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In vitro enamel erosion and abrasion-inhibiting effect of different fluoride varnishes

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ABSTRACT

Objective: To investigate the erosion and abrasion inhibiting effect of CPP-ACP/NaF and xylitol/NaF varnishes.
Methods: Bovine enamel samples (n = 40) were exposed to the following treatments (n = 10): NaF varnish (Duraphat®, positive control); CPP-ACP/NaF varnish (MI varnishTM); xylitol/NaF (Profluorid®) or distilled and deionized water (MilliQ®, negative control). The samples were submitted for 3 days to 4 cycles/day of erosion (5 min in Sprite Zero) and 2 cycles of abrasion/day after the first and last erosive challenge, with a toothbrush machine and slurries of a placebo toothpaste for 15 s (50 strokes/s). Among the cycles and after the last daily cycle, the specimens remained in artificial saliva. The change in the enamel surface was evaluated by using 3D non-contact optical profilometry with surface roughness (Ra and Sa values) and tooth structure loss (TSL) measurements. Scanning electron microscopy (SEM) assessed the enamel topographic characteristics. Differences in the Ra, Sa and TSL among treatments were tested using one-way ANOVA followed by the Tukey test.
Results: All varnishes promoted better results for Ra and Sa values than the negative control ($p = 0.0001$), without difference among them ($p > 0.05$). However, CPP-ACP/NaF varnish stimulated fewer TSL (7.09 ± 0.70 μm) compared to NaF varnish (10.33 ± 1.36 μm, $p = 0.002$), xylitol/NaF varnish (9.96 ± 0.41 μm, $p = 0.007$) and the negative control (18.38 ± 3.32 μm, $p = 0.0001$).
Conclusion: A single-application of fluoride topical varnishes was effective in reducing enamel wear. The CPP-ACP/NaF varnish had the best effect against enamel loss from an erosion-abrasion challenge.

1. Introduction

Dental erosion is defined as an irreversible loss of tooth tissue by exogenous or endogenous acids without bacterial involvement (Huysmans, Chew, & Ellwood, 2011), accompanied by a progressive softening of the superficial and near-surface layer of enamel. (Huysmans et al., 2011; Lussi & Carvalho, 2014, 2015; Shellis, Barbour, Jesani, & Lussi, 2013). This softening surface turns less resistant, increasing the susceptibility to physical wear such as toothbrush abrasion (Ganss, Lussi, & Schlueter, 2014; Rios et al., 2006). Thus, the application of high concentrations of fluoride, especially varnish formulations, has been described to decrease the development of tooth enamel erosion and increase abrasion resistance (Lippert, 2014; Sar Sancakli, Austin, Al-Saqabi, Moazzez, & Bartlett, 2015).

The potential of sodium fluoride (NaF) and stannous fluoride to prevent dental erosion with efficacy has been described in the literature (Algarni et al., 2015; Sorvari, Meurman, Alakuijala, & Frank, 1994; Vieira, Jager, Ruben, & Huysmans, 2007). Recently, other products than fluoride, as casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) and Xylitol, have been studied to observe their protective effect against erosion or abrasion.

CPP-ACP is the Recaldent™ technology based on amorphous calcium phosphate (ACP) stabilized by casein phosphopeptides (CPP). The benefits of CPP-ACP nanocomplexes are the high concentration of calcium and phosphate ions that promote enamel remineralisation. In the presence of fluoride ions, there is the

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production of an ACFP phase (CPP-ACP addicted with fluoride ion), which may contribute to remineralisation when the fluoride ions are disassociated (Cross, Huq, & Reynolds, 2007; Reynolds et al., 2008). Whereas the xylitol is a non-acidogenic sweetener (Makinen, 2010) able to form complexes with calcium ions (Makinen, 2010; Miake, Saeki, Takahashi, & Yanagisawa, 2003). Due this property, some authors believe that xylitol might enhance remineralisation and inhibit the dissolution of calcium and/or phosphate ions from enamel structure (Chunmuang, Jitpukdee-bodintra, Chuenarrom, & Benjakul, 2007; Miake et al., 2003; Vongsavan, Surarit, & Rirattanapong, 2014). However, the role of xylitol has not been enough demonstrated as a preventive or remineralising agent (Tuncer, Onen, & Yazici, 2014)

Taking into account that in the researched literature no studies evaluating, at the same time, the effect of CPP-ACP/NaF and Xylitol/NaF varnishes with regard to erosion and abrasion, the purpose of the present study was to investigate the enamel erosion and abrasion-inhibiting effect of NaF fluoride varnishes with or without CPP-ACP and Xylitol.

2. Material and methods

2.1. Null hypothesis

The null hypothesis of this study was that the single application of CPP-ACP/NaF and Xylitol/NaF varnishes does not reduce alterations in roughness and tooth structure loss after an erosion and abrasion challenge.

2.2. Specimen preparation

Bovine teeth were prepared to obtain 40 enamel samples ($4 \times 4 \times 2$ mm). The teeth were stored in 4% formaldehyde (pH 7.0) solution until cut phase. The specimens were cut using an Isomet low-speed saw cutting machine (Buehler Ltd., Lake Bluff, Illinois, United States) with two diamond discs (Extec Corp., Enfield, Connecticut, United States), which were separated by a 4 mm-thick pacer. The block surfaces were polished using water-cooled silicon carbide paper 600 and 1200 (Extec Corp., Enfield, Connecticut, United States), followed by a 1-μm diamond abrasive slurry (Extec Corp., Enfield, Connecticut, United States). After each stage of polishing, the samples were cleaned in an ultrasonic device with distilled and deionized (DD) water (Milli-Q®, Merck Millipore Corporation, Darmstadt, Germany) for 5 min. The prepared blocks were covered with a humid soft paper towel to maintain at 100% of humidity up to the beginning of the experiments.

2.3. Baseline profile and groups allocation

Baseline surface microhardness (SMH) of the blocks (389.71 ± 38.97 kg/mm^2) was obtained using a microhardness tester (HVS-1000, Time Group Inc., Beijing, China) with a Knoop diamond (50 g, 5 s, 5 indentations spaced 100 μm) to assure that the hardness values were compatible of health dental enamel. Baseline roughness of the enamel surface was also measured ($Ra = 0.17 \pm 0.017$ μm and $Sa = 0.40 \pm 0.04$ μm), using a 3D non-contact chromatic confocal optical profilometry (Nanovea PS50 Optical, NANOVEA Inc., Irvine, California, United States). An area of 1 mm × 1 mm was acquired in the centre of each sample. Three linear horizontal measurements (500 μm) were performed and the average was used to determine Ra1 (surface linear roughness in the sound window). Three scans areas (250 μm × 250 μm) were acquired and the average was used to determine the Sa1 (surface roughness in sound window).

Then, the enamel blocks were randomly allocated to the following groups (n = 10): G1 = NaF varnish (5% NaF, Duraphat®, Colgate Oral Pharmaceuticals, New York, New York, United States, positive control); G2 = CPP-ACP/NaF varnish (2% CPP-ACP and 5% NaF, MI varnish™, GC America, Alsip, Illinois, United States); G3 = Xylitol/NaF varnish (1% Xylitol and 5% NaF, Profluorid®, Voco, Cuxhaven, Niedersachsen, Germany); and G4 = DD water (negative control).

The sample size calculation was based on the difference in the proportion of tooth structure loss (TSL) in each varnishes group (NaF varnish, "gold standard"; CPP-ACP/NaF varnish and Xylitol/NaF varnish) compared to negative control observed in a pilot study (unpublished data). Assuming a difference of proportions of 50% between test and control groups, based on a two-sided test, considering a power of 80% and $\alpha = 0.05\%$, a sample size of 11 blocks allocated into each group of treatment was required to complete the study. With the estimative of 10% of lost, at least 12 blocks for each group should be selected.

2.4. Experimental protocols

Half of the surface of the specimens was covered with acid-resistant nail varnish in order to create an unexposed area (right side of enamel surface, sound area) and the exposed area (left side) was used for the treatment according to the group.

The varnishes were applied once in a thin layer using a microbrush, a single researcher has made the application, with the same standard amount of the product for each specimen. After treatment, the specimens were immersed in artificial saliva (pH 7.0, 30 ml/specimen) for 6 h in order to simulate the clinical situation (Fernandez, Tenuta, Zarate, & Cury, 2014). The artificial saliva consisted of 1.5 mmol/L Ca, 0.9 mmol/L P, 150 mmol/L KCl, and 0.05 μg F/mL in 0.1 mol/L Tris buffer, pH 7.0 (Nassur et al., 2013). Then, the fluoride varnishes was removed using a scalpel blade and acetone with water (1:1); total removal was confirmed using a microscope (40× magnification).

All specimens were then submitted to 3-day of erosion/abrasion cycles: the erosion was performed with freshly opened bottles of Sprite Zero (Coca-Cola Company, Atlanta, Georgia, United States, pH 2.58, 30 ml/specimen) (Magalhaes, Levy, Rios, & Buzalaf, 2010), 4 times daily, for 5 min each. After the erosion protocol, the specimens were rinsed in distilled and deionized water for 5 s. Additionally, the specimens were abraded twice daily (Attin & Hornecker, 2005), after the first and last erosive challenge, using a mechanical toothbrush machine (Buehler Ltd., Lake Bluff, Illinois, United States) and fresh slurries (0.5 ml/specimen) of a non-fluoridated (placebo) toothpaste (toothpaste/water ratio 1:3) for 15 s (50 strokes/s), with a weight of 200 g.

Between erosion and abrasion, the specimens were rinsed with distilled and deionized water for 5 s and transferred again to artificial saliva. After the last day of erosive challenge, the specimens were also stored in artificial saliva overnight. A fresh artificial saliva solution was replaced daily in order to avoid oversaturation. The experiment was carried out at 37 °C.

2.5. 3D non-contact profilometry analysis

The surface topography of the specimens was analysed by the 3D non-contact chromatic confocal optical profilometry (Nanovea PS50 Optical, NANOVEA Inc., Irvine, California, United States). The measurements of capture were performed with a chromatic confocal sensor with a white light axial source, a scan velocity of 2 μ/s and a refraction index of 10,000. After each experiment, an area of 1 mm x 1 mm was obtained in the centre of the samples in the same way as at the baseline measurement. The 3D non-contact profilometry technique was used to determine: i) surface roughness (linear – Ra; and volumetric – Sa) at baseline (1)