting System and Exakt Mikrogrinder, Exakt-Apparatebau, Norderstedt, Germany; P800 and P1200 silicon carbide abrasive paper, Leco, St. Joseph, MI, USA) to prepare an experimental area of at least 3×3 mm. Specimens were stored in 100% humidity until use.

Mandibular mouth appliances with buccal aspects were made from cold-cured acrylic for each participant and were intraorally retained by braces [19]. A total of three dentine and three enamel specimens each were recessed in the buccal aspects of the appliances. The specimen surfaces were coplanar with the surface of the buccal aspect of the appliance. One half of the experimental area was covered with a light-curing acrylic (Technovit 7230 VLC, Kulzer-Exakt, Wehrheim, Germany) and served as a reference area for profilometry. After covering, specimens were scrutinised under a microscope (×10 magnification, 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 [20, 21] for at least 2 weeks. Before insertion into the mouth, the appliances with the specimens were immersed in 70% ethanol for 30 min.

Table 1 Flow rate, pH and buffering capacity of stimulated and unstimulated saliva of all participants

Participant	1	2	3	4	6	7	8	Reference values [17]
Unstimulated saliva								
Flow rate (ml/min)	0.65	0.65	0.62	1.13	0.59	0.34	0.45	>0.25
pH	7.44	6.93	6.98	8.39	7.97	8.45	6.41	>6.0
Buffering capacity	6.44	4.91	6.42	7.15	7.01	6.81	5.21	>4.25
Stimulated saliva								
Flow rate (ml/min)	2.13	2.75	1.75	3.37	2.61	3.72	3.47	>1.0
pH	8.91	8.06	8.09	8.29	8.16	8.35	8.10	>7.0
Buffering capacity	7.48	6.90	7.21	7.31	7.24	7.26	5.81	>5.75

Procedure and responsibilities

The study used a crossover design with three treatment periods of 7 days each. Three different solutions were used:

Negative control	Placebo, no fluoride, no tin and neutral pH
Positive control	Commercially available AmF/SnF_2 mouth rinse (Meridol®), pH 4.2
Experimental solution	0.05% *w/w* SnF_2 (409 ppm Sn^{2+}, 125 ppm F^-)
	0.16% *w/w* amine fluoride (Olaflur, 125 ppm F^-)
	0.36% *w/w* $SnCl_2$ (1,900 ppm Sn^{2+})
	0.64% *w/w* amine fluoride (Olaflur, 500 ppm F^-)
	0.11% *w/w* NaF (500 ppm F^-)
	pH 4.5

The order of treatments was different for all participants and was randomly assigned by generating a randomisation table with "randommethod.htm" [22, 23].

Participants were extensively trained in all procedures (in particular, the intraoral rinsing procedures with the solutions, the incorporation and the cleaning of the mouth appliances and the tap water rinsing procedure after the demineralisation); they also received written instructions and a schedule. Appliances were worn during the day and night with the exception of meals and drinks. After meals or drinks, 15 min elapsed before reinsertion.

Procedures usually started at 8.30 a.m. with an erosive demineralisation. For demineralisation, the mouth appliances were extraorally immersed for 6×5 min per day in 200 ml 0.05 M citric acid (pH 2.3, room temperature, citric acid monohydrate, Merck, Darmstadt, Germany). This solution was renewed at the beginning of each day. After demineralisation, appliances were rinsed with tap water for 1 min before reinsertion. Between each demineralisation, appliances were worn for approximately 1.5 h. After the first demineralisation per day, participants wore the appliances and rinsed once with 10 ml of the respective solution for 30 s. After rinsing, the solution was spit out, and the participants did not rinse with water afterwards. Every evening, appliances were placed for 1 min into 0.1% chlorhexidindigluconate solution (Chlorhexamed Fluid 0.1%, GlaxoSmithKline Consumer Healthcare GmbH & Co. KG, Buehl, Germany) to avoid plaque formation on specimens. All application times were measured with stopwatches. Individual oral hygiene was performed with a provided toothpaste (Elmex® anticaries toothpaste, 0.125% F as Olaflur; pH 4.6, GABA International AG, Therwil Switzerland) and without appliances in situ. Before the beginning of the experiment and between the treatment periods, a 5-day washout period was included.

Tissue loss measurement

Specimens were carefully removed from the appliances after each treatment period. Directly after removal, dentine specimens were treated with collagenase solution for 96 h at 37°C in order to remove the exposed organic matrix [24] prior to the tissue loss measurement (100 U/ml; collagenase from *Clostridium histolyticum* type VII (collagen digestion activity: 1,680 U/µg, Sigma Aldrich, St Louis, MO, USA) solved in a remineralisation solution (4.08 mM H_3PO_4, 20.10 mM KCl, 11.90 mM Na_2CO_3 and 1.98 mM $CaCl_2$, pH 6.7; Merck, Darmstadt, Germany) [25]).

The acrylic cover was carefully removed from all enamel and dentine specimens, and the surfaces were checked for acrylic remnants or damage. Tissue loss was measured profilometrically with a Perthometer S8P (Mahr, Göttingen, Germany). Procedures have been described previously in detail [8, 24]. On each sample, three traces were made at intervals of 0.25 mm, with each trace 1.75 mm in length. Traces were interpreted with a special software (Perthometer Concept 4.0, Perthen Mahr, Göttingen, Germany). Two regression lines were constructed on each trace: one on the reference area and one on the experimental area, both 0.3 mm in length with a distance of 0.3 mm to the edge between the reference and experimental area. The midpoints of both regression lines were calculated by Perthometer Concept software. The vertical distance between the midpoints was defined as tissue loss.

In the case of distinct substance loss, the reproducibility (tenfold tracing of one sample) was ±0.8 µm; in case of

slight substance loss, the reproducibility was ±0.9 µm. The repeat analysis of one trace showed a standard deviation of ±0.1 µm. The variance of a profile of one specimen was 0.3±0.5 µm.

Statistics

Statistical analyses were performed at the end of the study. No interim analysis was planned or performed.

The tissue loss values (expressed in micrometre) obtained from the three enamel and dentine specimens, respectively, were clustered per participant; their mean was used for further statistical analysis.

All statistical procedures were performed with Statistical Package for the Social Sciences (SPSS) 15.0 for Windows (SPSS, Chicago, IL, USA). For all data, no significant differences from the normal distribution (Kolmogorov–Smirnov test) were found. For comparisons of mean of groups and for comparisons of groups per participant, a variance analysis with Tukey's post hoc test was performed. The level of significance was set at 0.05.

Results

All participants completed the study. Three of eight participants reported an astringent feeling on the mucosa and a dull feeling on the teeth after using the experimental solution. Due to severe protocol deviation, one participant (No. 5) was excluded. Additionally, one dentine specimen in the negative control group, three enamel and one dentine specimen in the positive control group, and one enamel specimen in the experimental solution group could not be analysed because the acrylic coverage on the reference area was lost during the intraoral period: a total of 59 enamel and 61 dentine specimens were analysed. Results per participant are displayed in Table 2. Relative tissue loss values are shown in Fig. 1.

For enamel, tissue loss was highest in the negative control group (54.8±8.6 µm), and was significantly reduced by both tin-containing solutions ($p \leq 0.001$). Application of the commercially available positive control led to a tissue loss of 24.5±14.4 µm. Tissue loss after application of the experimental solution was notably lower compared with the positive control group (9.7±4.1 µm, $p \leq 0.05$). Based on the defined clinical difference (20 µm; 40% reduction compared to the negative control group), the experimental solution achieved this aim in all participants, whereas application of the positive control solution achieved this reduction only in five of seven participants.

For dentine, mineral loss was 48.5±13.0 µm in the control group. Use of the tin-containing solutions significantly reduced tissue loss ($p \leq 0.05$). Values were 32.8±9.6 µm in the positive control group and 26.2±6.7 µm in the experimental solution group. Between the positive control and experimental solution groups, no significant difference was found. A clinically relevant reduction was achieved by application of the experimental solution in four of seven participants and by application of the positive control solution in only two of seven participants.

Discussion

This study investigated the efficacy of an experimental tin-containing solution, which conformed to the directives of oral hygiene products in both concentration and pH, on erosive tissue loss in enamel and dentine in situ. The composition of the experimental solution was optimised based on the results of own previous in vitro studies [7, 8] and a series of preliminary studies, in which different ratios between tin and fluoride were tested. To allow such variations of the active agents, independent sources for tin (stannous chloride) and fluoride (amine and sodium fluoride) were used. Amine fluoride was necessary because stannous ion-containing solutions at a pH of 4.5 are prone

Table 2 Tissue loss (micrometre, mean) for each participant after 7 days of extraoral demineralisation and in situ application of placebo solution (negative control), positive control mouth rinse and experimental solution

Participant	1	2	3	4	6	7	8
Enamel							
Negative control	46.2	63.5	65.7	44.7	49.0	60.9	53.4
Positive control	8.4 (82%)	34.2 (46%)	3.4 (95%)	20.4 (54%)	39.4 (20%)	26.7 (56%)	38.9 (27%)
Experimental solution	6.7 (86%)	8.4 (87%)	6.9 (89%)	9.8 (78%)	14.4 (71%)	16.1 (74%)	5.2 (90%)
Dentine							
Negative control	66.5	52.0	28.4	62.2	41.1	45.2	44.0
Positive control	31.4 (53%)	37.5 (28%)	20.6 (27%)	28.3 (55%)	33.9 (18%)	50.9 (−13%)	27.0 (39%)
Experimental solution	30.4 (54%)	16.5 (68%)	22.0 (23%)	24.5 (61%)	37.3 (9%)	29.3 (35%)	23.6 (46%)

Values in braces represent the percentage of reduction of tissue loss compared to the negative control group

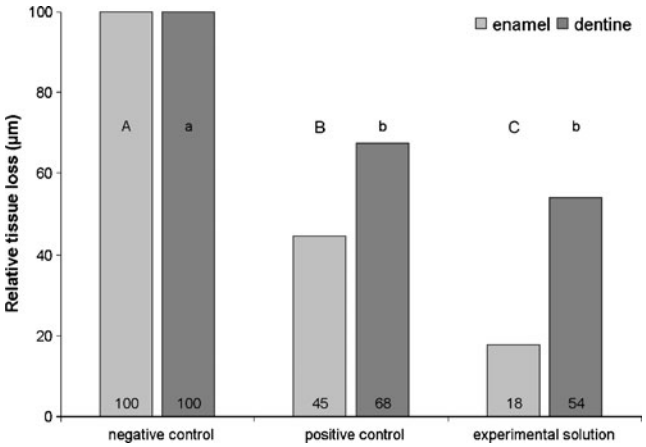

Fig. 1 Relative tissue loss (percent) in enamel and dentine after 7 days of extraoral demineralisation (6×5 min per day) and in situ treatment (1×30 s per day) with negative control (placebo solution), positive control (commercially available mouth rinse) and experimental solution. Columns sharing the same letter (enamel: *upper case letters*, dentine: *lower case letters*) are not significantly different ($p>0.05$)

to form precipitates. Amine fluoride, however, has the ability to stabilise such solutions, even at a relatively high pH [26, 27]. Regarding the toxicity of tin at concentrations used in the experimental solution, no adverse effects were anticipated, since concentrations up to 0.6% do not appear to pose a risk for systemic reactions [28]. The positive control solution was chosen because it is a commercially available tin-containing fluoride mouth rinse and has shown notable efficacy under mild erosive conditions in vitro [6].

The study design with six erosive impacts per day appears relatively strong but should reflect situations that can occur in patients at high risk for dental erosion with multiple acidic challenges per day [1, 29]. The application duration of 1×30 s per day was chosen according to current recommendations for mouth rinses.

Regarding the results of the three groups, the highest tissue loss was found in the negative control group for dentine and enamel. The results were comparable to a previously performed in situ study using the same erosive protocol [18].

For enamel, both tin-containing solutions were able to reduce tissue loss. The reduction of tissue loss was, in accordance with results found in the literature [8, 30, 31], distinctly higher after application of the highly concentrated experimental solution (82%) than of the less-concentrated positive control mouth rinse (55%). These results correspond quite well to those obtained by Wachtel [30]. He found a reduction in solubility of dental enamel in acid by approximately 50% after application of a 300-ppm Sn^{2+} solution (comparable to the reduction after application of the 409-ppm Sn^{2+}-containing positive control mouth rinse) and of 80% after application of a 1,500-ppm Sn^{2+} solution (comparable to the reduction after application of the 1,900-ppm Sn^{2+}-containing experimental solution).

The study by Wachtel [30] has also shown that the application of solutions with a tin concentration higher than 1,500 ppm did not lead to a further increase in efficacy and that a reduction to 750 ppm Sn^{2+} did not result in a forfeit of efficacy. This is of special interest, since three of the eight participants complained about a dull feeling on the tooth surfaces and about astringent sensations on the mucosa after using the experimental solution but not after using the positive control mouth rinse. Such feelings are often found after using preparations containing high concentrations of polyvalent metal cations in the oral cavity [32, 33]. Furthermore, it is possible that such relatively high concentrations of tin can lead to a discolouration of the teeth, although no staining was observed in the present study. Therefore, it would be worthwhile to optimise the concentration of tin to a level at which no changes in feeling will occur in the oral cavity with contemporaneous preservation of efficacy. Further in vitro and in situ dose finding studies could give more information about appropriate concentrations.

For dentine, the experimental solution and the positive control mouth rinse showed similar results. For an explanation of these results, the histology of experimental dentine erosion must be considered. In this tissue, the organic fraction is exposed to acid impacts, and this demineralised organic matrix can reach considerable thickness, particularly under harsh erosive conditions such as those in the present study. With an increase in thickness, this matrix slows erosion progression, as it acts as a diffusion barrier for acids [34]. It is probable that the matrix also has an impact on the effects of therapeutic measures. Little information exists about the reaction between tin and dentine. One in vitro study has shown that after acid etching and application of a highly concentrated stannous fluoride solution, globular precipitates form on the demineralised organic matrix [35]. To what extent tin can be incorporated under cyclic erosive conditions into the organic fraction or reaches the demineralisation front beneath the organic matrix cannot be answered at this time. It is quite conceivable, however, that some amounts of tin and fluoride were retained in the organic matrix and did not reach the demineralisation front. Thus, partial retention resulted in a similar concentration of tin and fluoride at the demineralisation front after using the less-concentrated positive control solution and the more concentrated experimental solution, respectively, and resulted in an equalised efficacy. Whether such thick organic matrices are formed in vivo is questionable. Studies should be performed in which the efficacy of symptomatic measures is investigated without these organic structures.

Regarding the individual data (Table 2), inter-individual variations were considerably higher in the positive control group than in the experimental solution group, at least for

the data obtained from enamel. Various factors could play a role in such inter-individual variation: differences within the individual biological factors, e.g. saliva composition and the different nature of the teeth that were used for specimens, as well as variations in study performance despite the fact that these procedures were standardised, and the participants were intensively trained in all procedures. The higher tin and fluoride concentrations masked all of these variations. Similar results were found in a previous in situ study that investigated the efficacy of various intensities of fluoridation measures (toothpaste fluoridation vs. combined fluoridation with toothpaste, mouth rinse and highly concentrated gel) [18]. Inter-individual differences were less pronounced with increasing fluoridation intensity and higher applied cumulative fluoride amounts per day.

In conclusion, the application of highly concentrated tin-containing fluoride solutions is a promising strategy for symptomatic therapy of dental erosion. However, to achieve a better acceptance of the solution without any sensations on the teeth or mucosa, the concentration of the active agents should be optimised without reducing efficacy. The optimal dose should be elucidated in further dosing studies.

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

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