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Grayson W. Marshall Jr.
University of California, San Francisco

Michal Staninec
University of California, San Francisco

Yasuhiro Torii
University of California, San Francisco

Sally J. Marshall
University of California, San Francisco

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COMPARISON OF BACKSCATTERED SCANNING ELECTRON MICROSCOPY AND MICRORADIOGRAPHY OF SECONDARY CARIES

Grayson W. Marshall, Jr.(*), Michal Staninec, Yasuhiro Torii, and Sally J. Marshall
Department of Restorative Dentistry, University of California, San Francisco
707 Parnassus Avenue, San Francisco, CA 94143-0758

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Abstract

Carious lesions are usually studied using light microscopy and /or microradiography which require preparation of thin sections. Backscattered scanning electron microscopy (BSEM) has received little attention although it provides information similar to that obtained with microradiographs, with the potential for higher resolution. Recently, microscopes have been introduced that can be used to study wet or nonconducting specimens, offering techniques for studying specimens without desiccation or preparation of thin sections. This investigation sought to determine if secondary carious lesions have the same characteristics when studied by microradiography as when using the "wet" BSEM mode. Microradiographs were made of thin sections from restored teeth with secondary caries induced in an artificial caries system. The thin sections were also studied by BSEM with a partial pressure in the specimen chamber to prevent specimen charging. Comparisons of the lesion size and shape were made using the two methods. Lesion depth measurements in enamel were the same; lesions that penetrated into dentin appeared to be of similar size and shape, but lesion depths measured by BSEM were slightly greater (paired t-test, $p < .05$). This was a result of cracks at the carious enamel-dentin interface that probably developed during storage of the samples.

Variations in the surface enamel rod structure and the development of subsurface lesions were apparent. Several zones were also apparent in the carious dentin, demonstrating loss of dentinal tubule detail in the depth of the lesion, collapse of tubules, and hypermineralized regions near the advancing front of the lesion. Several additional samples of natural carious teeth were examined. They demonstrated the characteristic structural features of the carious process. This method appears to have considerable promise for the study of such lesions.

Key Words: Backscattered scanning electron microscopy, Secondary caries, Microradiography, Enamel, Dentin.

* Address for correspondence
Grayson W. Marshall, Jr.
Department of Restorative Dentistry
University of California, San Francisco
San Francisco, CA 94143-0758
(415) 476-9119

Introduction

Backscattered electron imaging of carious lesions had received little attention until recent work (Boyde and Jones, 1983; Jones and Boyde, 1987) demonstrated the potential for this technique in the study of carious lesions in enamel and dentin. Pearce and Nelson (1989) showed that detailed structural information on carious lesions in human enamel could be obtained with this method, and that the signal emanated from about the first 4 μm from the surface, appearing sensitive to changes in mineral density. The images were similar to those obtained with microradiography for enamel lesions, but offered much higher resolution. This recent progress stems from the improvement in backscattered electron detectors; solid state 4-quadrant detectors were used in the preceding studies.

Progress has been made in allowing wet and non-conducting specimens to be studied in the SEM, following the work of Robinson (1975) and the extensions of it by Danilatos (1988) that permit wet or environmental SEM. These differentially pumped systems allow the specimen to be maintained at a higher pressure than the electron optical column. Thus, it should be possible to use backscattered electron studies for carious lesions in uncoated moist, or embedded, dried, and sectioned teeth. This study sought to determine if microradiography and backscattered electron images from uncoated sections used in microradiography gave comparable information for secondary carious lesions in enamel and dentin. Secondary caries can be defined as a carious process associated with the tooth/restorative material interface, widely recognized as a major problem associated with restorative dental treatment (Letzel et al, 1989).

Materials and Methods

Details of sample preparation have been described by Staninec et al (1988). Briefly, class V preparations were made and restored with amalgams (Tytin, Kerr Co., Romulus, MI) on opposing surfaces of freshly extracted noncarious unrestored human molars. The teeth were coated with nail varnish, leaving the amalgam restorations and a 1 mm border surrounding the restoration exposed, and then were mounted in an artificial caries chamber. The entire assembly was sterilized and tested for sterilization by incubation of the teeth with brain heart

Table 1. Lesion Depths (Average ± Standard Deviation)

Region	N	BSEM Depth (mm)	Microradiographic Depth (mm)	Statistical Significance*
Enamel	33	0.54 ± 0.11	0.55 ± 0.10	No
Dentin	33	0.73 ± 0.17	0.72 ± 0.16	Yes

*paired t-test, p less than 0.05.

infusion broth (Difco, Detroit, MI) containing 3% sucrose, for 2 days at 37°C. After the no bacterial growth condition was established, the system was accepted as sterile. The broth was drained and replaced with fresh broth inoculated with Streptococcus mutans OMZ 176. The teeth were incubated for 50 days at 37°C with broth changes made about every 4 days.

Following incubation, the teeth were removed from the chamber, cleaned, rinsed, and embedded in clear orthodontic resin (L.D. Caulk, Milford, DE). This resin was an autocuring polymethylmethacrylate powder and liquid system. The embedding technique was used to ensure the integrity of the restoration and tooth during sectioning for microradiography. Little or no penetration of the dentin or enamel occurred with this technique. Sections were prepared with a diamond saw and reduced to approximately 100 μm thicknesses by wet grinding on 600 grit abrasive paper.

Microradiographs of the sections were made by exposing the sections to a soft x-ray source (Faxitron, Hewlett Packard, McMinnville, OR) for 60 min at 15 kV and 3 mA. The microradiographs were photographed and the glass slides examined directly in a measuring microscope equipped with a digital reader (Genie Measuring Microscope, Gaertner, Chicago, IL). The depth of the lesions at the occlusal and gingival aspects of the restorations were measured at 30X adjacent to the restoration used as a reference point.

The same sections were studied using a wet SEM at 15-30 kV (ISI SX-40A, ISI, Inc., Milpitas, CA) equipped with a Robinson scintillator backscatter detector at specimen chamber pressures of 0.1-0.5 torr to suppress charging. Micrographs, at approximately the same magnifications made from the microradiographs, were obtained and lesion depths measured using a calibrated 10 X magnifier (Peak Lupe, GC, Japan).

The photomicrographs obtained from the microradiographs and by BSEM were compared for general similarities and differences in the appearance of the lesions. The depths of the lesions, measured at the gingival and occlusal aspects of the amalgam-tooth interface, were compared using paired t-tests ($p < 0.05$). Since the thin sections were fragile, it was difficult to prepare them with a high polish. However, the grinding marks obscured microstructural detail. After measurements of lesion depth were made, several samples were metallographically prepared by polishing through 1 μm polishing paste. These samples were then studied at higher magnification. In addition, freshly extracted teeth with natural primary or secondary caries were hemisected and polished in the same way and examined directly without dehydration or coating to gain additional information on the carious microstructure observable with this method. Wet specimens were studied at specimen chamber pressures up to 2 torr.

Results

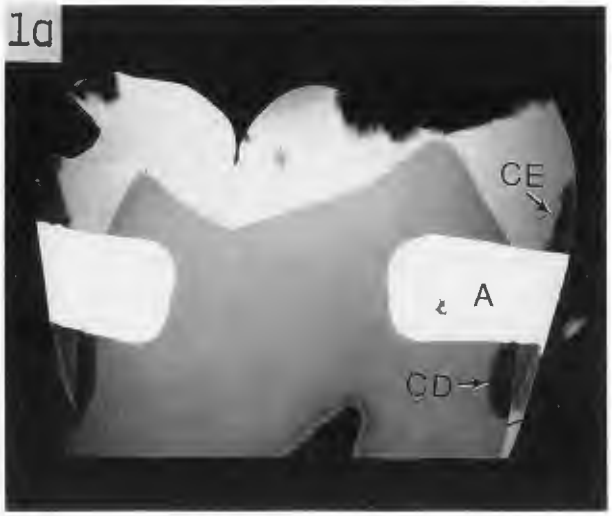
Fig. 1 shows a low magnification view of the microradiograph and backscattered electron micrograph of a typical section. At the occlusal aspect of each restoration, the carious lesion can be seen as a broad dark band in the microradiograph and as a lighter band of the same shape in the BSEM micrograph. The lesion at the gingival aspect of the restorations (lower region) also has the same shape in each method of viewing. However, the lesion has penetrated through the enamel and spread to include a larger portion of the dentin. This penetration into dentin was seen in all cases for the lower lesion, a result of the much thinner band of enamel covering the dentin at this site compared to the more occlusally located lesion that always remained in enamel. The portion of the lesion that extended into the dentin had essentially the same appearance in the microradiograph and BSEM micrograph. This can be seen in Fig. 2, a comparison of the microradiograph and BSEM micrograph at higher magnification. The dentin lesion is very dark, seen with either method, while the lesion in the enamel portion is only a slightly darker shade than the surrounding sound enamel in the BSEM. The lesion confined to enamel-only is shown in Fig. 3, as seen by microradiograph and BSEM. Again, the shape and size of the lesions appear to be identical, although the lesion appears much darker in the microradiograph.

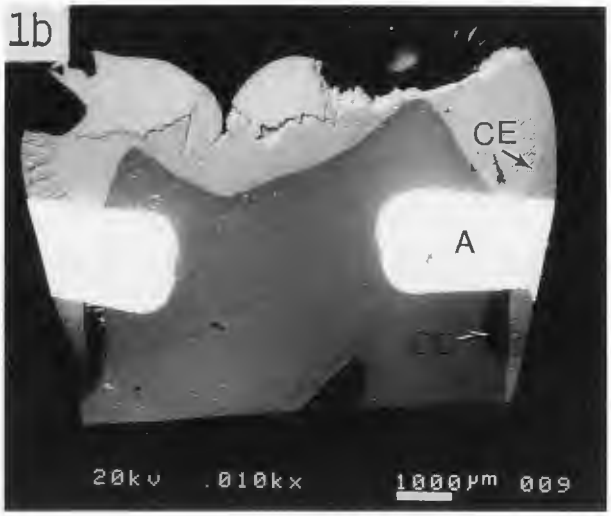
In order to provide a quantitative method of comparing the lesion characteristics, measurements of the depths of the lesions along the tooth-amalgam interface were made.

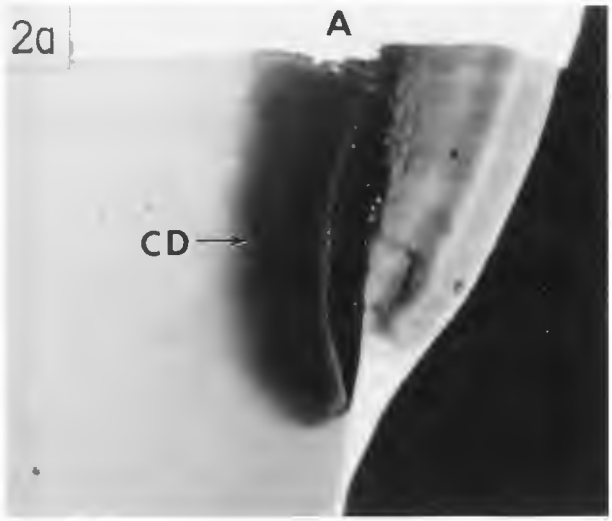
Figure 1. Microradiograph (a) and BSEM (b) of same section at low magnification. CE = carious enamel lesion; CD = carious lesion that penetrated through enamel into dentin; A = amalgam restoration.

Figure 2. Comparison of microradiograph (a) BSEM (b) of an artificial carious lesion in dentin. Shape of lesion is the same. Cracks and scratches are apparent in BSEM, but not microradiograph.

Figure 3. Artificial carious lesion in enamel. Size and shape of lesion is the same in microradiograph (a) and BSEM (b). Enamel lesion is much lighter in BSEM and grinding scratches are seen.


1a
CE
A
CD


1b
CE
A
20kv .010kx 1000µm 009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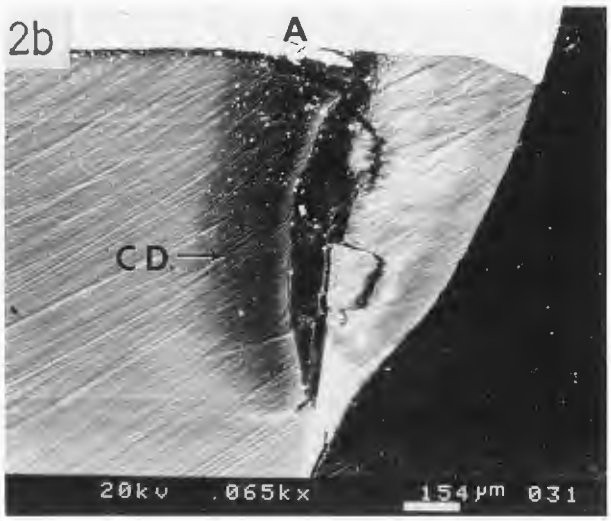
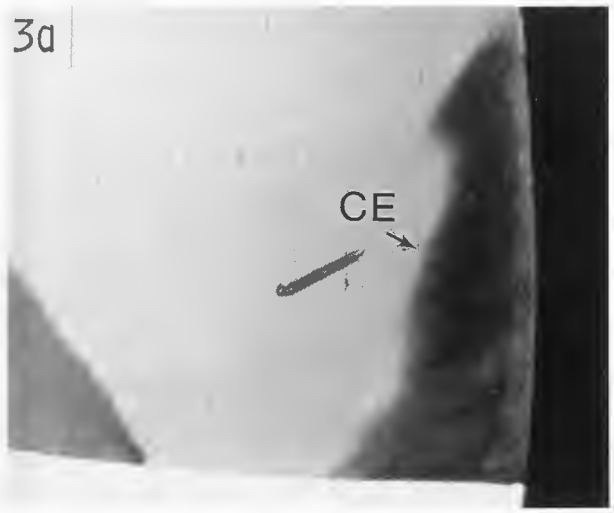
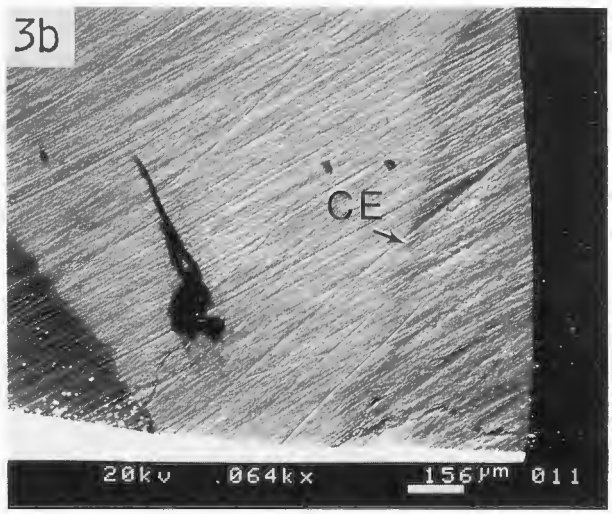
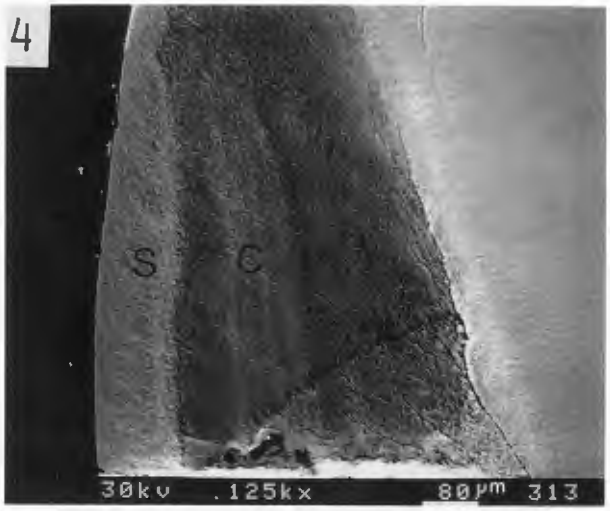
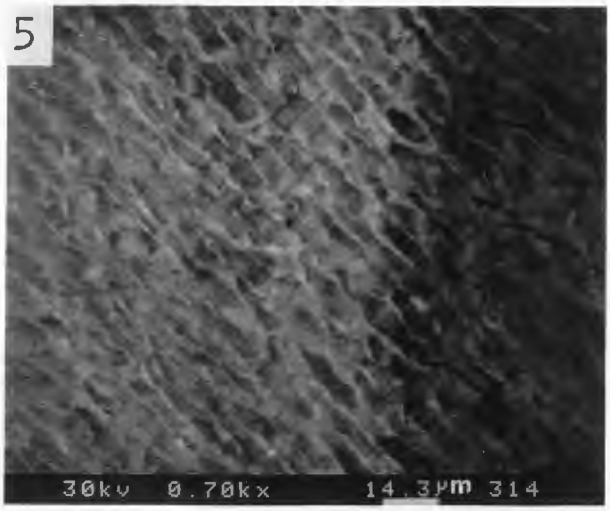

2b
A
CD
20kv .065kx 154µm 031


3a
CE


3b
CE
20kv .064kx 156µm 011





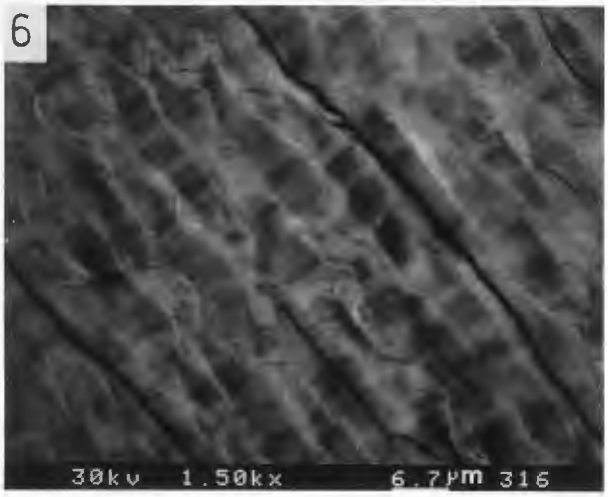


Figure 4. Low magnification BSEM view of artificial carious lesion in enamel after polishing. Intact surface zone (S) and carious zone (C) of the subsurface lesion are seen.

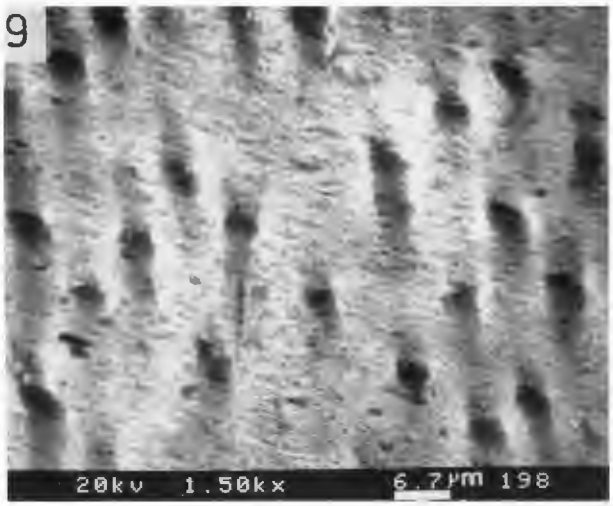
The results in Table 1 show that for the lesions measured in enamel the apparent depths of the lesions were the same and there was no significant difference between them. Similar depth measurements of lesions that penetrated into the dentin and were at the gingival aspect of the tooth-amalgam interface are also in Table 1. The depth measurements were very close for these lesions, but the measurements were slightly greater for the BSEM micrographs, and the difference was statistically significant (paired t-test, $p < 0.05$).

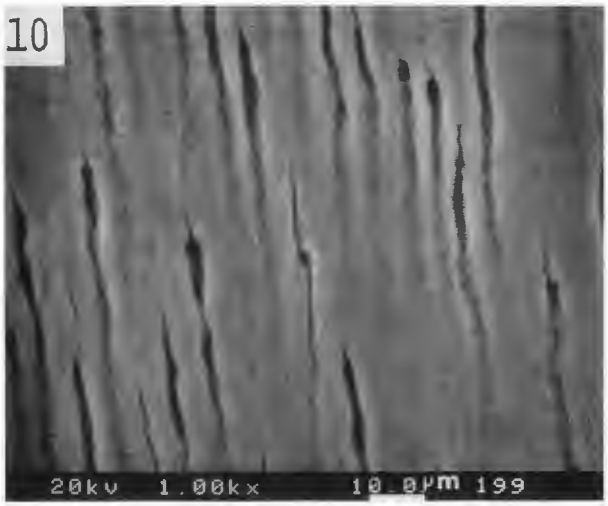
Figure 4 demonstrates the appearance of the lesion at low magnification, after polishing. After removal of the scratches caused by grinding the classic subsurface appearance of the lesion was readily apparent . At higher magnification (Fig. 5) the transition from the relatively intact surface to the carious lesion can be seen. Cross striations along the enamel rods were apparent just outside the obvious dark zone of demineralization, and hypermineralized prism peripheries (bright areas) were also seen. The cross striations continue into the dark region as shown in Figure 6.

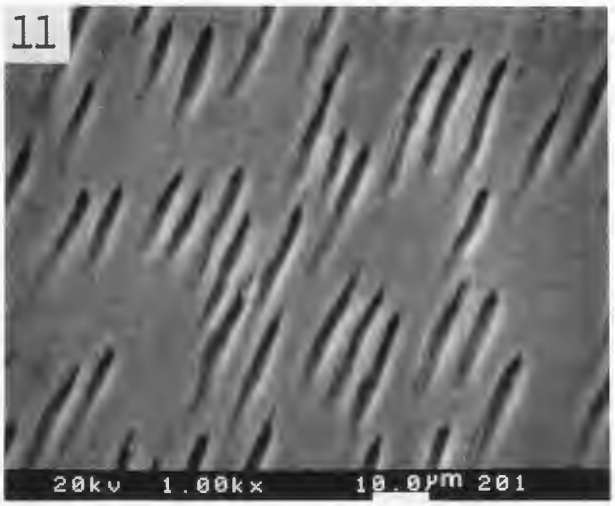
Figure 5. At higher magnification the transition region between relatively intact and carious zone of enamel; cross striations along the enamel rods, some of which appear to have hypermineralized peripheries.

Figure 6. Details of the cross striations in the enamel rods of the carious zone are shown.

Figure 7. Various zones associated with artificial secondary carious dentin lesion. Body of lesion shows loss of dentin tubules (B); occluded tubules (O) in less affected zone; tubules with clefts (C) and normal tubules (N) in advance of the lesion.







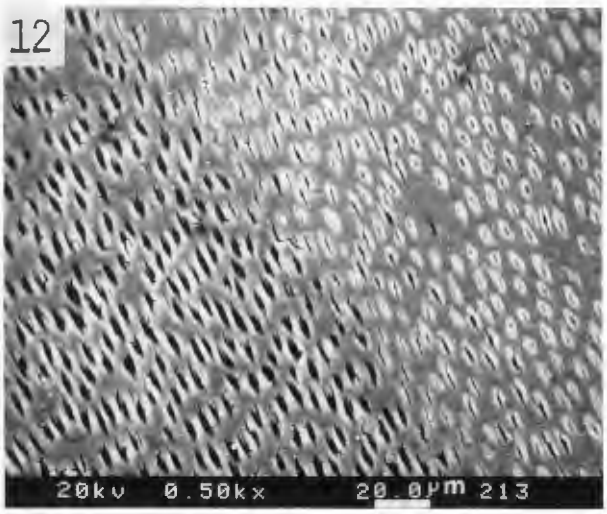


Figure 8. Transition zone between carious dark region (top) and transparent dentin in a longitudinally sectioned tooth with a natural carious lesion.

Figure 9. Higher magnification of dark zone showing demineralized matrix of the dentin tubules.

Figure 10. The transparent zone at high magnification showing partial occlusion of tubules.

Figure 11. Normal dentin.

Figure 12. Transition zone between transparent dentin (top right) and surrounding dentin from a tooth with natural caries. Surrounding dentin was less mineralized and exhibited clefts in tubules.

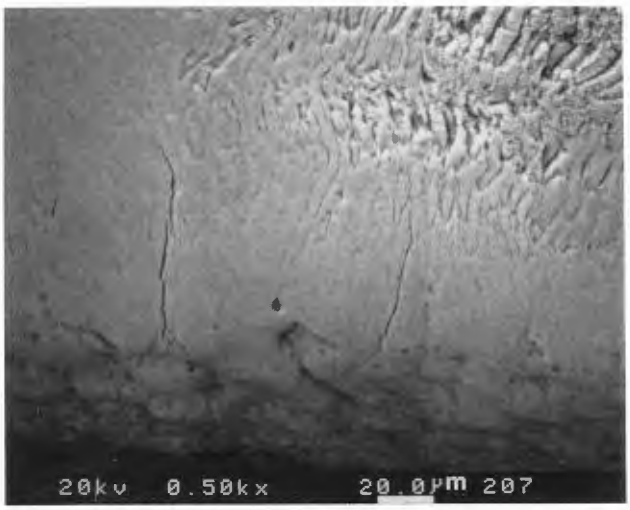


Figure 13. Sectioned tooth showing the enamel side of the naturally carious DEJ as well as cross striations in enamel rods.

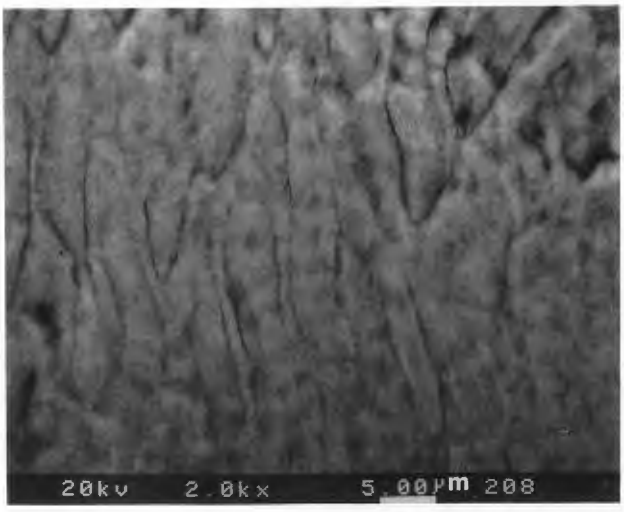


Figure 14. Higher magnification of polished enamel rods showing cross striations and openings between some of the rods.

After polishing, the lesions in dentin demonstrated several distinct zones as shown in Figure 7. In the most demineralized portion of the dentin lesion (left side) the tubular structure was lost. In advance of this zone, tubules appeared to be occluded. Proceeding toward more normal dentin, a thin zone of tubules showing clefts and partial flattening could be seen. Finally, a region of normal appearing tubules occurred (right side).

Since the sections prepared for microradiography were very fragile, additional observations of the carious features were observed in samples with natural caries. In contrast with the artificial carious sample, the lesions studied generally showed a broad region of transparent hypermineralized dentin, indicating the slow progression of the lesion. These regions appeared as roughly triangular or as a series of triangular regions with the base along the carious dentino-enamel junction (DEJ) and the apex toward the pulp chamber. Just below the zone of obvious destruction, preceding the transparent zone, was a dark zone of demineralization representing the carious front. Figure 8 shows this region of transition. Tubular structure can be seen in both zones. Figure 9 shows the dark zone at higher magnification, demonstrating a structure consisting largely of the remaining collagen matrix with much of the mineral removed. In Figure 10 the features of the lighter transparent zone, in which the majority of the tubules are occluded, are seen. Figure 11 shows the structures in the normal dentin well away from the lesion for comparison. Figure 12 shows the transition between transparent dentin (center right) and surrounding dentin in another sample. Interestingly, the several zones seen here contrasted roughly with the occluded and clefted zones seen on a much more limited scale in the artificial lesions.

The enamel side of another carious lesion is shown in Figure 13 and, once again, cross striations of enamel rods and openings between rods can be seen at this and higher magnifications (Figure 14).

Discussion

The lesions induced in dentin and enamel at the tooth amalgam interface for study by micro-radiography were readily apparent using backscattered electrons in the uncoated samples studied at specimen chamber pressures of about 0.1 to 0.5 torr. This pressure in the BSEM specimen chamber effectively suppressed charging on all parts of the sample, including the embedding media. It also suggested that samples could be studied by simply sectioning a carious tooth and viewing it directly prior to any dehydration or coating.

There were some minor differences in the appearance of the lesions as seen by microradiography and the backscattered electrons. The carious enamel lesion induced along the tooth-amalgam interface appeared to have the same size and shape in the microradiographs and BSEM photomicrographs, but was darker and more distinct in microradiographs. This effect is due, in part, to the relatively low accelerating voltage of 15-20 kV used initially in this study, and to the fact that the samples were not highly polished. At higher accelerating voltages, the enamel lesion becomes more distinct, as shown by Pearce and Nelson (1989). They estimated that the optimum accelerating voltage for enamel carious lesions was about 30 kV. This effect is shown in Figures 4-6. However the lower accelerating voltage was used in part of this study to optimize the appearance of the dentin lesions. In addition, the microradiographic sections were not initially polished because they were extremely fragile and we wished to avoid any additional artifacts that might be introduced by further handling. This limited polishing until measurements were completed and, when viewed by BSEM, left the samples with clear evidence of the grinding scratches that had been introduced during the wet grinding procedure. Such scratches and other defects are not discernible in the microradiographs. In addition, the BSEM micrographs demonstrated many cracks and

fractures in the teeth and defects in the embedding media that went unnoticed in the microradiographs.

Lesions that had penetrated through the enamel and into dentin along the tooth enamel interface had very similar appearances, but their depths were somewhat greater, as measured from the BSEM micrographs. It should be noted that this difference was significant using the paired t test, but would not be if the data were pooled. Assuming that the difference is real there are two possible explanations. First, the BSEM and microradiographs do not sample the same depths of the section, since the BSEM signal emanates from a few micrometers from the surface, while microradiography samples the entire 100 μm thickness of each section. Secondly, the BSEM frequently revealed areas in which the embedding media had separated from the sample, leaving a hole between the carious but apparently structurally rigid enamel, and the more delicate carious dentin. Such defects might go unnoticed in the microradiographs but are clearly seen in the BSEM. However, since there was a prolonged storage period prior to the BSEM study, with additional handling of the samples associated with the study, the defects could have been created during this period. In this instance, such defects would be expected to occur at the weakest part of the specimen, the carious dentin-enamel junction, where such defects were found. Thus, we believe that during storage, gradual shrinkage of the carious dentin occurs, causing a gap at the carious DEJ. The pulling away from the junction causes the dentinal lesions to actually be slightly deeper, as seen and measured in the BSEM.

After highly polishing the sample, the structural features of the carious process became more evident. Other authors have emphasized the need for high polish to remove topographical contrast (Boyde and Jones, 1983; Jones and Boyde, 1987; Pearce and Nelson, 1989). Once this was done, the typical structures seen in carious enamel, the formation of subsurface lesions, hypermineralization of enamel rod peripheries, and the enhancement of cross striations along the enamel rods after carious attack were noted (Jones and Boyde, 1987; Pearce and Nelson, 1989).

Polished carious dentin has not been extensively studied by BSEM. However, it is well known that tubules occlude, probably as a result of mineral formation during carious attack that causes transparent dentin formation. (Ogawa et al, 1983). Cleft formation as a result of carious attack on dentin has also been reported by Jones and Boyde (1987).

It appears, then, that all characteristic features found during carious attack of enamel and dentin can be examined in detail on uncoated or moist samples. Some artifacts have been observed while studying moist samples. Since the specimen is held at a partial pressure, water is gradually sublimated. The most striking result of this, seen in our study, was propagation of pre-existing cracks or gradual separation of weak interfaces. Some cracks propagated over 1000 μm during the course of observation. Additional studies are needed to define the best way to identify and limit artifacts developing during observation. Nevertheless, the "wet BSEM" technique appears to offer considerable promise for the study of natural and artificially-induced carious lesions. It offers essentially the same information on mineral changes as microradiography, but at higher resolution. In the future, it should be possible to eliminate much of the laborious specimen preparation needed for microradiography because the same information has become available without the need to prepare thin sections. Finally, the technique appears applicable to the study of the carious process in all of its forms, i.e. natural, artificial, primary, or secondary caries of enamel and dentin.

Acknowledgements

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Discussion with Reviewers

S.J. Jones: Could the authors please clarify the benefit gained from examining the "wet", uncoated specimens (other than preparation time) compared with polymethylmethacrylate embedding?

Authors: At the magnifications used, this technique eliminates the necessity of a conductive coating on the dentin specimens as well as the steps necessary for embedding. The major advantage of examination in the wet mode is that once the specimen is dried, embedded, and coated artificial carious lesions cannot be manipulated. One of the reasons for developing this technique was to make it possible to monitor the progress of lesions under various manipulations.

R. Becker: Which of the figures is a wet SEM image and which could not be obtained by "dry" backscattered SEM?

Authors: All micrographs were taken in the wet mode, which eliminated the necessity for any coating procedures. Micrographs in which the specimens had not been previously dried are Figures 8-14. Similar

photomicrographs might be obtained from the enamel in the dry condition without a coating procedure but they would probably contain charging artifacts and have inferior resolution. To the best of our knowledge none of the micrographs of dentin or dentin lesions could be obtained without drying and coating.

D.G.A. Nelson: Did the authors try to use any techniques to prevent dehydration and shrinkage in the dentin thin sections before microradiography and BSEM?
Authors: The microradiographic portion of the study was done first and all specimens were dehydrated prior to the BSEM studies. However, during the remaining portions of the study using sectioned teeth all specimens were maintained in a moist condition prior to BSEM study in the wet mode.

D.G.A. Nelson: Would the use of pressures higher than 0.5 torr, or higher humidity resolve the cracking problem for dentin in BSEM?
Authors: We anticipate that pressures in excess of 5 torr would resolve the dehydration problem. In the pressure range between 0.5 to 5 torr the extent of benefit is unknown and is currently under investigation in the range of 0.5 to 2 torr, which is the highest pressure we have successfully used. Nevertheless it should be understood that image quality decreases as the pressure increases because of electron scatter.

S.J. Jones: Did the movement of unsupported cracked enamel, as well as the shrinkage of the dentin, also contribute to the greater apparent depth of the dentinal lesion?
Authors: The contribution of fractured enamel is probably not very great because the measurements of lesion depth were generally not in the vicinity of the enamel fractures.

J.P. LeGeros and R.Z. LeGeros: You reported that crack propagation reached a magnitude of 1000μm over the period observed. Since crack propagation velocities are important physical characteristics, do you have an estimate of the observation period?
Authors: Such cracks were seen over an observation period of about 20 minutes. Although we have not attempted to measure the velocity, our impression is that the velocity is very high and we only recorded the start and finish of a propagation event.