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Combined effect of a fluoride-, stannous- and chitosan-containing toothpaste and stannous-containing rinse on the prevention of initial enamel erosion–abrasion

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ABSTRACT

Objectives: The aim of this study was to assess the preventive effect of a fluoride-, stannous- and chitosan-containing (F/Sn/chitosan-) toothpaste (TP) on initial enamel erosion and abrasion.

Methods: In total, 150 human premolar enamel specimens were ground, polished and divided into 5 toothpaste/rinse groups ($n = 30$): (G1) placebo-TP/tap water, (G2) sodium fluoride (NaF-) TP/tap water, (G3) F/Sn/chitosan-TP/tap water, (G4) F/Sn/chitosan-TP/Sn-rinse, (G5) NaF-TP/NaF-rinse. The 8-day erosion–abrasion cyclic treatment (one cycle/day) consisted of incubating the samples in artificial saliva (30 min), then submitting the samples to toothbrush abrasion (2 min incubation in toothpaste slurry; brushing with 20 toothbrush strokes) and rinsing (2 min; 10 ml) with the respective solution: tap water (G1–G3), Sn-rinse (G4) or NaF-rinse (G5). Afterwards, the samples were submitted to erosion (2 min; 30 ml 1% citric acid, pH = 3.6). Surface microhardness (SMH) was measured initially and after every abrasion and erosion treatment. Enamel substance loss was calculated after each abrasion. Non-parametric ANOVA followed by Wilcoxon rank tests were used for analysis.

Results: G1 presented the greatest SMH decrease, while G4 presented the least SMH decrease ($p < 0.001$). G3 had a similar SMH decrease to G2 and G5. Substance loss was significantly lower in G4 than all other groups ($p < 0.05$), closely followed by G3. Both G2 and G5 showed similar calculated enamel substance loss to G1.

Conclusion: The treatment with F/Sn/chitosan-TP and tap water provided a similar SMH decrease to both NaF-TP groups, but significantly lower substance loss. F/Sn/Chitosan-TP and Sn-rinse showed a better preventive effect, which promoted less SMH decrease and reduced substance loss.

Clinical significance: The toothpaste containing fluoride, stannous and chitosan shows promising results in reducing substance loss from erosion and abrasion. The combination of this toothpaste with the stannous-containing rinse showed even better prevention against erosion–abrasion.

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1. Introduction

Enamel erosive demineralisation starts with a partial loss of enamel mineral, which causes an initial surface softening and roughening. When the tooth surface is further exposed to acid, erosive demineralisation can eventually develop into enamel substance loss.[1–3] This process is accentuated when surface softening occurs in combination with subsequent abrasive forces of the tongue[4] or tooth brushing,[3] because the mechanical abrasion partially removes the softened enamel layer even when fluoridated toothpastes are used.[5,6] Still, fluoridated toothpastes have shown some reduction in enamel substance loss, possibly due to the precipitation of loosely bound (non-specifically adsorbed) non-stoichiometric calcium fluoride-like (CaF_2-like) material onto the eroded tooth surface.[7–11] However, this preventive effect is restricted due to the rapid dissolution of the CaF_2-like layer.[12]

Successful results on erosion prevention have been observed with products that contain fluoride in combination with polyvalent metal ions, such as titanium and stannous.[13] In the case of products containing stannous (Sn), a significantly greater preventive effect has been shown with these products.[14–18] This is probably due to the reaction between Sn and the dental hard tissue, forming Sn-containing salts that are more stable with respect to acidic dissolution.[19,20] Additionally, Sn-containing products form amorphous deposits on the enamel surface and allow Sn to incorporate into the eroded enamel.[21]

Another compound that also has a preventive potential for dental erosion is chitosan.[22] Chitosan is a polysaccharide derived from chitin, which is a natural compound found in the shells and exoskeleton of crustaceans.[23] It has been previously used in industry as a pharmaceutical excipient,[23] but has potential benefits in dentistry, providing protection against enamel demineralisation *in vitro*[24] and *in vivo*.[25] When present in a toothpaste, chitosan also showed promising results in dental erosion and toothbrush abrasion prevention.[26] Recently, a toothpaste combining fluoride, stannous and chitosan (F/Sn/chitosan) was developed and proposed for the prevention of dental erosion and abrasion.

In a previous *in vitro* erosion–abrasion experiment, the use of the F/Sn/chitosan-containing toothpaste promoted significant protection against enamel substance loss.[27] Similarly, in an *in situ* experiment, the samples brushed with this toothpaste had a 46% reduction in enamel substance loss compared to the placebo group.[28] However, these studies tested the F/Sn/chitosan-containing toothpaste in a more severe erosion–abrasion experimental model (6×2 min/day acidic challenges; 0.5% citric acid; pH 2.5; and 2×15 s/day toothbrush treatments), and no studies have been conducted so far focusing on the preventive effect of the toothpaste in an initial enamel erosion–abrasion model. Because bulk substance loss is an irreversible condition, prompt preventive measures, during the early stages of erosion, are essential to reduce tooth wear. Therefore, the aim of this *in vitro* study was to assess the efficacy of the fluoride-, stannous- and chitosan-containing toothpaste on preventing initial enamel erosion and abrasion. The null hypothesis tested was that the F/Sn/chitosan-containing toothpaste has no effect regarding surface microhardness (SMH) decrease or enamel substance loss.

2. Materials and methods

2.1. *Sample preparation*

A total of 150 enamel samples were prepared from human premolars' lingual surface. The teeth were randomly selected from a pool of extracted teeth stored in 2% chloramine solution. Patients were informed about the use of their teeth for research purposes and consent was obtained. The lingual surfaces were embedded in acrylic resin (Paladur, Heraeus Kulzer GmbH, Hanau, Germany) and serially abraded (LabPol 21, Struers, Ballerup, Denmark) with water-cooled silicon carbide paper discs (from grit #500 to #4000) in order to obtain a smooth flat enamel surface. This procedure eliminated the top 200 μm enamel surface layer. The samples were polished for a further 60 s (LabPol 6, Struers) with 3 μm grain diamond paste under constant cooling (DP-Stick P, Struers), and then stored in a mineral solution (1.5 mmol/l $CaCl_2$, 1.0 mmol/l KH_2PO_4, 50 mmol/l NaCl, pH = 7.0).[29] Immediately prior to the experiment, the samples underwent a further 60 s of polishing with 1 μm grain diamond paste under constant cooling.

2.2. *Experimental groups and test products*

The samples were randomly distributed into 5 groups ($n = 30$/group) according to the test products used:

- Placebo toothpaste and tap water rinse (Group 1);
- Sodium fluoride (NaF) toothpaste and tap water rinse (Group 2);
- F/Sn/chitosan toothpaste and tap water rinse (Group 3);
- F/Sn/chitosan toothpaste and Sn rinse (Group 4);
- NaF toothpaste and NaF rinse (Group 5).

The toothpastes and rinses are described in Table 1. They were coded and the examiner was blinded to the products. The toothpastes were experimental formulations, containing equal composition and abrasivity, but differed with respect to the active ingredients. The NaF rinse and the NaF toothpaste were experimental. The latter was similar to the F/Sn/chitosan toothpaste, but did not contain the other active ingredients. The placebo toothpaste was similar to both (NaF and F/Sn/chitosan toothpaste), but contained no active ingredient.

The pH of the rinses and toothpaste slurries was measured using a pH electrode. Toothpaste slurries were prepared daily by mixing 20 g of toothpaste with 40 g of artificial saliva. The artificial saliva was prepared in the laboratory: 1.25 mM $Ca(NO_3)_2 \cdot 4H_2O$, 0.90 mM KH_2PO_4, 129.91 mM KCl, 59.93 mM Tris buffer and 2.2 g/L porcine gastric mucine; pH 7.4.[30] It was prepared weekly, stored at −20 °C and a fresh aliquot was thawed daily at 25 °C and used to incubate the samples and to prepare the toothpaste slurries.

2.3. *Erosion–abrasion cyclic treatment*

The samples were submitted to an 8-day erosion-abrasion cyclic treatment, with one cycle/day (Fig. 1). One experimental cycle consisted of measuring the initial enamel surface microhardness (SMH), then incubating the samples in artificial