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Efficacy of stannous, fluoride and their their combination in dentin erosion prevention *in vitro*

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Abstract: The aim of this study was to compare the protective effects of solutions containing stannous (Sn), fluoride (F) and their combination in the prevention of dentin erosion. Forty bovine root dentin specimens (4′4′2 mm^3) were prepared and randomly assigned to 4 groups (n = 10): $SnCl_2$ (800 ppm/6.7 mM Sn), NaF (250 ppm/13 mM F), NaF/$SnCl_2$ (800 ppm/6.7 mM Sn; 250 ppm/13 mM F), and deionized water (DIW) as a negative control. An acquired pellicle was formed on dentin samples by incubation in clarified, pooled, stimulated human saliva for 24 hours. The specimens were subjected to 5 daily cycles, each consisting of 5 of min demineralization (0.3%/15.6 mM citric acid, pH 2.6, 6′/day) and 60 min of re-mineralization in clarified human saliva. Thirty minutes after the 1st, 3rd and 5th demineralization episodes of each day, the specimens were treated with one of the test solutions for 2 min. Surface loss was measured via optical profilometry. Mixed-model ANOVA followed by Tukey's test were used for the statistical analysis. Sn, F, and their combination significantly reduced the dentin surface loss by 23%, 36%, and 60% compared with DIW, respectively. All groups were significantly different ($p < 0.05$). The combination of Sn and F significantly reduced the amount of dentin surface loss compared with all other groups. The F group also significantly reduced surface loss compared with Sn and DIW, followed by the Sn group, which showed significantly greater protection compared with the DIW control. The daily use of a combined fluoride and stannous solution is promising for preventing dentin erosion.

Keywords: Dentin; Erosion; Fluorides.

Introduction

The prevalence of dental erosion has recently increased as a result of the increased consumption of dietary acids.[1,2] Enamel is the substrate most commonly associated with erosion; however, coronal and root dentin may also be affected if exposed to the oral environment. The decline of edentulism over time[3] and the increase in gingival recession[4] are important factors contributing to dentin exposure. Dentin erosion can lead to dentin hypersensitivity, poor esthetic appearance, functionality issues with teeth, and eventual pulp exposure at advanced stages.[2,5,6] Therefore, different approaches have been investigated to prevent dentin erosion.

The protective effect of fluoride-containing solutions has been demonstrated in *in vitro* and *in situ* studies.[7,8,9,10,11] The topical application of fluoridated solutions (112-450 ppm F, such as NaF) may protect against dental erosion through the formation of a CaF_2-like layer on the tooth surface.[10,12] Stannous-containing solutions were also found to prevent dental erosion by forming acid-resistant precipitates on the tooth surface.[9,13,14] Studies that used energy-dispersive X-ray spectroscopy showed that even at lower concentrations (400 ppm), Sn may react with hydroxyapatite and can be incorporated into the tooth structure to create a more acid-resistant substrate.[15] Stannous fluoride solutions have been shown to provide substantial protection against erosion.[9,15] However, there is little information on the effects of Sn and F ions, either individually or combined, on the prevention of dentin erosion in the presence of an acquired pellicle (AP).

Accordingly, the aim of this study was to compare the anti-erosive effects of solutions containing F, Sn, or both on dentin using an *in vitro* erosion cycling model and including the effects of the AP. Our tested null hypothesis was that treatment with solutions containing $SnCl_2$ and/or NaF would not provide significant protection against dentin erosion.

Methodology

Experimental design

Our experiment followed a complete randomized design testing the effects of solution treatment at 4 levels, Sn, F, Sn+F -containing solutions and DIW as a negative control. The experimental units were polished bovine dentin specimens (n = 10 per group). The response variable was surface loss (in µm) measured at the end of the cycling procedure.

Specimen preparation

Forty bovine root dentin slabs (4'4'2 mm^3) were cut from bovine incisors using a microtome (Isomet Buehler, Lake Bluff, USA). They were embedded in acrylic resin blocks (10×10×8 mm^3) (Varidur Buehler, Lake Bluff, USA), flattened using sequential water-cooled abrasive discs 500-, 1,200-, 2,400- and 4,000-grit Al_2O_3 papers; MD-Fuga, (Struers Inc., Cleveland, USA), polished 1-µm diamond suspension (Struers Inc., Cleveland, USA) and sonicated in a detergent solution (Micro-90, International Products Corporation, Burlington, USA). Specimens with any visual cracks or defects were discarded. Adhesive TC414 unplasticized polyvinyl chloride tape (TapeCase Ltd., Wheeling, USA) was placed on the specimens to create reference surfaces, leaving a central surface area of 4'1 mm^2 in the dentin exposed to the cycling procedure. The specimens were then randomly assigned to three test groups ($SnCl_2$, NaF, or both) and one control group (DIW) (n = 10).

Solutions preparation

The NaF solution contained 13 mM F (250 ppm) (NaF, CAS#7681-49-4, Sigma-Aldrich, Saint Louis, USA). The Sn solution contained 6.7 mM Sn (800 ppm) ($SnCl_2$, CAS#7772-99-3, Sigma-Aldrich, Saint Louis, USA) stabilized by 10.5 mM sodium gluconate (AC18139, FisherSci, Fair Lawn, USA). The Sn+F solution was a combination of both solutions (6.7 mM Sn as $SnCl_2$ and 13 mM F as NaF). All solutions were pH-adjusted to 4.5 using HCl or KOH. The erosive solution used was 0.3% (15.6 mM) citric acid (C1857, Sigma-Aldrich, Saint Louis, USA), pH 2.6.

Saliva collection

Human whole stimulated saliva was collected (IRB approval, #0304-58) 1 h after breakfast from six healthy donors with no active caries, periodontal disease, or hyposalivation and who were not taking any medications. Salivary secretion was stimulated by chewing a gum base for 1 min, and saliva was collected directly into 50-mL ice-chilled tubes for 2 h. After collection, the saliva samples were pooled and immediately centrifuged at 14,000 *g* for 20 min at 4°C. The supernatant was separated from the pellet, pooled and stored at -80°C.

Cycling procedures

The specimens were incubated in previously thawed clarified human saliva (CHS) (2 mL/specimen) for 24 h under gentle agitation to form the AP. Each cycle consisted of 5 min immersion in 0.3% citric acid (pH 2.6, 4 mL/specimen), followed by 60 min in CHS (2 mL/specimen). This procedure was repeated 6×/day for 5 days. The saliva was renewed 3×/day, whereas

the acid was renewed after each erosion episode. Solution treatments (4 mL/specimen) were performed for 2 min at 30 min after starting the 1st, 3rd and 6th remineralization periods. The specimens were rinsed with DIW for 10 s only after acid exposure, and the excess water was gently dried with absorbent paper (Kimwipes, Neenah, USA). All of the experimental procedures were conducted at room temperature. The specimens were independently treated throughout the experiment using 12-well plates.

Surface profilometry

After cycling, the tape was removed from the specimens, and the amount of surface loss was analyzed. An area 2 mm long (X) × 1 mm wide (Y) was scanned with an optical profilometer (Proscan 2000 Scantron, Venture Way, Taunton, United Kingdom). The scan covered the treated area and the protected reference surfaces on both sides. The step size was set at 0.01 mm and the number of steps at 200 in the X-axis; in the Y-axis, these were set at 0.05 mm and 20, respectively. Dedicated software was used to calculate the surface loss of the treated area by subtracting the average height of the test area from the average height of the two reference surfaces (Proscan Application software v. 2.0.17, Scantron).

Statistical analysis

Mixed-model ANOVA was used to test the effects of the solution treatments (DIW, F, Sn, Sn+F) on dentin surface loss. Pair-wise comparisons were made using Tukey's method to control the overall significance level at 5%.

Results

The DIW control showed significantly higher surface loss compared with the test groups, followed by Sn, F and Sn+F ($p = 0.05$ - $p < 0.0001$). Sn+F, Sn and F reduced dentin surface loss by 60%, 23%, and 36% compared with DIW, respectively (Figure).

Discussion

This study investigated the effect of stannous- and/or fluoride-containing solutions on the prevention of dentin erosion in the presence of human saliva. Saliva is the most important biological factor in the prevention of dental erosion[2] and should be considered when designing *in vitro* erosion studies. The roles of saliva in erosion prevention include buffering and diluting acids, providing the ions needed for re-mineralization, and forming the AP, a natural semi-permeable membrane that inhibits acid diffusion.[2,16] Therefore, dentin samples were incubated in CHS for 24 h, thereby enabling a mature AP to form prior to the cycling procedure. Moreover, to simulate remineralization and AP re-formation, the specimens were stored in CHS when not in the demineralization or treatment solutions. Citric acid was chosen because it is present in most commercially available acidic beverages.[9,14,17,18] The frequency and duration of the erosive challenge (5 min, 6×/day) and incubation in treatment solutions (2 min, 3×/day) were similar to previous studies to facilitate further indirect comparisons[15,18] and to provide clinical relevance. Solution treatments were performed after the first, third and sixth erosive challenges to mimic exposure to the test solutions after the main meals (breakfast, lunch and dinner, respectively). The present experimental model did not include toothbrush abrasion because our focus was on the interaction between the testing solutions and the dental pellicle, acids, and saliva at the surface of the dentin. The presence of abrasive forces, while more clinically relevant, could potentially mask the effects of these factors.

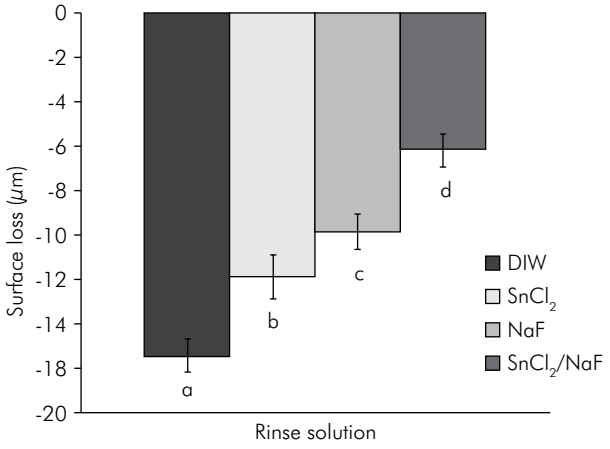

Figure. Mean (SD) erosive surface loss of dentin as a function of the treatment solution. Different letters represent statistically significant differences ($p < 0.05$).

The Sn solution reduced surface loss by 23% compared with the control, which is consistent with previous studies.[8] A distinct coating on the enamel surface has been detected using SEM after the topical application of Sn-containing solutions. This coating has been shown to persist after 2 min of exposure to 1% citric acid (pH 2.3).[14] In addition to precipitate formation and incorporation into enamel, stannous has been suggested to interact with pellicle proteins and enhance their protective effect against acid erosion.[19] Although previous studies were conducted on enamel, observations from the current study suggested a similar effect of Sn on dentin. Nevertheless, further research is needed to more thoroughly understand the mechanisms involved in the protective action of Sn on the dentin surface.

F solution reduced the surface loss of dentin by 36% compared with DIW, which is significantly more than Sn but less than their combination. The protective effect of F on dentin could be due to fluoride retention in intertubular, intratubular and peritubular dentin after topical application,[7,20,21] which is readily available during an erosive attack. An *in situ* study revealed that a NaF solution (500 ppm F, pH 4.5) provided 23% protection against erosion.[9]

The anti-erosive effect of the Sn+F solution has been shown in several studies.[9,14,18] Its protection is likely due to the interaction of both ions with hydroxyapatite and the subsequent formation of complex precipitates containing Sn_2OHPO_4, $Sn_3F_3PO_4$, $Ca(SnF_3)_2$ and CaF_2.[22] This layer acts as a protective barrier against acid diffusion and is considered to be more resistant to acid attack than that formed by fluoride only (CaF_2).[14] Therefore, the combination of both ions appears to have a superior effect than either alone. In agreement with our results, Ganss *et al*.[7] showed a similar treatment ranking order: NaF was better than $SnCl_2$ but less than their combination.

Observations from this study indicated that the frequent use of F and Sn ions at concentrations of 250 ppm and 800 ppm, respectively, are effective in the prevention of dentin erosion. Consequently, Sn-containing solutions may be recommended for individuals at high risk for dentin erosion, including patients with gingival recession or a highly acidic diet. Independent sources of Sn and F ions were obtained using the formula $SnCl_2$/NaF (rather than SnF_2) to provide the most effective Sn/F ratio (800 ppm Sn/250 ppm F) without adverse effects.[23] The Sn and F concentrations used were based on commercially available mouth rinses and were similar to those in previous reports.[7,11,24,25] Furthermore, the pH for all solutions was adjusted to 4.5 to compare the treatment solutions and to maintain product stability.[7,11,24,25] The stannous-containing solutions could be prepared only as acidic formulations because stannous is highly reactive at a neutral pH and will react to form complexes ($SnF_2(OH)_2$ and SnO_2) after a short duration.[26] Moreover, previous studies employed different fluoride compounds, such as SnF_2 and AmF, with the latter also being used to stabilize $SnCl_2$ in solution.[9,14,18] In the current study, NaF was the only source of fluoride, and Na gluconate was used to stabilize stannous. Despite the differences in the composition, the efficacy of $SnCl_2$-containing solutions used in the current experiment was similar to solutions used in previous studies.

Conclusion

We conclude that under conditions simulating the frequency of daily acid exposure with the recommended routine use of a daily mouth rinse, solutions that contain $SnCl_2$ (800 ppm Sn) or NaF (250 ppm F) significantly prevented the acidic erosion of dentin. More importantly, the combination of both Sn and F ions provided a significantly greater anti-erosive effect compared with either ion in isolation.

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