Microscopic appearance of enamel white-spot lesions after acid etching
Charles Q. Lee*/Zia Shey**/Charles M. Cobb*

Ultrastructural changes in surface characteristics of enamel white-spot lesions were compared with changes in the adjacent clinically sound enamel after they were etched with 30% phosphoric acid. Ten human permanent first molars exhibiting natural white-spot lesions were used as study specimens. The lesion surfaces and their adjacent sound enamel were etched with 30% phosphoric acid for 60 seconds. Specimens were then evaluated by polarized light microscopy, scanning electron microscopy, and energy-dispersive spectroscopy. The acid etching produced a porous surface on both the white-spot lesion and the surrounding sound enamel. However, the lesion surface appeared to be more resistant to acid and dissolved less than adjacent enamel. This difference in acid solubility produced a step-like appearance between a white-spot lesion and its adjacent enamel surface. Energy-dispersive spectroscopy demonstrated no difference in relative calcium-phosphorus ratios among the acid-etched white-spot lesion, acid-etched sound enamel, and unetched sound enamel. (Quintessence Int 1995;26:279-284.)

Introduction

It has been generally accepted that enamel white-spot lesions represent various degrees of surface and subsurface demineralization. Considerable research has focused on the formation of the surface zone during development of incipient enamel caries. The surfaces of early enamel white-spot lesions have been shown to remain relatively unaffected, with approximately an 8% mineral loss, while the subsurface lesion areas exhibit mineral loss ranging from 20% to 90%. A number of theories have been proposed for this observation. According to Darling and Ripa et al, structural differences between the inner and outer enamel may account for the formation of the surface zone. In an artificial caries system, it was demonstrated that the formation of the surface zone is highly dependent on the concentration of the acid-gelatin mixture. Moreno and Zahradnik and Silverstone et al have suggested that some degree of remineralization may occur at the enamel surface during the formation of a carious lesion, thus increasing the mineral content of the surface zone. However, Gray and Francis, Peace and Bibby, and Zahradnik et al attributed the relative lack of acid solubility of surface enamel during caries formation to certain macromolecules, such as protein, which may slow down the decalcification process. Robinson et al reported that the movement of mineral ions into carious lesions is improved by removal of organic material in the surface zone. According to Takuma, the enamel surface contains more fluoride and calcium, which could play a role in the production of the surface zone. Aoba and Yagi studied the crystallinity of the surface zone in dental caries and found that apatite crystallinity was higher in the surface zone than in the adjacent enamel. Hicks and Silverstone studied the histopathologic effects of acid etching on carieslike

* Department of Oral Biology and Endodontics, University of Missouri-Kansas City, School of Dentistry, Kansas City, Missouri.
** Department of Pediatric Dentistry and Biomedical Science, UMDNJ-New Jersey Dental School, Newark, New Jersey.

Reprint requests: Dr Charles Q. Lee, Department of Endodontics, University of Missouri-Kansas City, School of Dentistry, 650 East 25th Street, Kansas City, Missouri 64108.
lesions and sound enamel and reported that the alterations in both tissue substrates are similar.

Clinically, when restorative procedures are performed acid etching is used for bonding of resins to tooth surface. When such restorations involve an early white-spot lesion, the enamel caries is either removed or etched and covered with the bonding resin. This study examined the ultrastructural changes in the surface of enamel white-spot lesions and their adjacent sound enamel after they were etched with 30% phosphoric acid.

Method and materials

Ten permanent human first molars that exhibited enamel white-spot lesions were selected. These lesions were at least 5 mm in diameter buccolingually and located at the proximal surface. All teeth were stored in 85% alcohol at 4°C until used. These teeth were cleaned and soft tissue was removed; they were then examined under a dissecting microscope to assure that there were no surface cavitations. Each tooth was cut occluso cervically into four sections with a Mikrotrenn hard tissue microtome (Hofer Andreas). Each section included a part of white-spot and its adjacent sound enamel. The specimens were randomly assigned to one of four experimental groups.

Group I sections were etched and prepared for polarized light microscopy (PLM). Group II sections were etched and prepared for scanning electron microscopic (SEM) examination and energy dispersive spectroscopy (EDS). Group III and group IV sections served as unetched controls for PLM, SEM, and EDS evaluation.

Prior the acid etching, the sections were coated with an acid-resistant varnish to prevent a possible contact between the acid and the cut surfaces. The enamel surface was also coated with varnish except for a window area consisting of a lesion and 1 mm of peripheral sound enamel. The sound enamel, as well as the white-spot, was subsequently etched for 60 seconds with 30% phosphoric acid. Specimens were then washed with deionized water for 60 seconds and dried in a desiccator for 24 hours.

Prior to SEM examination, specimens were lightly sputter coated with carbon for the qualitative examination by EDS. The EDS was accomplished at a magnification of x 1,100 using 15 kV and a 100-nm spot for 200 live seconds. All specimens for SEM were dried by desiccation for 48 hours, subsequently vacuum coated with gold-palladium, and examined under an AMRAY 1200 B SEM. Specimens examined by PLM were cut to a thickness of 100 um with a Mikrotrenn hard tissue microtome and polished to a thickness of 80 um with silicon carbide wet paper. Water and quinoline were used as imbibition media to identify the various zones of incipient lesions.

Results

Under PLM the unetched enamel white-spot lesion exhibited a distinct surface and subsurface zones after imbibition in water (Fig 1). When sections were imbibed with quinoline, the surface and subsurface zones merged and disappeared. However, a positive birefringent dark zone and negative birefringent translucent zone became apparent (Fig 2).

After the sections were etched with 30% phosphoric acid, the negative birefringent surface zone remained relatively unchanged, although it exhibited a slight decrease in thickness (Fig 3). Although etching for 60 seconds might alter outer layers of the surface zone, it disrupted the existing continuity with the surrounding enamel. Scanning electron microscopic examination of the enamel surface prior to etching showed the surface to be relatively smooth. In contrast, the surface of unetched enamel white-spot lesions exhibited a rough topography with porosity (Fig 4). When the surfaces of etched enamel white-spot lesions and adjacent sound enamel were compared, they were very similar (Fig 5).

As a result of acid etching, three types of topographic patterns previously described by Silverstone19 were observed in surfaces of both enamel white-spot lesions and sound enamel. In pattern I the prism's center was preferentially etched, creating pores of various sizes that resembled a honeycomb. The pattern II of etched enamel was observed when dissolution of the prism's periphery resulted in projection of the prism core toward the surface. Occasionally an irregular, yet porous surface, which did not resemble the typical etched patterns, was observed; this could only be classified as pattern III (Fig 6 to 8).

In spite of morphologic similarities in the acid-etch patterns between sound and white-spot enamel surfaces, the degree of damage was different. Regardless of surface porosity, there was a relative difference in the acid solubility of the enamel white-spot lesion and that of sound enamel. Therefore the surface of the white-spot enamel lesion was more elevated than the surrounding sound enamel (Fig 9 and 10).

Energy-dispersive spectrographic analysis indicated
Fig 1. Polarized light microscopy of a ground section of a white-spot lesion prior to acid etching. Note the surface and subsurface zones of demineralization after imbibition in water. (Original magnification X 35.)

Fig 2. Polarized light microscopy of a ground section of a white-spot lesion prior to acid etching. Note the positive birefringent dark zone after imbibition with quinoline. (Original magnification X 35.)

Fig 3. Polarized light microscopy of a ground section of a white-spot lesion after etching with 30% phosphoric acid and examined in water. Note the distinct surface zone of demineralization. (Original magnification X 25.)

Fig 4. Scanning electron micrograph of an enamel white-spot lesion prior to acid etching. Note the surface porosity. (Bar = 10 μm.)

Fig 5. Scanning electron micrograph of the etched surface of both a white-spot lesion and its surrounding etched sound enamel. Note the similarities in the pattern of enamel rod etching. (Bar = 10 μm.)
Fig 6 Scanning electron microscopic appearance of etched sound enamel. Pattern I etching: the center of the prism has been dissolved. (Bar = 10 μm.)

Fig 7 Scanning electron microscopic appearance of etched sound enamel. Pattern II etching: note the projections of the prism’s core. (Bar = 10 μm.)

Fig 8 Scanning electron microscopic appearance of etched sound enamel. Irregular porosities resembling Pattern III. (Bar = 10 μm.)

Fig 9 Scanning electron microscopic appearance of an etched white-spot lesion and its surrounding sound enamel. Note that the surface of the lesion appears to be elevated compared with its adjacent enamel surface. (Bar = 10 μm.)

Fig 10 High magnification SEM of an etched white-spot lesion and its surrounding enamel showing a steplike appearance caused by a difference in their acid solubility. (Bar = 10 μm.)
the relative ratio of calcium (Ca) to phosphorus (P) remained the same among the acid-etched white-spot lesions, acid-etched sound enamel, and unetched sound enamel (Fig 11).

Discussion

The ultrastructural changes that occur in the enamel surface during caries development are not fully understood. Microradiodensitometric evaluations of the caries lesion surfaces show a relatively unaffected surface zone. The mineral loss of the surface zone is about 20% and the pore volume is between 1% and 5%. The subsurface region exhibits a pore volume of 25% or more. The relative lack of acid solubility of the enamel surface has been attributed to macromolecules (such as protein) and trace elements that protect the enamel surface and to a difference between structures of the inner and outer enamel. It is generally accepted that the surface zone of enamel white-spot lesions resembles the surface zone of artificial carieslike lesions when observed by polarized light and micro radiography. In studies by Featherstone et al. and more recently by Guo et al., artificial carieslike lesions have been frequently used as a model for demineralization and remineralization.

We suspected that the biologic behavior of artificial carieslike lesions may be somewhat different from that of enamel white-spot lesions. The SEM results revealed a difference between the two lesion types as manifested by the relative differences in acid solubility between the enamel white-spot lesions and their surrounding sound enamel. The different solubility appeared to leave the white-spot lesions elevated above the surface of the surrounding normal enamel that was also acid etched. However, within both the white-spot lesions and the surrounding sound enamel, the typical patterns of enamel rod etching were noted. This would imply that normal enamel and white-spot lesion surfaces have different rates of acid solubility, an observation that has not been reported in similar experiments using the artificial caries model. Although the general acid-etched surface topography resulting from this study was different from that previously described, the patterns of enamel rod etching were similar to those described by Hicks and Silverstone and Silverstone.

A logical explanation for the difference in acid dissolution between white-spot lesions and normal enamel would be their chemical dissimilarity. However, the EDS results indicated the same relative Ca-P ratios in all three test areas, suggesting that Ca and P may not be the major factors dictating the topographic difference. A second explanation for the difference is a surface hypermineralization of white-spot lesions, which would render them more resistant to acid.
dissolution. However, the EDS electron beam interacts with the subsurface structure to an average depth of 100 μm. The elemental peaks, therefore, represent an average and relative quantitation of each element constituting the structure examined. Thus, it might be expected that the Ca-P ratio for normal enamel and white-spot enamel would be very similar, i.e., approximately 1.7 to 1.0.

**Summary**

The current study showed that the surface of enamel white-spot lesions was more resistant to acid etching than was adjacent sound enamel, resulting in a morphologic step-like difference between the two surface areas. Further studies are needed to understand the difference between the surface of natural and artificial carieslike lesions.

**References**