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EFFECT OF FLUORIDE ON CARIOGENIC ORAL MICROORGANISMS

(AN *IN VITRO* STUDY)

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The effect of sodium fluoride and sodium monofluorophosphate at concentrations of 1, 5, 10, 50, 100 and 1000 mg/l in phosphate buffer (pH 6.5) as well as in UHT milk were studied on cultures and suspensions of *Streptococcus mutans*, *Lactobacillus acidophilus* and *Candida albicans*. Using serial tenfold dilutions up to 10^{-7} of 24–48 hour cultures, a subsequent 0, 60 and 120 min incubation caused no decrease in the number of CFUs. Growth kinetic studies in the Bioscreen biophotometer (Labsystem, Finland) revealed that sodium fluoride in different concentrations (from 0.875 mg/l up to 500 mg/l) influenced the growth dynamics of *S. mutans* and *C. albicans*: the exponential phase flattened out at the highest fluoride concentrations (500 mg/l) present in the growth media. The lag phase of *C. albicans* became longer. The results of these experiments indicate that sodium fluoride administered at higher concentrations than the usual caries preventive dosage made the generation time of cariogenic oral bacteria and fungi longer, slowing down their multiplication.

Keywords: fluoridated milk, oral microorganisms, *Streptococcus mutans*, *Lactobacillus acidophilus*, *Candida albicans*

Introduction

In the microbial ecology of dental caries *Streptococcus mutans*, *Streptococcus sobrinus*, *Streptococcus mitis*, *Lactobacillus acidophilus* and *Actinomyces viscosus* are

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considered to play a role in the development of enamel (coronal) caries. In addition, *Candida albicans* has shown a positive association with caries experience in several studies [1, 2, 3].

The efficient use of fluorides in caries prevention initiated a multitude of studies on the direct and indirect effects of fluoride, investigating its specific effects on the growth, colonization, macromolecular synthesis and glycolysis of oral bacteria. Fluoride may effect the development of biofilms by modifying the selective deposition of salivary macromolecules on enamel [4, 5]. Studies on oral microorganisms testing their susceptibility to fluorides showed that the bactericidal effect was dependent on the concentration of fluorides (μg/ml), on different pH values, and on various fluoride salts used. The results of these studies indicated no selective effect of fluorides on cariogenic oral bacteria [4, 6]. However, in the available literature no data on the effects of fluorides on oral bacteria using different vehicles could be found.

Based on recently published evidence, milk, as a vehicle for fluoride, proved to be effective in the prevention of dental caries [7].

Considering the occurrence of cariogenic bacteria in milk, based on literary data, *Streptococcus salivarius, Lactobacillus acidophilus* and *Lactobacillus casei* are the microorganisms most commonly associated with milk [8]. Among other cariogenic microorganisms, the effect of immune bovine milk on *S. mutans* in human dental plaque has been investigated by Filler et al. [9]. A reduction in numbers of *S. mutans* was found and colonies were smaller, than when using milk without antibodies. Vacca-Smith et al. [10] reported that milk components may reduce the adhesion of streptococci to hydroxiapatite.

Concerning the effect of fluoridated milk on oral microorganisms, literary data are limited. In connection with experimentally induced rat caries, Stösser et al. [11] could report no change in dental plaque composition when consuming milk or fluoridated milk. The percentage of caries–inducing streptococci was high, but was not influenced by milk or fluoride administration.

In a short-term human study investigating the effect of fluoridated schoolmilk on the microbial changes in dental plaque and saliva of children, no change in the total bacterial score was found, however, a decrease in the proportion of *S. mutans* in plaque has been assessed [12].

The aim of the present study was to investigate the effect of fluoridated milk on oral cariogenic microorganisms *in vitro*, and to compare the effect of different fluoride concentrations and different fluoride compounds in milk and in phosphate buffer as vehicles.

Materials and methods

Strains of microorganisms

Streptococcus mutans strain HG882, *Lactobacillus acidophilus* strain HG1149 and *Candida albicans* strain HG392 were kindly donated by Professor J. J. de Soet, Department of Oral Microbiology, Academic Centre of Dentistry, Amsterdam, The Netherlands. These strains were used throughout the experiments.

Culture media and cultivation methods

S. mutans was grown in Todd Hewitt Broth (THB) and on Mitis Salivarius Agar (MSA) [13], (Difco Laboratories Ltd), *L. acidophilus* was cultured in Brain Heart Infusion Broth (BHIB) and on Rogosa agar (RA) [14], (OXOID Ltd.), at 37 °C for 48 hours under anaerobic conditions, provided by OXOID Anaerobic Generating Kits in Biomerieux GasPak jar. The cultivation of *C. albicans* took place in Sabouraud's Dextrose Broth and on Sabouraud's Dextrose Agar (SDA), (OXOID Ltd.), at 37 °C for 24–48 hours aerobically.

Chemicals and vehicles

The effect of both sodium fluoride (NaF) and sodium monofluorophosphate (Na_2PO_4F=MFP)(Reanal, Hungary) was examined at different concentrations and in different vehicles on *S. mutans, L. acidophilus* and *C. albicans* test strains. For vehicles ultrapasteurized UHT milk with 1.5% fat content and phosphate buffer (PBS) pH 6.5 and pH 5.5 were used.

Serial dilution and microplating methods

The previous cultivation of the lyophilized test strains took place over 48 hours in BHIB for *L. acidophilus*, THB for *S. mutans* and SDB for *C. albicans*. For further investigations dilutions from these test cultures up to 10^{-2} for the fluoridated PBS and 10^{-3} for the fluoridated milk were done. All the solutions from the two different vehicles with the proper fluoride concentrations of both sodium fluoride and sodium monofluorophosphate were prepared. The effects of the following fluoride concentrations were studied: 1 mg/l (1 ppm), 5 mg/l (5 ppm), 10 mg/l (10 ppm) and 50 mg/l (50 ppm).

Equal amounts of each bacterial dilution were added to each concentration of both sodium fluoride and sodium monofluorophosphate. To reveal the effect of fluoride on the strains the same amount of the bacteria and fungi were added to PBS solutions

pH 6,5 and milk without fluoride. A subsequent 0, 60, 120 min. incubation time was investigated. For counting the germ content of each tube a serial tenfold dilution up to 10^{-7} was used. Five drops (10 μl/each) from each dilution were dropped onto appropriate plates depending on the cultivation property of the test strains. After counting the colony forming units (CFU) on the proper plates the CFU/ml content of the original tube was defined at the given time, according to the given fluoride type, vehicle type and fluoride concentration [15].

All measurements were carried out five times, and the mean values were calculated.

Phenol coefficient method

To control the reliability of our method the effect of phenol was also investigated on our test strains under the same conditions. 0, 1, 5, 10, 50, 100 and 500 mg/l sodium fluoride and sodium monofluorophosphate as well as 1:60, 1:80, 1:100, 1:120, 1:140 and 1:200 dilutions of phenol in PBS pH 6.5 were inoculated with 100 μl of the 10^2 dilution of the 24 hours liquid culture of the microorganisms. After five and ten minutes incubation time at room temperature 10 μl aliquots from each tube were placed onto appropriate agar surfaces and subsequently incubated for 48 hours as previously described. Finally quantitative growth was assessed. The influence of the different concentrations of fluorides and the different concentrations of phenol was compared.

All the procedures were repeated three times.

Growth kinetic studies

For studying the growth kinetics of oral microorganisms under the influence of fluoride Bioscreen biophotometer (Labsystem, Finland) was used. The growth curves of *S. mutans* and *C. albicans* were revealed with and without sodium fluoride and sodium monofluorophosphate at different concentrations (from 0,875 mg/l up to 500 mg/l) in THB for *S. mutans*, SDB for *C. albicans.* Killing curves were registered in distilled water and PBS pH 6.5; pH 7.0 for *S. mutans*, in distilled water and PBS pH 6.5 and pH 5.5 for *C. albicans*. The microplates were incubated at 37˚C and the measurement of turbidity was made automatically for 24 hours. The dilutions were twofold, the total amount of solutions in the wells of the microplate was 200 μl and each well was inoculated with 20 μl of a 0.5 McFarland density of the proper liquid culture media containing the test strains.

All measurements were carried out three times, and the mean values were defined.

Table I

The effect of sodium fluoride and sodium monofluorophosphate on the viable counts of Streptococcus mutans *strain HG882 in phosphate buffered saline, pH 6.5, during 2-hour exposure at room temperature*

Fluoride concentration	Exposure time, h		
	0	1	2
mg/l	Colony forming unit / ml*		
	NaF		
0	6.3×10^2	1.1×10^3	1.4×10^3
1	7.5×10^2	1.3×10^3	1.3×10^3
5	3.3×10^2	1.2×10^3	7.5×10^2
10	3.2×10^2	1.5×10^2	1.2×10^3
50	1.9×10^3	6.4×10^2	4.4×10^2
	Na_2PO_4F		
0	6.3×10^2	1.1×10^3	1.4×10^3
1	3.5×10^2	8.9×10^2	2.6×10^2
5	3.3×10^2	1.6×10^3	2.2×10^3
10	7.0×10^2	4.1×10^2	5.2×10^2
50	1.0×10^3	7.8×10^2	2.9×10^2

* Average of 5 drops (10 μl each)

Results

Serial dilution and microplating method

Studying the influence of fluoride on the viable counts of all microorganisms tested in all vehicles at room temperature during a period of two hours, neither sodium fluoride nor sodium monofluorophosphate caused a considerable decrease in the viable number of microbes (Tables I and II). Despite the lack of consistency in the results, it is clear that none of the fluorides in the applied molecular forms showed a remarkable

bactericidal effect on oral cariogenic microrganisms in the examined concentrations and during the observation period used.

Table II

The effect of sodium fluoride and sodium monofluorophosphate on the viable counts of Streptococcus mutans *strain HG882 in ultrapasteurised UHT 1.5% fatty milk during 2-hour exposure at room temperature*

Fluoride concentration mg/l	Exposure time, h		
	0	1	2
	Colony forming unit / ml*		
	NaF		
0	$2{,}1\times10^3$	$2{,}3\times10^3$	$2{,}1\times10^3$
1	$1{,}5\times10^3$	$1{,}3\times10^3$	$2{,}8\times10^3$
5	$2{,}0\times10^3$	$2{,}3\times10^3$	$2{,}1\times10^3$
10	$6{,}6\times10^2$	$8{,}6\times10^2$	$9{,}8\times10^2$
50	$5{,}8\times10^2$	$5{,}6\times10^2$	$8{,}2\times10^2$
	Na_2PO_4F		
0	$2{,}1\times10^3$	$2{,}3\times10^3$	$2{,}1\times10^3$
1	$1{,}8\times10^3$	$2{,}0\times10^3$	$1{,}9\times10^3$
5	$1{,}4\times10^3$	$1{,}6\times10^3$	$1{,}8\times10^3$
10	$6{,}4\times10^2$	$7{,}0\times10^2$	$1{,}0\times10^3$
50	$6{,}8\times10^2$	$6{,}6\times10^2$	$1{,}0\times10^3$

* Average of 5 drops (10 µl each)

Phenol coefficient method

The results of the methods discussed above raised the question whether fluorides in these molecular forms have any effects within a few minutes on our test strains. Therefore the influence of the different concentrations of fluorides and the different concentrations of phenol were compared.

None of the concentrations of fluorides – neither sodium fluoride nor sodium monofluorophosphate – killed the microbes of the three different species (*S. mutans, L. acidophilus, C. albicans*) during the 5 and 10 minutes of examination period.

While 1:100 dilution of phenol was necessary to destroy the cells of both *S. mutans* and *L. acidophilus* within 5 minutes, no living microbes were detected on the Sabouraud′s plate from the culture of *C. albicans* during the same incubation time by using 1:80 dilution of phenol. On Table III the data of *S. mutans* are shown.

Table III

The effect of NaF, Na_2PO_4F and phenol on the survival of bacteria of Streptococcus mutans *strains HG882 during 5 and 10 minutes of exposure*

		Exposure	
		5 minutes	10 minutes
		Growth	
Na F in PBS pH 6.5	1 mg/l	+	+
	5 mg/l	+	+
	10 mg/l	+	+
	50 mg/l	+	+
	100 mg/l	+	+
	500 mg/l	+	+
Na_2PO_4F in PBS pH 6.5	1 mg/l	+	+
	5 mg/l	+	+
	10 mg/l	+	+
	50 mg/l	+	+
	100 mg/l	+	+
	500 mg/l	+	+
Phenol PBS, pH 6.5	1:60	-	-
	1:80	-	-
	1:100	-	-
	1:120	+	-
	1:140	+	+
	1:200	+	+
Control		+	+

Growth kinetic studies

Based on the results of the tube dilution method, indicating that fluoride did not show any bactericidal effects on cariogenic microbes, investigations were continued on the effect of fluoride (both sodium fluoride and sodium monofluorophosphate) on the growth kinetics of *C. albicans* and *S. mutans*. Analyzing the killing curves of *C. albicans* and *S. mutans* in both the phosphate buffer and distilled water, there was no difference between the initial number of microbes and the number of microbes of the last measurement (data not shown). Analyzing the growth curves of both *C. albicans* in SDB with or without sodium fluoride at different concentrations and of *S. mutans* in THB under the same conditions it was revealed that sodium fluoride influenced the growth dynamics of these microbes. As Fig. 1 shows, the exponential phase of *S. mutans* flattened out at the highest fluoride concentrations present in the growth media. 500 mg/l concentration of sodium fluoride was the amount which altered the growth curve (Fig. 1). *C. albicans* grew slowly in SDB and this typical growth was not modified by low concentrations of fluoride, while 500 mg/l fluoride flattened the exponential phase of the culture and the lag phase became longer (Fig. 2). Under the microbiological conditions used in this experiment sodium monofluorophosphate seems to be indifferent on the growth dynamics of *C. albicans* and *S. mutans* (Figs 3 and 4).

Due to the high density of milk the growth-phase changes of microorganisms in milk containing different amounts of fluoride could not be examined under this system.

Discussion

The results of the experiments – in agreement with most of the literary data [5, 6] – show that fluoride in sodium fluoride and sodium monofluorophosphate molecular forms did not exert a prompt effect on the viable counts of *S. mutans* strain HG882, *L. acidophilus* strain HG1149 and *C. albicans* strain HG392 even at high concentrations in phosphate buffered saline of different pH and in milk.

However, as the investigations of the growth kinetics of microorganisms in enriched media similar to milk indicate, a long exposure to high concentrations of sodium fluoride can make the exponential phase of all strains longer. In other words, the generation time of *S. mutans* and *C. albicans* becomes longer in the prolonged presence of fluoride in this molecular form. Analysing the growth curve of *S. mutans* and *C. albicans* with or without sodium monofluorophosphate at different concentrations suggest that fluoride in this molecular form does not influence the growth dynamics of these cariogenic microbes.

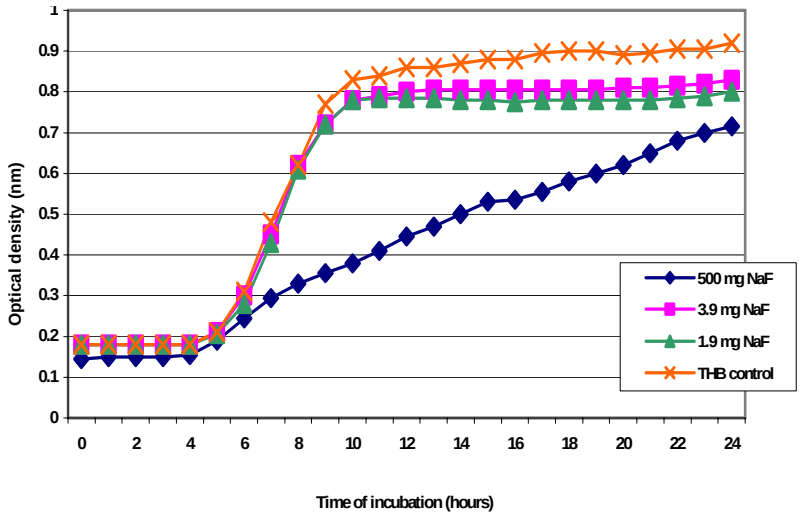

Fig. 1. Growth curves of *Streptococcus mutans* in the presence of different concentrations of NaF

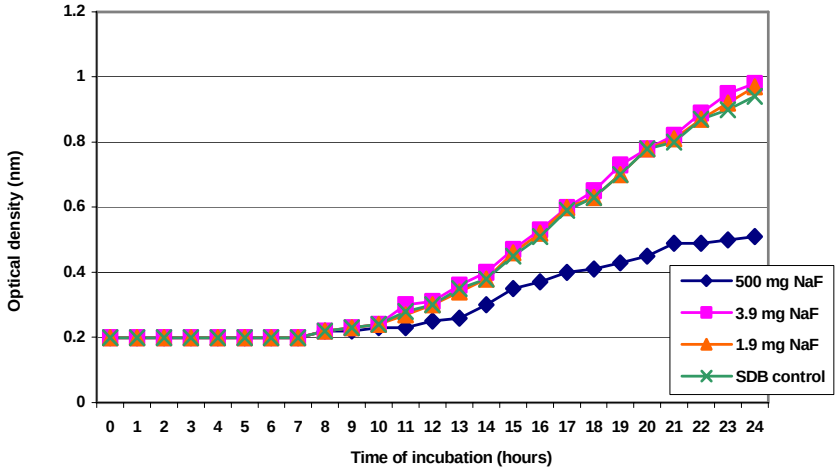

Fig. 2. Growth curves of *Candida albicans* in the presence of different concentrations of NaF

Milk may be a candidate for one of the vehicles since it has been shown to change the hydrophobic bacterial surfaces to hydrophilic [16], consequently, milk-treated bacteria are supposed to have larger water envelope in which an accumulation

of fluor as in a water phase of multiple disperse system can occur. Fluor should probably be in such a complex, from which nascent fluor is continuously released.

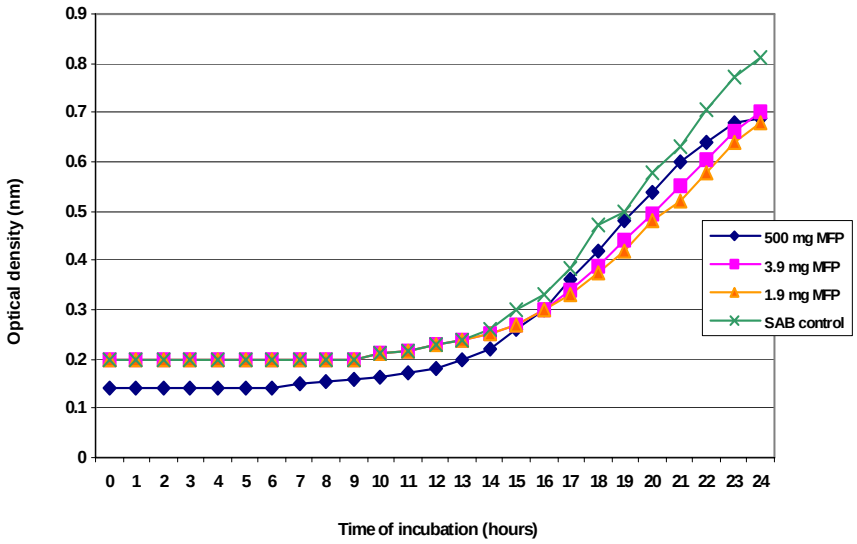

Fig. 3. Growth curves of *Streptococcus mutans* in the presence of different concentrations of Na_2PO_4F (MFP)

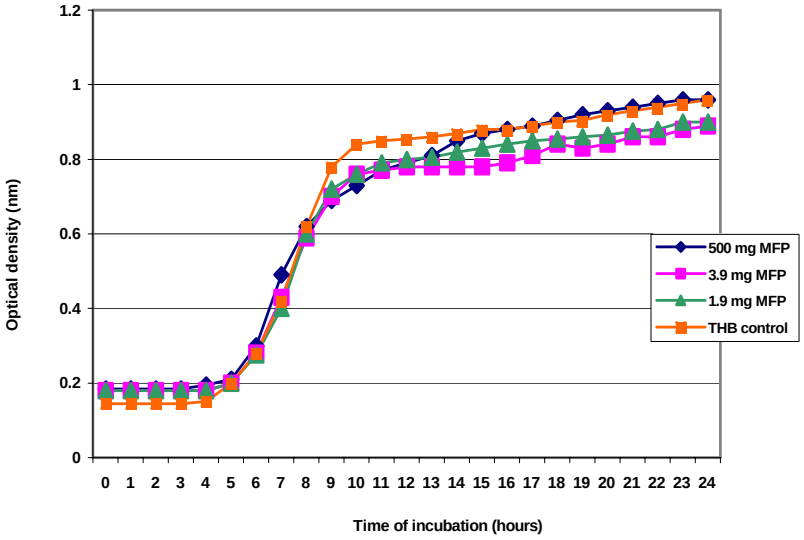

Fig. 4. Growth curves of *Candida albicans* in the presence of different concentrations of Na_2PO_4F (MFP)

As a conclusion, it might be supposed that repeated prolonged exposure of microorganisms in the oral cavity to fluoride may result in a decrease in the number and ratio of the main cariogenic microrganisms.

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