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# Effects of regular and highly fluoridated toothpastes in combination with saliva substitutes on artificial enamel caries lesions differing in mineral content

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## ARTICLE INFO

### Article history:

Accepted 8 February 2012

### Keywords:

Enamel  
Subsurface lesion  
Lesion morphology  
Demineralization  
Remineralization  
Microradiography  
Toothpaste  
Fluoride  
Octacalcium phosphate  
Saliva substitute

## ABSTRACT

**Objective:** For patients with hyposalivation fluorides are supportive to prevent caries lesions. Remineralization of subsurface lesions might be improved by toothpastes containing 5000  $\mu\text{g F}^-/\text{g}$  compared with those having 1400  $\mu\text{g F}^-/\text{g}$ . This could be influenced by the degree of baseline mineralization. Therefore, this in vitro study evaluated the effects of fluoride toothpastes differing in fluoride concentration in combination with de- and remineralizing saliva substitutes using two lesion types.

**Design:** Specimens with shallow (SL;  $\Delta Z$  (SD): 1915 (543)  $\text{vol}\% \times \mu\text{m}$ ) or deep lesions (DL; 5804 (427)  $\text{vol}\% \times \mu\text{m}$ ) were either stored in mineral water [saturation with respect to octacalcium phosphate ( $S_{\text{OCP}}$ ): 0.5], demineralizing experimental (Exp,  $S_{\text{OCP}}$ : 0.3), demineralizing commercial (Glandosane,  $S_{\text{OCP}}$ : 0.3), or remineralizing saliva substitute (modified Saliva natura;  $S_{\text{OCP}}$ : 1.9) for five weeks (37 °C). Either one of three brushing procedures was performed additionally three times daily: no brushing, Elmex anticaries toothpaste (E; 1400  $\mu\text{g F}^-/\text{g}$ ), Duraphat toothpaste (D; 5000  $\mu\text{g F}^-/\text{g}$ ). Mineral parameters before and after storage were evaluated using microradiographs.

**Results:** Storage in Exp as well as Glandosane induced a significant demineralization ( $p < 0.05$ ; relatively more pronounced in SL than DL). Additional brushing in particular with D reduced these effects. Storage alone in modified Saliva natura remineralized specimens ( $p < 0.05$ ).

**Conclusions:** Under the in vitro conditions chosen shallow lesions seem to be more susceptible for demineralization compared with deeper ones when stored in an undersaturated (with respect to OCP) saliva substitute. The highly fluoridated toothpaste seemed to be more beneficial than a regular one.

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## 1. Introduction

Fluoride toothpastes are widely used to prevent dental caries. Most of the randomized clinical trials have been limited to

toothpastes with 2800  $\mu\text{g F}^-/\text{g}$ , which have shown higher efficacy to prevent caries formation and progression than toothpastes with 1500  $\mu\text{g F}^-/\text{g}$ .<sup>1</sup> Toothpastes with fluoride levels up to 5000  $\mu\text{g F}^-/\text{g}$  have been developed for 'high-risk individuals'. Their higher efficacy to prevent caries compared

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doi:10.1016/j.archoralbio.2012.02.010

to toothpastes with lower fluoride concentrations has been affirmed through studies showing improved re-hardening of root lesions *in vivo*<sup>2</sup> as well as a reduced caries incidence (DFS) in caries-active adolescents.<sup>3</sup>

*In vitro* enamel remineralization has frequently been studied in superficial lesions (~75 µm), even though caries lesions *in vivo* may be considerably deeper.<sup>4</sup> For superficial caries lesions an *in vitro* pH-cycling study revealed that fluoride enhanced remineralization reaches a plateau around 500 µg F<sup>-</sup>/g, whereas for deeper lesions a dose response has been found up to 1500 µg F<sup>-</sup>/g.<sup>5</sup> A recent *in vitro* pH-cycling study, however, showed that remineralization of advanced enamel lesions (of up to 150 µm) was improved when using higher fluoride concentrations (5000 µg F<sup>-</sup>/g versus 1500 µg F<sup>-</sup>/g).<sup>6</sup>

Given that patients suffering from hyposalivation frequently experience high caries incidence,<sup>7</sup> the daily application of highly fluoridated products (e.g., toothpaste above 1500 µg F<sup>-</sup>/g or fluoride gel) combined with meticulous oral hygiene and a non-cariogenic diet is recommended.<sup>8</sup> Saliva substitutes were originally developed to alleviate oral complaints.<sup>9,10</sup> Over the last few years, however, some commercially available saliva substitutes have been associated with demineralizing or neutral effects on dental hard tissues, whereas others have been seen to remineralize.<sup>11,12</sup> A previous study on enamel lesions showed that highly fluoridated toothpastes (e.g., 5000 µg F<sup>-</sup>/g) had an inhibitory effect on further demineralization when specimens were stored in a demineralizing saliva substitute. Furthermore, the remineralizing effects of modified Saliva natura were not enhanced by brushing with highly fluoridated toothpastes when using a remineralization model.<sup>13</sup> This previous study, however, only examined superficial lesions up to approximately 110 µm.

Thus, the purpose of the present study was to assess the effects of daily tooth-brushing with fluoride toothpastes (5000 µg F<sup>-</sup>/g versus 1400 µg F<sup>-</sup>/g) in combination with three saliva substitutes on shallow and deep enamel lesions *in vitro*. Glandosane, a commercially available and potentially demineralizing product that is still commonly used, was studied in comparison to an experimental demineralizing carboxymethylcellulose (CMC)-based solution (Exp), a modified remineralizing saliva substitute (Saliva natura), and mineral water as a control. The null hypothesis was that both shallow lesions (SL) and deep lesions (DL) reveal no significant re- or demineralization after being treated as described for two and five weeks. However, we presumed that SL would show a significantly lower inhibition of demineralization when stored in a demineralizing saliva substitute and lower remineralization when stored in remineralizing saliva substitute compared with DL. Furthermore, we hypothesized that significantly higher demineralization/lower remineralization will occur with the standard fluoride toothpaste compared with the highly fluoridated one.

## 2. Materials and methods

### 2.1. Enamel specimens and lesion formation

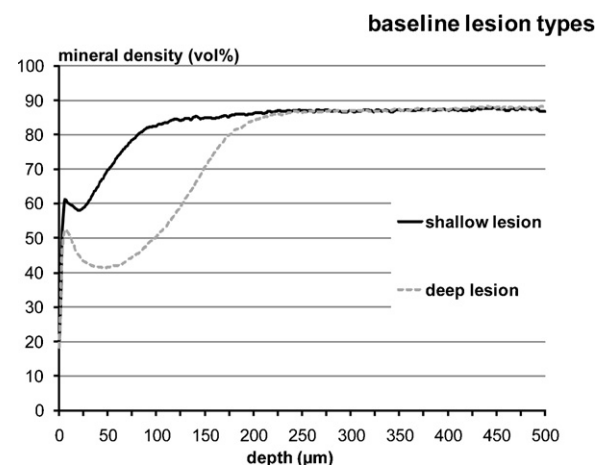
Ninety-six recently extracted permanent bovine central incisors were used in these experiments. From each crown,

three specimens (6 mm × 4 mm × 4 mm) were prepared from the labial aspect under running tap water using a diamond-coated band saw (Exakt 300CL; EXAKT Apparatebau, Norderstedt, Germany). Subsequently, the 288 enamel specimens were embedded in epoxy resin (Technovit 4071; Heraeus Kulzer, Wehrheim, Germany), and the natural surface was kept free from resin. The specimens were ground flat and hand-polished to 4000 grit (silicon carbide; Struers, Copenhagen, Denmark), thereby removing the outer enamel layer (approximately 200 µm). Prepared specimens were stored at 100% relative humidity at 4 °C until further use.

One-quarter of each specimen's surface was covered with an acid-resistant nail varnish (Lycra Flex + Silk; Astor, Paris, France) and flowable composite (Tetric Flow; Ivoclar Vivadent, Ellwangen, Germany) to serve as a sound enamel control. Artificial lesions were prepared by immersion in five litres of a solution containing 6 µmol/l methylhydroxydiphosphonate, 3 mmol/l calcium chloride dihydrate, 3 mmol/l potassium dihydrogen phosphate, and 50 mmol/l acetic acid (Merck, Darmstadt, Germany) at pH 4.95 in an incubator (37 °C; BR 6000; Heraeus Kulzer) for 7 days (shallow lesions, SL; *n* = 120; Fig. 1) or 26 days (deep lesions; DL; *n* = 168). The solution was not stirred or replaced during the demineralization period. The pH value was monitored daily (pH-electrode GE 100 BNC connected to pH-meter GMH 3510; Greisinger, Regenstauf, Germany), and slight elevations were corrected with hydrochloric acid to ensure that the pH level was between 4.92 and 4.98 during the demineralization period. Standard buffer solutions (Sigma-Aldrich, Steinheim, Germany) with nominal pH values of 4.0 and 7.0 ± 0.01 were used to calibrate the pH-meter.

### 2.2. Experimental solutions

Glandosane (carboxymethylcellulose-based; G; Cell Pharm, Hanover, Germany), which is a known demineralizing saliva substitute,<sup>11,12</sup> was tested in this study (pH 5.35; Table 1). Furthermore, an experimental citric acid buffered carboxymethylcellulose (CMC)-based solution (Exp, pH 6.31) was



**Fig. 1 – Mean mineral density profiles with mineral distribution at baseline (i.e., after demineralization) for the two lesion types. Shallow lesions (SL) are represented by the black line and deep lesions (DL) by the grey dotted line.**

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