Scanning Microscopy

Volume 1 | Number 4 Article 48

8-25-1987

A Scanning Electron Microscopy Study of Approximal Premolar Surfaces from Fifteen Year Old Children Living in a Fluoridated Community

Lisbeth Holmen
The Technical University of Denmark

Scanning Microscopy, Vol. 1, No. 4, 1987 (Pages 2019-2024) 0891-7035/87$3.00+.00
Scanning Microscopy International, Chicago (AMF O'Hare), IL 60666 USA

A SCANNING ELECTRON MICROSCOPY STUDY OF APPROXIMAL PREMOLAR SURFACES FROM FIFTEEN YEAR OLD CHILDREN LIVING IN A FLUORIDATED COMMUNITY

Lisbeth Holmen[a*,b]

a) Department of Structural Properties of Materials
The Technical University of Denmark
Building 307, DK-2800 Lyngby, Denmark

b) Department of Cariology and Endodontics
Royal Dental College, Copenhagen, Denmark

(Received for publication February 25, 1987, and in revised form August 25, 1987)

Abstract

The aim of the present study was to examine approximal enamel surfaces in the scanning electron microscope (SEM). Thirty premolars developed and erupted in an 'optimally' fluoridated (1 ppm) community had clinical indications of dental fluorosis and early signs of dental caries corresponding to the approximal contact area. The SEM-examination disclosed a spectrum of posteruptive changes ranging from isolated surface fractures to carious dissolution, abrasion and calcified microbial deposits. Conclusively, we found that teeth developed and erupted in areas with fluoridated water undergo similar posteruptive alterations due to prevailing environmental conditions as reported in teeth from low fluoride areas.

Key words: enamel caries, development of enamel, ultrastructure of dental fluorosis, water fluoridation, calcified microbial deposits.

Address for correspondence:
*Department of Structural Properties of Materials
The Technical University of Denmark
Building 307, DK-2800 Lyngby
Denmark
Phone No. 02 882488, ext. 2314

Introduction

Addition of fluoride to the drinking water in order to prevent dental caries has been based mainly on the possible systematic influence of fluoride during tooth formation. Thus the mechanisms of the cariostatic action of the fluoride have been linked to 1) the improvement of the apatite crystallinity, which is believed to decrease crystal solubility and reactivity (Driessens, 1973), 2) a higher mineralization of the enamel surface by acquisition of fluoride during the preeruptive maturation stage (Weatherell et al., 1977), and 3) by changing the size and morphology of the tooth crowns (Cooper and Ludwig, 1965; Aasenden and Peebles, 1974; Goose and Roberts, 1979).

In the light of widespread use of water fluoridation, it is interesting that only one study has compared the light microscopical features of approximal 'white spot' caries lesions in fluorosed teeth with those observed in teeth developed in a low fluoride area (Kidd et al., 1981). Essentially these authors found that the histological features were very similar, but they also noted a tendency towards enhanced approximal wear in the fluorosed teeth. More recently conducted scanning electron microscopical (SEM) studies have deepened our understanding of the ultrastructural surface changes preceding the clinically visible 'white spot' lesion in teeth from low fluoride areas (Thylstrup and Fejerskov, 1981; Thylstrup and Fredebo, 1982; Haikel et al., 1983; Thylstrup et al., 1983; Holmen et al., 1985).

It was therefore considered relevant to study the extent to which similar subclinical changes can be identified in teeth developed in areas with elevated concentrations of fluoride in the water supplies. This study describes the macroscopical and SEM observations on approximal surfaces of human premolars formed and erupted in children living in an 'optimally' fluoridated community (1 ppm).

Materials and Methods

The material consisted of 30 randomly selected human premolars extracted in 15-year-old children who were undergoing orthodontic treatment. The patients were all born and raised in a fluoridated (1 ppmF^-) community in Winnipeg, Manitoba. Following extraction, the teeth were placed in a 5% hypochlorite solution for 2 h in order to remove organic surface coatings. The specimens were then rinsed in 3-4 batches of distilled sterile water for 48 h under constant stirring and subsequently cleaned ultrasonically for 30 s. The teeth were photographed using a stereomicroscope, dehydrated, critical-point dried, evacuated to stable vacuum at 10^{-6} Torr (Edwards Model E 12E) and coated with a 5nm platinum layer (Nanotec-SEM preparation). The specimens were kept under stable vacuum prior to examination in the scanning electron microscope (JEOL ISM -U3 SEM).

Results

All teeth were characterized by a diffuse reduction in enamel translucency without air-drying, indicating dental fluorosis. According to the classification system proposed by Thylstrup and Fejerskov (1978), these changes corresponded to score 3. In addition to the signs of dental fluorosis, a wide range of approximal surface changes were found corresponding to microbial stagnation areas in the periphery of the interproximal contact area.

Detailed examination of these areas under the stereomicroscope showed, in the majority of the teeth (38 surfaces), localized areas with more distinct opacity (Figure 1). In contrast to the surrounding enamel, these areas had a more dull appearance. The enamel in the contact areas was always translucent and shiny even after prolonged air-drying. In the remaining 11 teeth it was only possible to identify the characteristic translucent contact area at this level of examination.

Scanning electron microscopic examination

Examination at low magnification of the unerupted part of the approximal enamel surfaces disclosed a microanatomical variation identical to that of normal teeth (Thylstrup and Fredebo, 1982; Holmen and Thylstrup, 1984), ranging from regular smooth surfaces with hardly detectable perikymata patterns (Figure 2a) to irregular wavy perikymata patterns with rows of Tomes' process pits. In addition, developmental irregularities such as surface overlapping projections and focal holes were to be found in all specimens. In contrast, there were no signs of localized or focal loss of the unerupted part of the surface. High-power magnification of the unerupted enamel surfaces showed a regular arrangement of crystals and crystal groups which were characterized by their uniform, rounded appearance (Figure 2b). The crystals and the groups of crystals were separated by a distinct network of spaces giving the impression of a very porous microstructure of the unerupted part of the enamel.

Examination in the SEM of a typical surface with a marked approximal facet and localized dull-whitish areas cervical to the facet, as macroscopically illustrated in Figure 1, shows marked surface abrasion corresponding to the approximal facet area (AF, Figure 3). Occasionally larger parts of the outer surface were fractured away in the facet area exposing the interior enamel structure. The individual fracture lines correspond to the striae of Retzius and were often rounded. In the bottom of the cavity the pattern of enamel rods can be discerned. In addition, isolated microcavities were found in all specimens especially corresponding to the periphery of the abraded approximal facet (Figure 3). Detailed examination of a smooth part of the facet (Figure 4a) showed exposure of rod and interrod areas, in addition to more dense areas in which fine wear striations can be discerned. High power examination of the dense areas (Figure 4b) showed groups of crystals which seemed to have fused together. The crystal arrangement was more irregular than observed on the unerupted part of the enamel (Figure 2b) and localized loss of crystals was frequently observed (compare Figure 2b with 4b).

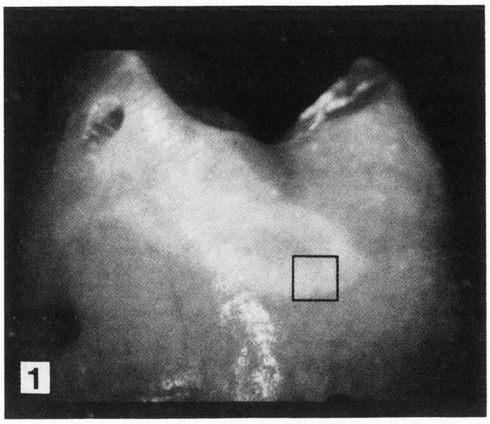


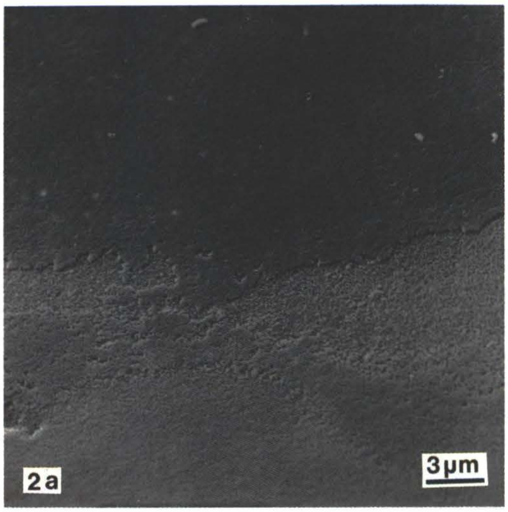
Figure 1: Approximal surface of maxillary premolar with slight dental fluorosis and a well-defined 'white spot' lesion.

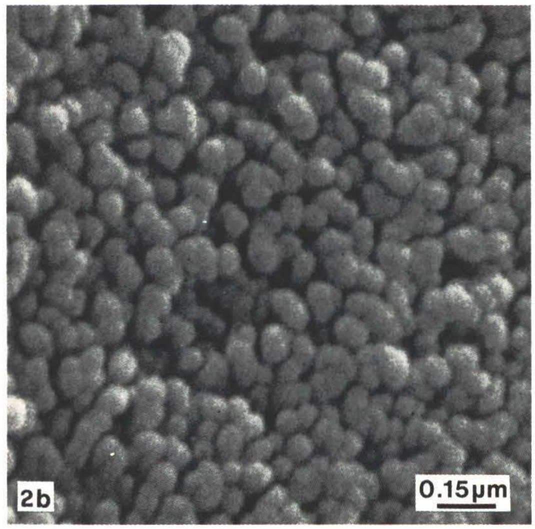
The bottoms of the cavities in the approximal contact area were highly irregular, with crests and valleys (Figure 5a). However, the most protruding parts of the exposed surface appeared smooth, with a crystal arrangement equivalent to that of the surrounding dense enamel surface of the approximal facet. In contrast, examination of the deeper and more protected parts of the surface profile disclosed loosely packed and highly irregularly arranged crystals (Figure 5b).

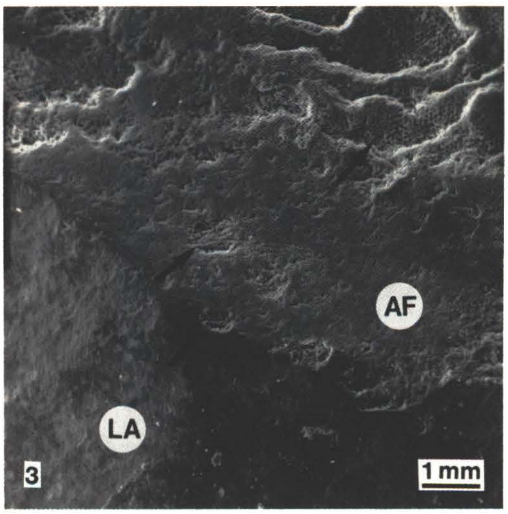
Detailed SEM-examination of the dull-whitish area cervical to the facet (Figure 6a) showed extensive signs of surface dissolution as larger parts of the outer microsurface were dissolved and fractured away. Furthermore, the dissolution had made the arcade-shaped rod peripheries more visible. Examination at the crystal level (Figure 6b) revealed that the crystals had lost their regular arrangement, uniform shape and size, which gave the impression of more loosely bound crystals and crystal-like material in this area. This observation was very consistent and was thus seen in smooth areas as well as in areas with developmental irregularities.

In specimens which appeared macroscopically sound, it was possible without exception to observe minute changes of the external microsurface cervical to the interproximal facet. These changes were consistent in the entire lesion area and similar to the surface reactions observed in visible lesions with partly dissolved individual crystals and disintegration of crystal groups.

In addition to the enamel surface findings, it was common to encounter large amounts of calcified microbial remnants in all specimens (Figure 7a). They were obviously situated all over the erupted approximal surfaces and it was possible to detect the impressions of the formerly present bacteria.



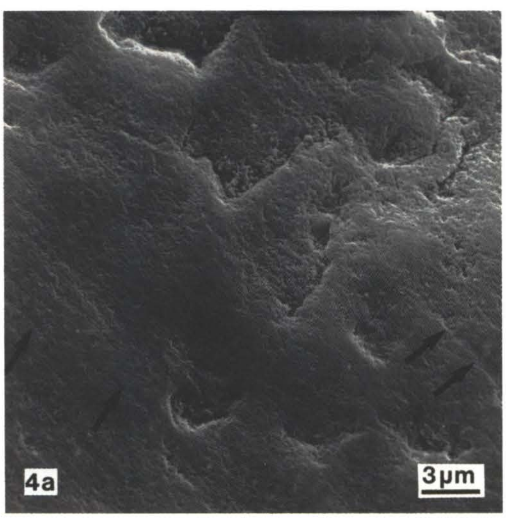


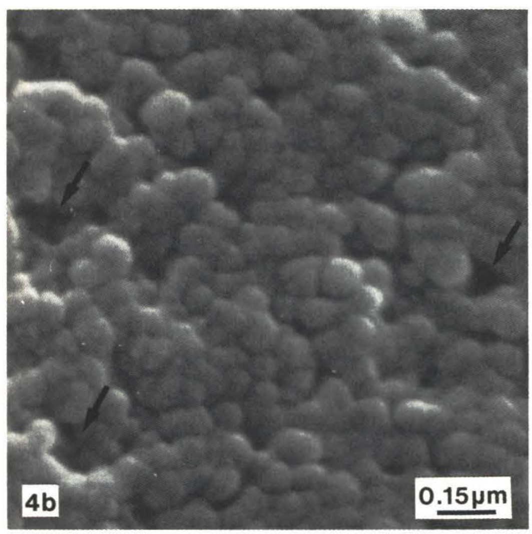


Figures 2a,b: Micrographs of an unerupted enamel surface at different levels of examination. (a) Low magnification showing a relatively smooth surface layer and (b) detailed view of the uniform and individual crystals and crystal groups in this area.

Figure 3: Micrograph of the framed area in Figure 1. Note the flattened approximal facet (AF) with localized losses of outer enamel surface (arrows) in contrast to the lesion area (LA).

Figures 4a,b: Micrographs from the approximal facet (AF) in Figure 3. (a) The surface is dominated by wear (arrows show the fine wear-striation) and a smoothening out of irregularities. (b) Crystals and crystal groups from (a). Note groups of tightly packed crystals separated by a delicate network of spaces, and localized loss of crystals (arrows).





High resolution examination of the calcified substance revealed crystal-like material in addition to substances which, at this level of examination, appeared structureless (Figure 7b).

Discussion

This study found that teeth from children born and raised in an 'optimally' fluoridated area had very mild signs of dental fluorosis indicating a slightly enhanced subsurface porosity (Thylstrup and Fejerskov, 1978) and were, therefore, not more highly mineralized than teeth developed in areas with a low fluoride content in the drinking water. Furthermore, the SEM observations showed that the external apatite crystals from the unerupted part of the enamel gave hardly any evidence of being larger and more perfect than those found in comparable areas of normal enamel after application of identical preparation procedures and SEM techniques (Holmen and Thylstrup, 1984; Holmen et al., 1985). More important, the width of the intercrystalline spaces gave no impression of a more dense external surface layer than normally found in the SEM (Holmen and Thylstrup, 1984). It is obvious, however, that a more profound analysis of this observation requires a detailed and systematic quantitation of intercrystalline spaces.

With respect to the erupted part of the approximal surfaces, it was possible in this study to demonstrate initial subclinical caries dissolution as well as manifest 'white spot lesions' including macroscopically visible localized loss of the enamel surface layer. The macroscopically observed dull-whitish appearance of the areas cervical to the approximal facet thus reflected the direct dissolution of the enamel surface as seen in the SEM which has more recently been shown to be a characteristic feature of active enamel caries lesions (Thylstrup and Fejerskov, 1981; Thylstrup and Fredebo, 1982; Haikel et al., 1983; Holmen et al., 1985). In areas without such macroscopically visible changes, particularly in the vicinity of the facet area, the present study also demonstrated early signs of dissolution at the ultrastructural level of examination. It is therefore reasonable to assume that these observations corresponded to those previously reported on stages of caries dissolution preceding the 'white spot' lesions (Thylstrup and Fredebo, 1982; Holmen and Thylstrup, 1984; Holmen et al., 1985).

It is tempting to believe that the elevated porous structure of the fluorosed teeth, in addition to demineralization prior to establishment of interproximal contact, was responsible for the observed enhancement of interproximal wear, as also suggested by Thylstrup and Fejerskov (1978) and Kidd et al. (1981). This hypothesis is supported by the presence of a multitude of localized microcavities in the periphery of the approximal contact areas being indications of extensive abrasion of the surface layer. The mechanical removal of the surface in this area thus leads to exposure of the underlying porous tissue which further promotes interproximal wear.

In short, this study gives reason to believe that teeth developed and erupted in children living in a fluoridated area in principle show the same spectrum of structural changes previously observed in teeth from low fluoride areas (Thylstrup and Fredebo, 1982; Thylstrup et al., 1983).

The present macroscopical and ultrastructural results showed, therefore, that fluoride incorporated in the enamel during tooth formation as well as the continuous presence in the oral fluids, did not totally protect the enamel surface from the initiation of caries development. The cariostatic mechanism of fluoride in water fluoridated areas is therefore merely related to an inhibition of lesion progression. This assumption is in accordance with more recent analysis of clinical data from water fluoridated areas (Groeneveld, 1986).

In contrast to hitherto described SEM-findings in approximal surfaces from non-fluoridated areas (Thylstrup and Fejerskov, 1981; Thylstrup and Fredebo, 1982; Thylstrup et al., 1983) this study demonstrated the presence of considerable amounts of calcified microbial remnants equally distributed on the surface.

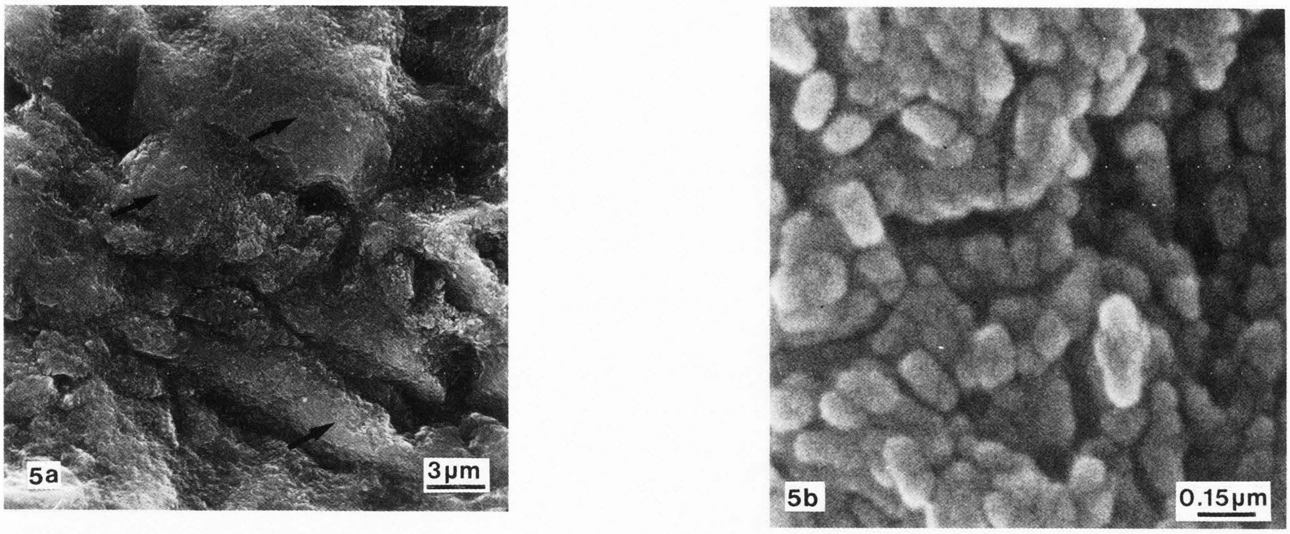
It is interesting, therefore, that Houwink and Backer Dirks (1966) found a slight increase in clinically detectable calculus in areas with elevated concentration of fluoride in the drinking water. This phenomenon is presumably linked to the elevated fluoride content in the oral fluids, which has been noted in children residing in fluoridated areas, particularly after rinsing with the fluoride-containing local water (Bruun and Thylstrup, 1984).

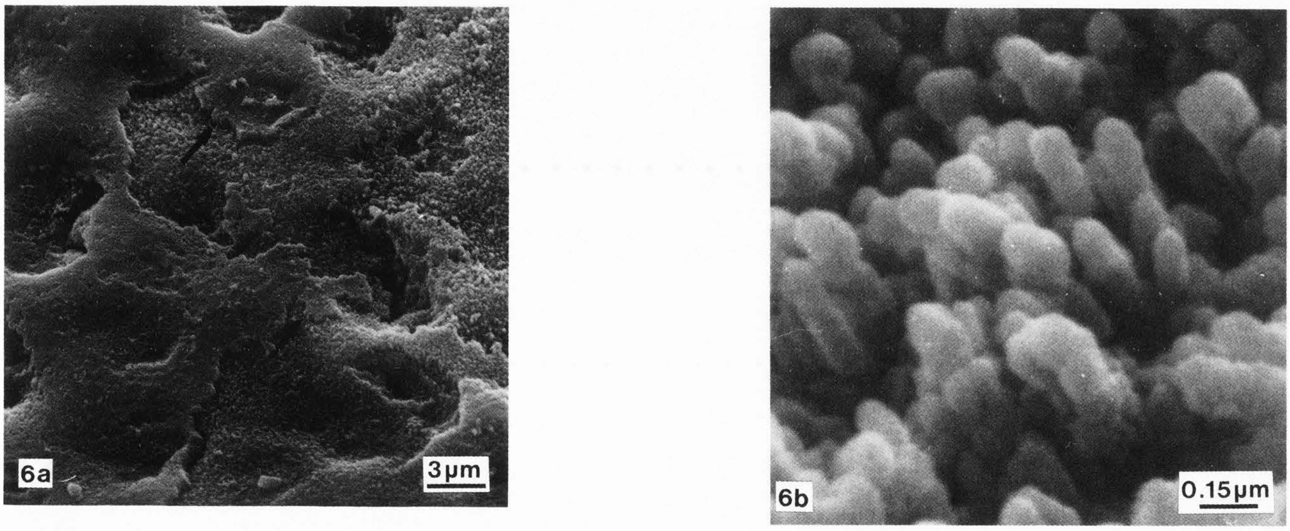
The crystal-like material observed in this study may therefore be a result of a complicated interaction of fluoride with the calcium/phosphate system in the liquid phase surrounding the teeth. The elevated tendency to plaque calcification may presumably be of some importance in the matter of cariostatic ability of fluoride as the fluoride containing calcium phosphate deposits probably act as a mineral reservoir and thereby reduce the net-loss of mineral from the teeth during pH-lowering.

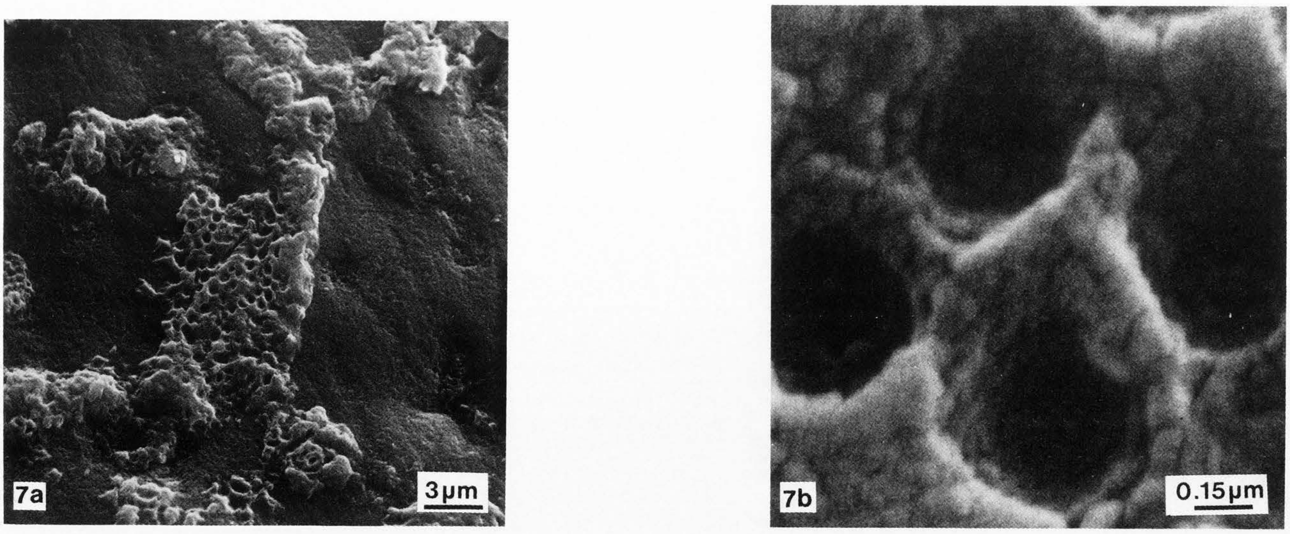
Figures 5a,b: Detailed view of a microcavity below the flattened approximal facet (AF) in Figure 3. (a) Note the highly irregular bottom with relatively smooth crests (arrows). (b) High resolution micrograph showing loosely packed and irregularly arranged crystals and crystal groups from the deepest part of the bottom profile.

Figures 6a,b: (a) Surface features from the lesion area (LA) in Figure 3. Note initial direct dissolution of the external enamel surface (arrows). (b) High power magnification of (a), illustrating alterations in crystal arrangement and size compared to Figure 2b.

Figures 7a,b: (a) Micrograph of calcified intermicrobial substances. (b) High magnification of the crystalline material between the impression of formerly present bacteria.


5a
3μm
5b
0.15μm


6a
3μm
6b
0.15μm


7a
3μm
7b
0.15μm

Acknowledgements

The author would like to thank Professor George Bowden, Dr. Boyar, and the staff of the Department of Orthodontics, Faculty of Dentistry, University of Manitoba, for being most helpful in sending the specimens for this study, which was supported by the Danish Medical Research Council, grant No. 12-5738.

References

Aasenden R, Peebles TC (1974) Effects of fluoride supplementation from birth on human deciduous and permanent teeth. Arch.Oral Biol. 19, 321-326.

Bruun C, Thylstrup A (1984) Fluoride in whole saliva and dental caries experience in areas with high or low concentrations of fluoride in the drinking water. Caries Res. 18, 450-456.

Cooper VK, Ludwig TG (1965) Effect of fluoride and of soil trace elements on the morphology of the permanent molars in man. NZ Dent. J. 61, 33-40.

Driessens FCM (1973) Fluoride incorporation and apatite solubility. Caries Res. 7, 297-314.

Goose DH, Roberts EE (1979) Possible influence of fluoridation on tooth crown size. J. Dent. Res. 58, 1562-1563.

Groeneveld A (1986) Clinical observations on caries and water fluoridation. In: Tooth Development and Caries, Vol. II, Driessens FCM, Wöltgens JHM (eds), CRC Press, Florida, 147-183.

Haikel Y, Frank RM, Voegel JC (1983) Scanning electron microscopy of the human enamel surface layer of incipient carious lesions. Caries Res. 17, 1-13.

Holmen L, Thylstrup A (1984) Variations in 'normal' enamel surfaces as visualized in the SEM. In: Tooth Morphogenesis and Differentiation II, Ruch JV, Belcourt AB (eds), Edition Inserm, Paris, 283-294.

Holmen L, Thylstrup A, Øgaard B, Krag F (1985) A scanning electron microscopic study of progressive stages of enamel caries in vivo. Caries Res. 19, 355-367.

Houwink B, Backer Dirks O (1966) Dental calculus and fluoridation of the water. Adv. Fluorine Res. Dent. Caries Prevent. 14, 149-156.

Kidd EAM, Thylstrup A, Fejerskov O (1981) The histopathology of enamel caries in fluorosed deciduous teeth. Caries Res. 15, 346-352.

Thylstrup A, Featherstone JDB, Fredebo L (1983) Surface morphology and dynamics of early enamel caries development. In: Demineralization and Remineralization of the Teeth, Leach SA, Edgar WM (eds), IRL Press, London, 165-184.

Thylstrup A, Fejerskov O (1978) Clinical appearance of dental fluorosis in permanent teeth in relation to histologic changes. Community Dent. Oral Epidemiol. 6, 315-328.

Thylstrup A, Fejerskov O (1981) Surface features of early carious enamel at various stages of activity. In: Tooth Surface Interactions and Preventive Dentistry, Rölla G, Sönju T, Embery G (eds), IRL Press, London, 193-205.

Thylstrup A, Fredebo L (1982) A method for studying surface coatings and the underlying enamel features in the scanning electron microscope. In: Surface and Colloid Phenomena in the Oral Cavity: Methodological Aspects, Frank RM, Leach SA (eds), IRL Press, London, 169-184.

Weatherell JA, Deutsch D, Robinson C, Hallsworth AS (1977) Assimilation of fluoride by enamel throughout the life of the tooth. Caries Res. 11, Suppl. 1, 85-115.

Discussion with Reviewers

S. Risnes: Is it necessary to critical-point dry an enamel specimen from which the surface organic material has been removed?
Author: It is not absolutely necessary to critical-point dry in this case, but we have learnt from experience that if this process is omitted, it is very difficult (if not impossible) and time consuming to evacuate the specimens to stable vacuum (10^{-6} Torr) prior to the coating procedure. If the specimens are still degassing, the coating layer will become unevenly distributed on the surface.

S. Risnes: How do you explain the patchy (with distinct boundaries) loss of the microsurface shown in Fig. 6a? Can the same phenomenon be observed in Fig. 2a from the unerupted part?
Author: The patchy appearance of the microsurface is caused in the first place by caries demineralization, subsequently by fracturing away of this partly dissolved external microlayer, presumably due to functional use (i.e. mastication). No, this phenomenon has not been observed in the unerupted parts of the enamel surfaces. Occasionally unerupted enamel surfaces, in teeth from low fluoride areas as well as from areas with water fluoridation, display minor areas with loss or absence of microsurface, which in low magnification could offer points of resemblance with the patchy pattern shown in Fig. 6a. However, high power magnification always reveals large differences in crystal arrangement (compare Fig. 2b with Fig. 6b) reflecting the differences of the cause of this phenomenon. The explanation for the absence of parts of the microsurface in unerupted enamel surfaces could be preparation damage.