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In vivo Study on the Prevention of Postradiation Caries

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Abstract. Postradiation caries is usually prevented by the application of topical fluorides (F) at high concentrations. The aim of this study was to develop an optimal preventive program for postradiation caries by evaluating the effects of F concentration and application procedures in subjects with radiation-related xerostomia. Six ground enamel slabs were mounted on each side of the lower denture of each of 7 xerostomia patients. Four procedures were used: no F exposure (control), neutral F gel applied every 2nd day or weekly, and a daily rinse with a F mouthwash for a period of 6 weeks. The enamel slabs were analyzed at weekly intervals by scanning optical monitoring, longitudinal microradiography, and scanning electron microscopy. In addition, hardness measurements were performed on the slabs. F analyses of the enamel slabs were done prior to their insertion in the appliances and after 6 weeks of intraoral exposure. In the control experiments severe demineralization of enamel occurred within 6 weeks. Application of F gel or the use of the F mouthrinse resulted in a significant inhibition of the demineralization process. Of the procedures evaluated, F gel applied every 2nd day was the most effective in preventing the onset of postradiation caries.

Postradiation caries, a rapidly progressing highly destructive type of dental caries, is a common side effect of radiation treatment [Del Regato, 1939; Frank et al., 1965; Karmiol and Walsh, 1975]. According to Dreizen et al. [1977], caries lesions can be seen within 3 months and extreme damage of the dentition within 1 year after the start of radiotherapy. In several clinical studies it has been shown that almost complete caries prevention can be achieved in irradiated patients by the daily application of topical fluoride (F) agents combined with a program of strict oral hygiene [Wescott et al., 1975; Dreizen et al., 1977; Katz, 1982; Markitziu et al., 1982; Horiot et al., 1983; Meyerowitz et al., 1986].

The aim of this study was to develop an optimal preventive program for postradiation caries by evaluating the effects of F concentration and application procedures in subjects who have had irradiation treatment for carcinomas of the head and neck.

Materials and Methods

Subjects

Seven edentulous subjects (3 women and 4 men) suffering from radiation-related xerostomia participated in this study. The subjects received an average radiation dose of 55 Gy (range 50–66 Gy) at a level of 2 Gy/day, 5 days/week from a ⁶⁰Co source. The mean age of the subjects was 67.3 years (range 55–73 years). All subjects wore full dentures. The severity of xerostomia was measured by wiping the oral cavity after swallowing with a water-absorbant gauze which was weighed before and after saliva collection. The tests were performed on 3 different days between 10.00 and 10.30 h, and the subjects were not allowed to take food or beverages for 2 h before the test [Vissink et al., 1983].

Experimental Design

In this study a recently developed in vivo model for the investigation of xerostomia-related dental caries was used [Jansma et al., 1988]. Both the left and right molars of the lower denture of each subject were replaced by a metal sample holder [de Bruyn et al., 1985]. Each holder contained six human enamel slabs. The slabs could be removed and replaced by unscrewing the occlusal part of

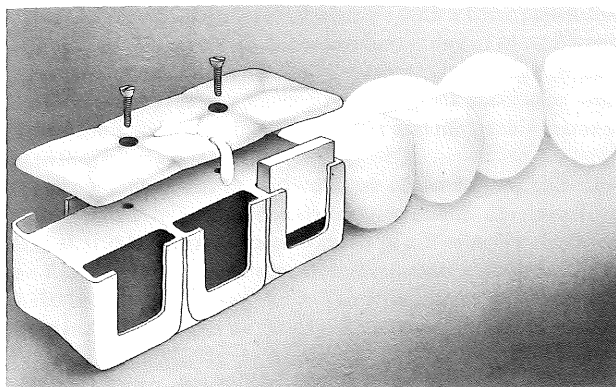


Fig. 1. Sample holder mounted in a lower denture. The slabs can be removed and replaced by unscrewing the occlusal part of the holder.

the holder (fig. 1). About 9 mm² of each slab was exposed to the oral environment.

In all subjects four experiments were performed: procedure A: no F therapy (control); procedure B: 1% neutral NaF gel applied for 5 min every 2nd day; procedure C: 1% neutral NaF gel applied for 5 min once a week, and procedure D: rinsing with 10 ml of a F-containing mouthwash (0.05% NaF; Prodent, Amersfoort, The Netherlands) for 1 min once daily. The subjects received information sheets with instructions for each of the four procedures. The NaF gel was applied with a squeeze bottle (one drop per enamel specimen). The subjects were not allowed to clean the enamel slabs. They were instructed to keep their dentures in tap water during the night.

The enamel slabs were analyzed at weekly intervals using longitudinal microradiography (LMR), scanning optical monitoring (OM), scanning electron microscopy (SEM), and hardness measurements (HM). F analyses of the enamel slabs were done prior to their insertion in the appliances and after 6 weeks of intraoral exposure. Each experiment extended over 6 weeks, and the next was started after an interval of 2 weeks. In each experiment 12 enamel slabs per subject were examined: 4 for LMR and OM, 6 for HM and SEM, and 2 for F analyses. Slabs used for SEM were replaced by acrylic blocks.

Enamel Slab Preparation

The facial enamel surfaces of noncarious human mandibular permanent incisors were partially ground flat on 1,200-grit silicon carbide paper, polished on a Kent Mark II polisher (Engis, Maidstone, England) using Hypress diamond compounds (Engis), and cut in rectangular slabs (3 × 4 × 1.5 mm) by means of a water-cooled diamond saw (Horico, Berlin, FRG). For LMR and OM the lingual aspects of the enamel slabs were ground on 220-grit silicon carbide paper to obtain planoparallel slabs with a thickness of 340 ± 20 μm. All enamel slabs were embedded in cold-cure polymethyl methacrylate (de Trey, Wiesbaden, FRG) and ultrasonically cleaned in tap water for 10 min. Care was taken to keep the experimental facial sides free from acrylic resin.

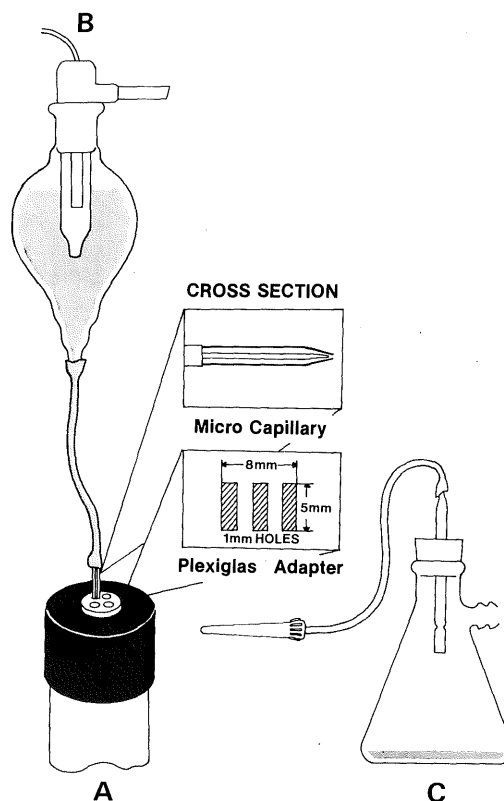


Fig. 2. Apparatus for the measurement of F. A: Selective F electrode; B: reference calomel electrode; C = suction apparatus.

LMR and Scanning OM

By means of dental impression paste (President Regular Body; Coltène, Altstätten, Switzerland) the enamel slabs for LMR and OM were embedded in polymethyl methacrylate sample holders which fitted in both the LMR and the OM experimental setup. In this manner an enamel slab could exactly be repositioned in its individual holder at each measuring interval to be scanned with LMR and OM at the same discrete surface position. LMR was performed as described by de Josselin de Jong [1986] and de Josselin de Jong et al. [1987, 1988]. For OM the optical caries monitor as described by ten Bosch et al. [1980, 1984] and Borsboom and ten Bosch [1982] was applied. Both methods were performed before the start of each experiment and at weekly intervals for the duration of the investigation.

Scanning Electron Microscopy

The enamel slabs were washed under running tap water to remove surface debris and glued on aluminum stubs with fast-curing epoxy resin. When transverse examinations were required, these enamel slabs were also fractured. A thin Au layer (approximately 15 nm) was sputtered on the slabs. Scanning electron micrographs were taken at weekly intervals with a JEOL type 3C (Tokyo, Japan) scanning electron microscope operating at 25 kV.

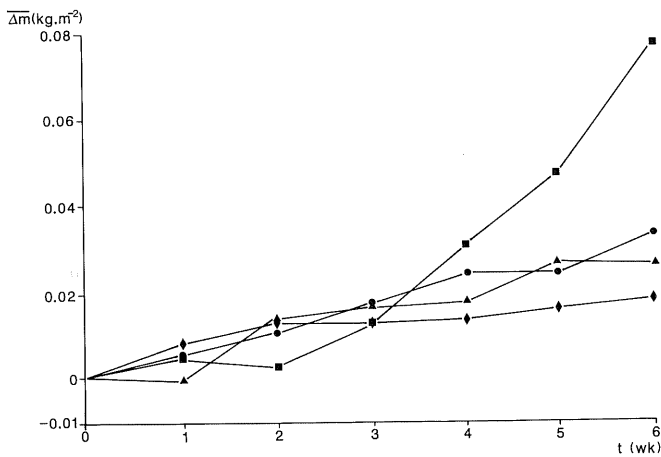


Fig. 3. Mineral loss, Δm ($\text{kg} \cdot \text{m}^{-2}$), of the enamel samples as a function of the time of demineralization (weeks) in the four experiments. Median values ($n = 7$). ■ = No F therapy; ◆ = F gel 1/2 days; ● = F gel 1/week; ▲ = F mouthrinse 1/day.

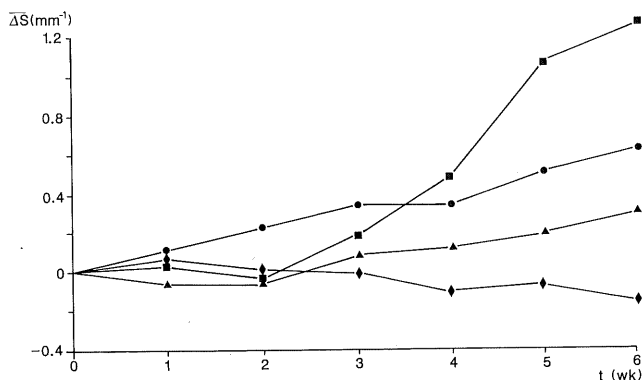


Fig. 4. Change in optical scattering, ΔS (mm^{-1}), as a function of the time of demineralization (weeks) in the four experiments. Median values ($n = 7$). ■ = No F therapy; ◆ = F gel 1/2 days; ● = F gel 1/week; ▲ = F mouthrinse 1/day.

Hardness Measurements

Microhardness measurements were performed with a Leitz Durimet miniload hardness tester with a Knoop diamond (Leitz, Wetzlar, FRG) at a load of 100 g, applied for 20 s. Five indentations were made in a definite pattern in the central area of each enamel slab. The measurements were performed at weekly intervals.

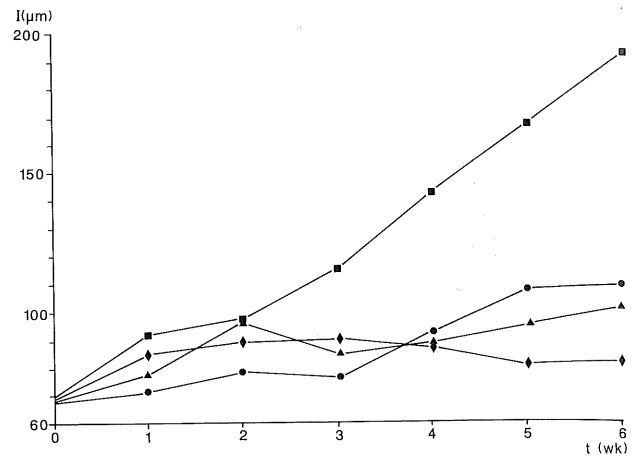


Fig. 5. Indentation length (I , μm) as a function of the time of demineralization (weeks) in the four experiments. Median values ($n = 7$). ■ = No F therapy; ◆ = F gel 1/2 days; ● = F gel 1/week; ▲ = F mouthrinse 1/day.

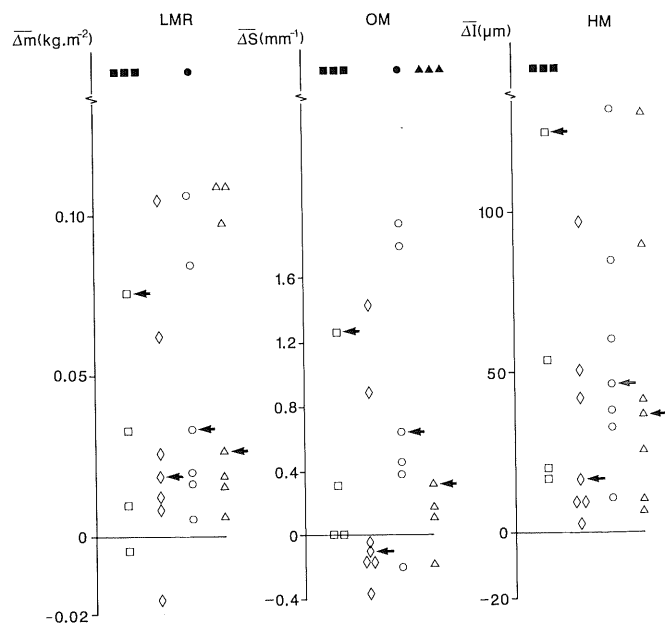


Fig. 6. Range of median values as obtained with LMR, OM, and HM at week 6 ($n = 7$). Δm ($\text{kg} \cdot \text{m}^{-2}$), ΔS (mm^{-1}) and ΔI (μm) indicate the change in, respectively, mineral content, optical scattering and indentation length. □, ■ = No F therapy; ◆ = F gel 1/2 days; ○, ● = F gel 1/week; △, ▲ = F mouthrinse 1/day. Arrows point to the median values as shown in figure 3-5; ■, ●, and ▲ = severely damaged enamel slabs.

Biopsy Procedures and F Analysis

Three successive acid etch biopsies were performed on the ground enamel surface of each enamel slab prior to insertion in the intraoral device. Biopsy sites were demarcated by placing an adhesive tape with a circular hole of 1 mm diameter on the enamel surface. Then 0.4 μl of 1 M perchloric acid was deposited on the de-

marked biopsy site and absorbed after 5 s with a filter paper disc which was placed in a polyethylene tube containing 25 μ l total ionic strength adjustment buffer (Orion Research, Cambridge, Mass., USA). The etched area was washed twice in quick succession with 0.4 μ l total ionic strength adjustment buffer and the washings transferred to the polyethylene tube.

The F concentrations in 5- μ l volumes of the etching solutions were determined by a microanalytical technique developed by Vogel et al. [1983]. The apparatus consists of a F-selective electrode (Orion Research; fig. 2, A) and a calomel reference electrode (fig. 2, B) linked to a microcapillary tube. The P concentrations were determined in 10- μ l volumes by the analytical technique developed by Chen et al. [1956] using a Spectronic 2000 spectrophotometer (Bausch and Lomb, Rochester, N.Y., USA). The mass enamel in the etching solutions was calculated by assuming that enamel contains 18.0% P [S  remark and Samsahl, 1961] and expressed in micrograms. The enamel F concentrations were adjusted to standardized depths of 5 μ m [Retief et al., 1980]. After the enamel slabs were exposed to the intraoral environment for 6 weeks, three successive acid etch biopsies were again carried out on demarcated biopsy sites immediately adjacent to the initial biopsy site, and the enamel F concentrations were again adjusted to standardized depths of 5 μ m.

Statistical Analysis

As a result of the deterioration of the enamel blocks in the oral environment of the xerostomic patients, exact measurements for LMR, OM, and HM were sometimes not possible. These measurements were not omitted, but were considered as being extreme in an untoward direction. The median was used as the summary statistic for measurements from a single subject in a given week. An overall comparison of the results obtained after 6 weeks was accomplished by the generalized signed-rank test [Fidler and Nagelkerke, 1986]. When the results were significant at the 5% level, this test was followed by a pairwise signed-rank test (one-tailed at the 1% level). At an one-tailed 5% level, the latter was used to compare per experiment the results after 6 weeks to those prior to the exposure of the enamel blocks to the oral environment. Logarithms of the adjusted enamel F concentrations were used in the statistical analysis. For each enamel block and for each etch depth (0-5, 5-10, 10-15 μ m) the F acquired by the enamel was calculated by subtracting the adjusted baseline enamel F concentration (week 0) from the adjusted experimental enamel F concentration (week 6). The data were analyzed by means of a multivariate analysis of variance using the SYSTAT statistical package [Wilkinson, 1986].

Results

The subjects participating in this study suffered from moderate to severe xerostomia with the mean \pm SD of the amount of saliva in the oral cavities being 414 ± 218 mg. Healthy subjects who do not use drugs and who have not been exposed to radiation therapy have 1,800-3,000 mg saliva in their oral cavities [Vissink et al., 1983].

Plaque accumulation on the enamel slabs was ob-

Table 1. Treatment differences (p values; one-tailed signed-rank test)

Evaluation method	F gel 1/2 days vs. F gel 1/week	F gel 1/2 days vs. F mouthrinse 1/day	F gel 1/week vs. F mouthrinse 1/day
LMR	0.02	0.05	> 0.20
OM	0.01	0.01	> 0.20
HM	0.11	0.08	> 0.20

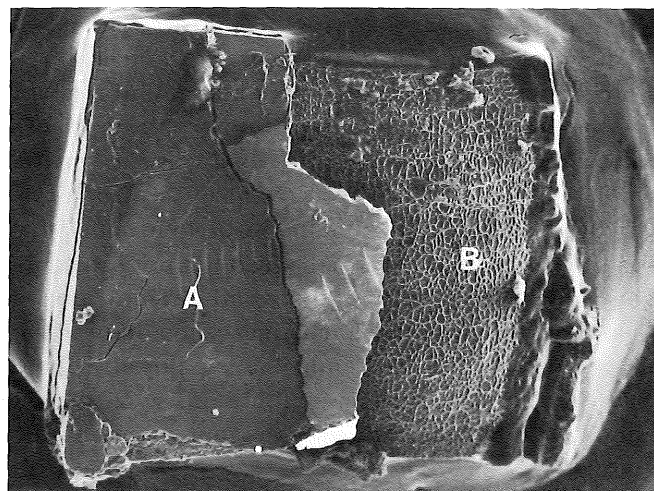


Fig. 7. Overall view of an untreated enamel slab after 6 weeks. A = Presence of a 'relatively' unaffected enamel layer which seems to be loosening of the deeper part of the slab; B = complete absence of enamel and exposure of the underlying dentin. The cracks in the slabs are artefacts (bar 1 mm).



Fig. 8. F-treated enamel slab after 6 weeks. Porosity of enamel surface and starting crater formation (double arrow) can be observed. Single arrows mark the borderline of the exposed part of the slab. Cracks are artefacts in the sample (bar 1 mm).

Table 2. Median F concentration (ppm F) of the enamel slabs at week 0 (n = 49)

Etch depth μm	Minimum value	Percentile			Maximum value
		25th	50th	75th	
0-5	106	263	303	350	662
5-10	189	247	275	339	656
10-15	189	266	299	329	592

served within 1 day. The composition of the oral flora was comparable to the flora which might be expected in postradiation subjects with natural teeth [Weerkamp et al., 1987]. The mineral loss in Δm ($\text{kg} \cdot \text{m}^{-2}$) of the slabs exposed to the control and experimental disciplines is shown in figure 3. The changes in optical scattering ΔS (mm^{-1}) are depicted in figure 4, and the results of the microhardness tests are presented in figure 5. The ranges of variations of the median obtained with LMR, OM, and HM are shown in figure 6. In the control experiment (procedure A), significant demineralization of the slabs was measured with LMR, OM, and HM. Application of F for 6 weeks (procedures B-D) resulted in significant inhibition of the demineralization process. Evaluation by LMR and OM showed that the application of F gel every 2nd day was significantly more effective in reducing the demineralization process than the other F therapies (table 1). HM showed no significant differences among the three F therapies (table 1).

Great variations in the surface morphology of the enamel slabs were observed by SEM. After 3 weeks porous enamel surfaces, starting crater formation, and hollowing of prism cores were observed in most of the control enamel slabs after exposure to the oral environment. The caries process proceeded progressively, resulting in severely demineralized slabs after 6 weeks (fig. 7). In some of the slabs loss of enamel resulted in the exposure of dentin. Enamel slabs exposed to the F therapies showed a reduction in enamel demineralization when compared with the control slabs, but no clear differences were seen between the applied F therapies. In most slabs only the initial stage of enamel demineralization was observed (fig. 8).

The median adjusted F concentration of the enamel slabs at the three depths studied prior to the

Table 3. Median F uptake per enamel block, $[\text{F}^-_{6\text{w}}]/[\text{F}^-_{0\text{w}}]$, under the different experimental conditions

Experiment	$[\text{F}^-_{6\text{w}}]/[\text{F}^-_{0\text{w}}]$	95% confidence limits
No F	1.5*	1.2-2.0
F gel 1/2 days	4.1**	3.5-5.0
F gel 1/week	3.1	2.6-3.7
F mouthrinse	3.7	3.1-4.4

* $p < 0.001$ (no F vs. F experiments); ** $p < 0.05$ (F gel 1/2 days vs. F gel 1/week).

insertion in the intraoral device (week 0) is given in table 2. The adjusted baseline enamel F concentrations at the three etch depths were not significantly different ($p > 0.10$). The adjusted experimental enamel F concentrations increased in the 44 enamel slabs exposed to the control and F therapies for 6 weeks ($p < 0.01$). Enamel demineralization was so severe in 5 of the enamel slabs that acid etch biopsies could not be performed. This increase varied significantly among subjects ($p < 0.001$), among the various procedures ($p < 0.001$), and at different depths ($p < 0.05$), but the interactions between the three parameters, subjects, procedures, and etch depths were not significant ($p > 0.10$).

The increase of enamel F concentration at the first etch depth was estimated to be 1.2 times greater than the increase at the third etch depth. The increase in enamel F concentration depends only slightly on etch depths and can conveniently be described by one number. The 'median increase per enamel slab' observed in the different treatment procedures is presented in table 3. In the control slabs the enamel F concentration increased by a factor of 1.5; on exposure to the F therapies the factors ranged from 3.1 to 4.1. The differences between the control and F procedures were significant ($p < 0.001$). The factor obtained for the application of F gel every 2nd day was significantly different from the factor for the weekly application of the F gel ($p < 0.05$).

Discussion

Previous studies carried out in our laboratory showed that the enamel F concentrations in adjacent sites on unground surfaces varied significantly [Benediktsson et al., 1982]. Removal of the surface enamel

by grinding resulted in enamel F concentrations in adjacent sites being not significantly different. This was the reason that ground enamel slabs were used in the present study. Intradental control was, therefore, possible, and the F acquired at each etch depth was obtained by subtracting the adjusted baseline enamel F concentrations (0 weeks) from the corresponding adjusted experimental F concentrations (6 weeks).

It is apparent from the results that application of F resulted in a significant reduction of demineralization. With both LMR and OM it was shown that F gel applied every 2nd day was significantly more effective than the other F therapies. The differences in the LMR and OM values (positive versus negative values; F gel $\frac{1}{2}$ days; fig. 3, 4 and 6) may be interpreted as slight depositions of organic material on the surface. This translucent layer did not influence the LMR data, while it diminished optical scattering \bar{S} . F analysis demonstrated a significantly higher F uptake after F gel applied every 2nd day compared to the weekly gel therapy. Hardness measurements, however, did not show significant differences between all therapies, although there was a tendency which indicated a greater inhibitory effect of F gel applied every 2nd day. This may be explained by the fact that indentation length on whole samples is mainly a qualitative parameter for the outer enamel region. In case of subsurface lesions, the indentation length does not give details of the hardness changes below the surface nor in different regions of the lesion [Featherstone et al., 1983].

Although F gel applied every 2nd day was the most effective therapy in this study, slight demineralization of enamel was still observed. The necessity for additional oral hygiene measures is stressed by this observation. This is in accordance with reported data that complete postradiation caries prevention can be accomplished with the use of high-dose F therapies and strict adherence to oral hygiene procedures [Dreizen et al., 1977; Reynolds et al., 1980; Markitziu et al., 1982; Katz, 1982; Meyerowitz et al., 1986]. F mouthrinses used once daily, which are easy to perform, could result in a much higher degree of patient compliance [Katz, 1982]. In the present study the daily use of F mouthrinses was only effective in the subjects with moderate demineralization of the enamel slabs. In all other subjects the use of F mouthrinses once daily was inadequate to prevent demineralization. It is, however, difficult to define the caries susceptibility at the onset of the preventive treatment. Therefore,

the application of a F gel every 2nd day and a strict oral hygiene regimen are recommended as an optimal procedure to inhibit the onset of postradiation caries.

From this study it may be concluded that F gel applied every 2nd day was the method of choice among those tested for preventing the onset of postradiation caries.

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