

did not significantly differ between them, but they had significantly greater SL than F+Sn (2.68 ± 1.62) and F+Sn+LPP (2.10 ± 0.95). F+Sn and F+Sn+LPP did not differ from each other. Sodium fluoride alone did not exhibit a significant anti-erosive effect. The combination between sodium fluoride and stannous chloride reduced enamel erosion, regardless of the presence of the linear chain sodium polyphosphate.

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A Hand-Held Optical Reflectometer to Measure Initial Erosion on Dentine

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Previous studies have shown that a hand-held optical reflectometer can measure initial erosion on enamel, but this was yet to be tested on dentine. This *in vitro* study aimed to assess initial erosion on dentine with the optical reflectometer. We ground and polished premolar roots ($n = 30$) to obtain dentine specimens, which were sorted into 3 groups: tap water (TW), sodium fluoride (500 ppm F) solution (NaF) and Elmex® Erosion Protection (800 ppm tin and 500 ppm F) solution (EP). This cyclic experiment consisted of 5 erosion cycles. In each cycle, we immersed the dentine specimens in the respective solution (10 ml, 30°C, shaking; 2 min), washed them for 20 s, and then immersed them in citric acid (0.65%, pH 3.6, 30 ml, 25°C, shaking) for a total erosion time of 1, 2, 4, 6, and 8 min. Reflection intensity was measured using an optical reflectometer, and the amount of calcium released by the dentine specimens was quantified with an atomic absorption spectrometer. Between-group differences in relative surface reflection intensity (rSRI) and calcium release were tested using Kruskal-Wallis and post-hoc Mann-Whitney tests, association between the two variables was tested using Spearman's correlation. We observed dentine erosion in all groups to different degrees. After 8 min erosion, the lowest rSRI loss was observed in EP (median 85%), followed by NaF (91%) and TW (94%; $p < 0.01$). Likewise, the lowest calcium release values were observed for EP (median 38.1 nmol/mm^2), followed by NaF (55.0 nmol/mm^2) and TW (70.6 nmol/mm^2 ; $p < 0.001$). Additionally, the good correlation between rSRI and calcium release (correlation coefficient -0.70) shows that the optical reflectometer can measure and differentiate initial erosion on dentine.

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Modification of Acquired Pellicle with Cystatins Reduces Initial Enamel Erosion

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Cystatin-B was identified as an acid-resistant protein in the acquired enamel pellicle; it could therefore be included in oral products to protect against erosion. However, its cost is high. Recently, a cystatin derived from sugar cane (canecystatin-5) was cloned and recombinantly expressed. This study evaluated the effect of pellicle modification, by incorporation of cystatin-B or canecystatin-5, on the protection against initial enamel erosion *in vitro*. Seventy-five bovine enamel specimens (4x4 mm) were divided into 5 groups: 1) deionized water (control), 2) 0.5% mucin + 0.27% casein solution, 3) 0.025 µg/µL cystatin-B solution, 4) 0.025 µg/µL canecystatin-5 solution, and 5) 0.025 µg/µL canecystatin-5 solution applied before the formation of the acquired pellicle. Stimulated saliva was collected from three volunteers and used to form an acquired enamel pellicle on the specimens for 2 h. Specimens (groups 1–4) were exposed to the protein solutions with stirring at 30°C for 2 h. For group 5, blocks were exposed to canecystatin-5 solution before the pellicle was formed. All specimens were then incubated in 0.65% citric acid (pH 3.4) for 1 min at 30°C. Treatment was done once/day for 3 days. Surface hardness was analyzed at baseline and after days 1 and 3 and percentage of surface hardness change (%SHC) was calculated. Data were analyzed by ANOVA and Tukey's test ($p < 0.05$). At day 1, treatment with cystatin B ($35.1 \pm 9.9\%$) and canecystatin-5 ($35.2 \pm 6.6\%$) before pellicle formation significantly reduced % SHC compared with control ($46.9 \pm 6.7\%$). At day 3, all treatments with cystatins (54.5 ± 8.6 , 55.5 ± 10.7 and $53.1 \pm 9.3\%$ for cystatin-B, canecystatin-5 and canecystatin-5 before pellicle formation, respectively) significantly reduced % SHC compared with control ($67.6 \pm 9.4\%$). In addition, treatment with canecystatin-5 before pellicle formation significantly reduced % SHC compared with the combination mucin/casein ($64.4 \pm 9.4\%$). Thus, canecystatin-5 seems to be a good candidate to be added to oral products to protect against erosion.

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