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Comparative analysis of the protective effects of fluoride compounds on dental erosion in mouse model

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Abstract

Background Dental erosion development is affected by various factors such as types and amounts of acids, drinking habits and lifestyle choices. To prevent the onset and progression, identification of early erosive lesions as well as increased knowledge of the preventive treatment possibilities is of the importance. The aim of this study is to compare the protective effects of various fluoride compounds against dental erosion utilizing an established mouse model.

Methods Three groups of ten young CD-1 mice were provided cola drink ad libitum during six weeks. Fluoride solutions containing metal components, TiF_4 (0.5 mol/l, pH 1.2, 9500 ppm F) and SnF_2 (0.5 mol/l, pH 2.6, 9500 ppm F), and a non-metal fluoride, NaF (0.5 mol/l, pH 8, 9500 ppm F) were applied to the molars under sedation twice a week. Additionally, one positive (acidic drink) and one negative (distilled water) control group were included. Mandibular molars were thereafter dissected and analyzed using scanning electron microscopy (SEM). The first molars were transversely ground, observed by SEM, and tooth height and dental hard tissue loss were measured. Further, pulp structure was described.

Results The application of metal fluorides, TiF_4 and SnF_2, resulted in the formation of a protective coating layer on the molars. The overall protective effects of fluoride compounds on the development of dental erosion were evident in increasing succession from NaF, TiF_4 to SnF_2. Molars applied NaF showed a 6% reduction in tooth tissue loss compared to the untreated positive control molars. TiF_4 and SnF_2 treated molars continued to display decreased tooth tissue loss by 37% and 67%, respectively.

Conclusions The metal fluorides offer superior protection against dental erosion compared to the traditional fluoride compound. The results particularly emphasize the protective effect of SnF_2, which was most effective in preserving enamel structure and minimizing dentin exposure. This suggests that SnF_2 could be an effective option for preventing dental erosions.

Keywords Dental erosion, Fluoride, NaF, SnF_2, TiF_4

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Background

Dental erosion is of a multifactorial etiology, including various extrinsic and intrinsic acid factors, such as acidic drinks or food, or frequent exposure to gastric acid [1]. The prevalence of dental erosion is high – reported rates in permanent teeth range from 20 to 45% [2]. Moreover, the incidence of dental erosion is increasing, particularly among younger individuals. This trend is partly attributed to changes in diet and lifestyle [3, 4]. A common consequence of advanced dental erosion is irreversible tooth loss and exposed dentin and dentinal tubules. This may cause dentin hypersensitivity, leading to persistent pain and discomfort [5, 6].

To minimize patients' discomfort and to preserve the tooth structure, different treatment options may be offered. However, restorative treatment can be extensive, demanding, and invasive [7, 8]. Therefore, various fluoride treatments are recommended as part of a prophylactic and non-operative approach for individuals at risk. The application of fluoride to the oral cavity generates a protective CaF_2 (calcium-fluoride) layer on the enamel surface. This layer serves as a reservoir of calcium ions, which help buffer or neutralize hydrogen ions from acids [9]. Under acidic conditions, fluoride from CaF_2 is integrated into the tooth's mineral to form fluorapatite or fluor hydroxyapatite, which enhances resistance to further dissolution and reduces sensitivity [9]. The use of fluoride products, such as NaF (sodium fluoride), is optimized with high concentrations and low pH formulas to form CaF_2 deposits. However, these high-concentration fluoride products are typically not available over the counter [10]. Furthermore, in vitro studies have demonstrated that pretreating enamel with either SnF_2 (stannous fluoride) or TiF_4 (titanium tetrafluoride) reduces or inhibits the erosive effects of acids [11–16]. The formation of a "glaze-like layer" on dental enamel exposed to TiF_4 solution is considered crucial in minimizing enamel dissolution [17–20]. Similarly, in vitro studies on SnF_2 have shown the formation of a protective surface coating [21, 22] and the incorporation of Sn into the enamel under erosive conditions [23, 24]. This prophylactic treatment was first explored in vivo by Young et al., 2006, though it remains controversial [25, 26]. In addition, little focus has been given to the effects on the dentin-pulp-complex, when tooth surfaces are treated with various fluoride treatments. The pulp is important in defense processes against bacterial invasion, reactionary tissue deposition due to caries and tooth wear, sensing changes early and hence limiting the chances for progression of an unwanted stimulus and having a proprioceptive function that limits the masticatory load. It is due to its confinement, cellular composition, the high sensory nerves and the rich microcirculatory units that makes the pulp not only unique but also allows for the structural changes in order to react rapidly to the different stimuli [27].

Since the loss of dental hard tissue is irreversible, human studies on dental erosion raise ethical concerns. Our recent study established a mouse model tailored for extrinsic dental erosion, where lesions of varying severities can be induced and analyzed [28]. This technique involves examining transversely ground molars using scanning electron microscopy (SEM) to document dental erosion and its depths, even in small dentition such as mouse molars [28]. The aim of the present in vivo study is to evaluate and compare the protective effects of different fluoride compounds against dental erosion using the established mouse model. We also explored the SEM-images at hand for any visible structural changes in pulp. To the best of our knowledge, this is the first in vivo study to explore the effects of various fluorides on dental erosion. The hypothesis is that the metal fluorides, TiF_4 and SnF_2, provide better protection against dental erosion compared to the traditional fluoride compound, NaF.

Methods

Animal model

Fifty phenotypically young female mice (CD-1 strain, 7 weeks old, 30 ± 5 g body weight) were included in this study. Prior to the experiment, the mice were provided with standard laboratory fodder and water ad libitum. They were maintained on a 12-h light: dark cycle at 21 °C with a relative humidity of 65%. The experiment adhered to Norwegian regulations and legislation (Norwegian Regulation on Animal Experimentation of 2015 based on the EU Directive on the Protection of Animals Used for Scientific Purposes 2010/63/EU and the Norwegian Animal Welfare Act of 2009). This study was authorized by the Norwegian Food Safety Authority (FOTS ID 28734) and the experiment was conducted at the department for comparative medicine, Faculty of Medicine, University of Oslo.

Fifty mice were randomly assigned to five experimental groups (Fig. 1). Four groups were provided with an acidic drink (Coca-Cola, phosphoric acid, pH = 2.27), while one group was given distilled water (negative control). Each cage was supplied with two 250 ml bottles of the respective drinks, which were replaced three times per week. Prior to the study, the pH of the cola drink in a 250 ml experimental bottle was monitored over three days, showing no significant change in pH. Throughout the experiment, all animals were provided with softened laboratory fodder and drinks ad libitum, and the consumption of drinks per cage was recorded. Three experimental groups provided with acidic drink received topical applications of fluoride solutions: TiF_4 (0.5 mol/L, pH 1.2, 9500 ppm F), SnF_2 (0.5 mol/L, pH 2.6, 9500 ppm

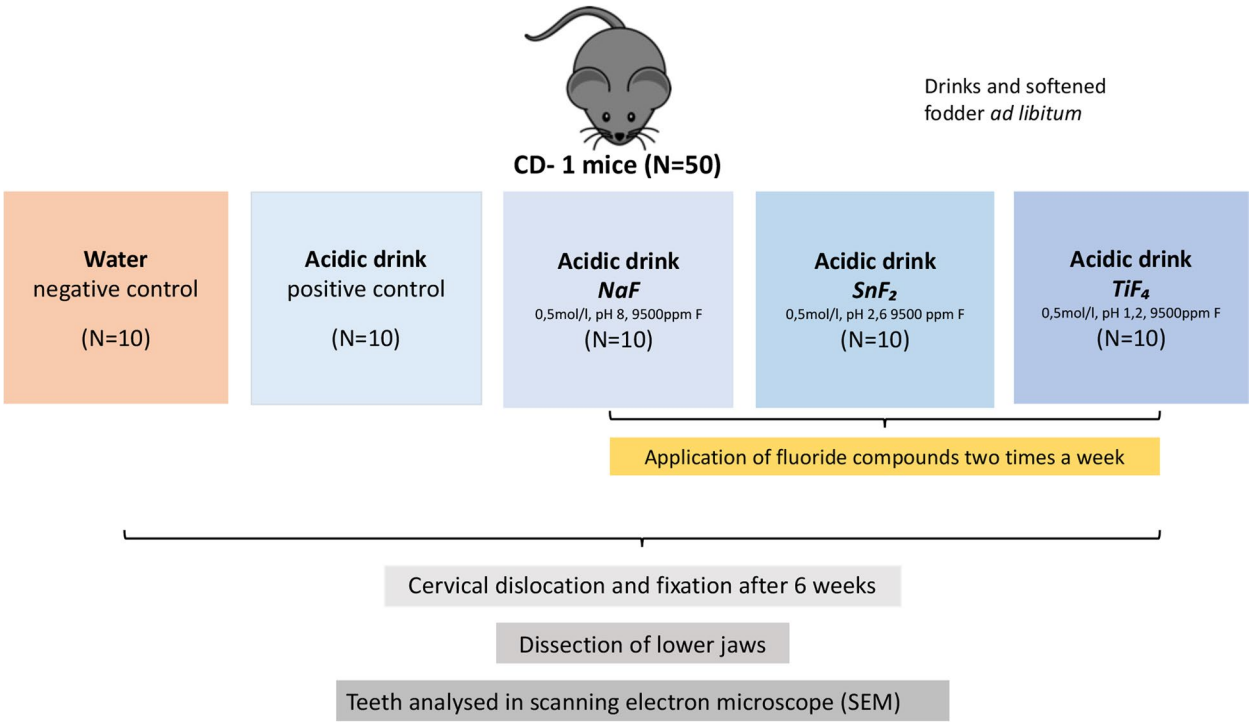

Fig. 1 Enrollment Flowchart for Study Animals. Fifty mice were allocated into five experimental groups. Four groups received acidic drink (Coca Cola, phosphoric acid, pH = 2.27), and one group was given distilled water as a negative control. Three of the groups had their molar teeth treated with fluoride compounds: TiF_4 (0.5 mol/l, pH 1.2, 9500 ppm F), SnF_2 (0.5 mol/l, pH 2.6, 9500 ppm F), and NaF (0.5 mol/l, pH 8, 9500 ppm F). After the experimental period of six weeks, all animals were sacrificed by cervical dislocation, and the teeth were prepared for observation in SEM

F), and NaF (0.5 mol/L, pH 8, 9500 ppm F) on the surface of their molar teeth. These solutions were produced in a laboratory at NIOM (Nordic Institute of Dental Materials) [29]. All parameters were checked after manufacturing and prior to experiment. Mice in the three treatment groups were transferred to an induction chamber and sedated shortly with an inhalation anesthetic (Isoflurane, concentration 2.5 – 3.0%) [30]. Each mouse was closely monitored during the procedure, and fluoride solution was applied only after the loss of the righting reflex. The application time was limited to 10–15 s, and mice were returned to their cages once fully active. The fluoride treatments were applied using a micro brush twice a week for five weeks. Following the six-week experimental period, all animals were sacrificed by cervical dislocation, and their heads were fixed in 70% ethanol.

Scanning electron microscopy

The mandibular molars were dissected and fixed in 70% ethanol. The isolated teeth were thoroughly cleaned by dissection and gentle brushing under running tap water. The specimens were then air-dried overnight and mounted on brass cylinders using cyanoacrylate glue. They were sputter-coated with a 30 nm layer of platinum and observed using a Tabletop Microscope TM4000Plus (Hitachi, Tokyo, Japan) operated at 10 kV.

The jaw segments containing all three molars were embedded in Epon and grinded transversely under a stereomicroscope using 800 and 1200 grit 3 M waterproof silicon carbide paper (3 M, St. Paul, MN, USA). The ground surfaces were further polished by grinding the specimens against the backside of the 3 M waterproof silicon carbide paper with 0.05 µm particle size alumina powder (Buehler Micropolish, Buehler, Lake Bluff, IL, USA) in water. After careful brushing under running tap water and removal of excess water, the teeth were etched for 45 s in 1% nitric acid, air-dried overnight, sputter-coated with a 30 nm layer of platinum, and observed using scanning electron microscopy (SEM). The grinding method used to achieve a transversal ground plane for SEM observation has been previously described [28].

Measurements and statistical analysis

SEM images of the transversely ground and etched planes were utilized for measuring the lingual tooth height and lingual enamel/dentin loss. In addition, a tendency for degree of structural changes in dental pulps were detected. The level of disarranged and porous structure was graded as 0 (none), 1 (mild), 2 (moderate) or 3

(severe), and scored for all 5 groups involved in the current study. Mean values and standard deviations were calculated using Microsoft Excel (Microsoft Office Excel, 2020). Significant differences of pulp estimation were analyzed using one-tailed *t*-test with unequal variance. Otherwise, the measurement data were tabulated and analyzed with the Statistical Package for Social Sciences (SPSS) version 22.0 for Windows (SPSS Inc., Chicago, Illinois, USA). One-way analysis of variance (ANOVA) followed by the Tukey post-hoc test, as well as independent t-tests, were employed to evaluate the data. *P*-values < 0.05 were considered statistically significant. In the sample size calculation for a "One-way analysis of variance (ANOVA)" involving five groups, it was found that at least 39 teeth per group were necessary to achieve a power of 80% with a significance level of 0.05, given a medium effect size.

Results

Unlike the negative control teeth (Figs. 2a-d & 3a), mandibular molars exposed to acidic drinks showed substantial changes, particularly in the lingual and occlusal aspects. These teeth displayed a rounded cuspal morphology and pronounced erosion (Figs. 2e-t & 3b-e). A consistent pattern of erosion was observed, marked by a distinct erosive step that clearly separated the unaffected cervical region from the affected occlusal region of the lingual enamel (Fig. 2e-t). Above this step, towards the occlusal surface, the enamel on all lingual cusps was eroded, resulting in exposed dentin. However, the extent of erosion varied among the experimental groups treated with different fluoride compounds (Figs. 2i-t & 3c-e) when compared to the positive control molars (Figs. 2e-h & 3b), which were exposed to cola drinks but did not receive any fluoride treatment. In all experimental groups, the cervical portion of the lingual enamel, situated below the erosive step, remained unaffected and retained its typical four-layered enamel structure (Figs. 3b-e & 4b).

Among the four groups provided with cola drink, the positive control molars (Figs. 2e-h & 3b), which did not receive any treatment, exhibited the most significant erosion. The enamel on the lingual parts of these molars was completely eroded, exposing the dentin. The lingual aspect of these teeth displayed continuous dentin on all cusps, with only minor enamel remnants present in the grooves between the cusps (Fig. 2h). The extensive erosion of dentin in the pulpal direction led to a loss of the curved outline characteristic of the lingual tooth surface (Fig. 3b). The lingual tooth height was reduced by 35% (458 µm vs. 705 µm) in the positive control molars compared to the negative control (Table 1). Additionally, the lingual enamel/dentin loss was 145 ± 19 µm (Table 1) compared to the negative control, measured as shown in Fig. 3h.

Molars treated with NaF exhibited less erosion compared to the positive control group (Table 1). On the lingual aspect of these teeth, while most of the enamel

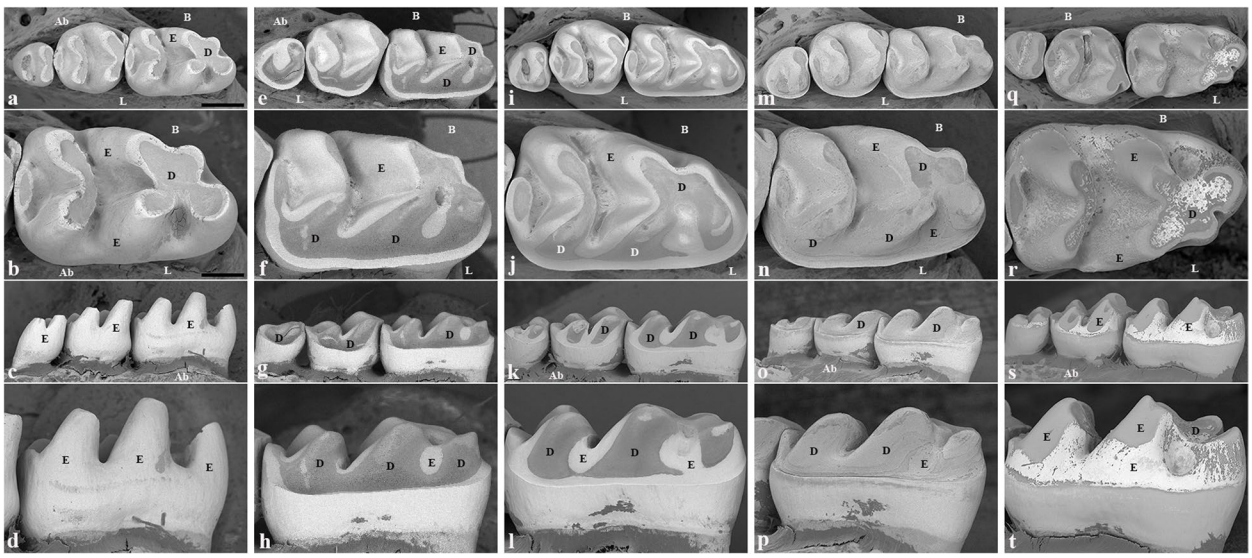

Fig. 2 SEM images of *mandibular* molars from negative control (**a**-**d**), positive control (**e**–**h**), NaF (i-l), TiF_4 (**m**-**p**), and SnF_2 (**q**-**t**) mouse. Occlusal (**a**, **b**, **e**, **f**, **i**, **j**, **m**, **n**, **q**, **r**) and lingual (**c**, **d**, **g**, **h**, **k**, **l**, **o**, **p**, **s**, **t**) view of the molars. (**b**, **f**, **j**, **n**, **r**) Higher magnification of occlusal view of mandibular first molar in panels **a**, **e**, **i**, **m**, **q**, respectively. (**d**, **h**, **l**, **p**, **t**) Higher magnification of lingual view of mandibular first molar in panels **c**, **g**, **k**, **o**, **s**, respectively. The bar represents 500 µm in panels **a**, **c**, **e**, **g**, **i**, **k**, **m**, **o**, **q**, **s**, and 250 µm in panels **b**, **d**, **f**, **h**, **j**, **l**, **n**, **p**, **r**, **t**. E = enamel, D = dentin, Ab = alveolar bone, B = buccal side, L = lingual side

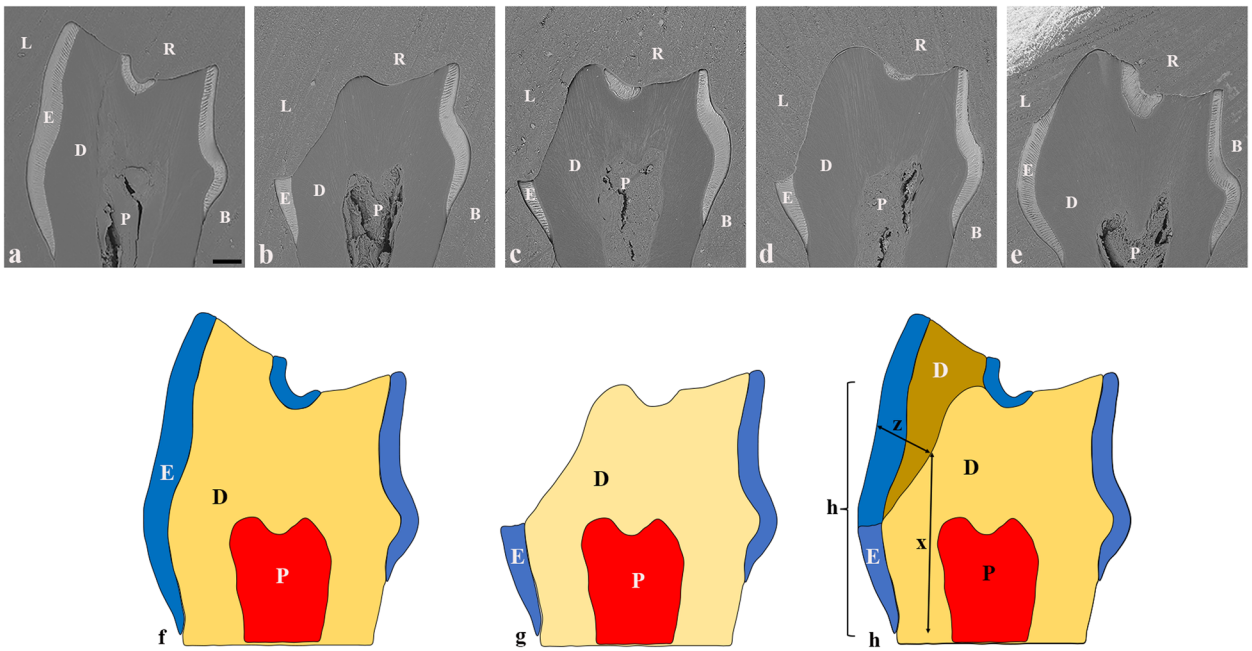

Fig. 3 SEM images (**a**-**e**) and schematic representation (**f**–**h**) of transversely ground planes of mandibular first molars. SEM images of transversely ground planes of mandibular first molars from negative control (**a**), positive control (**b**), NaF (**c**), TiF_4 (**d**), and SnF_2 (**e**) mouse. A separate schematic presentation of transversely ground planes of negative (**f**) and positive (**g**) control mandibular first molar. A collective presentation of ground molar planes (**h**) as a method to compare the experimental groups and to illustrate the measurement techniques used. X indicates the extent of lingual enamel/dentin loss attributable to erosion, while Y represents the vertical distance from the lingual step to the reference point for X. Z represents the lingual tooth height, measured from the enamel-cementum junction to the highest point on the lingual aspect of the tooth. The dimensions shown correlate with the findings presented in Table 1. The bar represents 100 µm in panels a-e. E = enamel, D = dentin, P = pulp, R = resin, B = buccal side, L = lingual side

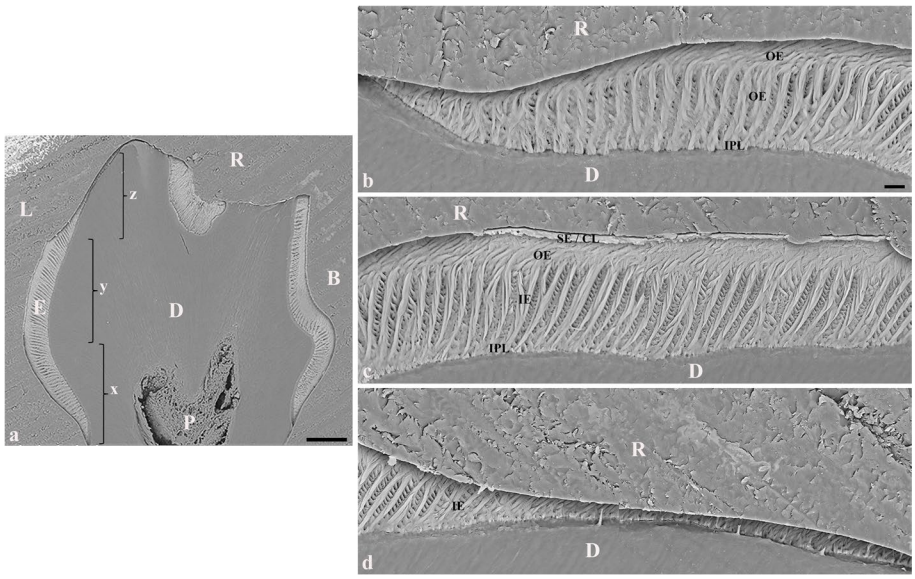

Fig. 4 SEM images of transversely ground planes of mandibular first molar from SnF_2 mouse. (**b**, **c**, **d**) Higher magnification of cervical lingual enamel from the area in (**a**) marked with x, y, and z, respectively. The bar represents 100 µm in panel a, and 10 µm in panels b-d. E = enamel, D = dentin, P = pulp, R = resin, B = buccal side, L = lingual side, IPL = inner prism-free layer, IE = inner enamel, OE = outer enamel, SE/CL = superficial enamel/coated layer

Table 1 Dimensions of lingual tooth height and enamel/dentin loss

	Negative ctr	Positive ctr	NaF	TiF_4	SnF_2
Lingual tooth height (h)	705 ± 43	458 ± 38	494 ± 29	534 ± 26	615 ± 41
Lingual enamel/dentin loss (z)	-	145 ± 19	138 ± 24	92 ± 21*	48 ± 13*

The values represent measurements taken from SEM images of transversely ground planes, as shown in Fig. 3. Measured dimensions (mean ± SD, µm) of mandibular first molar lingual tooth height and enamel/dentin loss are presented. Letters in parentheses (h and z) refer to the letters in Fig. 3h

(-) Not applicable

(*) Significant difference

$p < 0.05$, when compared to positive control

was still eroded, there was a slightly greater presence of enamel in the grooves between the cusps (Fig. 2j-l). The erosion of dentin in the pulpal direction led to the loss of the characteristic curved outline of the lingual tooth surface (Fig. 3c). The lingual tooth height in the NaF-treated molars was reduced by 30% (494 µm vs. 705 µm) compared to the negative control (Table 1). Additionally, the lingual enamel/dentin loss measured 136 ± 24 µm (Table 1), which represents a 6% reduction in tooth tissue loss compared to the untreated positive control molars (Table 1).

The application of metal fluorides, TiF_4 and SnF_2, resulted in the formation of a protective layer on the molars (Figs. 2m-t, 3d,e & 4a,c) and generally provided better protection against the erosion of dental hard tissues compared to NaF (Table 1). Focusing on TiF_4, our results indicated that all treated molars exhibited rounded morphology and extensive erosion (Fig. 2m-p). Some enamel remnants were observed on the eroded lingual aspect (Fig. 2p), and the entire tooth surface, except for the enamel under the erosive step, was covered by a glaze- like layer (Fig. 2p). However, the depth of erosion in the exposed dentin, as judged by the curved outline of the lingual tooth surface, which was predominantly covered by the glaze (Fig. 3d), was less pronounced compared to the positive control (Fig. 3c). The lingual enamel/dentin loss in the TiF_4-treated molars was measured at 92 ± 21 µm (Table 1), representing a 37% reduction in tooth tissue loss compared to the untreated positive control molars (Table 1). Additionally, the lingual tooth height in the TiF_4-treated molars was reduced by 24% (534 µm vs. 705 µm) compared to the negative control (Table 1).

The most effective protection against dental erosion was observed in molars treated with SnF_2 (Figs. 2 & 3). Although the protective effect slightly varied among the mice, this was the only experimental group where the enamel on the lingual aspect of the tooth was preserved to some extent, even above the characteristic erosive step. This treatment also resulted in the formation of a coating layer on the molars (Figs. 2t, 3e & 4a,c). When examining the lingual aspect of the tooth (Fig. 4a), it was noted that this coating layer did not cover the most cervical part of the enamel (Fig. 4b), approximately at the level of the characteristic erosive step. However, the enamel above this level displayed the coating layer (Fig. 4c), which then disappeared at the enamel occlusal to this, indicating that this is the active erosive area where both the coating layer and the enamel are eroded (Fig. 4d). The coating layer did not penetrate deeper into the enamel, such as the outer enamel, but was confined to the superficial enamel (Fig. 4c). The lingual tooth height in the SnF_2-treated molars was reduced by only 13% (615 µm vs. 705 µm) compared to the negative control (Table 1). Additionally, the lingual enamel/dentin loss in the SnF_2-treated molars was measured at 48 ± 13 µm (Table 1), representing a 67% reduction in tooth tissue loss compared to the untreated positive control molars (Table 1).

The scoring results for pulp porosity were base lined against negative controls (Fig. 5A, labelled 0 and 1). Contrastingly, the dental pulps of the positive controls displayed a more disarranged and porous structure (Fig. 5A, labelled 2 and 3). Hence, the level of porosity was graded accordingly from 0 (non) to 3 (severe) and scored for all five groups involved in current study. Table 2 summarizes the differences based on *p*-values calculated for compared groups. The results show similar pulp structures in both TiF_4 and SnF_2 with no significant difference compared to the positive control (Fig. 5B). However, dental pulp of teeth treated with NaF did display a significant difference, revealing a more homogeneous composition, as observed in negative controls (Fig. 5B).

Discussion

Erosive tooth wear is increasingly recognized as a key condition in the dental community due to its rising prevalence, changes of lifestyle and the growing elderly population retaining their teeth for life [1]. The primary symptoms, such as dentin hypersensitivity, imbalanced occlusion with functional loss, and aesthetic issues, underscore the necessity for effective preventive strategies. Consequently, early diagnosis of dental erosion is crucial, alongside a thorough understanding and implementation of appropriate preventive interventions. This

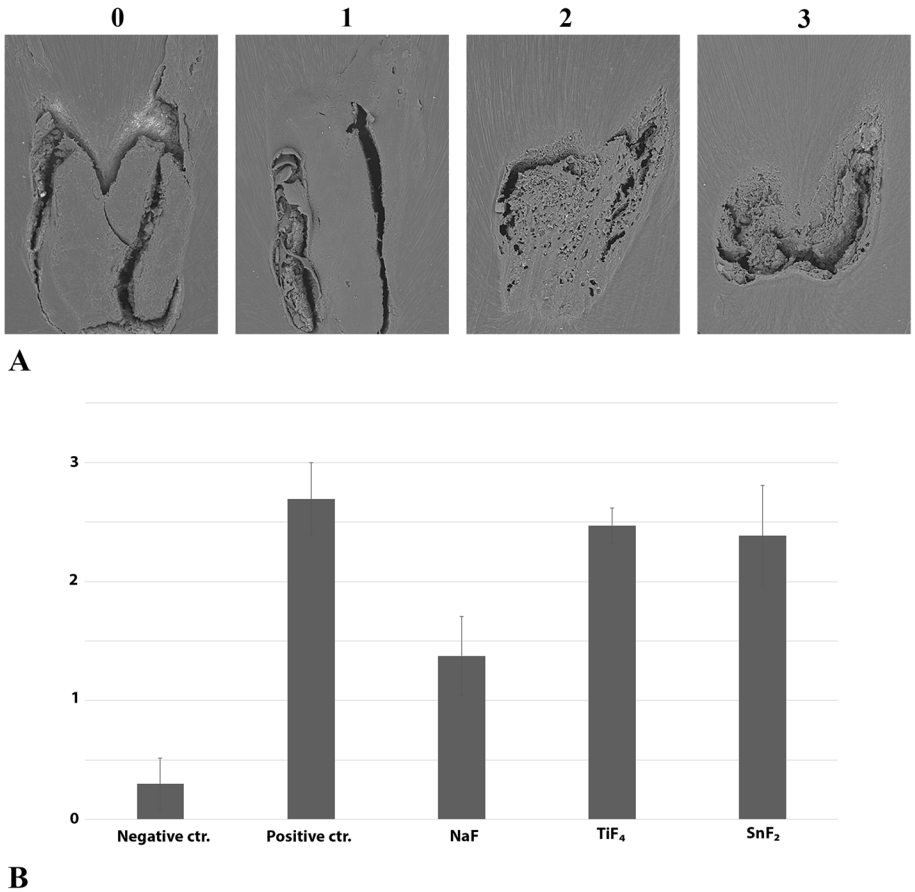

Fig. 5 SEM images of pulp tissue (**A**) and statistical representation of the scoring for pulp porosity (**B**) SEM images of transversely ground planes of mandibular first molars for pulp tissue from negative control (**A**) labelled 0 and 1, and positive control (**A**) labelled 2 and 3. The labels represent grading of the level of porosity observed (0 = non, 1 = mild, 2 = moderate, 3 = severe). Statistical representation of the scoring results (**B**) of the 5 groups: negative control, positive control, NaF, TiF_4 and SnF_2

Table 2 Difference in pulp porosity between groups

Compared groups		p-value
Pos. ctr	**Neg. ctr**	**1,15342E-07***
Pos. ctr	NaF	0,000748851*
Pos. ctr	TiF_4	7,91811E-08*
Pos. ctr	SnF_2	2,38572E-06*
Neg. ctr	NaF	0,000154425*
Neg. ctr	TiF_4	0,219,675,031
Neg. ctr	SnF_2	0,198,488,092
NaF	TiF_4	0,00013374*
NaF	SnF_2	0,00282218*
SnF_2	TiF_4	0,391,019,344

To determine significant difference between the compared groups, *p*-values (sig. diff < 0.05*) were calculated

heightened focus on prevention aims to mitigate the adverse effects of erosive tooth wear and enhance the overall quality of dental care for affected individuals. Moreover, the importance of salivary composition and salivary flow rate are fundamental factors in the prevention of erosive tooth wear. The protective activity where saliva dilutes, neutralizes, clears and buffers acids together with the role in the remineralization process by providing calcium, phosphate and fluoride is essential [31].

Several studies have investigated the potential effects of fluoride on the development of dental erosion, however, they are limited to in vitro and in situ approaches. Similar erosion patterns to those described in this work have been previously observed in both rats [32–34] and mice [28, 35]. This study represents the first in vivo experiment to examine the protective effects of different fluoride compounds on the development of dental erosion. The use of animal models, particularly mice, are widely accepted for studying oral pathology. In this study, we employed a standardized in vivo model, extensively detailed in our previous research, which has proven effective for investigating even the initial stages of

erosive lesions [28]. Therefore, we believe that this model facilitates a controlled and ethical approach to studying the protective effects of fluoride compounds on dental erosion.

The protective effects of fluoride compounds on the development of dental erosion were evident in increasing succession from NaF, TiF_4, to SnF_2 (Figs. 2 & 3). The results particularly emphasize the superior protective effect of SnF_2 against dental erosion, as it maintained more of the enamel structure and reduced dentin exposure compared to other fluoride treatments. Stenhagen et al. [36] explored the impact of daily fluoride mouth rinsing on enamel erosive/abrasive wear in situ. The findings indicated that SnF_2 provided the best protection, followed by TiF_4, with NaF offering minimal protection. Furthermore, in this study the estimation of the glaze/coating was measured to be 1.0 µm (range 0,1- 2,4 µm) on the specimens treated with SnF_2 and 0.6 µm (range 0.2 µm-1.2 µm) on those treated with TiF_4 solution. Hence, indicating that SnF_2 was thicker and probably more resistant against abrasion [36]. Additionally, an in situ study examining the protective effects of a toothpaste containing SnF_2 and a solution containing SnF_2 demonstrated that the solution had a significantly better protective effect [37]. Various studies have also shown the presence of globular particles following SnF_2 treatment. These particles are believed to consist of a calcium fluoride-like agent and tin phosphate [38, 39]. Rykke et al., found that the amount of pellicle material that aggregated on teeth treated with SnF_2 was double that of untreated surfaces [40]. This indicates a different protein adsorption potential on the tooth surfaces treated with SnF_2. Recent in situ studies have discovered that the pellicle formation developed according to the availability of Sn^{2+}—cations, provide a significant protection against severe erosion [41, 42]. Our findings, together with previous studies, underscore the effectiveness of SnF_2 in providing a robust protective barrier against dental erosion, highlighting its potential for use in preventive dental care. However, unfavorable effects from the use of compounds with stannous has been described, e.g., discoloration of the teeth, astringent sensation and a dull feeling on the tooth surface [43].

In addition, it is worth mentioning the erosion inhibiting effect of NaF with the accumulation of CaF_2 on the enamel surface. Research has demonstrated the best effect of NaF with high concentrations, long application period and low pH [44]. This can be considered an influencing factor in our study where the application period of the solution was limited to 10–15 s and with a pH level of 8.

The use of TiF_4 in dental treatments is controversial. TiF_4 can oxidize and form TiO_2 (titanium dioxide), which has been shown to potentially induce DNA strand breaks and chromosomal damage, raising concerns about its genotoxicity. Due to these concerns, the European Food Safety Authority (EFSA) stated in 2021 that TiO_2 should no longer be used in food additives [45]. However, research on the genotoxicity of TiO_2 presents conflicting results, indicating that more studies are needed to fully understand its safety [46]. In contrast, $SnCl_2$ (stannous chloride) was re-evaluated in 2018, and the findings confirmed that stannous compounds are neither carcinogenic nor genotoxic [47]. This re-evaluation supports the continued use of SnF_2 in dental applications, highlighting its safety and effectiveness compared to TiF_4. Further research is warranted to resolve the conflicting data on TiO_2 and to solidify the understanding of the long-term safety of various fluoride compounds used in dental care.

In this study we also scored for the heterogeneity in pulp-structure as seen as level of porosity in SEM-images (Fig. 5A, graded 0–3). We strongly suspect this to be a result of pulp irritation, and hence an inflammatory reaction involving the vascular structure of the pulp [27]. Our results indicate that teeth treated with NaF displayed less severe porosity than both SnF_2 and TiF_4. This mild effect on pulp-tissue by NaF may be due to its low acidity (pH 8) and hence should be given more attention in future studies as it may be relevant for clinical considerations.

The most cervical part of the experimental mouse lingual enamel remained unaffected, a finding consistent with previous observations [28]. Since the enamel is not uniformly eroded along the entire lingual surface exposed to acid, we have speculated that the gingiva may cover and protect the cervical region. Our current study supports this hypothesis, as the SnF_2 coating layer did not cover this part of the enamel (Fig. 4a). This suggests that the gingiva indeed shields the cervical enamel, preventing both the formation of the SnF_2 coating layer and exposure to acidic conditions. Interestingly, despite severe lesions on the lingual surfaces, the buccal aspects of the teeth did not exhibit dental erosion at all. If this is due to a well-developed musculus masseter, covering the buccal aspect, is uncertain. We speculate that the rapid and frequent movement of the mouse tongue may play a crucial role here and surpass the potential protective effect from the secreted saliva, unlike in humans. Indeed, previous studies have shown that tongue movements can intensify dental erosion [48]. Therefore, the mechanical action of the tongue may contribute to the localized nature of the erosion observed, with the lingual surfaces being more susceptible due to their direct contact with acidic solutions and tongue movement.

In this study, the transversal ground planes of mouse molars facilitated detailed measurements of tooth erosion. As previously discussed, achieving an ideal

transversal ground plane through the correct cusps of mouse molars is technically challenging due to their small size, which may affect the accuracy of the measurements [28]. Nevertheless, it is unlikely that this variation would obscure the significant differences in tooth height and enamel/dentin loss observed between the groups following erosion and subsequent treatment. This is supported by previous measurements in wild-type mice and NOD mice using the same method, which yielded similar values (Table 1) [28, 35]. Furthermore, while there may be alternative methods for performing these measurements, as illustrated in Fig. 3, we found the chosen method to be suitable for the present study. Further research should be conducted based on current findings, i.e., to explore the preventive effect of different mouth rinses for home use with a lower fluoride concentration and application frequency, and the long-term effect on dental erosion. In addition, even more severe dental erosion could be studied.

Conclusions

The present study demonstrated the protective effects of fluoride compounds on the development of dental erosion, with effectiveness increasing from NaF to TiF_4, and most notably to SnF_2. The results particularly highlight the superior protective effect of SnF_2, which better preserved enamel structure and minimized dentin exposure compared to other fluoride treatments. However, it is important to note that while SnF_2 provided the best protection, it was not completely effective under extreme acidic conditions, such as the continuous consumption of acidic drinks over several weeks, as modeled in this study.

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Authors' contributions
Conceived and designed the experiments: J.M.H.B., A.T., A.S., A.M., T.P.U., Q.K. Performed the experiments: J.M.H.B., A.T. Analyzed the data: J.M.H.B., A.S., Q.K. Wrote the paper: J.M.H.B., A.T., A.S., A.M., T.P.U., Q.K. All authors reviewed the manuscript.

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Data availability
The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate
The experiment adhered to Norwegian regulations and legislation (Norwegian Regulation on Animal Experimentation of 2015 based on the EU Directive on the Protection of Animals Used for Scientific Purposes 2010/63/EU and the Norwegian Animal Welfare Act of 2009). This study was authorized by the Norwegian Food Safety Authority (FOTS ID 28734) and the experiment was conducted at the department for comparative medicine, Faculty of Medicine, University of Oslo.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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References

1. Lussi A, Carvalho TS. Erosive tooth wear: a multifactorial condition of growing concern and increasing knowledge. Monogr Oral Sci. 2014;25:1–15.
2. Schlueter N, Luka B. Erosive tooth wear - a review on global prevalence and on its prevalence in risk groups. Br Dent J. 2018;224(5):364–70.
3. Mulic A, Vidnes-Kopperud S, Skaare AB, Tveit AB, Young A. Opinions on Dental Erosive Lesions, Knowledge of Diagnosis, and Treatment Strategies among Norwegian Dentists: A Questionnaire Survey. Int J Dent. 2012;2012: 716396.
4. Jaeggi T, Lussi A. Prevalence, incidence and distribution of erosion. Monogr Oral Sci. 2014;25:55–73.
5. Seong J, Claydon N, Macdonald E, Garner S, Newcombe RG, West N. A randomised clinical trial to determine the abrasive effect of the tongue on human enamel loss with and without a prior erosive challenge. J Dent. 2017;58:48–53.
6. Warreth A, Abuhijleh E, Almaghribi MA, Mahwal G, Ashawish A. Tooth surface loss: A review of literature. Saudi Dent J. 2020;32(2):53–60.
7. Peutzfeldt A, Jaeggi T, Lussi A. Restorative therapy of erosive lesions. Monogr Oral Sci. 2014;25:253–61.
8 da Belmar Costa M, Delgado AHS, de Pinheiro Melo T, Amorim T, Mano Azul A. Analysis of laboratory adhesion studies in eroded enamel and dentin: a scoping review. Biomater Investig Dent. 2021;8(1):24–38.
9. Wiegand A, Bichsel D, Magalhães AC, Becker K, Attin T. Effect of sodium, amine and stannous fluoride at the same concentration and different pH on in vitro erosion. J Dent. 2009;37(8):591–5.
10. Petzold M. The influence of different fluoride compounds and treatment conditions on dental enamel: a descriptive in vitro study of the CaF(2) precipitation and microstructure. Caries Res. 2001;35(Suppl 1):45–51.
11. van Rijkom H, Ruben J, Vieira A, Huysmans MC, Truin GJ, Mulder J. Erosion-inhibiting effect of sodium fluoride and titanium tetrafluoride treatment in vitro. Eur J Oral Sci. 2003;111(3):253–7.
12 Willumsen T, Ogaard B, Hansen BF, Rølla G. Effects from pretreatment of stannous fluoride versus sodium fluoride on enamel exposed to 0.1 M or 0.01 M hydrochloric acid. Acta Odontol Scand. 2004;62(5):278–81.
13. Ganss C, Schlueter N, Hardt M, Schattenberg P, Klimek J. Effect of Fluoride Compounds on Enamel Erosion in vitro: A Comparison of Amine. Sodium and Stannous Fluoride Caries Research. 2007;42(1):2–7.
14. Ganss C, Klimek J, Brune V, Schürmann A. Effects of two fluoridation measures on erosion progression in human enamel and dentine in situ. Caries Res. 2004;38(6):561–6.
15. Schlueter N, Ganss C, Mueller U, Klimek J. Effect of Titanium Tetrafluoride and Sodium Fluoride on Erosion Progression in Enamel and Dentine in vitro. Caries Res. 2007;41(2):141–5.

16. Hove LH, Holme B, Young A, Bjørg TA. The erosion-inhibiting effect of TiF4, SnF2, and NaF solutions on pellicle-covered enamel in vitro. Acta Odontol Scand. 2007;65(5):259–64.
17 Mundorff SA, Little MF, Bibby BG. Enamel dissolution. II. Action of titanium tetrafluoride. J Dent Res. 1972;51(6):1567–71.
18. Wei SH, Soboroff DM, Wefel JS. Effects of titanium tetrafluoride on human enamel. J Dent Res. 1976;55(3):426–31.
19. Gu Z, Li J, Söremark R. Influence of tooth surface conditions on enamel fluoride uptake after topical application of TiF4 in vitro. Acta Odontol Scand. 1996;54(5):279–81.
20. Büyükyilmaz T, Ogaard B, Rølla G. The resistance of titanium tetrafluoride-treated human enamel to strong hydrochloric acid. Eur J Oral Sci. 1997;105(5 Pt 2):473–7.
21. Hove L, Holme B, Øgaard B, Willumsen T, Tveit AB. The protective effect of TiF4, SnF2 and NaF on erosion of enamel by hydrochloric acid in vitro measured by white light interferometry. Caries Res. 2006;40(5):440–3.
22. Ganss C, Schlueter N, Hardt M, Schattenberg P, Klimek J. Effect of fluoride compounds on enamel erosion in vitro: a comparison of amine, sodium and stannous fluoride. Caries Res. 2008;42(1):2–7.
23. Hjortsjö C, Young A, Kiesow A, Cismak A, Berthold L, Petzold M. A Descriptive in vitro Electron Microscopic Study of Acidic Fluoride-Treated Enamel: Potential Anti-Erosion Effects. Caries Res. 2015;49(6):618–25.
24. Schlueter N, Hardt M, Lussi A, Engelmann F, Klimek J, Ganss C. Tin-containing fluoride solutions as anti-erosive agents in enamel: an in vitro tin-uptake, tissue-loss, and scanning electron micrograph study. Eur J Oral Sci. 2009;117(4):427–34.
25. Young A, Thrane PS, Saxegaard E, Jonski G, Rolla G. Effect of stannous fluoride toothpaste on erosion-like lesions: an in vivo study. Eur J Oral Sci. 2006;114(3):180–3.
26. Hjortsjö C, Jonski G, Thrane P, Saxegaard E, Young A. The effects of acidic fluoride solutions on early enamel erosion in vivo. Caries Res. 2009;43(2):126–31.
27. Yu C, Abbott PV. An overview of the dental pulp: its functions and responses to injury. Aust Dent J. 2007;52(1 Suppl):S4–16.
28. Tulek A, Saeed M, Mulic A, Stenhagen KR, Utheim TP, Galtung HK, et al. New animal model of extrinsic dental erosion-Erosive effect on the mouse molar teeth. Arch Oral Biol. 2018;96:137–45.
29. Hove LH. The inhibiting effect of TiF_4, SnF_2 and NaF on erosion-like lesions in-vitro and in-situ [Doctoral dissertation]. Oslo: University of Oslo; 2007.
30. Navarro KL, Huss M, Smith JC, Sharp P, Marx JO, Pacharinsak C. Mouse Anesthesia: The Art and Science. ILAR J. 2021;62(1–2):238–73.
31. Hara AT, Zero DT. The potential of saliva in protecting against dental erosion. Monogr Oral Sci. 2014;25:197–205.
32. Sorvari R, Pelttari A, Meurman JH. Surface ultrastructure of rat molar teeth after experimentally induced erosion and attrition. Caries Res. 1996;30(2):163–8.
33. Sorvari R, Kiviranta I, Luoma H. Erosive effect of a sport drink mixture with and without addition of fluoride and magnesium on the molar teeth of rats. Scand J Dent Res. 1988;96(3):226–31.
34. Sorvari R, Kiviranta I. A semiquantitative method of recording experimental tooth erosion and estimating occlusal wear in the rat. Arch Oral Biol. 1988;33(4):217–20.
35. Tulek A, Mulic A, Refsholt Stenhagen K, Galtung HK, Saeed M, Utheim TP, et al. Dental erosion in mice with impaired salivary gland function. Acta Odontol Scand. 2020;78(5):390–400.
36. Stenhagen KR, Hove LH, Holme B, Tveit AB. The Effect of Daily Fluoride Mouth Rinsing on Enamel Erosive/Abrasive Wear in situ. Caries Res. 2012;47(1):2–8.
37. Hove LH, Stenhagen KR, Holme B, Tveit AB. The protective effect of SnF2 containing toothpastes and solution on enamel surfaces subjected to erosion and abrasion in situ. Eur Arch Paediatr Dent. 2014;15(4):237–43.
38. Ellingsen JE. Scanning electron microscope and electron microprobe study of reactions of stannous fluoride and stannous chloride with dental enamel. Scand J Dent Res. 1986;94(4):299–305.
39. Wei SHY, Forbes WC. Electron Microprobe Investigations of Stannous Fluoride Reactions with Enamel Surfaces. J Dent Res. 1974;53(1):51–6.
40. Rykke M, Ellingsen JE, Sönju T. Chemical analysis and scanning electron microscopy of acquired pellicle formed in vivo on stannous fluoride treated enamel. Scand J Dent Res. 1991;99(3):205–11.
41. Johannes N, Hertel S, Stoffel V, Hannig C, Basche S, Schmitt V, et al. Impact of pH-adjusted fluoride and stannous solutions on the protective properties on the pellicle layer in vitro and in situ. Sci Rep. 2024;14(1):3378.
42. Kensche A, Buschbeck E, König B, Koch M, Kirsch J, Hannig C, et al. Effect of fluoride mouthrinses and stannous ions on the erosion protective properties of the in situ pellicle. Sci Rep. 2019;9(1):5336.
43. Schlueter N, Klimek J, Ganss C. Efficacy of tin-containing solutions on erosive mineral loss in enamel and dentine in situ. Clin Oral Investig. 2011;15(3):361–7.
44. Saxegaard E, Rolla G. Fluoride acquisition on and in human enamel during topical application in vitro. Scand J Dent Res. 1988;96(6):523–35.
45 Additives EPanel oF, Flavourings, Younes M, Aquilina G, Castle L, Engel K-H, et al. Safety assessment of titanium dioxide (E171) as a food additive. EFSA J. 2021;19(5):e06585.
46. Kirkland D, Aardema MJ, Battersby RV, Beevers C, Burnett K, Burzlaff A, et al. A weight of evidence review of the genotoxicity of titanium dioxide (TiO(2)). Regul Toxicol Pharmacol. 2022;136: 105263.
47 Additives EPoF, Nutrient Sources added to F, Younes M, Aggett P, Aguilar F, Crebelli R, et al. Re-evaluation of stannous chloride (E 512) as food additive. EFSA J. 2018;16(6):e05295.
48. House RC, Grisius R, Bliziotes MM, Licht JH. Perimolysis: unveiling the surreptitious vomiter. Oral Surg Oral Med Oral Pathol. 1981;51(2):152–5.

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