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In vivo remineralization of enamel at the buccal and proximal sites using two fluoride regimens

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Abstract

The purpose of this in vivo study was first to design a model that theoretically would be superior to models currently used for assessing the demineralization and reprecipitation phenomena in white spot lesions. This model then would be used to compare the effectiveness that a control regimen and 2 low concentration fluoride regimens would have with respect to the remineralization of artificial white spot lesions located at both the buccal and proximal sites.

A cross-over design was implemented in which each subject participated in three 2-week regimens: (1) brush twice daily with optimally fluoridated water; (2) brush twice daily with 0.243% sodium fluoride dentifrice; and (3) brush twice daily with 0.243% sodium fluoride dentifrice plus rinse twice daily with 0.022% sodium fluoride solution. The treated artificial enamel lesions were analyzed quantitatively using polarized light microscopy. The in vivo model was unique and reliable. There was significant ($P < 0.01$) remineralization of the white spot lesions in all media but there were no significant differences ($P > 0.05$) detected at either site or with the 3 test regimens.

Fluoride has been recognized as a valuable therapeutic agent to provide partial protection against dental caries for approximately one-half century. Delivered in optimal amounts in the community water supply, fluoride provides a caries reduction of 60% (Mellberg and Ripa 1983). Fluoride mouthrinses, used weekly or biweekly on school-based preventive programs or daily at home, have been shown to reduce the incidence of dental decay by 30% (Carlos 1985). Similar reductions have been found with fluoride dentifrices (Stookey 1985). Regardless of the modality of delivery, fluoride is recognized by the dental profession as a valuable agent to provide partial protection against dental decay.

Fluoride has been shown to increase the resistance of enamel to acids, increase the maturation rate of enamel, and interfere with the metabolism of microorganisms (Mellberg and Ripa 1983). Recent evidence suggests that the primary mechanism of action of fluoride may be its ability to facilitate the remineralization of white spot lesions (Silverstone 1982). It has been established that a low concentration, high frequency regimen is more cariostatic than a high concentration, low frequency regimen. The greatest reduction in caries occurs on smooth surfaces; however, the effectiveness of fluoride on the buccal or lingual surfaces compared to the proximal surfaces is relatively unknown.

For approximately 20 years, cariologists have been developing in vivo models for investigating the demineralization and reprecipitation phenomena on dental tissue specimens. These models have been reported to have various shortcomings. Polarized light microscopy may be the most sensitive and descriptive analytical technique for evaluating the histological changes in white spot lesions (Theuns and Groenveld 1977; Wefel and Harless 1984; Silverstone 1985).

A unique model was designed to assess the demineralization and reprecipitation phenomena in white spot lesions. This model then would be used to compare the effectiveness between a control regimen and 2 low concentration fluoride regimens with respect to the remineralization of artificial white spot lesions located at both the buccal and proximal site.

Materials and Methods

The 5 human volunteers selected for this study consisted of 4 males and 1 female ranging in age from 24 to 45 years with an average age of 30 years. A visual-tactile screening examination revealed that the volunteers had past histories of dental decay but all were currently caries free. All volunteers selected: required a full veneer crown on the mandibular first or second molar, or had space in the area allowing for the insertion of a prostheses; had a DMFT equal to or above 12; exhibited an acidogenic microflora during a microelectrode plaque pH test; and did not have medical or dental

conditions that may effect the carious process. Subjects required a resting pH value greater than 6 and a pH response to a 10-ml 5% sucrose solution of at least 0.5 pH units (Jensen and Schachtele 1983).

The appliances used were either a full veneer crown or a removable prostheses (Fig 1). Receptacles of approximately 2 x 4 mm, angulated apically to increase their depth, were prepared at the buccal and proximal site for the placement of enamel sections. The proximal receptacle was located below the contact point in the area in which proximal carious lesions commonly occur. This location would not allow plaque to be removed during brushing or mastication.

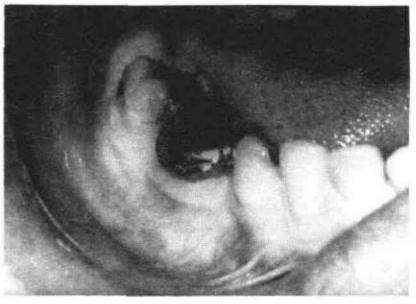

FIG 1. Intraoral view showing the buccal and interproximal receptacles of a full veneer test crown. The enamel section was located below the contact point simulating a natural oral phenomenon.

Artificial caries-like lesions were prepared using newly erupted human premolars. The entire tooth was painted with varnish except for an area of approximately 1 x 6 mm on the buccal and/or lingual middle one-third surface of their crown. The teeth then were suspended into a 17% undialysized acidified gel system containing calcium, phosphate, and fluoride at a pH of 4.2. After the lesion formed, enamel sections were cut and then polished to an average thickness of 125 μm. Each enamel section was photomicrographed using polarized light microscopy in 5 imbibition media: water, air, Thoulet's media 1.41 and 1.47, and quinoline. They were trimmed and secured with varnish into their designated receptacle. Only the teeth that yielded 6 or more acceptable sections were used in this study.

A cross-over design was implemented. Each subject participated in three 2-week test regimens as follows: (1) brush with 1 ppm of fluoridated water; (2) brush with 0.243% sodium fluoride dentrifice (Crest Dentrifice — Proctor and Gamble; Cincinnati, OH); and (3) brush with 0.243% sodium fluoride dentrifice plus rinse with 0.022% sodium fluoride mouthrinse (Listermint Mouthrinse — Warner-Lambert; Morris Plains, NJ).

The regimen sequence which each subject followed was determined randomly. The subjects were instructed to brush and/or rinse twice per day and to avoid flossing the proximal test site. For regimen 3, the rinsing and brushing were performed at different times during the day. The subjects also were required to avoid all other fluoride products throughout the experiment and to continue with their normal dietary habits.

After each 2-week test session, the appliances were removed from the mouth. The enamel sections then were removed from their receptacles, photomicrographed, and new enamel sections were placed into the appliance. The changes which took place in the internal pore volume of the surface zone, body, and dark zone of each enamel section were quantitated by one investigator who traced the boundary of the 3 zones with a GP-6-50 digitizer (Science Accessories Corp — Southport, CT; Fig 2).

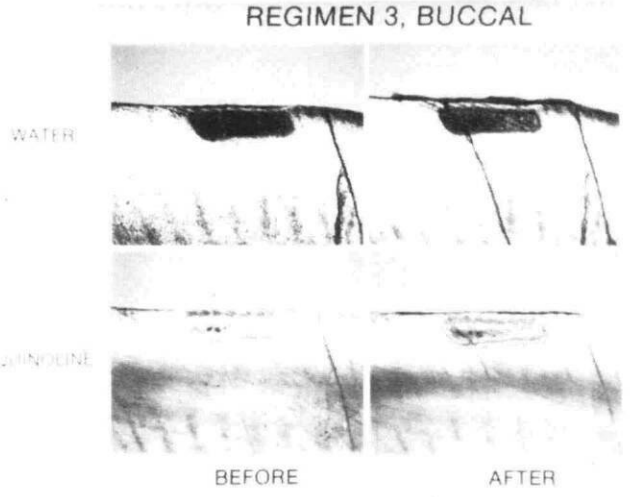

FIG 2. Photomicrographs of an undecalcified ground section through an enamel lesion treated in regimen 3 (sodium fluoride dentrifice plus sodium fluoride mouthrinse) at the buccal site. The surface zone (S) and dark zone (D) have substantially increased in size at the expense of the body (B).

Figure 3 (next page) illustrates the design devised for this experiment. Two teeth were assigned to each subject. The enamel sections were removed and new ones were placed at the end of each 2-week session. Therefore, for each session there was an enamel section placed from each tooth at the buccal and proximal site. This enabled us to account for the tooth within a subject variability by an ANOVA test. The section-to-section variability was minimized by randomly assigning the 6 sections from each tooth to the 2 x 3 factorial of sessions and positions. The change in area after 2 weeks of oral exposure for the surface zone, body, and dark zone at both sites for all 5 imbibition media was computed.

Results

The carious-like lesions had substantial remineralization in all regimens at both sites after 2 weeks of intraoral exposure. Tables 1, 2, and 3 (next page) reveal that the remineralization of the caries-like lesions in all media were insignificant ($P > 0.05$) between the regimens and sites but were significant ($P < 0.01$) between subjects.

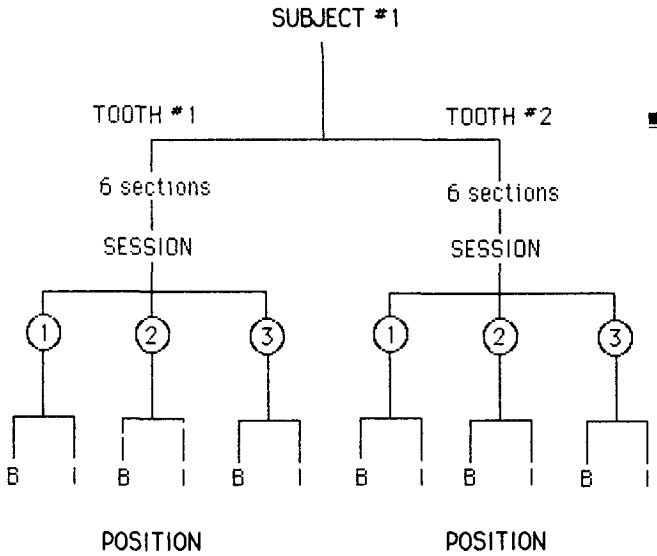

FIG 3. Diagram of the experimental model. Two teeth were assigned to each subject. For each session, 1 section from each tooth was placed at both the buccal (B) and proximal (P) sites.

TABLE 1. ANOVA Test Used to Detect Changes in the Area of the Surface Zone Using Water Media

Source	*DF*	*SS*	*MS*	*F Value*	*PR>F*
Model	14	1.495	0.107	6.23	0.000
Source	44	0.737	0.017		

Source	*DF*	*SS*	*F Value*	*PR>F*
Area	1	0.801	46.71	0.000
Subject	4	0.363	5.29	0.002
Tooth (subject)	4	0.306	4.46	0.004
Session	2	0.000	0.00	0.998
Position	1	0.000	0.00	0.978

TABLE 2. ANOVA Test Used to Detect Changes in the Area of the Body Using Water Media

Source	*DF*	*SS*	*MS*	*F Value*	*PR>F*
Model	14	0.432	0.031	4.91	0.000
Source	44	0.276	0.006		

Source	*DF*	*SS*	*F Value*	*PR>F*
Area	1	0.003	0.50	0.484
Subject	4	0.380	15.10	0.000
Tooth (subject)	4	0.030	1.17	0.336
Session	2	0.006	0.46	0.632
Position	1	0.000	0.06	0.816

TABLE 3. ANOVA Test Used to Detect Changes in the Area of the Dark Zone Using Quinoline Media

Source	*DF*	*SS*	*MS*	*F Value*	*PR>F*
Model	14	29.358	2.097	2.22	0.022
Source	44	41.560	0.944		

Source	*DF*	*SS*	*F Value*	*PR>F*
Area	1	4.658	4.93	0.032
Subject	4	15.594	4.13	0.006
Tooth (subject)	4	1.368	0.36	0.834
Session	2	4.345	2.30	0.112
Position	1	0.219	0.23	0.633

Of the 3 zones evaluated, the most significant remineralization ($P < 0.001$) occurred in the surface zone (Table 1). The greatest increase was 33.9% in regimen 1 at the buccal site and the lowest increase was 16.3% in regimen 2 at the proximal site (Table 4 – next page). The surface zone increased by 25.6% at the buccal site compared to 21.2% at the proximal site (Table 5 – next page).

Table 2 shows that the area of the body decreased in water media at an insignificant level ($P > 0.484$). The data obtained in water media was consistent and reproducible as compared to the data obtained in air and Thoulet's media. Therefore, changes in the body will be discussed in water media only.

The control regimen was less effective than the fluoride regimens in reducing the area of the body at the buccal site. However, the control regimen was more effective than the fluoride regimens at the proximal site. The mean reductions in the body for the fluoride regimens were 18.6% buccal and 15.1% proximal, whereas for the control regimen they were 13.5% buccal and 16.1% proximal (Table 6 – next page).

Approximately 90% of the enamel lesions did not form a dark zone after 6 weeks of exposure in the acidified gel system. After the 2-week test sessions, approximately 80% of the enamel lesions either formed or had an increase in the area of the dark zone (Table 7 – next page). Although the dark zone significantly increased ($P < 0.03$) in area, there were no significant differences ($P > 0.05$) detected among the 3 test regimens at either site (Table 3).

Discussion

Wefel and Harless (1984) reported that the acidified gelatin gel system, which was used in this study, is currently the best system for producing artificial lesions that mimic natural lesions. The best method to analyze changes in the enamel sections is by polarized light microscopy. Theuns and Groenveld (1977) observed that photomicrographs will show changes in optical properties before they are observable on microradiographs. Silverstone (1985) also reported that polarized light microscopy is a more sensitive and descriptive analytical technique than microradiography.

The prevention of dental decay on the smooth surfaces appears to be becoming an increasing concern since dental decay in pits and fissures is effectively inhibited by the placement of sealants. This model, therefore, may be commonly used to compare simultaneously the buccal and proximal sites because the methodology is reproducible. Only 1 of the 60 enamel sections used was lost (Wefel et al. 1987).

TABLE 4. Per Cent Change in the Area of the Surface Zone in All Regimens at the Buccal and Proximal Site

Regimen	*% change and standard deviation*	
	Buccal	*Proximal*
1	34.0 ± 24.1	21.1 ± 12.4
2	23.4 23.6	16.3 11.2
3	20.2 ± 23.7	26.8 ± 22.1

Mean values for all human volunteers.

TABLE 6. Per Cent Change in the Area of the Body in Water at the Buccal and Proximal Site

Regimen	*% Change and Standard Deviation*	
	Buccal	*Proximal*
1	13.5 ± 10.2	16.1 ± 11.6
2	17.9 9.2	15.7 8.6
3	19.3 ± 13.7	14.4 ± 13.7

Mean values for all human volunteers.

Previous studies have used screening criteria that were limited to evaluating the oral cavity of the human volunteers, reviewing their past and present medical and dental health conditions, and evaluating their compliance. Additional screening criteria was included, aimed at estimating the cariogenic status of the experimental group. However, the ANOVA test revealed significant differences ($P < 0.02$) between the subjects for the remineralization of white spot lesions in all imbibition media (Tables 1, 2, 3). The development of tests that would accurately and consistently determine the cariogenic status of an individual may reduce the subject variance. For instance, taking the incidence of new decay over a 1-year period is probably the most accurate method to determine the cariogenic status of an individual. However, to do this requires a relatively long time period and ethical problems may occur if radiographs are used as part of the diagnosis.

The significant variability ($P < 0.02$) between the subjects was not a surprising finding. Similar experiments have reported a substantial variability in groups of 3-11 subjects (Koulourides 1974; Mobley 1981; Featherstone et al. 1982). Stookey et al. (1985) reported a significant variability ($P < 0.001$) between subjects in 4 experiments with each using approximately 20 subjects. By not controlling and testing for the variability among subjects, the validity of similar studies may be questionable. It is therefore important to include in the experiment a cross-over design to control for subject variability. Also, the subject variance should be accounted for in the total variance, thereby increasing the sensitivity of the experiment.

Stookey et al. (1985) reported reproducible results in their intraoral test model and correlations have been made between various analytical techniques (Featherstone et al. 1982; Wefel and Harless 1984). Additional research is required to determine if the methods and materials used in other model systems are reproducible, such as in studies using natural carious lesions or artificial caries-like lesions analyzed by polarized light microscopy or microradiography.

TABLE 5. Per Cent Increase in the Area of the Surface Zone and Per Cent Decrease in the Area of the Body at the Buccal and Proximal Site

Regimen	*% Change*				
	Surface Zone	*Body*			
	Water	*Air*	*Water*	*Th 1.41*	*Th 1.47*
Buccal	25.6	3.3	17.1	21.8	37.0
Proximal	21.2	1.7	15.4	14.6	27.0
% difference	4.2	1.6	1.6	7.1	10.0

Mean values for all human volunteers.

TABLE 7. Number of Lesions Which Either Formed or had an Increase in the Size of the Dark Zone

Regimen	*Position*	
	Buccal	*Proximal*
1	7 of 9 (77.8%)	8 of 10 (80.0%)
2	9 of 10 (90.0%)	8 of 10 (80.0%)
3	8 of 10 (80.0%)	8 of 10 (80.0%)

Mean values for all human volunteers.

Similar studies have used artificial caries systems that have been shown to develop lesions with a thin and porous surface layer (Wefel and Harless 1984). Interestingly, only a few investigators commented on or illustrated the characterization of their artificial lesions. None of the studies directly compared the artificial lesions with natural lesions. Furthermore, only one of them used polarized light microscopy (Wefel et al. 1987). Compared to natural lesions, these "porous" lesions would acquire more fluoride in less time, thereby increasing the chance of obtaining statistically significant results. In this study, the caries-like lesions were substantially remineralized in all regimens after 2 weeks of oral exposure. The authors would find it interesting to measure the fluoride uptake into the enamel for the 3 regimens and then compare these results to those obtained in clinical trials and to other in vivo human experiments.

The authors expected to find the least degree of remineralization, if any, with the control regimen which consisted of brushing twice daily with optimally fluoridated water. The ANOVA test revealed no significant differences ($P > 0.05$) between the control regimen and

the fluoride regimens. In fact, the surface zone in the control regimen increased in area more than the dentifrice regimen and the dentifrice plus mouthrinse regimen.

Another example of remineralization occurring in the control regimen is illustrated in the data obtained for the dark zone. Similar to the surface zone, the dark zone is a product of remineralization. The results in Table 7 show that the number of lesions which either formed or had an increase in the size of the dark zone was comparable for all 3 regimens.

There were no additional benefits obtained using the sodium fluoride (NaF) mouthrinse and NaF dentifrice combination as opposed to using the NaF dentifrice alone. Perhaps patients who have a highly cariogenic microflora and frequently snack on cariogenic foods would obtain a cost-benefit ratio indicating the daily use of an NaF mouthrinse. Additional research is required to determine which patient's preventive dental plan should be supplemented with an NaF mouthrinse. Until this information is available, dental practitioners should recommend the use of an NaF mouthrinse for caries-active or high-risk patients, and particularly for medically or mentally compromised patients.

Controlled human clinical trials (Ast et al. 1956; Backer-Dirks 1961) in optimally fluoridated water communities have reported comparable reductions in caries between the buccal-lingual and proximal surfaces. In one study, Backer-Dirks (1974) measured the caries reduction on permanent teeth in 15-year-old children in fluoridated and nonfluoridated communities. He reported fluoride to be 13% more effective at the buccal-lingual surfaces than at the proximal surfaces. Jensen (1984) has shown that proximal plaque has a lower resting pH and a prolonged minimum pH response to a sucrose challenge with a retarded return toward resting pH levels. Clinical observations of the subjects consistently revealed greater amounts of plaque and debris at the proximal than buccal-lingual surfaces.

The above-mentioned comments suggest that proximal surfaces are more susceptible to dental decay than buccal-lingual surfaces. Although insignificant ($P > 0.05$), there appears to be a trend for greater remineralization at the buccal site. Perhaps extending the timeframe of the test sessions beyond 2 weeks may make this finding significant.

It can be concluded that the subjects used in this experiment could remineralize white spot lesions located at either the buccal or proximal site without the aid of topical fluoride therapy. The next step is to test the anticaries efficacy of various preventive regimens in high-risk or caries-active individuals who would benefit most from topical fluoride therapy. However, problems may arise in obtaining human consent for a control group and with compliance of this particular subject type.

Conclusions

1. The in vivo model developed to assess the demineralization and reprecipitation phenomena in artificial white spot lesions using polarized light microscopy appears to be unique and reliable.
2. There was a substantial difference ($P < 0.01$) between subjects for the remineralization of white spot lesions in all media.
3. The white spot lesions substantially remineralized in all regimens after 2 weeks of oral exposure.
4. There were no significant differences ($P > 0.05$) found between the control regimen and 2 low-concentration fluoride regimens in their efficacy to remineralize the white spot lesions.
5. There were no significant differences ($P > 0.05$) found between the degree of remineralization in white spot lesions located at the buccal and proximal sites.

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Ast DB, Smith DJ, Wachs B, Cantwell KT: Newburgh-Kingston caries-fluorine study XIV. Combined clinical and roentgenographical dental findings after 10 years of fluoride experience. J Am Dent Assoc 52:314-25, 1956.

Backer-Dirks O: Longitudinal dental caries study in children 9-15 years of age. Arch Oral Biol 6:94-108, 1961.

Carlos JP: Fluoride mouthrinses, in Clinical Uses of Fluorides, Wei SHY, ed. Philadelphia; Lea and Febiger 1985.

Featherstone JDB, ten-Cate JM, Shariati M, Arends J: Comparison of artificial caries-like lesions by quantitative microradiography and microhardness profiles. Caries Res 17:385-91, 1983.

Jensen ME, Schachtele CF: Plaque pH measurements by different methods on the buccal and approximal surfaces of human teeth after a sucrose rinse. J Dent Res 62:1058-61, 1983.

Koulourides T, Phantumvanit P, Munksgaard EC, Housch T: An intraoral model used for studies of fluoride incorporation in enamel. J Oral Path 3:185-96, 1974.

Mellberg JR, Chomicki WG: Fluoride uptake by artificial caries lesions from fluoride dentifrices in vivo. J Dent Res 62:540-42, 1983.

Mellberg JR, Ripa LW: Fluoride in Preventive Dentistry: Theory and Clinical Applications. Philadelphia; Quintessence Pub Co 1983.

Mobley MJ: Fluoride uptake from in situ brushing with a stannous fluoride and a sodium fluoride dentifrice. J Dent Res 60:1943-48, 1981.

Silverstone LM: Fluorides and remineralization, in Clinical Uses of Fluorides, Wei SHY, ed. Philadelphia; Lea and Febiger 1985.

Stookey GK: Are all fluorides the same? in Clinical Uses of Fluorides, Wei SHY, ed. Philadelphia; Lea and Febiger 1985.

Stookey GK, Schemehorn BR, Cheetham BL, Wood GD, Walton GV: In situ uptake from fluoride dentifrices by carious enamel. J Dent Res 64:900-903, 1985.

Theuns HM, Groeneveld A: Polarizing microscopy of sound enamel. Caries Res 11:293-300, 1977.

US Department of Health and Human Services: National Dental Caries Prevalence Study — The prevalence of dental caries in United States children. Washington, DC; US Department of Health and Human Services 1979/1980.

Wefel JS, Harless J: Comparison of artificial white spots by microradiography and polarized light microscopy. J Dent Res 63:1271-75, 1984.

Wefel JS, Maharry GJ, Jensen ME, Harless JD: Development of an intraoral single-section remineralization model. J Dent Res 66:1485-89, 1987.

Going to the dogs

There's a war going on in California over who should clean dog's teeth.

According to a recent *Wall Street Journal* article, pet groomers who charge about $5 to clean a dog's teeth say there is a veterinarian-backed conspiracy to drive them out of the dog tooth-care business. The vets, who get about $100 for dog-tooth prophys, say that allowing pet groomers to clean dogs' teeth is like letting hairdressers perform surgery.

State officials, insistent that dogs' teeth should be cleaned only by licensed vets, sent in an undercover pooch to break up what they considered an illegal dog tooth cleaning operation.

The groomer and dog tooth cleaner involved promptly sued the state, noting that the state's business and veterinary-medicine codes do not prohibit groomers from cleaning dogs' teeth.

A Superior Court judge ruled that there is no wrongdoing in brushing, flossing, or even scraping a dog's teeth with metal dental instruments. He did, however, grant a state-sought injunction prohibiting groomers from using ultrasonic tooth-cleaning devices or metal scrapers above or below the gumline.

The size of the dog-tooth cleaning business is hard to estimate. According to the WSJ, a good dog tooth cleaner can easily see a hundred patients a month. There are also 3000 vets in California–including a few who specialize in dog dentistry.

Both sides do agree on one thing: dogs that get their teeth cleaned regularly have fresher breath.