The Protective Effect of the Experimental TiF4 and Chitosan Toothpaste on Erosive Tooth Wear in Vitro

Monique Francese
Universidade de São Paulo
Isabela Gonçalves
Universidade de São Paulo
Mariele Vertuan
Universidade de São Paulo
Beatriz Souza
Universidade de São Paulo
Ana Magalhães (acm@usp.br)
Universidade de São Paulo

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Abstract

This study evaluated the protective effect of an TiF_4 and chitosan toothpaste on erosive tooth wear (ETW) *in vitro*. Bovine samples were randomly assigned to toothpastes (n=12): 1) TiF_4 (1400 ppm F^-), 2) 0.5%. chitosan (75% deacetylation, 500 mPas), 3) TiF_4 (1400 ppm F^-) plus 0.5%. chitosan (75% deacetylation, 500 mPas), 4) Placebo, 5) Erosion Protection (Elmex® - GABA, Suíça, 1400 ppm F^-). Twelve samples were only eroded. All samples were submitted to erosive pH cycles and the groups 1-5 to abrasive challenges using toothpastes' slurries plus 45 s of treatment, for 7 days. The final profile was overlaid to the baseline one for the ETW calculation (µm). The data were subjected to Kruskal-Wallis/Dunn tests. TiF_4 toothpastes, regardless of the presence of chitosan, were able to significantly reduce ETW compared to placebo, while chitosan alone was similar to placebo for both tissues. The toothpastes containing TiF_4 were even superior to the commercial Elmex® toothpaste on enamel wear, which were also significantly different from placebo. TiF_4 and Elmex® toothpastes minimized the impact of brushing on eroded surface compared to the other groups. In conclusion, TiF_4 toothpastes, regardless the presence of chitosan, showed to be effective in minimizing ETW *in vitro*.

Introduction

Erosive tooth wear (ETW) is the cumulative superficial loss of mineralized tooth substance due to physical or chemical-physical process (erosion, attrition, abrasion), in which erosion is the predominant etiological factor, associated with mechanical disturbs, as toothbrushing [1, 2]. The dentition naturally undergoes some wear overtime; however, the rate of wear should be extremely slow to maintain healthy the tooth morphology and functions throughout the lifetime [3]. Tooth wear can be defined as pathological if it is beyond the physiological level relative to the individual's age, depending on its severity (dentin exposure) and if it interferes with the self-perception of well-being due to the presence of pain, function and/or aesthetics compromises [2, 4].

The increase in the prevalence and clinical detection of ETW in recent decades has attracted the attention of the dental community around the world [5–7]. The older age groups generally show high levels of wear [8] and severe ETW may have impact on the quality of life of the affected individuals [9].

Among the various strategies to control ETW, the most tested one is the application of fluorides [10–12] especially those containing Sn^{2+} or Ti^{4+}, polyvalent metals that interact with the tooth structure, forming a more acidic resistant layer compared to CaF_2 induced by the application of conventional fluorides as NaF [11, 13]. The daily application of a solution containing TiF_4/NaF has promising results compared to those obtained with the commercial fluoridated solution ($SnCl_2$ and NaF/AmF), clinically indicated for ETW, under *in vitro and in situ* models [13–15]. It has also shown to be more promising than a unique professional application of TiF_4 varnish [16]. TiF_4 incorporated into a toothpaste has also shown to reduce the ETW created by the association of erosive and abrasive challenges [17].

Another compound of interest to control ETW is chitosan. Chitosan is a natural polymer derived from chitin deacetylation that has the ability to interact electrostatically with the tooth structure and easily adsorbs to enamel forming a protective layer [18]. Toothpaste containing chitosan (Chitodent® - Helmuth Focken Biotechnik) is able to inhibit ETW, showing similarity to that containing NaF; however, its protective effect is reduced when brushing forces are applied [19, 20].

To overcome this issue, chitosan has been added to fluoridated toothpastes [21]. Fluoridated toothpastes containing tin (around 3500 ppm Sn^{2+} and 1400 ppm F^-), in the presence of chitosan (0.5%), have a better protective effect against erosive enamel wear than those with fluoride only [19, 21]. Chitosan can increase the retention of tin to enamel, which may, at least in part, explain its protective effect in association with F^- and Sn^{2+} [22].

Taking this idea in mind, it is expected improvement of the protective effect of TiF_4 toothpaste, already tested [17], with the inclusion of chitosan into the formulae. Recently, we have shown that solutions containing TiF_4/NaF and chitosan had a protective effect against enamel wear similar to the positive control (Elmex® GABA solution, containing Sn^{2+} and F^-) [23], while for dentin, no improvement in the effect of the fluoridated solution was seen with the addition of chitosan [24].

Therefore, the aim of this work was to evaluate the protective effect of TiF_4 and chitosan containing toothpaste on ETW *in vitro*. The null hypothesis is that no difference exists between TiF_4 toothpaste, with or without chitosan, with respect to the protective effect on ETW.

Material And Methods

Sample preparation

The study was approved by the Local Ethics Committee for Animal Use (number 007/2020). Besides, all methods were performed in accordance with the relevant guidelines (ARRIVE guidelines) and regulations.

Seventy-two bovine enamel and 72 root dentin samples were prepared from incisors stored in 0.1% thymol solution (pH 7.0). The roots were separated from the crowns and both were then separately coupled to a prefabricated silicone mold (Biopdi, São Carlos, Brazil) and embedded in autopolymerizing acrylic resin, allowing the exposition of the labial surface. The samples were polished using silicon carbide sandpapers (320, 600 and 1200 grades of Al_2O_3 papers; Buehler, Lake Bluff, USA). Afterwards, the baseline profile was measured by using a contact profilometer and two thirds of the samples' surfaces were protected with red nail polish (Risqué®, São Paulo, Brazil), to obtain two control areas [13, 14].

The samples were randomly assigned to 5 toothpastes (n=12): 1) experimental containing TiF_4; 2) chitosan; 3) TiF_4 plus chitosan; 4) placebo (no F and no chitosan), 5) commercial Erosion Protection (Elmex® - GABA, Suíça). Twelve samples were only eroded (control). Table 1 shows the details about the toothpastes.

Table 1
Composition of the toothpastes under study (according to information available in the label) and their respective pH values (slurries' pH, mean and S.D.).

Group	Toothpaste	Ingredients*	Active ingredients*	Fluoride content	pH
TiF_4	Bauru fórmulas (Bauru, São Paulo, Brazil)	Carboxymethyl cellulose (CMC), Glycerin, Methylparaben, Sorbitol, Abrasive silica, Titanium dioxide, Cocobetaine, Aqua qsp	Titanium tetrafluoride (TiF_4)	1400 ppm F^-	3.08± 0.09
Chitosan	Bauru fórmulas (Bauru, São Paulo, Brazil)	Carboxymethyl cellulose (CMC), Glycerin, Methylparaben, Sorbitol, Abrasive silica, Titanium dioxide, Cocobetaine, Aqua qsp	0.5% chitosan (75% deacetylation, 500 mPas)	Nothing	7.14 ± 0.20
TiF_4 plus chitosan	Bauru fórmulas (Bauru, São Paulo, Brazil)	Carboxymethyl cellulose (CMC), Glycerin, Methylparaben, Sorbitol, Abrasive silica, Titanium dioxide, Cocobetaine, Aqua qsp	Titanium tetrafluoride (TiF_4) and 0.5% chitosan (75% deacetylation, 500 mPas)	1400 ppm F^-	4.22 ± 0.03
Placebo	Bauru fórmulas (Bauru, São Paulo, Brazil)	Carboxymethyl cellulose (CMC), Glycerin, Methylparaben, Sorbitol, Abrasive silica, Titanium dioxide, Cocobetaine, Aqua qsp	Nothing	Nothing	6.97 ± 0.23
Elmex® Erosion Protection	GABA International AG, Grabetsmattweg, Switzerland	Glycerin, Sorbitol, Hydrated silica, Aroma, Cocamidopropyl Betaína, Sodium sacharine, Hydroxyethylcellulose, Aqua	Stannous chloride ($SnCl_2$), Amine fluoride (AmF), Sodium fluoride (NaF), 0.5% chitosan	1400 ppm F^-	4.55 ± 0.07

The chitosan toothpaste was prepared as described for the experimental solution [23, 24]. All toothpastes were diluted (1 part toothpaste to 3 parts deionized water by weight; hereafter named slurry) for the treatment. The pH of the slurries was measured in duplicate using a pH meter previously calibrated to pH 4.1 and 7.0 standards (Orion 3-star pH Bench Top, Thermo Electron Corporation, USA). The toothpastes were applied at their natural pH, since TiF_4 is less effective in preventing tooth erosion when its pH is buffered to a high value [25].

Erosive and abrasive cycling

The samples were subjected to daily erosive and abrasive challenges for 7 days [26, 27]. Erosion was induced 4 times a day by using 0.1% citric acid solution (pH 2.5) for 90 s (30 mL/sample) at 25º C. The samples were then washed in deionized water (5 s) and immersed in artificial saliva [28] (pH 6.8, 30 mL/sample) for 2 h between the erosive challenges at 25º C.

After the first and the last daily erosive challenges, the samples from G1 to G5 were subjected to abrasion for 15 s (except the erosion only - G6), using toothbrushing machine (Biopdi®, São Carlos, Brazil), toothbrush (5460 ultrasoft Curaprox®, Kriens, Switzerland, 1 toothbrush/sample) and the toothpastes' slurries (1:3 water, 15 mL/sample, 37º C) under standardized velocity (3 linear movements/s) and force (1.5 N) [26, 27]. Afterwards, the samples were kept for further 45 s in contact with the toothpastes' slurries to complete 1 min of treatment and washed using deionized water for 10 s. The samples were kept in artificial saliva overnight completing 24 h of cycling.

After 7 days, the nail polish was removed by using acetone solution and the ETW (final profile) was measured.

Contact Profilometry

ETW was determined using a contact profilometer (Mahr Perthometer, Göttingen, Germany). Five equidistant surface scans of each sample were performed (4.12 ± 0.59 mm for enamel and 6.22 ± 1.20 mm for dentin, 250 µm apart from each other) at the baseline and at the final measurement. To achieve the repeatability, the samples presented an identification mark (small drillings made with drill ¼, Jet Carbide, Kerr, Joinvile, Brazil) and two scratches to delimitate the exposed area. They were inserted into a metal device (x and y axes determination, reproducibility of 0.08 µm), to allow the stylus to be accurately repositioned at each measurement. The baseline profile was compared to the final profile for the calculation of the ETW by using the software Marh Surf XCR20. This analysis was done under 100% humidity for dentin [13, 14, 23, 24].

The scans were superposed (final profiles versus baseline profile) and the average depth of the under-curve area was calculated, considering the limit of detection of the system of 0.5 µm [13, 14, 23, 24].

Statistical analysis

The ETW data were statistically compared using Kruskal-Wallis followed by Dunn test, since no equality of variances was found (Bartlett's test). The software applied was Graph Pad Instat (San Diego, USA) and the level of significance was set at 5% [13, 14, 23, 24].

Results

The experimental TiF_4 toothpastes, regardless of the presence of chitosan, were able to significantly reduce enamel and dentin wear (about 80% of prevention) compared to placebo, while chitosan alone was similar to placebo for both tissues. The toothpastes containing TiF_4 were even superior to the commercial Elmex® toothpaste in reducing enamel wear, but they were similar in case of dentin, and both significantly reduced tooth wear compared to placebo ($p < 0.0001$). Both TiF_4 and Elmex® toothpastes were not significantly different compared to the erosion condition for enamel and presented lower values compared to erosion for dentin, which means that they significantly reduced the abrasive brushing effect on eroded tooth surface (Figures 1 and 2).

Discussion

The null hypothesis of this work was accepted, since the inclusion of chitosan did not improve the protective effect of TiF_4 toothpaste, which in fact was even better than the positive control at least for reducing enamel wear. The experimental toothpastes containing TiF_4 and chitosan demonstrated a protective effect of 89% for enamel and 78% for dentin compared to placebo, whereas the commercial Elmex® presented 43% and 71% of protective fraction, respectively.

According to previous studies, the protective capacity of TiF_4 on the enamel is justified by the action not only of fluoride, but also of titanium [29, 30]. TiF_4 has been added to several formulations (such as varnish and solution), proving to be an effective compound against tooth demineralization (both caries and dental erosion) when compared to formulations containing NaF *in vitro* and *in situ* [13, 15, 30–32] Titanium minimizes tooth demineralization, since it tends to complex with apatite, forming a “glaze” layer rich in titanium oxide and hydrated titanium phosphate, which is more acid-resistant than the layer of CaF_2 induced by the application of NaF [30]. In addition, TiF_4 induces greater CaF_2 precipitation than NaF due to its low pH [30].

Chitosan was added into the toothpaste containing TiF_4, to improve its protective effect, since the literature has shown that the addition of chitosan to fluoridated solutions and toothpastes leads to a reduction in ETW [23, 24, 33, 34]. A recent work has shown benefit of the association between TiF_4/NaF and 0.5% chitosan on the protection of enamel wear *in vitro* [24]. This biopolymer is able to adsorb to enamel, creating a positively charged and more hydrophobic surface [20, 35], providing a mechanical barrier against acids [20]. This mechanism justifies its role as a mechanical barrier against the penetration of acids, contributing to the inhibition of demineralization [36]. However, when applied isolated (without fluoride), its protective effect is reduced by brushing forces, as shown by our study and others [19, 20]. Our study also showed that the addition of chitosan to TiF_4 toothpaste did not improve the protection, regardless the tooth substrate, indicating that chitosan, under the tested conditions, may not interact with the tooth surface producing an organic layer as expected.

An interesting result found in this study is that the experimental TiF_4 toothpaste was superior to the commercial version indicated for controlling ETW in case of enamel. The commercial Elmex® Erosion Protection toothpaste has in its formulation: F (as AmF and NaF, 1400 ppm), Sn^{2+} (as $SnCl_2$, 3500 ppm), and chitosan (0.5%) [21, 22, 37]. Ganss et al. [22] observed, in an *in vitro* study, applying more frequent erosive challenges and a longer treatment time with toothpaste, 68% reduction in enamel wear with the use of Elmex® Erosion Protection toothpaste compared to placebo. Schlueter et al. [21, 37], using similar methodology, but *in situ*, reported a reduction of approximately 50% of enamel wear by the use of Elmex® Erosion Protection compared to placebo, which is in agreement with our work.

Tin, like titanium, has an interaction with the tooth structure, being incorporated into the tooth surface and creating a mechanical barrier together with fluoride precipitates. When tin is combined with chitosan, there is a synergistic effect due to the formation of tightly connected multilayers [12, 22, 37] acting as a shield for the deposition of Sn^{2+} and increasing the preventive effect of this complex structure against ETW. This scenario was not observed in the case of TiF_4.

Differently from enamel, studies involving dentin are scarce. Elmex® Erosion Protection toothpaste reduces dentine wear, but not at superior level compared to a conventional fluoride toothpaste [38]. Tin may react with the dentin surface regardless of the presence of the demineralized collagen layer. In cases in which the organic matrix is preserved, phosphoproteins might attract the tin ion, which is then retained in the organic matrix to some extent but also accumulates in the underlying mineralized tissue. Under the absence of the demineralized organic matrix layer, the reaction is by precipitation [39].

One factor that could have influenced the lack of synergic effect of chitosan and TiF_4 is the erosive challenge, since the benefit of metal fluorides has been more evident when erosive challenges are longer [12, 37]. Another important aspect is that the presence of abrasive silica in toothpaste can have limited the protective effect of chitosan associated with TiF_4, when compared to fluoridated gels that do not have abrasive that could interact with chitosan [20, 40]. It is also relevant to discuss that the effect of chitosan is also dependent of the low pH of the vehicle. In case of our study, the final pH value of toothpaste containing chitosan alone was close to neutral, which can reduce the protonation of the molecule and its protective effect, which should be considered. Previous works testing solution containing chitosan, at low pH, showed better effect on ETW [23, 24] than the tested toothpaste.

It is known that abrasive wear of eroded hard tissues is considered an adverse side effect of aggressive tooth brushing, which is determined mainly by the abrasiveness of toothpaste rather than by the toothbrush [41, 42]. Based on this, the beneficial effect of TiF_4 toothpaste, regardless of chitosan, may be not only due to the fluoride and titanium, but also to its low abrasivity. Thus, the need for further studies in the area is irrefutable, to analyze RDA/REA values of the experimental toothpastes and the effect of aggressive erosive challenges on their protection capacity. Other important point for the future is to buffer the toothpastes in order to have similar final pH (around 4.5) for all.

A limitation of the present study was the absence of human saliva, which is justified by the difficult to collect the amount needed for a pH cycling model of 7 days. Chitosan seems to have a great affinity to salivary proteins, reacting better with the tooth surface in the presence of those proteins [40, 43, 44]. Despite Luka et al. [45] showed no improvement of the protective effect of Sn^{2+}/F^{-}/chitosan toothpastes under the presence of mucin *in* vitro, the present result shall be confirmed under *in situ* model, a condition closer to *in vivo* situation, with the presence of human saliva that may interplay the action of active compounds on the tooth [46, 47]. Although chitosan did not have any protective effect when included into TiF_4 toothpaste, it increased the toothpaste pH closer to the pH of the commercial toothpaste, value more suitable for a daily use.

Conclusion

Based on the results, we conclude that TiF_4 toothpastes, regardless of the presence of chitosan, are effective in minimizing tooth wear caused by brushing of eroded surface, which shall be confirmed under clinical studies. TiF_4 toothpaste containing chitosan presented a higher pH than TiF_4 only, which is desirable, but chitosan does not improve the protective effect of the toothpaste, at least not under this model.

Declarations

Data Availability Statement

All data generated or analyzed during this study are included in this article.

Conflict of Interest

None.

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Author contributions

Monique Francese contributed to Methodology; Validation; Formal analyses; Investigation; Data curation; Writing-Original Draft; Writing-Review & Editing; Visualization; Isabela Gonçalves contributed to Investigation and Funding acquisition; MarieleVertuan contributed to Investigation; Beatriz de Souza contributed to Conceptualization; Methodology and Investigation; and Ana Magalhães contributed to Conceptualization; Methodology; Validation; Formal analyses; Resources; Data curation; Writing-Original Draft; Writing-Review & Editing; Visualization; Supervision; Project administration and Funding acquisition. All authors: final approval of the version to be published.

Ethics declarations

The study was approved by the Ethics Committee for Animal Use of Bauru School of Dentistry – USP (number 007/2020).

It follows the recommendations in the ARRIVE guidelines. All methods were performed in accordance with the relevant guidelines and regulations.

Competing interests

The authors declare no competing financial interests.

Informed Consent

Not needed.

References

1. Carvalho, T. S., Lussi, A. Chapter 9: Acidic Beverages and Foods Associated with Dental Erosion and Erosive Tooth Wear. *Monogr Oral Sci.***28**, 91-98. https://doi.org/10.1159/000455376 (2020).
2. Schlueter, N. et al.Terminology of Erosive Tooth Wear: Consensus Report of a Workshop Organized by the ORCA and the Cariology Research Group of the IADR. *Caries Res.***54**, 2-6. https://doi.org/10.1159/000503308 (2020).
3. Lussi, A. et al. The use of fluoride for the prevention of dental erosion and erosive tooth wear in children and adolescents. European archives of paediatric dentistry: official journal of the *Acad. Paediat. Dent.***20**, 517–527. https://doi.org/10.1007/s40368-019-00420-0 (2019).
4. Wetselaar, P., Wetselaar-Glas, M., Katzer, L. D., Ahlers, M. O. Diagnosing tooth wear, a new taxonomy based on the revised version of the Tooth Wear Evaluation System (TWES 2.0). *Oral. Rehabil.***47**, 703–712. https://doi.org/10.1111/joor.12972 (2020).
5. Hasselkvist, A., Arnrup, K. Prevalence and progression of erosive tooth wear among children and adolescents in a Swedish county, as diagnosed by general practitioners during routine dental practice. *Heliyon***11**, e07977. https://doi.org/1016/j.heliyon.2021.e07977 (2021).
6. Donovan, T., Nguyen-Ngoc, C., Alraheam, I. A., Irusa, K. Contemporary diagnosis and management of dental erosion. *Esthet. Restor. Dent.***33**, 78-87. https://doi.org/10.1111/jerd.12706 (2021).
7. Martignon, S. et al. Epidemiology of Erosive Tooth Wear, Dental Fluorosis and Molar Incisor Hypomineralization in the American Continent. *Caries Res.***55**, 1-11. https://doi.org/10.1159/000512483 (2021).
8. Bartlett, D., O'Toole, S. Tooth wear and aging. *Dent. J.***64**, S59–S62. https://doi.org/10.1111/adj.12681 (2019).
9. Mehta, S. B., Loomans, B. A. C., Banerji, S., Bronkhorst, E. M., Bartlett, D. An investigation into the impact of tooth wear on the oral health related quality of life amongst adult dental patients in the United Kingdom, Malta and Australia. *Dent.***99**, 103409. https://doi.org/10.1016/j.jdent.2020.103409 (2020).
10. Magalhães, A. C., Wiegand, A., Rios, D., Buzalaf, M., Lussi, A. Fluoride in dental erosion. *Oral Sci.***22**, 158–170. https://doi.org/10.1159/000325167 (2011).
11. Pini, N. I. P., Lima, D. A. N. L., Luka, B., Ganss, C., Schlueter, N. Viscosity of chitosan impacts the efficacy of F/Sn containing toothpastes against erosive/abrasive wear in enamel. *Dent.***92**, 103247. https://doi.org/10.1016/j.jdent.2019.103247 (2020).

12. Fiorillo, L., Cervino, G., Herford, A. S., Laino, L., Cicciù, M. Stannous Fluoride Effects on Enamel: A Systematic Review. *Biomimetics (Basel)***5**, 41. https://doi.org/10.3390/biomimetics5030041 (2020).
13. Souza, B. M., Lima, L. L., Comar, L. P., Buzalaf, M. A., Magalhães, A, C. Effect of experimental mouthrinses containing the combination of NaF and TiF_4 on enamel erosive wear *in vitro*. *Oral Biol.***59**, 621-624. https://doi.org/10.1159/000479038 (2014).
14. Castilho, A. R., Salomão, P. M., Buzalaf, M. A., Magalhães, A. C. Protective effect of experimental mouthrinses containing NaF and TiF_4 on dentin erosive loss *in vitro*. *Appl. Oral Sci.***23**, 486-490. https://doi.org/10.1590/1678-775720150127 (2015).
15. de Souza, B. M., Santi, L. R. P, Silva, M. S, Buzalaf, M. A. R., Magalhães, A. C. Effect of an experimental mouthrinse containing NaF and TiF_4 on tooth erosion and abrasion *in situ*. *Dent.***73**, 45-49. https://doi.org/10.1016/j.jdent.2018.04.001 (2018).
16. Magalhães, A. C. et al. Effect of a Single Application of TiF_4 Varnish versus Daily Use of a Low-Concentrated TiF_4/NaF Solution on Tooth Erosion Prevention *in vitro*. *Caries Res.***50**, 462–470. https://doi.org/10.1159/000448146 (2016).
17. Comar, L. P. et al. Effect of NaF, SnF(2), and TiF(4) Toothpastes on Bovine Enamel and Dentin Erosion-Abrasion *In Vitro*. *J. Dent.***2012**, 134350. https://doi.org/10.1155/2012/134350 (2012).
18. Kumar, D., Gihar, S., Shrivash, M. K., Kumar, P., Kundu, P. P. A review on the synthesis of graft copolymers of chitosan and their potential applications. *J. Biol. Macromol.***163**, 2097–2112. https://doi.org/10.1016/j.ijbiomac.2020.09.060 (2020).
19. Ganss, C., Lussi, A., Grunau, O., Klimek, J., Schlueter, N. Conventional and anti-erosion fluoride toothpastes: effect on enamel erosion and erosion-abrasion. *Caries Res.***45**, 581–589. https://doi.org/10.1159/000334318 (2011).
20. Ganss, C., Marten, J., Hara, A. T., Schlueter, N. Toothpastes and enamel erosion/abrasion - Impact of active ingredients and the particulate fraction. *Dent.***54**, 62–67. https://doi.org/10.1016/j.jdent.2016.09.005 (2016).
21. Schlueter, N., Klimek, J., Ganss, C. Randomised*in situ* study on the efficacy of a tin/chitosan toothpaste on erosive-abrasive enamel loss. *Caries Res.***47**, 574–581. https://doi.org/10.1159/000351654 (2013).
22. Ganss, C. et al. Efficacy of the stannous ion and a biopolymer in toothpastes on enamel erosion/abrasion. *Dent.***40**, 1036–1043. https://doi.org/10.1016/j.jdent.2012.08.005 (2012).
23. Souza, B. M., Machado, P. F., Vecchia, L. R., Magalhães, A. C. Effect of chitosan solutions with or without fluoride on the protection against dentin erosion *in vitro*. *J. Oral Sci.***128**, 495-500. https://doi.org/10.1111/eos.12740 (2020).
24. de Souza, B. M., Santi, L. R. P., João-Souza, S. H., Carvalho, T. S., Magalhães, A. C. Effect of titanium tetrafluoride/sodium fluoride solutions containing chitosan at different viscosities on the protection of enamel erosion in vitro. *Oral Biol.***120**, 10492. https://doi.org/10.1016/j.archoralbio.2020.104921 (2020).

25. Wiegand, A. et al. Effect of TiF_4, ZrF_4, HfF_4 and AmF on erosion and erosion/abrasion of enamel and dentin *in situ*. *Oral Biol.***55**, 223–228. https://doi.org/10.1016/j.archoralbio.2009.11.007 (2010).
26. Mosquim, V., Souza, B. M, Foratori, J. G. A., Wang, L., Magalhães, A. C. The abrasive effect of commercial whitening toothpastes on eroded enamel. *J. Dent.* **30**, 142–146. (2017).
27. Vertuan, M., de Souza, B. M., Machado, P. F., Mosquim, V., Magalhães, A. C. The effect of commercial whitening toothpastes on erosive dentin wear *in vitro*. *Oral Biol.***109**, 104580. https://doi.org/10.1016/j.archoralbio.2019.104580 (2020).
28. Klimek, J., Hellwig, E., Ahrens, G. Fluoride taken up by plaque, by the underlying enamel and by clean enamel from three fluoride compounds *in vitro*. *Caries Res.***16**, 156–161. https://doi.org/10.1159/000260592 (1982).
29. Tezel, H., Ergücü, Z., Onal, B. Effects of topical fluoride agents on artificial enamel lesion formation *in vitro*. *Quintessence Int.***33**, 347–352. (2002).
30. Comar, L. P. et al. Mechanism of Action of TiF_4 on Dental Enamel Surface: SEM/EDX, KOH-Soluble F, and X-Ray Diffraction Analysis. *Caries Res.***51**, 554–567. https://doi.org/10.1159/000479038 (2018).
31. Levy, F. M., Rios, D., Buzalaf, M., Magalhães, A. C. Efficacy of TiF_4 and NaF varnish and solution: a randomized *in situ* study on enamel erosive-abrasive wear. *Clinical Oral Invest.***18**, 1097–1102. https://doi.org/10.1007/s00784-013-1096-y (2014).
32. Comar, L. P. et al. TiF_4 and NaF varnishes as anti-erosive agents on enamel and dentin erosion progression *in vitro*. *Appl. Oral Sci.***23**, 14-18. https://doi.org/10.1590/1678-775720140124 (2015).
33. Pini, N. I., Lima, D. A., Lovadino, J. R., Ganss, C., Schlueter, N. *In vitro* Efficacy of Experimental Chitosan-Containing Solutions as Anti-Erosive Agents in Enamel. *Caries Res.***50**, 337–345. https://doi.org/10.1159/000445758 (2016).
34. Sakae, L. O. et al. An *in vitro* study on the influence of viscosity and frequency of application of fluoride/tin solutions on the progression of erosion of bovine enamel. *Oral Biol.***89**, 26-30. https://doi.org/10.1016/j.archoralbio.2018.01.017 (2018).
35. Busscher, H. J., Engels, E., Dijkstra, R. J., van der Mei, H. C. Influence of a chitosan on oral bacterial adhesion and growth *in vitro*. *J. Oral Sci.***116**, 493–495. https://doi.org/10.1111/j.1600-0722.2008.00568.x (2008).
36. Arnaud, T. M., de Barros, N. B., Diniz, F. B. Chitosan effect on dental enamel de-remineralization: an *in vitroJ. Dent.* **38**, 848–852. https://doi.org/10.1016/j.jdent.2010.06.004 (2010).
37. Schlueter, N., Klimek, J., Ganss, C. Effect of a chitosan additive to a Sn^{2+}-containing toothpaste on its anti-erosive/anti-abrasive efficacy–a controlled randomised*in situClin. Oral Invest.* **18**, 107–115. https://doi.org/10.1007/s00784-013-0941-3 (2014).
38. Aykut-Yetkiner, A., Attin, T., Wiegand, A. Prevention of dentine erosion by brushing with anti-erosive toothpastes. *Dent.***42**, 856–861. https://doi.org/10.1016/j.jdent.2014.03.011 (2014).
39. Ganss, C. et al. Mechanism of action of tin-containing fluoride solutions as anti-erosive agents in dentine - an *in vitro* tin-uptake, tissue loss, and scanning electron microscopy study. *J. Oral Sci.***118**,

376–384. https://doi.org/10.1111/j.1600-0722.2010.00742.x (2010).

40. Wasser, G., João-Souza, S. H., Lussi, A., Carvalho, T. S. Erosion-protecting effect of oral-care products available on the Swiss market. A pilot study. *Swiss Dent. J.***128**, 290–296. (2018).
41. Wiegand, A., Kuhn, M., Sener, B., Roos, M., Attin, T. Abrasion of eroded dentin caused by toothpaste slurries of different abrasivity and toothbrushes of different filament diameter. *Dent.***37**, 480–484. https://doi.org/10.1016/j.jdent.2009.03.005 (2009).
42. Wiegand, A. et al. Impact of toothpaste slurry abrasivity and toothbrush filament stiffness on abrasion of eroded enamel - an *in vitroActa Odontol. Scand.* **66**, 231–235. https://doi.org/10.1080/00016350802195041 (2008).
43. Machado, A. C. et al. Using fluoride mouthrinses before or after toothbrushing: effect on erosive tooth wear. *Oral Biol.***108**, 104520. https://doi.org/10.1016/j.archoralbio.2019.104520 (2019).
44. Machado, A. et al. Anti-erosive effect of rinsing before or after toothbrushing with a Fluoride/Stannous Ions solution: an *in situ* investigation: Application order of Fluoride/Tin products for erosive tooth wear. *Dent.***101**, 103450. https://doi.org/10.1016/j.jdent.2020.103450 (2020).
45. Luka, B., Arbter, V., Sander, S., Duerrschnabel, K. A., Schlueter, N. Impact of mucin on the anti-erosive/anti-abrasive efficacy of chitosan and/or F/Sn in enamel *in vitro*. *Rep.***11**, 5285. https://doi.org/10.1038/s41598-021-84791-9 (2021).
46. Buzalaf, M. A., Hannas, A. R., Kato, M.T. Saliva and dental erosion. *App.l Oral Sci.***20**, 493–502. https://doi.org/10.1590/s1678-77572012000500001 (2012).
47. Kensche, A. et al. Effect of fluoride mouthrinses and stannous ions on the erosion protective properties of the *in situSci. Rep.***9**, 5336. https://doi.org/10.1038/s41598-019-41736-7 (2019).

Figures

Figure 1

Box Plot of enamel wear (μm) after the experimental protocol according to each treatment group [median (interquartile range-II)]. Q1 - quartile1, Q3 - quartile 3. Kruskal-Wallis/Dunn test ($p < 0.0001$). Different letters show significant differences among the groups.

Figure 2

Box plot of dentin wear (μm) after the experimental protocol according to each treatment group [median (interquartile range-II)]. Q1 - quartile1, Q3 - quartile 3. Kruskal-Wallis/Dunn test ($p < 0.0001$). Different letters show significant differences among the groups.