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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

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ABSTRACT

Objective: To evaluate the influence of the viscosity and frequency of application of solutions containing fluoride (F) and stannous chloride ($SnCl_2$) on enamel erosion prevention.

Design: Bovine enamel specimens were randomly distributed into 12 groups (n = 10), according to the following study factors: solution (C: deionized water; F: 500 ppm F^-; F + Sn: 500 ppm F^- + 800 ppm Sn^{2+}); viscosity (low and high); and frequency of application (once and twice a day). Specimens were submitted to an erosive cycling model, consisting of 5 min immersion in 0.3% citric acid, followed by 60 min exposure to a mineral solution. This procedure was repeated 4×/day, for 5 days. Treatment with the experimental solutions was performed for 2 min, 1×/day or 2×/day. Enamel surface loss (SL) was determined by optical profilometry. Data were analyzed by 3-way ANOVA and Tukey tests ($\alpha = 0.05$).

Results: There were significant differences between the levels of the factor solution ($p < .001$), viscosity ($p < .001$) and in the interaction between solution and viscosity ($p = .01$). Regarding solution, the mean SL ± standard deviation for the groups was F + Sn (4.90 ± 1.12) < F (7.89 ± 1.19) < C (14.20 ± 1.69). High viscosity solutions demonstrated less SL than low viscosity; however, only when applied once a day ($p < .001$). Applying the solutions twice a day yielded lower SL than once a day, but only for the low viscosity solutions ($p = .003$).

Conclusions: Under the conditions of this short-term in vitro experiment, it could be concluded that increasing the viscosity of the oral rinse solutions reduced enamel loss by erosion; however, this effect was small and only observed when the solutions were applied once a day.

1. Introduction

The frequent contact of non-bacterial acids with the tooth surfaces can lead to dental erosion (Eccles, 1978), which has become a condition of growing concern among dental practitioners and researchers (Lussi & Carvalho, 2014). Data from in vitro and in situ studies have shown that topical fluoride application can protect the dental substrates against erosive attacks (Ganss, Neutard, von Hinckeldey, Klimek, & Schlueter, 2010; Scaramucci, Borges, Lippert, Frank, & Hara, 2013; Scaramucci et al., 2015; Wiegand et al., 2010). So far, promising results have been obtained with the use of stannous fluoride or the combination of fluoride and tin added as separate compounds (Ganss, Hardt et al., 2010; Scaramucci et al., 2015). This was attributed to the deposition of calcium fluoride-like material and other metal-rich deposits on the tooth surfaces, which would temporally protect the tissue against demineralization (Huysmans, Young, & Ganss, 2014; Magalhães, Wiegand, Rios, Buzalaf, & Lussi, 2011). Additionally, it was demonstrated that tin can incorporate into enamel and dentin, thus reducing their solubility (Ganss, Hardt et al., 2010; Schlueter, Hardt et al., 2009).

However, the effectiveness of the treatment with fluoride compounds may be limited, especially under frequent acid attacks. One way to overcome this limitation is by high frequency of application of the

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fluoridated products (Huysmans et al., 2014). Nevertheless, when delivered in vehicles that allow frequent applications, such as mouthrinses and dentifrices, fluoride does not sustain much substantivity in the oral cavity (Mason, Shirodaria, Sufi, Rees, & Birkhed, 2010). Although the anti-erosive effect of a solution containing 500 ppm of fluoride and 800 ppm of tin was previously observed with a single daily application (Ganss, Neutard et al., 2010), it was further demonstrated that applying this solution twice a day could improve its efficacy (da Silva, Ramos-Oliveira, Mantilla, & de Freitas, 2017). It was hypothesized that the use of solutions with higher viscosity could improve the retention of fluoride and metal cations on tooth surfaces (Pini, Lima, Lovadino, Ganss, & Schlueter, 2016), and this could ultimately allow a lower frequency of application.

To the authors' knowledge, there is little information available on how the viscosity of fluoridated solutions would influence their protection against dental erosion. One investigation evaluated the effect of increasing the viscosity of a fluoride plus tin-containing solution using varieties of chitosan with different molecular weights (Pini et al., 2016). The authors observed that the more viscous solution presented a higher anti-erosive effect, however, it should be noted that chitosan is a positively charged polymer that can behave not only as a thickening agent, but also as film-forming molecule (Tharanathan & Kittur, 2003). In this sense, it is unknown whether the improved anti-erosive effect observed could be related only to the increased viscosity of the solution. The use of non-ionic thickening polymers could help clarify this issue, as it is unlikely that they would strongly bind to hydroxyapatite (Aykut-Yetkiner, Wiegand, Bollhalder, Becker, & Attin, 2013), although the possibility of a weaker interaction, such as physical adsorption should not be disregarded (Umoren & Eduok, 2016).

In view of the foregoing, the aim of this present study was to evaluate the interaction between the viscosity and frequency of application of solutions containing fluoride and stannous chloride on protection against enamel erosion. Our null hypotheses were: 1. There would be no difference in enamel surface loss with the use of fluoridated solutions with different viscosity; 2. There would be no difference in enamel loss by applying the fluoridated solutions once or twice daily.

2. Materials and methods

2.1. Study design

This in vitro study was conducted with a completely randomized experimental design, with 3 experimental factors: experimental solutions (control, F and F + Sn), viscosity (low and high), and frequency of application (once or twice a day). The solutions were tested in an erosive cycling model, using enamel specimens from bovine incisors (n = 10). The response variable was enamel surface loss (in μm), measured with an optical profilometer at the end of cycling.

2.1.1. Specimen preparation

Bovine incisors were stored in 0.1% thymol solution (Sigma-Aldrich, St Louis MO, USA), under refrigeration at 4 °C, until the experiment began. Enamel fragments (4 mm × 4 mm × 2 mm) were prepared, using an automatic cutting machine (Isomet 1000 Precision Saw, Buehler, Lake Bluff, IL, USA). The fragments were embedded in acrylic resin (Varidur, Buehler) and the resulting blocks were ground flat with abrasive discs (400, 1200, 2400 and 4000 grit; Fepa P, Buehler), in a polishing machine (Ecomet, Buehler), under constant water cooling. At the end of the polishing procedure, the specimens were submitted to an ultrasonic bath with deionized water for 3 min.

2.1.2. Surface curvature assessment

After preparation of the specimens, they were submitted to profilometric analysis to select specimens with a curvature < 0.3 μm. This analysis was performed with an optical profilometer (Proscan 2100, Scantron, Venture Way, Tauton, UK). The instrument sensor scanned an area that was 2 mm long (X-axis) and 1 mm wide (Y-axis), located at the center of the specimen. The equipment was set to go through 200 steps in the X −axis, with each step measuring 0.01 mm. In the Y-axis, there were 20 steps measuring 0.05 mm each. The curvature was calculated based on the subtraction of the mean height of the future test area from the mean height of the two reference surfaces using a dedicated software (Proscan Application software v. 2.0.17). The surfaces of 120 specimens with maximum initial curvature of 0.3 μm and without cracks or any other defects, were protected with unplasticized polyvinyl chloride (UPVC) tapes, leaving a central area of 4 mm × 1 mm exposed for the subsequent tests.

2.1.3. Experimental solutions

For the F solutions, the concentration of F was chosen based on previous studies (Ganss, Hardt et al., 2010; Schlueter, Neutard, von Hinckeldey, Klimek, & Ganss, 2010). The solution was formulated with NaF (Sigma-Aldrich Co) and the pH was adjusted to 4.5 with HCl solution. For the F + Sn solutions, a commercially available mouthrinse was used (Elmex Erosion Protection, GABA Schweiz AG, Colgate-Palmolive Co, Therwil, Switzerland; 500 ppm F^-, 125 ppm F^- as AmF and 375 ppm F^- NaF; 800 ppm Sn^{2+}, as $SnCl_2$; pH 4.5). All the pH values were determined by using a calibrated pH electrode (HI2221, Hanna Instruments, Woonsocket, RI, USA).

The viscosity of the solutions was adjusted with polymer hydroxyethyl cellulose (HEC; Natrosol, Hercules Incoporated, Wilmington, DE, USA) and expressed in mPa.s. A preliminary test was performed to evaluate the concentration of HEC needed to achieve solutions with high viscosity. Although more viscous, these solutions had to be compatible with the mode of use of a mouthrinse, e.g., it had to be possible to swish the solution around the mouth. To determine the HEC concentration, solutions containing increasing HEC concentrations (0.1%, 0.15%, 0.2%, 0.25%, 0.3%, 0.35% and 0.4%) were evaluated regarding their dynamic viscosity. For this measurement, a SV-10 vibro viscometer (A&D, Toshima-ku, Tokyo, Japan) was used. The measurements were performed at 24 °C.

The dynamic viscosity of the solutions containing 0.1%, 0.15%, 0.2%, 0.25%, 0.3%, 0.35%, 0.4% and 0.45% were, respectively: 2.70; 4.04; 5.99; 8.24; 12.02; 17.51; 24.71; and 37.54. Based on these results (and on our premise that the solution had to be able to be swished around the mouth), we opted to use the concentration of 0.35% HEC in the high viscosity solutions (17.51 mPa.s of dynamic viscosity).

After adding the HEC to the high viscosity solutions, the viscosity of all experimental solutions was determined by using a similar methodology. Detailed description of the experimental groups is shown in Table 1.

2.1.4. Erosive cycling

The specimens were randomly allocated into the 12 experimental groups (n = 10), and submitted to a 5-day erosive cycling. During each day, the specimens were individually immersed in 4 ml of 0.3% citric acid solution (natural pH of 2.6) for 5 min, followed by 60 min exposure to 4 ml of a mineral solution (Scaramucci et al., 2013). This was repeated 4 times per day. For the groups treated with the test solutions

Table 1
Detailed description of the experimental groups.

	Description
Solutions	**C**: control (deionized water; natural pH)
	F: sodium fluoride solution (NaF; 500 ppm F; pH = 4.5)
	F + Sn: Elmex Erosion Protection (500 ppm F as NaF and AmF; 800 ppm Sn as $SnCl_2$; pH 4.5)
Viscosity	**Low** (water viscosity, no hydroxyethyl cellulose added)
	High (with addition of 0.35% hydroxyethyl cellulose)
Frequency	**Once a day**
	Twice a day