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Inhibition of enamel erosion by stannous and fluoride containing rinsing solutions

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Summary This *in vitro* study investigated the erosion-inhibiting properties of dental rinses during erosion in the presence of the salivary pellicle. The erosion inhibition by a Sn/F containing dental rinse (800 ppm Sn²⁺, 500 ppm F⁻, pH = 4.5) was compared with a fluoridated solution (500 ppm F⁻, pH = 4.5) and water (control). Calcium release and enamel softening were significantly reduced among enamel samples exposed to the Sn/F rinse (group SF) compared to those treated with the fluoride solution (group F) and the control ($p < 0.05$).

SEM showed slightly etched enamel interfaces in group SF, whereas the erosion was more pronounced in group F and even more severe in the control group. In conclusion, the Sn/F combination provided the best inhibition of erosion among tested solutions. This study demonstrates the application of different analytical tools for comparative erosion quantification. A strong correlation ($r^2 \geq 0.783$) was shown between calcium release and enamel softening during demineralization.

Introduction

Application of stannous compounds was reported for caries prevention more than 40 years ago when the antibacterial effect of tin was discovered and attributed to the biocidal activity of its ions (ANDRES ET AL. 1974, SVATUN & ATTRAMADAL 1978, MILLER ET AL. 1994, WADE ET AL. 1997). Furthermore, the erosion-inhibiting property of stannous compounds was later discovered in *in situ* (HOOPER ET AL. 2007, SCHLUETER ET AL. 2009b, 2011) and *in vitro* experiments (HOVE ET AL. 2007a, SCHLUETER ET AL. 2009a, SCHLUETER ET AL. 2009c, WIEGAND ET AL. 2009, SCHLUETER ET AL. 2010). Schlueter et al. (SCHLUETER ET AL. 2009c) showed significant protective potential of stannous and fluoride ions against dental erosion if combined together in the formulation. This *in vitro* study clearly demonstrated the importance of fluoride ions for tin interactions with enamel.

However, the study was performed without formation of the salivary pellicle layer. Moreover, reduction of tooth dissolution by applying SnF₂-containing dentifrice was also investigated *in vivo* (YOUNG ET AL. 2006). The data obtained in these studies differed according to erosive conditions and experimental designs. The basic observations were that in enamel the efficiency of different mouthrinses depends on the type of active compound applied, i. e. SnF₂, SnCl₂, TiF₄ or AmF (SCHLUETER ET AL. 2010).

Although many publications have investigated erosion inhibition by application of stannous compounds, most of them were focused on late erosive stages with severe substance loss in the range of micrometers. However, efficient preventive strategies are essential already at the early erosion phase. While softening of the enamel might be a reversible process and the dental tissue can be potentially remineralized, substance loss

ference between initial enamel microhardness (SMH_0) and the value after each erosive treatment (SMH_t) ($\Delta SMH = SMH_0 - SMH_t$) was used for the statistical data analysis. Indentations were performed with a load of 50 g for the hardness measurements.

Analysis of enamel wear

For each sample, six additional Knoop indentations with a load of 400 g were made on the original healthy enamel surface. The depth of these indentations was measured after each demineralization according to the previously published procedure (RAKHMATULLINA ET AL. 2011). Since the enamel surface was continuously etched during erosive challenges, median depth values were calculated only if at least four out of six indentations could be measured in each enamel sample. The median depth values of the entire treatment group (at each particular erosion time) were calculated if at least 75% of enamel samples could be analyzed in the group. The numbers of quantitatively measured indentations are also presented.

Statistical data analysis

A total of 90 enamel samples were included in the study, i.e. 30 samples in each of three treatment groups. Sample size calculations were based on previously performed *in vitro* studies. Statistical data analysis was performed using a nonparametric ANOVA model (F1_LD_F1) (BRUNNER ET AL. 2002) and pairwise Wilcoxon rank sum tests with the Bonferroni–Holm correction for multiple testing. The level of significance was set at 0.05. The correlation between calcium release and microhardness change in the three tested groups was calculated using Spearman rank coefficients (r^2) due to nonlinear functions. The correlation coefficients were determined using all samples in each group ($n = 30$) at all applied erosion times.

Results

Figure 1 shows the experimental design of the study. After the microhardness analysis of the initial healthy samples, they were incubated in human saliva and rinsed in one of the test solutions, followed by demineralization and subsequent anal-

ysis of the eroded enamel. This cycle was repeated eight times with monitoring of the erosion progression after each cycle. The slowest rate of calcium loss was detected in the enamel samples treated with Sn^{2+}/F^- -containing dental rinse (Fig. 2A). The kinetics of calcium dissolution in group F was found to be slower than in group C but significantly faster than in group SF. After the entire erosion duration (32 min), approximately 52 nmol/ mm^2 of calcium ions were released in group SF, whereas double (104 nmol/ mm^2) and triple the amount of calcium ions were released in groups F and C, respectively (Fig. 2A). A statistically significant difference in calcium loss was observed at all erosion times between groups SF and F, groups SF and C, and groups F and C ($p < 0.05$). Statistical data comparisons were also performed to evaluate values of calcium loss determined at sequential erosion durations, i.e. between 4 and 8 minutes, 8 and 12 minutes, etc. Calculations showed significant differences ($p < 0.05$) within every group for calcium dissolution between each subsequent demineralization treatment.

As a result of calcium release, enamel softening occurred in the three treatment groups (Fig. 2B). Less enamel hardness loss (softening) was measured in the enamel from group SF compared to the values in groups F and C. More specifically, a hardness loss of 216 in the Knoop hardness number (KHN) was measured in group SF after 32 minutes of erosion, while enamel in groups F and C lost approximately 271 KHN and 304 KHN, respectively (Fig. 2B). Statistically significant differences ($p < 0.05$) in enamel softening (at all erosion times) were established between groups SF and F, SF and C, and groups F and C. Strong correlations were observed between calcium release and enamel softening in the three treatment groups: $r^2 = 0.783$ in group SF, and $r^2 = 0.864$ and 0.793 in groups F and C, respectively.

To quantitatively assess enamel wear in the treatment groups, the depth of large indentations (400 g) was monitored after erosive treatments throughout the entire experiment (Fig. 3A). However, due to severe surface etching with erosion progression, reliable quantitative measurement of the indentation depth was not possible at longer erosion times (see Fig. 3B). For clarity of presentation, Figure 3A shows median depth values if $\geq 75\%$ of all indentations could be quantitatively measured in each group at each erosion time. In group SF, ap-

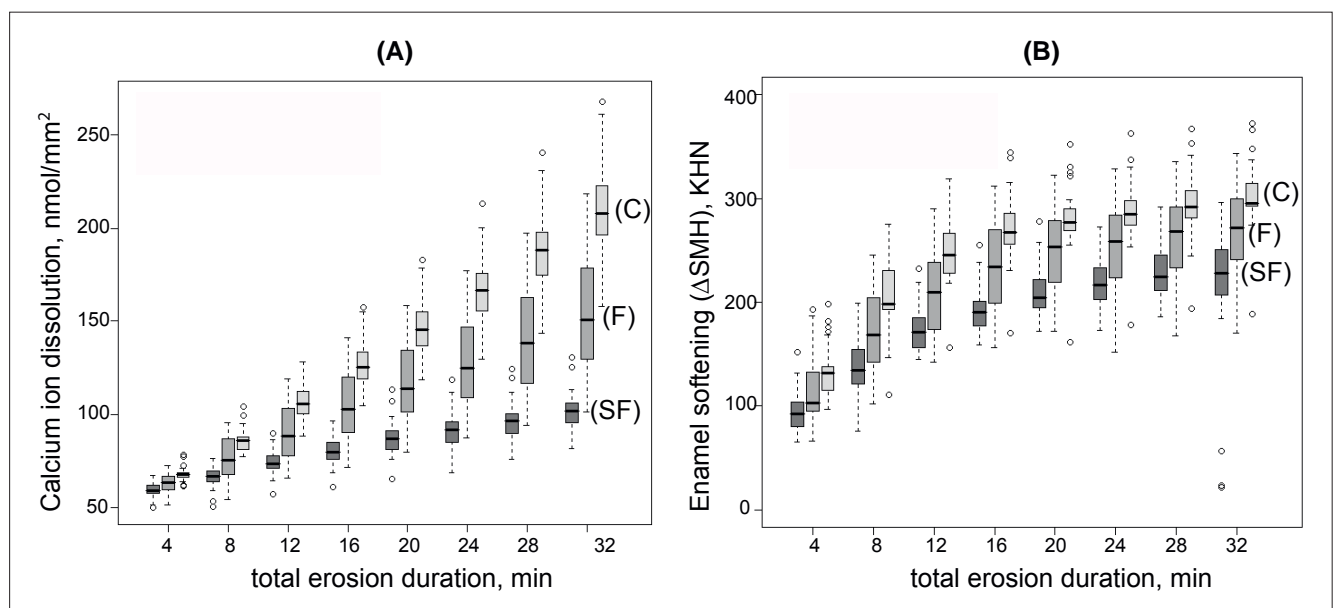


Fig. 2 (A) Calcium dissolution from enamel samples and (B) change of enamel hardness as a function of erosion time measured in the three treatment groups. (SF): Sn^{2+}/F^- dental rinse group (dark gray bars); (F): fluoridated rinse group (gray bars); (C): negative control group (light gray bars).

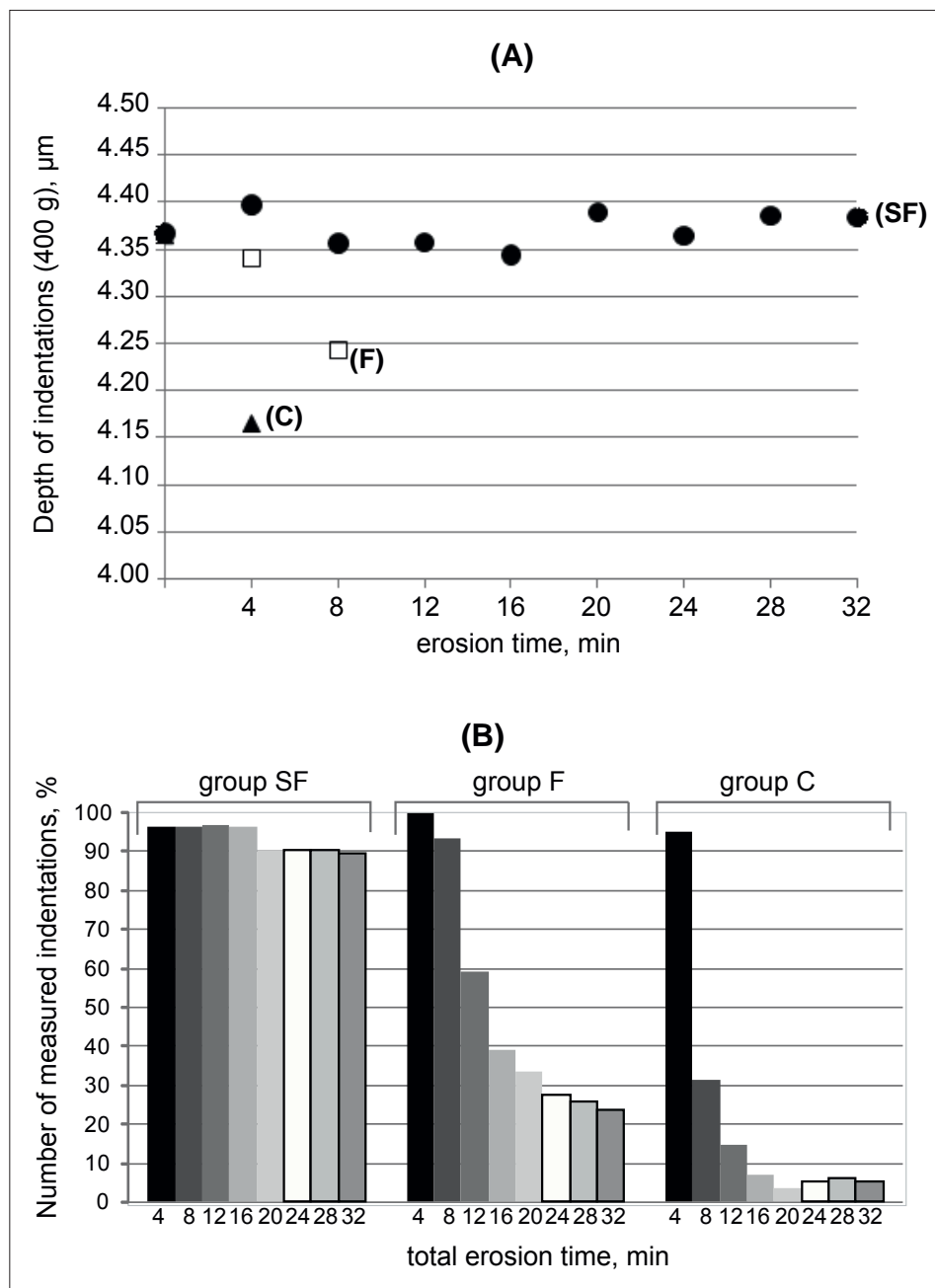


Fig. 3 (A) Change of indentation depth (400 g) after erosive cycles measured in the three treatment groups. (SF, ●): Sn²⁺/F⁻ dental rinse group; (F, □): fluoridated rinse group; (C, ▲): negative control group. Less than 75% of the enamel samples could be analyzed in group F at erosion times ≥ 12 minutes and in group C at erosion times ≥ 8 minutes, therefore the depth values are not presented for these two groups at mentioned erosion times. (B) Number of 400 g indentations in three groups (SF, F and C) that were quantitatively measured after erosive cycles; the total erosion time is given on the x axis.

proximately 90% of indentations could be measured even after a total of 32 minutes of erosive challenge (Fig. 3B), demonstrating no dramatic substance loss during the entire experiment (Fig. 3A). In contrast, a ~0.1 µm median depth decrease was measured in group F and ~0.2 µm of wear was detected in group C after 4 and 8 minutes of erosion, respectively (Fig. 3A). Only 60% of indentations could be analyzed in group F after 12 minutes of demineralization and only 30% could be measured in group C (Fig. 3B), thus the median depth values in groups F and C are not presented at all erosion times.

Structural assessment of the eroded enamel surfaces was performed using SEM. In Figure 4, SEM images of a representative sample from each group show different degrees of enamel erosion with the characteristic honeycomb surface topography. The surface of enamel samples in group SF (Fig. 4, SF) was less eroded than in group F (Fig. 4, F), indicating inhibition of erosion progression in enamel samples treated with the Sn²⁺/F⁻-containing dental rinse. The enamel surfaces in the control group (Fig. 4,

C) had highest degree of etching compared to the surfaces in groups F and SF. No sign of any amorphous precipitates (Sn²⁺ salts, CaF₂) was detected in any of the evaluated samples from the three treatment groups.

Discussion

Various analytical tools were used in this study for the monitoring of different enamel parameters during erosion progression. Measurement of calcium release into acidic solutions and change of enamel microhardness are methods usually applied for the assessment of dental erosion *in vitro* (MEURMAN ET AL. 1990, ATTIN ET AL. 1997, LUSSI ET AL. 2000, GANSS ET AL. 2005, HJORTSJO ET AL. 2010, SHELLIS ET AL. 2011). The linear rate of calcium release revealed statistically significant differences between the groups SF and F, SF and C, and F and C (Fig. 2A), clearly indicating the erosion-inhibiting property of the elmex® Erosion Protection mouthrinse. The application of the fluoridated

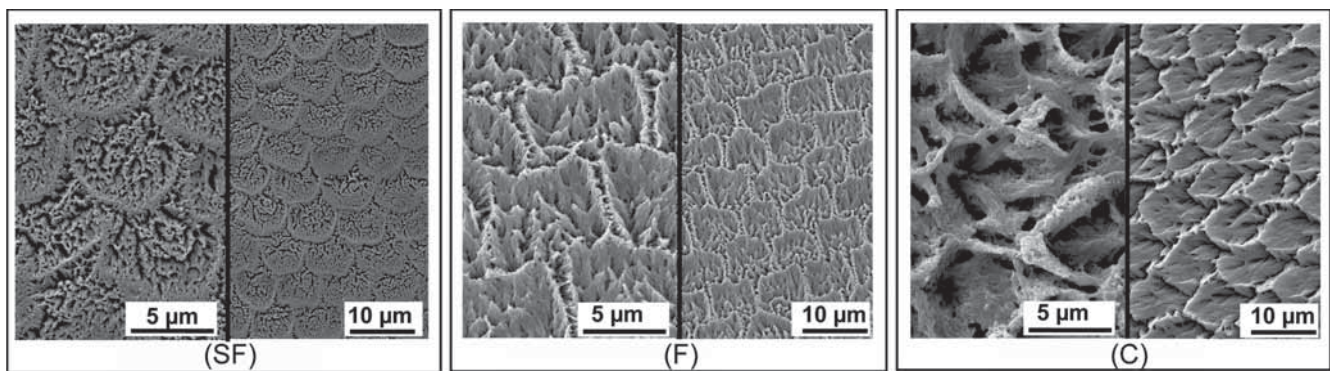


Fig. 4 SEM images of enamel surfaces in the three treatment groups after the entire erosive experiment. (SF): Sn²⁺/F⁻ dental rinse group; (F): fluoridated rinse group; (C): negative control group.

dated solution alone also slowed down the erosion progression in group F compared to group C (Fig. 2).

Calcium dissolution typically results in softening of the enamel first, followed by a gradual tissue wear if the calcium loss persists (ATTIN ET AL. 2003, BARBOUR ET AL. 2003). Similar to the results of the calcium release analysis, microhardness change in the three groups indicated the best erosion inhibition in the enamel treated with Sn²⁺/F⁻-containing mouthrinse (Fig. 2B), even in the presence of the salivary pellicle. The softening change over erosion time did not show a linear relationship as was observed for calcium release. Rapid loss of enamel hardness was measured within the first 12–16 minutes of erosion, followed by a stabilization period where hardness values measured within a treatment group were nearly constant. The observed tendency of the microhardness change (Fig. 2B) is related to the limitation of the technique. It is known that the hardness analysis provides a better detection of the initial softening stages of dental erosion, while it is limited in its erosion assessment at the advanced phases of substance loss (HARA & ZERO 2008, STENHAGEN ET AL. 2010). Most probably, this limitation of the technique is related to the histology of the enamel erosion. Thus, the enamel hardness decreases indeed during the first softening phase due to a loss of tooth mineral. Persistent demineralization results in the substance loss where the uppermost enamel tissue (bulk) is eliminated but the remaining tissue is softened and present on the interface (LUSSI ET AL. 2011). With longer acidic impact this softened tissue area reaches equilibrium and does not progress further, while bulk mineral undergoes further dissolution. Because microhardness tester assesses the uppermost surface layer, it measures this equilibrated softened tissue during the advanced erosion stages showing similar hardness values. Perhaps application of smaller loads for the microhardness analysis could be applied in order to improve the possibilities of the method. Nevertheless, a strong correlation was found between calcium release and change in enamel microhardness in all three groups ($r^2 = 0.783\text{--}0.864$). Remarkably, calcium loss follows linear function during first 30 minutes of *in vitro* erosion progression (GRAY 1962), thus both softening and initial substance loss phases can be successfully quantified by this method. The obtained results of calcium dissolution in this study correlated well with the cited research. However, the kinetics of calcium dissolution can differ from a linear fashion at the severe erosive stages (GRAY 1962). The microhardness analysis of the eroded enamel was best appropriate for the softening phase where decrease of the enamel hardness with erosion time followed nearly linear function (Fig. 2B).

The good protective potential of the Sn²⁺/F⁻-containing dental rinse against initial erosion progression was also supported by the analysis of tissue loss from the demineralized enamel samples. Longer enamel resistance towards acid-induced wear was quantitatively (Fig. 3A) and qualitatively (Fig. 3B) shown in group SF. The decrease in indentation depth (increase in substance loss) followed the trend of group C > group F > group SF, with a maximal enamel wear of ~0.2 μm in group C after 4 minutes of demineralization. The etching of the enamel surfaces could also be observed in SEM images of the treated samples (Fig. 4). The most eroded enamel surface was seen in control group C (Fig. 4, C), while the least eroded interface corresponded to the enamel samples in group SF (Fig. 4, SF), supporting the results of the microhardness and calcium release analyses.

In conclusion, all applied methods and achieved results showed significantly greater erosion-inhibiting properties with a dental rinse comprised of Sn/F compounds (500 ppm F⁻, 800 ppm Sn²⁺, pH = 4.5) compared to the F⁻-containing solution (500 ppm F⁻, pH = 4.5) or tap water. This effect was observed in the presence of the salivary pellicle layer, which corroborated the results of previous *in situ* (HOVE ET AL. 2008, GANSS ET AL. 2010, HUYSMANS ET AL. 2011) and *in vitro* (HOVE ET AL. 2007b) studies where different stannous containing dental products were used.

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Résumé

Les substances stanneuses sont utilisées dans les produits dentaires contre les caries et depuis peu aussi contre l'érosion dentaire. Ils peuvent également pénétrer dans le tissu dentaire érodé, ce qui le rend plus résistant à l'acide. L'efficacité des ions stanneux (Sn) dans l'inhibition de l'érosion est améliorée par la présence de fluorure (F). De nombreuses études ont démontré une progression plus lente de l'érosion si les produits dentaires contiennent à la fois de l'étain et des fluorures. La majorité des études faites ont été réalisées sous des conditions fortement érosives. Les résultats étaient une dissolution de l'émail dans l'ordre de grandeur du micromètre. Le but de cette étude était de tester l'efficacité inhibitrice d'un rinçage dentaire

is an irreversible event and leads to more severe tissue wear due to higher susceptibility of the softened demineralized tissues to abrasion by the tongue or a toothbrush (VORONETS ET AL. 2008; VORONETS & LUSSI 2010). Therefore, timely preventive measures are important in the early softening stages for the prevention of tooth wear in patients. This study investigated the efficiency of stannous and/or fluoride containing dental rinses in the prevention of early erosion involving softening and initial substance loss only. Therefore, short demineralization times were used in the experiment. In addition, the formation of an *in vitro* salivary pellicle layer on the enamel surface was included. It is well known that the salivary pellicle serves as a soft diffusion barrier between teeth and the surrounding environment (LENDEMANN ET AL. 2000). Although it can be removed under strong acidic etching, clinically relevant mild erosive conditions cannot eliminate the pellicle layer completely (HANNIG ET AL. 2005). Therefore, it remains unclear if the presence of this layer might affect the stannous interaction with enamel and thus the efficiency of Sn/F-containing mouth-rinse at the early softening stage. The latter is especially important as stannous uptake is also limited by the extent of enamel erosion, i.e. reduced enamel erosion equates to reduced stannous ion penetration (SCHLUETER ET AL. 2009a). Nevertheless, the authors hypothesized that the combination of stannous and fluoride compounds would improve the efficiency of a mouthrinse in the inhibition of early dental erosion even in the presence of the salivary pellicle layer.

Materials and Methods

Polished enamel specimens

Enamel specimens (n = 90) were prepared from caries-free human molar teeth extracted by dental practitioners in Switzerland. Before the extraction, the patients were informed about the use of their teeth for research purposes and consent was obtained. All teeth were stored in 1% chloramine T trihydrate solution after the extraction. Tooth crowns were separated from the roots using an Isomet® Low Speed Saw (Buehler, Düssel-dorf, Germany) and coated with red nail polish for determination of the exposed enamel area. The buccal sites of the specimens were embedded in the resin (Paladur, Heraeus Kulzer GmbH, Hanau, Germany) in two planar parallel moulds. The thinner mould (200 µm thick) was removed while the teeth in the thicker one (7.5 mm thick) were serially abraded under constant tap water cooling using a Knuth Rotor machine (LabPol 21, Struers, Copenhagen, Denmark) with silicon carbide paper discs of grain size 18.3 µm, 8 µm and 5 µm, 60 seconds each. The embedded enamel blocks were taken out of the moulds before being polished for 60 seconds with 3 µm diamond abrasive on a Struers polishing cloth (LaboPol-6, DP-Mol Polishing, DP-Stick HQ, Struers) under constant cooling. Between two polishing steps and after the final polishing, all slabs were sonicated for 1 minute in tap water and rinsed. Thus, all prepared specimens had a flat ground enamel area with a 200 µm cut off layer. Samples were stored in a mineral solution (1.5 mmol/l CaCl₂, 1.0 mmol/l KH₂PO₄, 50 mmol/l NaCl, pH = 7.0) and underwent further polishing with a 1 µm diamond abrasive (LaboPol-6, DP-Mol Polishing, DP-Stick HQ, Struers) for 60 seconds, immediately before the experiment.

Formation of salivary pellicle

Paraffin wax-stimulated saliva from 25 healthy donors was collected into ice-chilled vials, pooled and centrifuged (4000×g at 4°C for 15 min). Enamel specimens were immersed in

salivary supernatant for 2 hours under constant agitation (70 U/min) at 30°C (Salvis, Renggli AG, Rotkreuz, Switzerland). The centrifuged pooled saliva was stored at -80°C between experiments.

Test solutions and treatment procedure

Three test solutions were applied in the study: elmex® Erosion Protection dental rinse (800 ppm Sn²⁺, 500 ppm F, pH = 4.5, GABA Int. AG, Switzerland), a NaF reference solution (500 ppm F⁻, pH = 4.5) and water as a negative control. The NaF reference solution was prepared by dissolution of NaF in water followed by adjustment of the pH to 4.5. Tap water was used as a negative control rinse due to the absence of stannous and fluoride ions, constant pH of 7.4-7.5, and clinical relevance to normally used flushing fluid. Enamel samples were incubated for 2 minutes twice a day for 4 days in 10 ml of test solution: elmex® Erosion Protection rinse (**group SF**, n = 30), or NaF-containing rinse (**group F**, n = 30) or water (**group C**, n = 30) (Fig. 1). After treatment, samples were rinsed with tap water (10 s) and dried (5 s) with oil-free air.

Erosive challenge

The enamel specimens were immersed twice daily for 4 days in 30 ml of citric acid (0.65%, pH = 3.6) for 4 minutes under constant agitation (70 U/min, Salvis, Reussbühl, Switzerland) at 30°C (Fig. 1). Samples were removed from acidic solutions, rinsed with deionized water (10 s) and dried with oil-free air (5 s).

Analysis of calcium release by atomic absorption spectroscopy, measurement of enamel microhardness and scanning electron microscopy (SEM)

The detailed analytical procedures of the three following methods have been described in a previously published study (RAKHMATULLINA ET AL. 2011). Calcium release was standardized to the exposed enamel surface area in each sample. The dif-

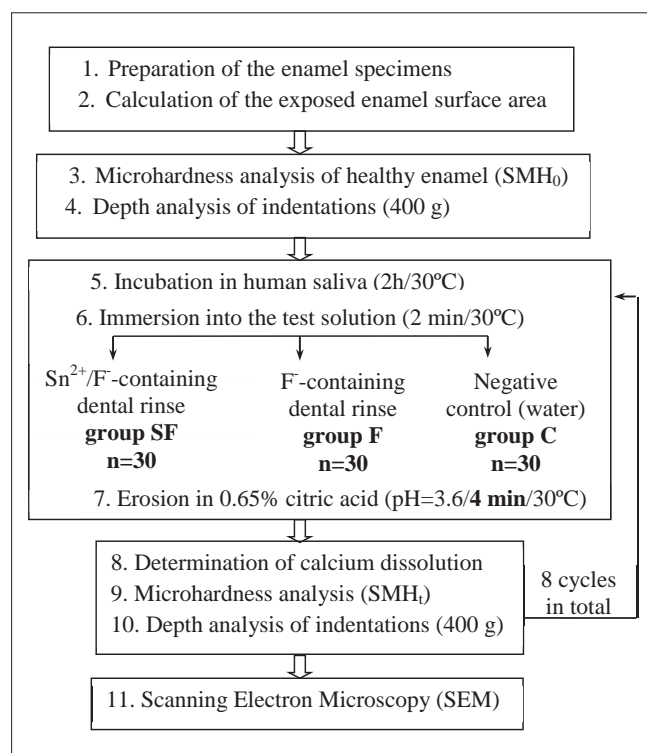


Fig. 1 Experimental design of the study.

stanneux (elmex® Erosion Protection) sur l'érosion dans les premiers stades érosifs (phase de dissolution), ainsi lors de perte de substance dentaire en présence de la pellicule salivaire. La pellicule salivaire est un film de protéines important à la surface des dents. Elle lubrifie la surface, agit comme bouclier en évitant le contact direct avec les composés acides et régule l'adhérence bactérienne aux tissus dentaires.

Dans cette étude, l'inhibition de l'érosion dentaire a été comparée en utilisant un rinçage dentaire contenant Sn/F (800 ppm Sn²⁺, 500 ppm F⁻, pH = 4,5), une solution fluorée (500 ppm F⁻, pH = 4,5) et de l'eau (témoin). Trois groupes de traitement ont été étudiés (n = 30/groupe). Le design expérimental de l'étude se composait de huit cycles. Dans chaque cycle, les spécimens préparés à partir d'émail de molaires humaines ont été soumis à une séquence de procédures: 1) immersion dans la salive et formation de la pellicule salivaire, 2) incubation dans l'une des solutions à tester et 3) déminéralisation dans l'acide citrique (pH = 3,6; 1%). Avant et après chaque cycle, les spécimens ont été analysés. Les variations de la perte de substance dentaire, la microdureté d'émail et la dissolution de calcium dans les solutions acides ont été suivies tout au long de l'expérience dans les trois groupes. En outre, des images MEB ont été prises de chaque spécimen d'émail après l'expérience.

Il a été constaté que la dissolution de calcium et le ramollissement de l'émail des spécimens d'émail exposés au rinçage Sn/F ont été significativement réduites (groupe SF), en comparaison avec ceux traités dans une solution fluorée (groupe F) et le témoin (p < 0,05). Presque aucune perte d'émail n'a été trouvée dans le groupe SF. Dans le groupe témoin, près de 0,2 µm d'épaisseur d'émail a été éliminée après 4 minutes de déminéralisation. Les images MEB ont montré un léger mordantage de la surface de l'émail dans le groupe SF, tandis que l'érosion a été plus prononcée dans le groupe F et encore plus sévère dans le groupe témoin. En conclusion, la combinaison Sn/F a assuré la meilleure inhibition de l'érosion initiale parmi les solutions testées. Cette étude démontre l'application de différentes méthodes pour la quantification de l'érosion. Une forte corrélation (r² ≥ 0,783) a été démontrée entre la dissolution de calcium et le ramollissement de l'émail lors de la déminéralisation.

Zusammenfassung

Zinnverbindungen werden schon länger in zahnärztlichen Produkten gegen Karies und seit Kurzem auch gegen Erosionen verwendet. Sie können in die erodierte Zahnhartsubstanz eindringen, wodurch diese säurebeständiger wird. Die Wirksamkeit der Zinnionen (Sn) in der Erosionshemmung wird durch die Anwesenheit von Fluorid (F) erhöht. Viele Studien haben

eine langsamere Progression der Erosion nachgewiesen, wenn zahnärztliche Produkte sowohl mit Zinn als auch Fluorid verwendet wurden. Der Grossteil der Forschungsarbeiten wurde unter starken erosiven Bedingungen durchgeführt, bei denen Schmelzverluste im Mikrometerbereich auftraten. Die vorliegende Studie setzte sich zum Ziel, die erosionshemmende Wirkung einer Zinn enthaltenden Mundspülung sowohl im Anfangsstadium der Erosion (bei der Erweichung des Schmelzes) als auch beim ersten Substanzverlust in Gegenwart der Pellikel zu untersuchen. Die Pellikel ist ein wichtiger Proteinfilm auf der Zahnoberfläche, der die Zähne gleitfähig macht, vor dem direkten Kontakt mit sauren Stoffen schützt und der die Bakterienadhäsion auf den Zahngeweben regelt.

In dieser Studie wurde die Erosionshemmung durch eine Sn/F-haltige Zahnspülung (800 ppm Sn²⁺, 500 ppm F⁻, pH = 4,5) mit einer fluoridierten Lösung (500 ppm F⁻, pH = 4,5) und Wasser (Kontrolle) verglichen. Dabei wurden drei Behandlungsgruppen (n = 30/Gruppe) untersucht. Das experimentelle Design der Studie bestand aus acht Zyklen. In jedem Zyklus wurden die aus menschlichen Molaren präparierten Schmelzprobekörper einer Sequenz von Prozeduren unterzogen: 1) Immersion im Speichel und Bildung der Pellikel, 2) Inkubation in einer der Testlösungen und 3) Demineralisation in Zitronensäure (pH = 3,6; 1%). Vor und nach jedem Zyklus wurden die Probekörper analysiert. Die Änderung der Mikrohärtigkeit, der Substanzverlust und die Kalziumfreisetzung in die sauren Lösungen wurden während des Experiments in den drei Behandlungsgruppen überwacht. Zusätzlich wurden nach dem Experiment von den Probekörpern Bilder im Rasterelektronenmikroskop (REM) angefertigt.

Es zeigte sich, dass die mit der Sn/F-haltigen Spülung behandelten Probekörper (Gruppe SF) eine signifikant reduzierte Kalziumfreisetzung und Schmelzerweichung im Vergleich zu den mit Fluoridlösung behandelten (Gruppe F) und der Kontrolle (p < 0,05) aufwiesen. Fast kein Schmelzverlust wurde in der Gruppe SF gefunden. In der Kontrollgruppe wurde die Dicke des Zahnschmelzes bereits nach 4 Minuten Demineralisation um 0,2 µm reduziert. Die REM-Bilder zeigten nur leicht angeätzte Schmelzoberflächen in der Gruppe SF, während die Erosion in Gruppe F ausgeprägt und bei der Kontrollgruppe am deutlichsten war. Abschliessend lässt sich festhalten, dass unter den getesteten Lösungen die Sn/F-Kombination die beste Hemmung initialer Erosionen zeigte. Diese Studie demonstriert zusätzlich die Anwendung verschiedener analytischer Methoden für die vergleichende Quantifizierung der Erosion. Es wurde eine sehr starke Korrelation (r² ≥ 0,783) zwischen Kalziumfreisetzung und Schmelzerweichung während der Demineralisation gefunden.

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