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In vivo Effects of Fluoride, Chlorhexidine and Zinc Ions on Acid Formation by Dental Plaque and Salivary Mutans Streptococcus Counts in Patients with Irradiation-induced Xerostomia

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Irradiation therapy including major salivary glands may result in xerostomia and enhanced susceptibility to dental caries. The present aim was to assess the ability of mouthrinses with F^-, Zn^{2+}, and chlorhexidine (CH), in various combinations, to reduce acidogenic potential of dental plaque and salivary mutans streptococcus counts (SMSC) in 7 patients with xerostomia secondary to irradiation. The patients rinsed twice daily for 3 weeks with the following test solutions: (1) 12 mmol/l NaF (F; control), (2) NaF + 20 mmol/l $ZnCl_2$ (F–Zn), and (3) NaF + 1.1 mmol/l CH (F–CH). Resting periods (F) of varying lengths were incorporated. Acid formation by dental plaque was monitored as plaque pH response to a sucrose mouthrinse, at the end of each test period, 4 h after mouthrinsing with test solution. Plaque pH was measured repeatedly at 2–8 sites in each patient before, and up to 60 min after the sucrose mouthrinse using touch microelectrodes. SMSC were determined using Dentocult SM-Strip mutans. Compared with F, F–CH significantly ($P \leqslant 0.02$) reduced acid formation by plaque and SMSC, whereas F–Zn did not affect acid formation or SMSC significantly. Pilot experiments in 4 patients showed mouthrinses with NaF + 0.55 mmol/l CH + 10 mmol/l Zn^{2+} to be ineffective, whereas NaF + 2.2 mmol/l CH was highly effective, but no better than F–CH. Twice daily mouthrinses with 12 mmol/l NaF in combination with 1.1 mmol/l CH may be an effective regimen to prevent post-irradiation caries.
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INTRODUCTION

IRRADIATION THERAPY including major salivary glands results in hyposalivation or xerostomia and as a consequence, caries susceptibility increases dramatically [1–3]. In addition, patients with hyposalivation tend to change their diets towards frequent consumption of soft, sugar-containing foods and frequent sipping of fluid, thereby increasing the caries challenge [4, 5]. Due to the high caries risk combined with the possibility for osteoradionecrosis after tooth extractions in patients receiving irradiation therapy [1, 2], effective, and easily applicable caries-prophylactic regimens are called for.

Dental caries is a demineralisation of the tooth surface caused by organic acids produced by dental plaque bacteria from fermentable carbohydrates. It has been shown that plaque pH in hyposalivatory patients reaches low values following a sucrose challenge, and that the pH is depressed for a prolonged period of time [6]. The low plaque pH is most probably due to delayed sugar and acid clearance [7] and concomitant shifts in the oral microbial flora towards increased numbers of acidogenic and aciduric microorganisms such as mutans streptococci (MS) and lactobacilli [4].

The cariostatic effect of fluoride is multifactorial [8], however, its modulating effects on de- and remineralisation processes of the tooth surface is assumed to be essential [9]. Saliva is undersaturated with respect to hydroxyapatite but supersaturated with respect to fluoridated hydroxyapatite when pH is in the range of 4.0–5.5, at which pH range demineralisation of hydroxyapatite may occur [10]. It is therefore of crucial importance that a sufficient level of fluoride is constantly available in the aqueous environment of the teeth, i.e. in saliva and plaque fluid, for inhibition of demineralisation and enhancement of remineralisation when plaque pH drops to such low values.

Daily use of fluoride gel in custom-fitted trays effectively decreases post-irradiation caries [3]. However, this regimen is laborious, and patient compliance is probably higher when mouthrinses are used. It was recently shown, using an intraoral model, that twice daily mouthrinses with 12 mmol/l sodium fluoride in addition to use of fluoridated toothpaste promote remineralisation in only 3 out of 6 patients with irradiation-induced hyposalivation [6].

It may thus be beneficial to combine the use of fluoride with antimicrobial agents such as chlorhexidine (CH) [11]

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and zinc ions [12] which reduce acid formation by dental plaque bacteria. Such combinations may reduce acid formation and thereby reduce the acid challenge.

A single mouthrinse with CH effectively depresses acid formation by dental plaque in healthy subjects [11]. Moreover, *Streptococcus mutans* and *Streptococcus sobrinus*, which are associated with caries progression in humans [13], are particularly susceptible to CH [14]. CH mouthrinses suppress the growth of these bacteria in dental plaque [15] and saliva [16] in humans. Zinc ions have a less pronounced inhibitory effect on acid formation [12] but reportedly accumulate in dental plaque after consecutive mouthrinses [17].

The aim of the present study was to assess the *in vivo* ability of mouthrinses with fluoride, chlorhexidine, and zinc ions, in various combinations, to reduce acidogenic potential of dental plaque and salivary mutans streptococcus counts (SMSC) in patients with xerostomia secondary to irradiation.

PATIENTS AND METHODS

Patients

7 dentate patients, 3 female and 4 male, aged 28–78 years (mean 59 years), were recruited from a group of 28 patients who had been treated with irradiation for oral cancer in hospitals in Oslo in 1989 and 1990. In all patients irradiation had involved major salivary glands with a dose equal to or more than 50 Gy. At the start of the study, xerostomia was confirmed by unstimulated and paraffin-stimulated salivary secretion rates of, respectively, 0.03 ± 0.04 ml/min and 0.08 ± 0.08 ml/min.

The local region's ethical committee gave approval to the study protocol, and informed, written consent was obtained from all patients (Declaration of Helsinki, 1975).

Test Solutions

Freshly prepared aqueous test solutions of the following agents were tested in all patients (see Table 1): (1) 12 mmol/l (0.05%) NaF (F; control solution;—Riedel de Haen AG, Seelze-Hannover, West Germany), (2) 12 mmol/l NaF + 20 mmol/l zinc chloride (F-Zn;—Merck Laboratories, Darmstadt, West Germany), and (3) 12 mmol/l NaF + 1.1 mmol/l chlorhexidine diacetate (F-CH; corresponding with a 0.1% chlorhexidine digluconate solution;—ICI Ltd., Macclesfield, U.K.). The following test solutions were tested in 4 patients: (4) 12 mmol/l NaF + 0.55 mmol/l chlorhexidine diacetate + 10 mmol/l zinc acetate (F-CH-Zn; Merck) and (5) 12 mmol/l NaF + 2.2 mmol/l chlorhexidine diacetate (F-2CH).

Experimental design

The study was performed according to a double-blind crossover design. The patients were given oral and written information about the procedures to be followed during the test and resting periods, and cooperation was confirmed after each period.

Each test period lasted for 3 weeks. The patients rinsed for 1 min in the morning and in the evening with 10 ml of a test solution, and they refrained from eating and drinking for at least 20 min following mouthrinses. They performed habitual mechanical oral hygiene and used a standardised amount of fluoridated (0.22% NaF, 0.1% F^-) toothpaste (Caridент) mornings and evenings. At least 30 min elapsed between toothbrushing and mouthrinsing with test solution. Resting periods of at least 3 weeks were incorporated between each test period. The resting periods were extended whenever SMSC had not reached pre-experimental levels. The patients rinsed with F alone during the resting periods. No special instructions were given concerning diet.

Acid formation by dental plaque was monitored as plaque pH response to a sucrose mouthrinse, at the end of each test period, 4 h after the last mouthrinse with test solution. The patients had not brushed their teeth in the morning, and they had refrained from eating and drinking, except for water, at least 2 h prior to measurements. Plaque accumulations at each site were assessed after the pH measurements, by the same examiner, according to the criteria of the Plaque Index score system [18].

At the end, unstimulated and paraffin-stimulated salivary secretion rates were determined. Unstimulated samples were collected during a period of 5 min. For collection of stimulated samples, the patients chewed on a piece of Parafilm (0.3 g; American Can Company, Greenwich, Connecticut, U.S.A.) for 1 min, the saliva produced was swallowed, and saliva samples were collected during the following 5 min of chewing. Saliva was collected using a pipette whenever the patients were unable to expectorate due to low salivary secretion rate. Stimulated saliva samples were used for determination of SMSC (see later).

pH measurements

The plaque pH response was monitored with Beetrode MEPH-1 touch microelectrodes (World Precision Instruments, Inc., New Haven, Connecticut, U.S.A.) [19, 20]. The microelectrode and a porous glass reference electrode (Beetrode MERE-1, W.P. Instruments) were connected to a battery-run Orion 290 A pH/ISE meter (Orion Research, Inc., Boston, Massachusetts, U.S.A.). A reference salt bridge was created by the patient having one finger dipped into a 3 mol/l potassium chloride solution, also containing the reference electrode.

The microelectrode had a diameter of 0.1 mm, allowing plaque pH measurements at discrete sites. pH was monitored repeatedly in dental plaque at 2–8 sites in each patient, before and 2, 5, 10, 15, 20, 30, 40, 50, and 60 min after a 1 min mouthrinse with 10 ml of a 10% sucrose solution. Resting

Table 1. Effects on plaque pH *response after 3 weeks of twice daily mouthrinses*

Test solutions	pH $area_{0-60}$*	pH $area_{2-30}$*	Min pH*	ΔpH*
F (12 mmol/l NaF)†	184 ± 26	83 ± 11	5.6 ± 0.3	1.5 ± 0.4
F-Zn (NaF + 20 mmol/l $ZnCl_2$)	196 ± 29	88 ± 13	5.8 ± 0.5	1.2 ± 0.4
F-CH (NaF + 1.1 mmol/l CH)	205 ± 39‡	97 ± 18§	6.0 ± 0.7‡	0.6 ± 0.2‖

*Mean ± S.D. ($n = 7$). †Control solution. ‡$P \leq 0.02$; §$P = 0.005$; ‖$P = 0.002$, significantly different from F (one-tailed paired t-test).

plaque pH was assessed by five consecutive measurements at each site before the sucrose mouthrinse. All measurements were carried out by the same examiner, and the pH-meter readings were noted by an independent assistant. The electrodes were calibrated against standard pH buffers at pH 7.00 and 4.01 before and after measurements at each time point. If the electrode had drifted by more than 0.1 pH unit in either buffer, the readings in between were adjusted according to calibration curves made prior to analyses of the data.

Determination of salivary mutans streptococcus counts

SMSC were determined using Dentocult SM-strip mutans test (Orion Diagnostica, Espoo, Finland) [21] which is based upon cultivation of MS from fresh paraffin-stimulated saliva on plastic strips in a liquid mitis-salivarius medium containing bacitracin. Following 2 days of incubation at 37°C, the number of MS adhering to the strip was assessed using a stereomicroscope.

Data analyses

Since NaF was the basis in all mouthrinses, F served as a control. Total area under the pH curves ($area_{0-60}$), area under the pH curves from time points 2–30 min ($area_{2-30}$), minimum (min) pH, and ΔpH (the difference between mean resting pH and min pH) were used as parameters for analyses of the pH data.

Areas under the pH curves were calculated in order to compare the pH responses over time. Total pH area was defined as the curve connecting pH measurements conducted at time intervals from time 0 (i.e. resting pH) to time 60 min, a horizontal line through pH 3.0, a vertical line through time 0, and a vertical line through time 60 min ($area_{0-60}$). Thus the smaller the pH drop, the larger the pH area and vice versa. Since mean resting pH varied between the regimens, analyses of pH curves from time points 2–30 min ($area_{2-30}$) were also performed. In addition, site-specific comparisons of differences between $area_{2-30}$ for control (F) and test solutions F–CH or F–2CH were performed.

The pH value used for each time point (Figs 1 and 2) was the mean of readings taken from between two and eight sites

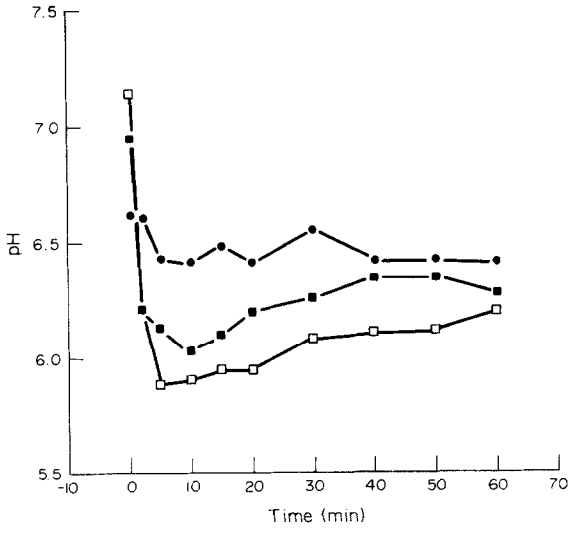

Fig. 1. pH response in dental plaque (mean of two to eight sites in each of 7 patients) following a sucrose mouthrinse after 3 weeks of twice daily mouthrinses with: □ F (12 mmol/l NaF; control solution), ■ F–Zn (NaF + 20 mmol/l $ZnCl_2$), and ● F–CH (NaF + 1.1 mmol/l CH).

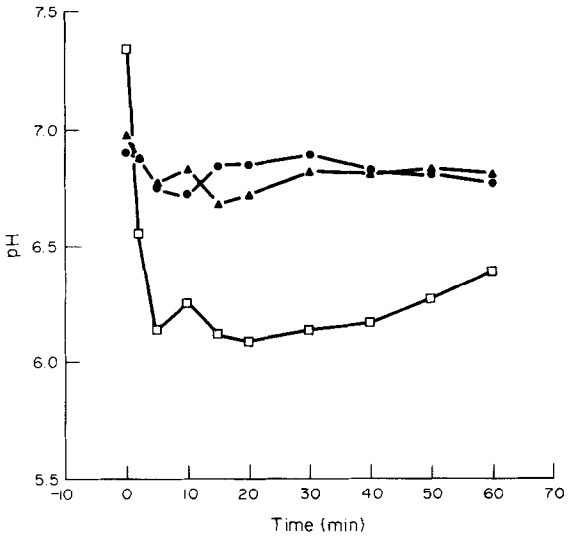

Fig. 2. pH response in dental plaque (mean of two to eight sites in each of 4 patients) following a sucrose mouthrinse after 3 weeks of twice daily mouthrinses with: □ F(12 mmol/l NaF; control solution), ● F–CH (NaF + 1.1 mmol/l CH), and ▲ F–2CH (NaF + 2.2 mmol/l CH).

in each of the 7 (or 4) patients. The one-tailed paired *t*-test was used for statistical analyses of differences between F and the test solutions.

The number of MS adhering to the Dentocult SM-Strip was assessed on a scale from 0 to 3. Classes 0 and 1 corresponded to $<10^5$ colony forming units (CFU)/ml saliva, class 3 corresponded to $>10^6$ CFU/ml saliva, and class 2 corresponded to a count between class 1 and class 3. Wilcoxon signed-rank test (one-tailed) was used for statistical analyses of the effects of F–Zn and F–CH compared with F on SMSC. The level of statistical significance was set at $\alpha = 0.05$.

RESULTS

Effects on acid formation

The mean pH response in dental plaque of all patients following a sucrose mouthrinse after 3 weeks of twice daily mouthrinses with F, F–Zn, and F–CH are shown in Fig. 1. The pH drop in plaque was significantly less after mouthrinses with F–CH compared with F, expressed by significantly greater pH areas ($P < 0.02$ for $area_{0-60}$; $P = 0.005$ for $area_{2-30}$), significantly higher min pH ($P = 0.02$), and significantly smaller ΔpH ($P = 0.002$, Table 1). The pH drop was slightly, but not significantly less after mouthrinses with F–Zn compared with F (Fig. 1, Table 1). However, great intra- and inter-individual differences in pH responses were observed (Table 2). The inhibitory effects after mouthrinses with F–CH and F–2CH were similar in 4 patients who completed both regimens (Fig. 2). This was confirmed by site-specific comparisons of differences between pH $area_{2-30}$ for F and F–CH or F–2CH. F–CH–Zn showed non-significant reduction in the plaque pH drop (data not shown) in 4 patients who completed this regimen.

Effects on salivary mutans streptococcus counts

SMSC varied between $<10^5$ (classes 0 and 1) and $>10^6$ (class 3) CFU/ml saliva after F-mouthrinses (Table 3). F–CH significantly ($P < 0.02$) decreased SMSC (Table 3). F–Zn decreased SMSC in 3 out of 6 patients (Table 3). F–CH–Zn

Table 2. Total plaque pH *area*$_{(0-60)}$*/minimum* pH* *after 3 weeks of twice daily mouthrinses*

Test solutions	Patients						
	A	B	C	D	E	F	G
F (12 mmol/l NaF)†	196/5.9	151/5.2	173/5.5	216/5.5	153/5.2	192/5.9	208/6.0
F–Zn (NaF + 20 mmol/l $ZnCl_2$)	179/5.6	227/6.3	164/5.3	216/6.0	155/5.2	209/5.9	220/6.3
F–CH (NaF + 1.1 mmol/l CH)	254/6.9	152/5.1	203/6.0	230/6.3	153/5.0	217/6.2	227/6.4

*Mean values of two to eight sites in each patient. †Control solution.

Table 3. Salivary mutans streptococcus counts after 3 weeks of twice daily mouthrinses*

Test solutions	Patients						
	A	B	C	D	E	F	G
F (12 mmol/l NaF)†	1	2–3	3	2	3	0	2
F–Zn (NaF + 20 mmol/l $ZnCl_2$)	0	2–3	1	2	2	0	2
F–CH (NaF + 1.1 mmol/l CH)‡	0	1	2	0	0	0	0

*Classes 0 and 1 correspond to $<10^5$ colony forming units (CFU)/ml saliva; class 3 corresponds to $>10^6$ CFU/ml saliva; and class 2 corresponds to a count between class 1 and class 3. †Control solution. ‡$P < 0.02$, significantly lower values than F (one-tailed Wilcoxon signed-rank test).

reduced SMSC by one or two classes in the 4 patients who completed this regimen (data not shown). Following mouthrinses with F–2CH, all of the patients ($n = 4$) had undetectable (class 0) levels of MS (data not shown).

Effects on plaque formation and salivary secretion rate

Mouthrinses with F–CH and F–2CH effectively decreased plaque accumulations (data not shown). Salivary secretion rates were within the same range during the study (data not shown).

Caries development

No systematic registration of caries development was performed but only 1 patient received fillings due to caries during the study period of 6 months.

Adverse responses

5 of the patients complained of the taste of F–Zn and F–CH and/or of a feeling of dryness in the mouth lasting up to 30 min after rinsing. The complaints were most pronounced after mouthrinses with F–Zn. 1 patient sensed a metallic taste of F–Zn lasting up to 2 h after rinses. 3 out of 4 patients complained of the taste and/or of a feeling of dryness in the mouth immediately after mouthrinses with F–CH–Zn and F–2CH.

DISCUSSION

Mouthrinses with 12 mmol/l sodium fluoride in combination with either 1.1 mmol/l (F–CH) or 2.2 mmol/l (F–2CH) CH reduced acid formation by dental plaque (Figs 1 and 2, Table 1). The two NaF–CH-containing mouthrinses were equally effective (Fig. 2). CH is the most potent antiplaque and antigingivitis agent currently available [22]. Moreover, both mouthrinses containing NaF–CH effectively decreased plaque formation even though plaque registration was a rough estimate of the plaque load since plaque accumulations were assessed after the pH electrode had been inserted into the plaque several times during the pH measurements. Hence, reduced amounts of plaque at the pH measurement sites may partly account for the observed inhibitory effects of the NaF–CH-containing mouthrinses on plaque pH responses.

All test solutions contained 12 mmol/l sodium fluoride which corresponds to the recommended daily 0.05% sodium fluoride mouthrinse. Plaque pH responses after fluoride rinses were less pronounced in the present study than reported for hyposalivatory patients by others [6]. In a recent study by Meyerowitz *et al.* [6] mean minimum pH in interproximal plaques which had not been treated with fluoride for at least 12 h, was 4.7 following a sucrose mouthrinse in irradiated patients, at the end of a 4 week period of twice daily mouthrinses with 12 mmol/l sodium fluoride. In the present study plaque had been exposed to fluoride 4 h prior to the pH measurements. Clearance of topically applied fluoride is reportedly retarded in patients with hyposalivation [23]. It is thus possible that fluoride exerted inhibitory effects on acid formation by plaque bacteria 4 h after the last mouthrinse [24]. Daily mouthrinses during a period of 1–2 months with 15 ml of a 48 mmol/l sodium fluoride solution have been shown to reduce plaque acid formation 8–12 h after the last NaF-mouthrinse; the inhibitory effect being most pronounced in subjects with low pre-experimental plaque pH minimum [25]. In the present study plaque pH was monitored preferably at interproximal sites. However, whenever interproximal surfaces were filled, plaque pH was monitored at mesiobuccal or buccal sites. This may partly explain the relatively high plaque pH values recorded [19]. Even though the pH drops were moderate, it should be noted that plaque pH had not yet reached resting levels 1 h after the sucrose challenge (Figs 1 and 2), at which time period plaque pH may return to resting values in young healthy subjects [20].

We chose a period of 4 h between mouthrinsing and pH measurements. The rationale for this is that a plaque antimetabolic agent should be efficient for at least 4 h, and preferably longer, if applied twice daily. It is assumed that binding of both CH and zinc ions to oral surfaces, i.e. mucosa, tooth pellicle, and plaque, and suitable release rates from their binding sites in an active form, and dose, are essential for the inhibitory effects of these agents on plaque pH response and plaque formation [22, 26]. There is evidence that CH and zinc ions bind to the same binding sites in the oral cavity [27]. The reduced salivary secretion rate in the present patients would be expected to favour oral retention of both CH and

zinc ions [7], as has been shown for topically applied fluoride [23]. Furthermore, zinc ions have been found to accumulate in plaque after consecutive mouthrinses in subjects with normal salivary output [17].

Previous studies in healthy subjects, using a surface glass electrode for pH measurements and a different study design, have shown that a single mouthrinse with 0.55 mmol/l CH [11] or 20 mmol/l zinc chloride [12] inhibits plaque acid formation up to 4 h after mouthrinsing. CH (0.44 mmol/l) and zinc ions (8.0 mmol/l), separately and in combination, have also been found to reduce the fall in plaque pH at 90 min following mouthrinses; the combination being more effective than the separate agents [28]. Although the zinc-containing mouthrinses were used twice daily during 3 weeks, neither F–Zn nor F–CH–Zn were found to be significantly better than F (Table 1; data not shown for F–CH–Zn). Possibly zinc ions with or without the addition of CH were less effective in the present patient group due to altered composition and/or diffusion properties of plaque and saliva. The present results may indicate that higher concentrations of antiglycolytic agents are required to inhibit plaque acid formation in xerostomic patients than in subjects with normal salivary output. F–Zn reduced SMSC in only 3 out of 6 patients (Table 3) which is in accordance with previous findings that ecological shifts of the oral microbial flora were not observed after the use of dentifrices containing fluoride and zinc citrate [29].

Various mouthrinse/gel treatments with sodium fluoride and CH have been found to reduce SMSC in patients with irradiation-induced hyposalivation [30–32]; the antibacterial action being attributed to the CH treatment. Sodium fluoride applied in gels or mouthrinses has not been found to reduce the level of MS in plaque [4, 6]. The present study showed that twice daily mouthrinses with 12 mmol/l sodium fluoride in combination with 1.1 mmol/l CH effectively decrease SMSC (Table 3). It should be realised, however, that use of the Dentocult SM-Strip mutans test does not necessarily assess the total viable counts after CH treatment since CH affects the adherence properties of the bacteria [33]. MS may be present in the medium without adhering to the strip, hence, the test may underestimate SMSC following CH treatment [33]. However, the test probably reflects the number of bacteria capable of adhering to tooth surfaces following CH treatment and thus seems useful for the present purpose.

The patients experienced a bad taste and dryness in the mouth after mouthrinses with zinc ions and/or CH. The adverse responses were most pronounced when zinc ions were included in the mouthrinses. The astringent effect of zinc ions is well known [34]. CH causes dryness of the oral mucosa presumably through precipitation of acidic salivary proteins on the mucin layer, thus reducing its lubricating effect [22]. Local adverse effects of CH such as the observed dryness and bitter taste, discolouration of teeth, fillings, and tongue, and taste disturbances are generally less pronounced when lower concentrations are used [22]. It is therefore of particular interest to assess the lowest concentration of CH effective in patients with xerostomia.

The present patients had received irradiation dose of major salivary glands which permanently reduce the salivary output [1–3]. In spite of that, only 1 of the patients experienced a caries problem which required filling therapy during the study period of 6 months. Restoration of caries lesions may decrease SMSC [35], however, this patient had low SMSC throughout the entire study period (Patient A, Table 3). Dental treatment was otherwise confined to professional toothcleaning in 2 patients. This was always performed at the start of a resting period in order to create similar experimental conditions during each test period.

Use of mouthrinses and toothpaste containing acidulated sodium fluoride (10.5 mmol/l) and CH (0.55 mmol/l) in combination have been shown to inhibit development of caries and gingivitis in schoolchildren [36]. Various regimens of topical applications of sodium fluoride and CH [32, 37], including daily mouthrinses with 12 mmol/l sodium fluoride in combination with 2.2 mmol/l CH [37], have previously been suggested for prevention of post-irradiation caries. The present results suggest that twice daily mouthrinses with 12 mmol/l sodium fluoride in combination with 1.1 mmol/l CH may prevent post-irradiation caries as well. This may be due to complementary modes of action of fluoride and CH; NaF–CH elevates plaque pH and fluoride exerts effects on de- and remineralisation processes of the tooth surface. In addition to an anticipated role as a caries-prophylactic agent, the regimen of twice daily mouthrinses with 12 mmol/l sodium fluoride in combination with 1.1 mmol/l CH may contribute to maintain gingival health [22] in patients with irradiation-induced xerostomia.

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