IN VIVO EVALUATION OF EFFECT FLUORIDE VARNISH ON BACTERIAL COLONIZATION OF TOOTH ENAMEL USING SCANNING ELECTRON MICROSCOPY

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SUMMARY: The purpose of this study was to assess the antimicrobial effect of Duraphat, a fluoride varnish. The Duraphat-treated human enamel specimens were mounted on one side of maxillary removable space-maintainers with the untreated ones on opposite sides as control specimens. After 24 hours and one week the amount and nature of plaque accumulation were examined under scanning electron microscope (SEM). The majority of the organisms were found to be coccal following the examinations made on specimens at 24 hours. T here seemed to be no significant difference in the amount of plaque accumulations between the experimental and control specimens at 24 hours. After one week however a thick layer of bacteria was observed to cover enamel surfaces, which was less dense on Duraphat-treated specimens compared to those of the control cases.

Key Words : Fluoride varnish, plaque formation, SEM.

INTRODUCTION

The mechanisms of action of fluorides have been investigated for many years. In many ways, fluorides represent an ideal agent as a public health measure against dental caries (a disease of microbial etiology); since: a) It stabilizes the tissues at risk (enamel and dentin), b) It reduces the advantage of aciduric organisms within the plaque and c) It does not change the normal oral flora (as would an antibiotic), but reduces the acidogenity of cariogenic bacteria (1).

There are also substantial evidence that fluorides, influence metabolism of plaque microbiota (2), possibly their adhesion to the enamel surface (3), inhibition of microbial enzyme system (1), reduction of growth rates of bacteria (4,5) and possible bactericidal effect (6).

One of the factors that are also considered to be important is the release of F^- ions when CaF_2 is dissolved, producing an antimicrobial effect (7).

In the initial stage of plaque formation, bacteria can adhere to the pellicle by means of interaction with the salivary proteins, or in some instances, by direct adherence to the enamel surface (8). In the absence of adequate oral hygiene, plaque continues to build up in area, thickness, wait and numbers and types of

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bacteria. Increase in plaque thickness is important in caries since it effects the rate and amount of acid production and diffusion into and out of plaque (9).

Fluoride varnishes which are developed as alternatives to the convential topical applications, mainly to prolong contact time between fluoride and teeth, are toxicologically safe, easy to use, and exhibit excellent cariostatic properties (10).

The product used by Schmidt (1964) was later marked as Duraphat and contains 5wt% sodium fluoride or 2.26wt% F - in a neutral colophonium base. Several studies have shown it to be effective in reducing caries (10).

The purpose of this investigation was to evaluate the effect of Duraphat on bacterial deposition on dental enamel surface in vivo using Scanning Electron Microscopy.

MATERIALS AND METHODS

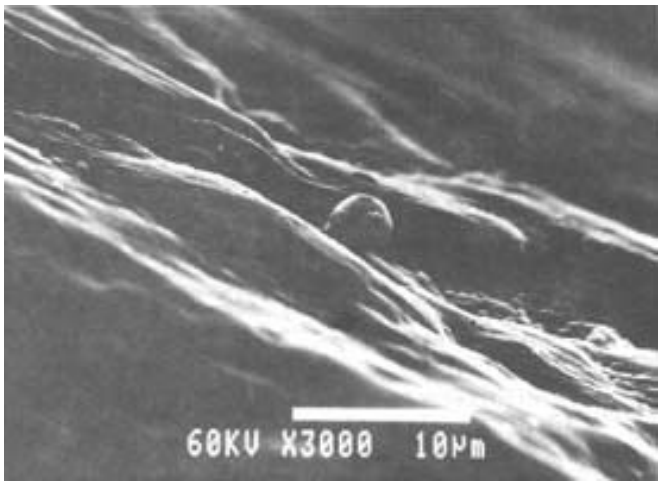
Cylindrical enamel sample blocks of 2 mm height and 4 mm diameter were sectioned from randomly selected smooth surfaces of extracted permanent human molars using a high speed cylindrical drill under coolant water. The samples were, then cleaned using flour of pumice prior to sterilization with ethylene-oxide for 8 hours. 20 maxillary appliances were constructed with a consecutive preparation of recesses, opposing each other on the palatal portion for the samples to be fitted in such a manner that the enamel blocks would keep the same level with the acrylic base when embedded in place with sticky wax.

Before both the experimental and control samples were fixed, subjects wore the appliances first with only Duraphat-treated enamel blocks embedded on one side and the opposite recess filled with wax for 24 hours. Following this procedure control samples were fixed into the initially wax filled recesses of the appliances. Having been divided into two equal groups the appliances, each containing both samples were worn again for 24 hours and one week to be removed after words for further examination under SEM. Children with maxillary appliances were instructed not to brush space maintainers and blocks, but to rinse them in tap water only (the drinking water was not fluorinated). Tooth brushing of the residual dentition was performed with conventional F - containing toothpaste.

Preparation for the SEM examination

Test and control specimens of enamel surface with adhering deposits were removed from the appliances to be kept in a 1% cacodylate buffer solution containing 1% gluter-

Figure 1: SEM micrograph of enamel worn in the mouth for 24 h. Bacteria population is comprised mostly in coccal forms. Orig. marg.x3000.



aldehyde and 4% formaldehyde at +4°C for 24 hours. As for the post-fixation procedure; after the fixative agents washed away with cacodylate buffer, samples were again kept for 2 hours in another solution (%1 osmium tetroxide (OsO_4) buffered with cacodylate buffer) at a low temperature room. Following the repeated cleansing procedure, with cacodylate buffer, samples were dehydrated in increasing concentrations (25%, 50%, 75%, 100%) of acetone. A fluorocarbon compound (Peldri II, pelcocat) was used to maintain optimal dehydration, that is to say, the removal of tissue fluid while avoiding any collapse in the internal structure of bacteria (11). The next step was to keep the samples in a low-vacuum field for 3 hours so as to remove the tissue adhered to Peldri II. Finally, the specimens were mounted on metal stubs, specially designed for the SEM investigations with a conductive silver paint and, then, coated with a 20 μm (20°C A) thick layer of gold with a sputter device (BIO-RAD SEM coating system).

Samples were examined under a JOEL-brand (SEM ASIDIO) scanning electron microscope operated at 40KV, 60KV and 80KV.

RESULTS

Although the deposit and bacterial accumulation on the specimens varied in appearance and thickness, the SEM micrographs presented, do seem to represent the surface of enamel specimens.

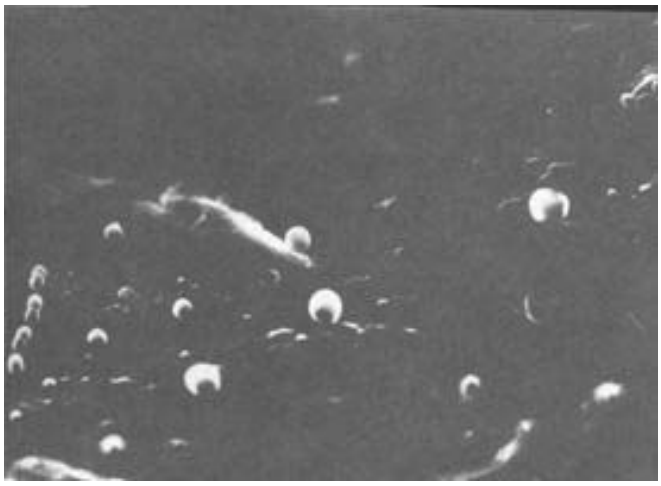
There were no patient-dependent factors noted in this study under the experimental conditions described.

Typical micrographs obtained with varied magnification for 24 hours (Figures 1, 2 and 3) and one week positioning (Figures 4, 5 and 6) *in vivo* are presented.

Hardly any detail was observable on the enamel surfaces after 24 hours (Figure 1). Most of the organisms on this specimens were coccal and epithelial cells and the amount of deposit was observed to vary considerably between individuals and specimens. There was no significant difference in amount of bacterial colonization on the experimental and control groups. Coccal bacteria were found to have clustered independently on the surface (Figure 2), whereas some other had formed agglomerates (Figure 3).

After one week, however, appearance was comparable to that of untreated control samples in general. On the Duraphat-treated surfaces, the plaque accumulation was not thick (Figure 5). and the interbacter-

Figure 2: Plaque formation on surfaces of intact human enamel treated with a Duraphat observed by SEM after exposure to oral environment for 24 h. Orig. marg. x5000.



ial matrix was as dense as the control specimens (Figure 6). The morphology of plaque accumulation sites was quite classical with a clearly distinguishable thread-like interplaque matrix in which many coccal and rod shaped microorganisms had dispersed (Figure 4).

After one week *in vivo*, a relatively thick plaque layer had covered the untreated enamel surface. Thin, filament-like structures had radiated from individual coccal bacteria and interwined with the filaments radiating from other bacteria (Figure 6).

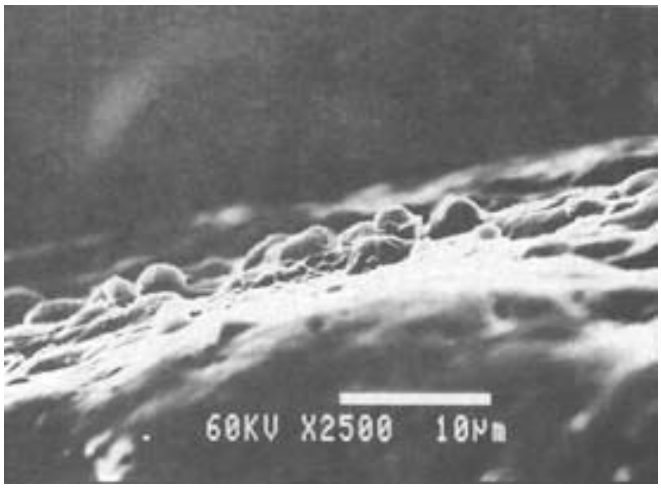
DISCUSSION

Scanning electron microscopy was used to observe the effect of a NaF varnish (Duraphat) on early plaque formation in this study. The use of SEM for the investigation of dental plaque and enamel surfaces offers both advantages and disadvantages. A considerable advantage of the SEM technique is that, the uncovered enamel surface can be observed at a magnification of x200000 besides, the plaque and bacteria. The disadvantage of SEM, on the other hand, is of course that, only the superficial portion of plaque (or deposit) is observable (7).

In the SEM studies, careful dehydration is of great importance so as to keep the plaque structure intact as much as possible ad to avoid a structural collapse of bacteria in vacuum. Previous studies have shown that many different techniques such as freeze drying (12), critical point drying (13) or fluorocarbon technique (11) were used for successful dehydration of biological samples, and thus becoming quite popular. To the authors knowledge, drying with Peldri II technique has not been previously published in dental plaque research.

Currently, mechanical methods for plaque control still remain as most effective for controlling bacterial accumulations on teeth. Many antimicrobial agents have been used in attempt to reduce plaque, however only a few have shown any degree of success. Chlorhexidine gluconate, SnF_2, amine fluoride and alexidine have been shown to be effective in reducing the total amount of bacteria on teeth (4). In previous studies, the agents were used at lower fluoride con-

Figure 3: SEM illustrating aggregates of bacteria, on an enamel surface treated with Duraphat after 24 h. Orig. marg.x2500.



centration in the form of mouthrinses rather than topically. This reported effect on plaque formation could therefore include effects both on the overall oral condition and on the tooth surfaces. In the present study, however, the fluoride varnish was applied in a higher concentration on the tooth specimens before insertion in the mouth. Therefore, as fluoride uptake in plaque from the oral environment was avoided, any observed effect could be ascribed to ions released from the test surface itself. Thus the observed lack of effect on bacterial colonization indicated that the fluoride compounds established a firm bond to the dental hard tissues with minimal amounts of fluoride release.

However, general appearance was comparable after one week. This could possibly be depending on Duraphat itself, which remained on the enamel surface during the overall experimental period. For Duraphat treated surfaces, it has been shown previously that CaF_2 will be leached away after about one week (14). This may be partly a reason why Duraphat is successful in reducing bacterial growth.

Dijkman and Nelson (7) have shown that the presence of CaF_2 presents at the enamel surface following topical fluoride treatment inhibits dental plaque formation substantially. However in the study of Zickert and Emilson (15) Duraphat had no significant effect on plaque accumulation and salivary levels of streptococcus mutants in children.

SEM observation following 24 hours in this study revealed no difference in the recovery of plaque microbiota. Yet, also many a number of studies have shown that NaF varnish (Duraphat) reduce carries significantly. This suggests that caries prevention cannot be explained by inhibition of bacterial growth.

According the Van Loveren's (16) proposal, daily fluoride treatments should be performed shortly before cariogenic challenge in order to serve an optimal antimicrobial effect. However, the subject is prone to further investigation.

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Figure 4: SEM micrograph of enamel worn in the mouth for 1 week. Bacterial population is comprised many coccal and rod shaped. Orig. marg.x4000.

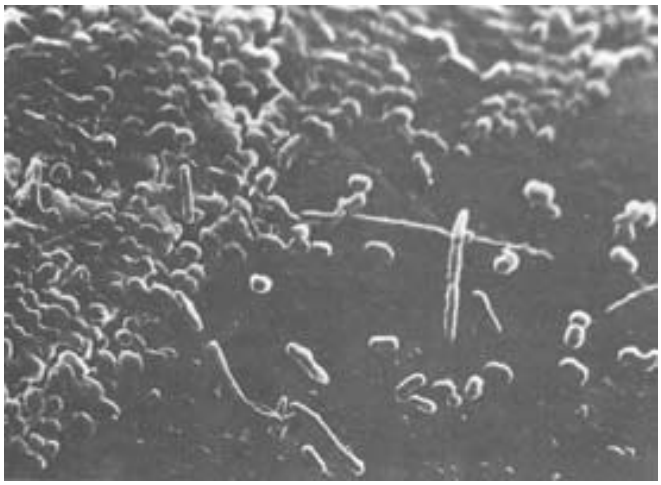


Figure 5: Plaque formation on surfaces of intact human enamel treated with a NaF lacquer observed by SEM after exposure to the oral environment for 1 week. Orig. marg.x4000.

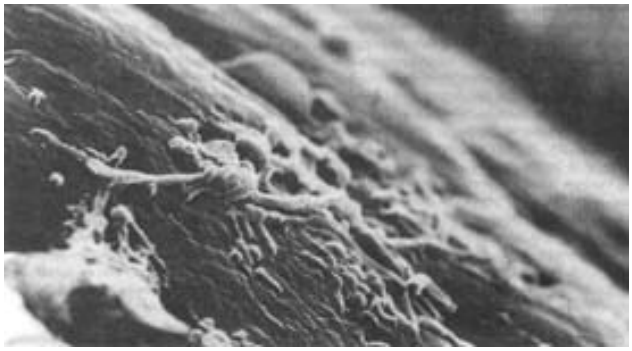
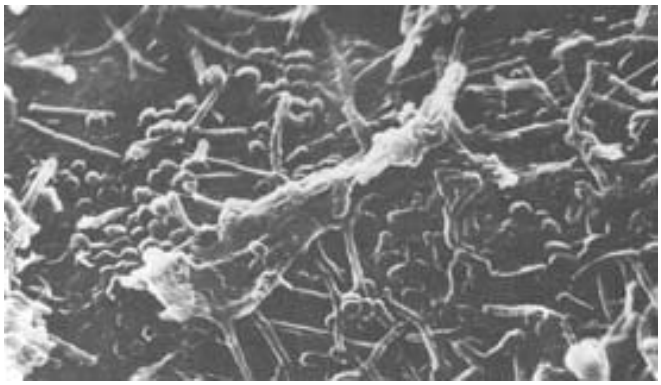


Figure 6: Plaque formation on surfaces of intact untreated human enamel observed by SEM after exposure to the oral environment for 1 week. Orig. marg.x4000.



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