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Effect of sodium fluoride on oral biofilm microbiota and enamel demineralization

Thomas Thurnheer[a,*], Georgios N. Belibasakis[b]

[a] Division of Oral Microbiology and Immunology, Clinic of Preventive Dentistry, Periodontology and Cariology, Center of Dental Medicine, University of Zürich, Zürich, Switzerland

[b] Division of Oral Diseases, Department of Dental Medicine, Karolinska Institutet, Stockholm, Sweden

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ABSTRACT

Objective: Fluoride is widely used as an anti-caries agent, e.g. in toothpastes and mouth rinses. However, the nature of the anti-caries action is not entirely clear. Mechanisms suspected to explain the cariostatic effect include inhibitory effects on acid formation by bacteria, inhibition of extracellular polysaccharide (EPS) production, inhibition of enamel demineralization and enhancement of remineralizaton or combination thereof. The aim of this study was to examine with the supragingival Zurich *in vitro* biofilm model the effect of fluoride in NaF formulation, on the microbiota and on demineralization.

Methods: Biofilms consisting of *Actinomyces oris*, *Candida albicans*, *Fusobacterium nucleatum, Streptococcus oralis*, *Veillonella dispar* and *Streptococcus sobrinus*, were grown anaerobically on sintered hydroxyapatite or bovine enamel disks, exposed to 200, 400, and 1400 ppm of NaF, or 0.1% chlorhexidine (positive control). The biofilms were harvested after 64 h and CFUs were assessed for total bacteria. Demineralization of enamel disks was measured by quantitative light-induced fluorescence.

Results: NaF did not affect the bacterial numbers. No enamel mineral loss was observed at 1400 and 400 ppm of fluoride, whereas the pH of the surrounding medium was increased to 5.5 and 5.0, respectively, compared to the untreated control (pH 4.5 and mineral loss ΔF of −32%). At 1400 ppm NaF the biofilm's EPS volume was also significantly reduced.

Conclusions: Administration of NaF completely prevented demineralization without affecting biofilm composition and growth. This protective effect may be attributed to the observed decrease in acid production or EPS volume, or to a shift in the de/remineralization balance.

1. Introduction

Sodium fluoride has been mostly used for a long time as anti-caries agent, e.g. in water fluoridation, toothpastes and mouth rinses. Already 70 years ago Bibby and Van Kesteren (1940) reported on the effects of sodium fluoride on streptococci and other oral bacteria. Since then, many reports covering this topic have been published (for reviews, see (Chong, Clarkson, Dobbyn-Ross, & Bhakta, 2014; Hamilton, 1990; Jenkins, 1999; Kalesinskas, Kačergius, Ambrozaitis, Pečiulienė, & Ericson, 2014; Marquis, 1995; Marquis, Clock, & Mota-Meira, 2003). The widespread use of fluoride has had a great impact on the prevention of caries. However, the nature of the anti-caries action is still not entirely clear. Mechanisms suspected to explain the cariostatic effect range from inhibition of the microbial carbohydrate metabolism resulting in reduced acid formation (Bowden, 1990; Hata, Iwami, Kamiyama, & Yamada, 1990), disruption of intracellular pH regulation due to effects on bacterial membranes (Hamilton & Bowden, 1996), inhibition of extracellular polysaccharide (EPS) production (Koo, Sheng, Nguyen, & Marquis, 2006; Shimura & Onisi, 1978) to inhibition of enamel demineralization (ten Cate & Featherstone, 1991; Zero, 2006;) and enhancement of remineralization (Fernández, Tenuta, Del Bel Cury, Nóbrega, & Cury, 2017; Kapoor, Indushekar, Saraf, Sheoran, & Sardana, 2016; Li, Wang, Joiner, & Chang, 2014). The aim of this study using the six-species "supragingival" Zurich *in vitro* biofilm model was to examine the effects of fluoride on the microbial composition of the biofilm, the pH, as well as enamel demineralization, when applied daily three times for 1 min only.

* Corresponding author at: Clinic of Preventive Dentistry, Periodontology and Cariology, Center of Dental Medicine, University of Zürich, Plattenstrasse 11, CH-8032 Zurich, Switzerland.

E-mail address: thomas.thurnheer@zzm.uzh.ch (T. Thurnheer).

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Table 1
Test solutions containing NaF and CHX in different concentrations.

No	Compound	Concentration	Remarks
1	NaF	1400 ppm F^- (74 mM)	equal to concentration in high-fluoride dentifrices (Davies et al., 2003)
2	NaF	400 ppm F^- (21 mM)	equal to concentration in low-fluoride dentifrices (Davies et al., 2003)
3	NaF	200 ppm F^- (11 mM)	Equal to concentration in mouth washes (Zero, 2006)
4	NaF	100 ppm F^- (5 mM)	
5	CHX	0.1%	Positive control
6	CHX	0.001%	
7	NaF + CHX	100 ppM + 0.001%	
8	Water		Negative control

2. Materials and methods

2.1. In vitro biofilm experiments

The procedures to produce six-species biofilms have been described in detail (Shapiro, Giertsen, & Guggenheim, 2002; Thurnheer, Gmür, Shapiro, & Guggenheim, 2003; Thurnheer, Rohrer, Belibasakis, Attin, & Schmidlin, 2014). In brief, *Actinomyces oris* OMZ 745, *Candida albicans* OMZ 110, *Fusobacterium nucleatum* KP-F2 (OMZ 596), *Streptococcus oralis* SK 248 (OMZ 607), *Streptococcus sobrinus* OMZ 176, and *Veillonella dispar* ATCC 17748^T (OMZ 493) were used for biofilm formation. Biofilms intended for culture analyses were grown in 24-well polystyrene cell culture plates on sintered hydroxyapatite disks that had been preconditioned for pellicle formation in whole un-stimulated pooled saliva (in the following termed saliva) for 4 h. The processing of batches of saliva has been described in detail by Guggenheim et al. (2001). To initiate a biofilm experiment disks were covered for the first 16 h with 1.6 ml of growth medium containing 70% saliva, 30% modified fluid universal medium (mFUM) (Guggenheim et al., 2001) supplemented with Sørensen's buffer (final pH 7.2) and 200 µl of a cell suspension prepared from equal volumes and densities of each strain. The medium was changed after 16 and 40 h. In order to remove non adherent micro-organisms, biofilms were dipped 3 x in saline after 16, 20 and 24 h as well as after 40, 44 and 48 h. These dips followed immediately after exposure to fluoride and control solutions

The carbohydrate concentration of mFUM was either 0.3% glucose (0 to 16 h of biofilm cultivation) or 0.15% glucose and 0.15% sucrose (16 h to 64 h). The pH of the pooled culture supernatants was measured at the beginning and after 16, 40, and 64 h. At the end of the experiment (64 h), biofilms were either harvested for culture analyses by vigorous vortexing in 1 ml of 0.9% NaCl or proceeded to staining and confocal laser scanning microscopy (CLSM) (see below). Total CFU were assessed by culture on Columbia Blood Agar supplemented with 5% whole human blood (Thurnheer et al., 2014).

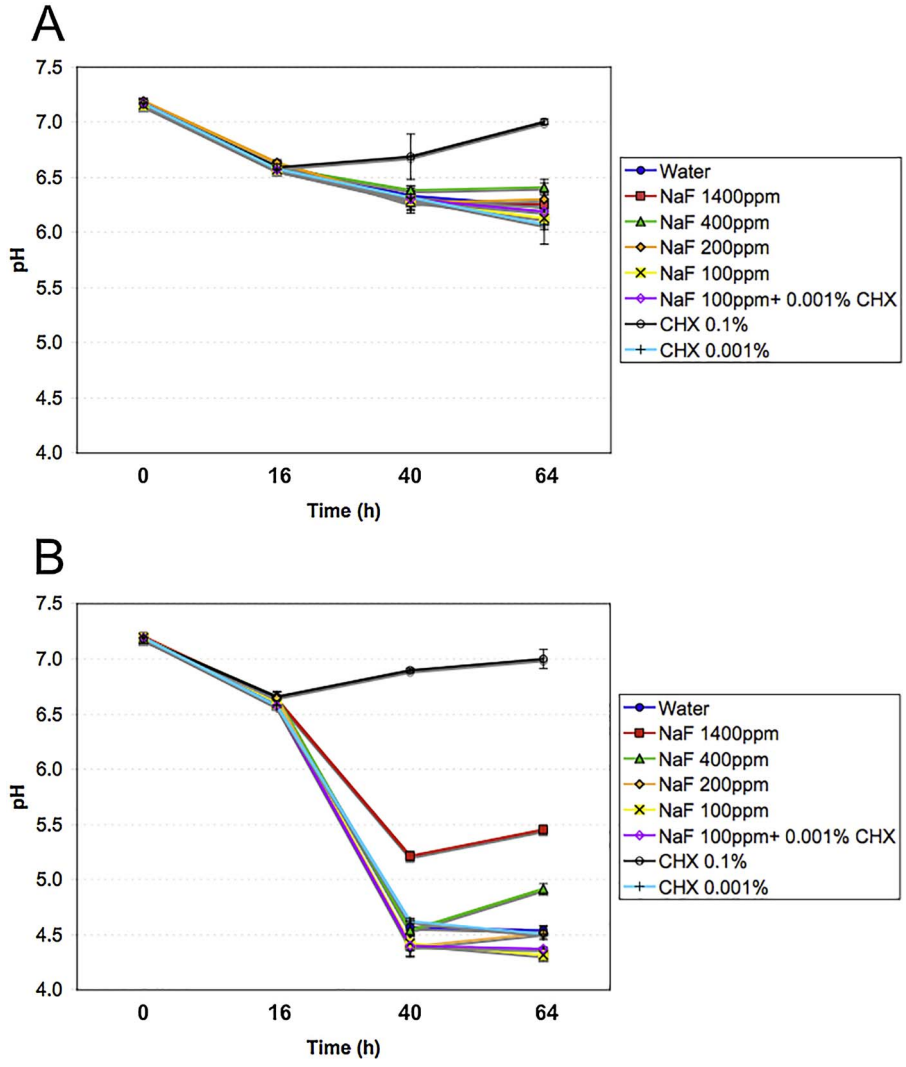

Fig. 1. pH of saliva/mFUM supernatants during biofilm growth in buffered media (A), and in weakly buffered medium (B).

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