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DENTIN: A DYNAMIC SUBSTRATE - A REVIEW

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Abstract

The structure of dentin is unusual in that the number and size of its tubules changes as one moves from the periphery toward the pulp chamber. Near the pulp, the tubules are very close together and the water content of this deep dentin is high. Near the enamel, the tubules are far apart, occupying less than 1% of the surface area. When enamel or dentin is cut, the surface becomes covered by an adherent layer of cutting debris called the smear layer. Its composition presumably reflects the composition of the underlying dentin. It is only about 1 μm thick but its presence modifies the function of the dentin a great deal. It lowers dentin permeability and therefore can be regarded as protective. However, it masks the underlying dentin and hence interferes with attempts to bond dental materials directly to dentin. If it is removed, the dentin becomes much more permeable and fluid shifts across the open tubules can cause sensitivity in vivo. As smear layers are very acid-labile, they often dissolve in oral fluids. Several attempts have been made to replace smear layers with acid resistant structures that accomplish the same function. Smear layer structure is being studied by using both scanning electron microscopy as well as electronic particle sizing equipment.

The close adaptation of dental materials to smear layers and to underlying dentin is currently an area of very active research. Removal of smear layers increases adaptation and bonding strength but may increase the incidence of pulpal inflammation if the bonding is not uniform or permanent. The dynamics of dentin are just beginning to be understood.

Key Words: Tubule density, dentin permeability, dentin, sensitivity, smear layers, dentin adhesives, dentin bonding.

Introduction

Normally, dentin is covered peripherally by enamel on the crown and by cementum on the root surfaces. Dentin is the only innervated hard tissue of teeth but it remains relatively insensitive as long as it is covered. However, dentin often becomes uncovered by traumatic loss of enamel (tooth fracture) or more commonly, dentin is slowly exposed as the gingivae recede, exposing soft cementum which is abraded by improper toothbrush habits. Coronal dentin is often exposed during the progression of dental caries. Such exposed dentin may become sensitive whether it is on the occlusal surface of the teeth or at the necks of teeth (Brännström and Aström, 1972; Nordenvall and Brännström, 1980; Addy et al., 1985; Absi et al., 1987).

Dentin is composed of a mineralized collagen matrix penetrated by long narrow parallel channels called dentinal tubules that are oriented perpendicular to the dentinoenamel junction. There are about 40,000 tubules per mm^2 in dentin (Garberoglio and Brännström, 1976) making it a very porous structure. One can easily calculate the percent of the dentin surface area occupied by tubules (Pashley, 1984). It varies from less than 1%, just beneath the enamel, to more the 22% near the pulp (A_t in Table 1). The water content or wetness of dentin reflects these intrinsic, structural differences. The increase in the percent of area occupied by tubules is due to the convergence of tubules upon the pulp chamber (Pashley, 1984). Around each tubule lumen is a cuff of hypermineralized dentin matrix termed peritubular dentin to distinguish it from intertubular matrix (Frank, 1959). Peritubular dentin contains (Fig. 1) little collagen matrix and is composed primarily of hydroxyapatite. As it is associated with each tubule, the area of dentin occupied by peritubular dentin (A_p in Table 1) increases as the tubules converge on the pulp. There is a corresponding decrease in the area occupied by intertubular dentin matrix (A_i in Table 1) as the pulp chamber is approached. These structural features contribute to the heterogeneity of dentin. When dentin is cut or shaped, a layer of cutting debris called the smear layer is created (Eick et al., 1970), which reflects the composition of the underlying matrix. As the composition of the underlying matrix may change as the dentin becomes thinner (i.e., near the pulp), it is not unreasonable to assume that the composition of the smear layer may also change as deeper dentin is cut. The thickness of smear layers is only about 1 μm (Figure 1). This

Table 1. Calculated values* for areas occupied by dentinal tubules (A_t), intertubular dentin (A_i), and peritubular dentin (A_p), at different distances from the pulp.

Distance from pulp	Number of tubules x 10^6 cm^{-2}	Radius of tubules x 10^{-4} cm	A_t	Areas[a] A_p	A_i
pulp	4.5	1.25	22.10	66.25[c]	11.65
0.1-0.5	4.3	0.95	12.19	36.58	51.23
0.6-1.0	3.8	0.80	7.64	22.92	69.44
1.1-1.5	3.5	0.60	3.96	11.89	84.15
1.6-2.0	3.0	0.55	2.85	8.55	88.60
2.1-2.5	2.3	0.45	1.46	4.39	94.15
2.6-3.0	2.0	0.40	1.01	3.01	95.98
3.1-3.5	1.9	0.40	0.96	2.86	96.18

* Data calculated from Garberoglio and Brännström (1976).

c There is no peritubular dentin at the pulpal surface, but it begins close to the pulpal surface.

a $A_t = \pi r^2 N(100)$ where N = number of tubules per cm^2. Note: A_t, although an area, is also the percent of surface area occupied by water.

$A_p = \pi N (R^2 - r^2) (100)$ where R = 2r and r = tubule radius.

$A_i = 100 - (A_p - A_t)$.

layer adheres to the underlying matrix with some tenacity. Smear layers cannot be rinsed or even scrubbed off such surfaces. The structural and functional implications of the smear layer will be discussed at length below.

Each dentinal tubule was produced by an odontoblast during the development of the tooth. An odontoblast process trails the cell body as the cell moved centripetally toward the pulp, laying down a collagen matrix. The cytoplasmic process maintains the patency of the tubule lumen. Considerable controversy exists as to how far the odontoblast process extends peripherally in the tubule. Many authorities maintain that it only extends about 25-30% of the length of the tubule. That is, that the peripheral two-thirds of the tubules are devoid of cytoplasmic process (Brännström and Garberoglio, 1972; Thomas and Carella, 1984). Others claim that the process extends from the cell body in the pulp chamber to the enamel, a distance of 3-3.5 mm (Maniatopoulos and Smith, 1983; LaFlesche et al., 1985).

There are several reasons why the extent of the process is important. There are no nerves in the peripheral dentin, yet it is extremely sensitive to a variety of stimuli (tactile, thermal, osmotic). If the odontoblast processes extend to the enamel, then they might transmit painful stimuli to pulpal nerves. If they do not extend to enamel then they cannot be stimulated directly and an alternate hypothesis would have to be developed. In the field of operative dentistry, the extent of the odontoblast process is also crucial to an understanding of how odontoblast cell bodies, which reside in the pulp chamber, can be injured by cutting dentin if there processes do not extend to the enamel. Much of the confusion may be due to investigators who erroneously mistake the presence of a basal lamina-like membrane lining the tubule lumen for the odontoblast process (Thomas, 1984, 1985). It can have the same tubular appearance as an odontoblast process, although it is hollow. Several authorities have argued that one cannot unequivocally identify a tubular structure in dentinal tubules as an odontoblast process using scanning electron microscopy (SEM) techniques. It is necessary to both identify the process using SEM and then confirm the presence of a plasma membrane in that same structure using transmission electron microscopy (TEM) before one can truly discern whether a tubular structure is the cytoplasmic process of odontoblasts (Thomas, 1985; Weber and Zaki, 1986). Even if the process did extend to near the enamel, its volume would be small since the diameter of the tubules decreases from 2.5 μm near the pulp to about 0.8 μm near the enamel (Table 1). When dentin is cut with dental drills, the odontoblast processes are severed (Dr. Richard Ten Cate, personal communication) and mixed with the smear layer created on the cut surface. If the odontoblasts do not extend to the enamel then this would not occur. However, it is not unusual for dentists to reduce dentin thickness to within one-third of its original thickness. This is the region where many investigators have observed odontoblast processes by SEM. At this point, there is no doubt that they are amputating cytoplasmic processes (Dr. Richard Ten Cate, personal communication). Thus, there are several theoretical reasons why one would expect the composition of smear layers crated on the deep dentin to be different from those created on superficial dentin. These differences flow from the unique structure of dentin. Indeed, once exposed, dentin must serve as a protective barrier to the diffusion of noxious bacterial products in saliva which can irritate pulpal soft tissues and trigger inflammatory reactions in the pulp chamber (Theilade and Theilade, 1976).

The barrier properties of dentin are unusual in

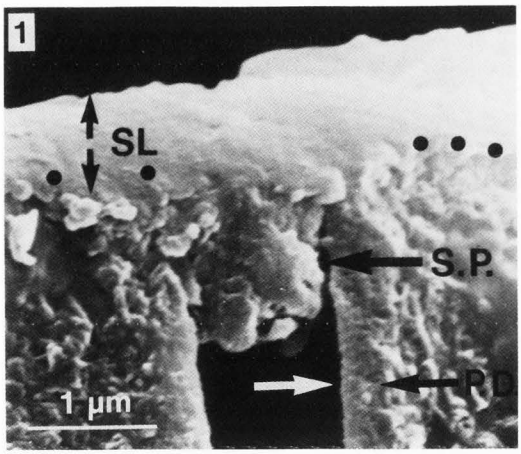


Fig. 1. Fractured surface of human dentin covered with a smear layer (SL). A single dentinal tubule is shown filled with a smear plug (S.P.) at its peripheral termination underneath the smear layer. The black dots indicate the approximate demarcation between the dentin matrix and the overlaying smear layer. Note the dentin around the tubule lumen (peritubular dentin or P.D.) has a different quality than the intertubular dentin on either side of peritubular dentin.

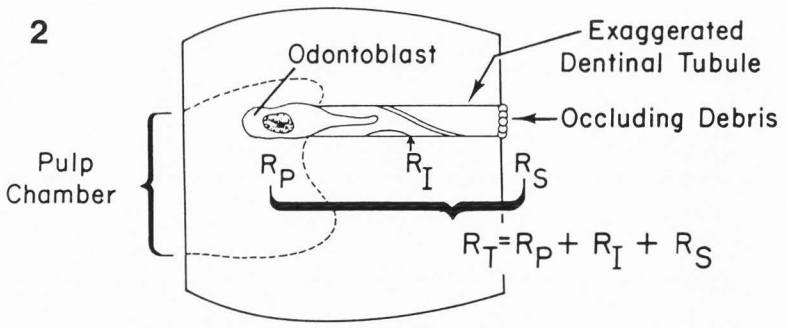


Fig. 2. The total resistance (R_T) to fluid movement across dentin is the sum of a surface resistance due to the smear layer (R_S), an intratubular resistance (R_I) and a pulpal resistance (R_P) (Pashley et al., 1978 a).

that they change as the thickness of dentin changes. The limited number of tubules (at least in superficial dentin) severely restricts diffusion. That is, only about 1% of the surface area is penetrated by tubule orifices. It is through these tubules that body fluids and materials dissolved in them, can permeate across dentin. The presence of a smear layer over the tubule orifice and especially the presence of "smear plugs" within each tubule (Fig. 1) also restricts diffusion (Pashley et al., 1978 a,b). Viewed from another perspective, the tubules within dentin represent millions of potential pathways to the pulp. The permeability characteristics of dentin reflect its lack of barrier properties. As the tubules through which solutes can diffuse across dentin are also the same channels that permit bulk fluid movement or convective transport, both convection and diffusion methods have been used to measure dentin permeability (Pashley 1985, Pashley et al., 1985). This review will discuss some of the relationships between dentin structure and its function with emphasis placed on the smear layer.

Dentin: Structure and Function

Dental Pain

The most popular theory of dentin sensitivity is termed the hydrodynamic theory (Brännström and Aström, 1972). That theory was developed to explain how peripheral dentin, which is devoid of nerves, is so sensitive. Brännström and his colleagues (Johnson and Brännström, 1974; Brännström et al., 1979; Brännström, 1981) performed numerous ingenious experiments both in vitro and in vivo which suggested that fluid shifts, in either direction through the tubules, caused deformation of mechanoreceptors near the pulpal termination of the tubules, which then caused pain. This helped to explain how a wide variety of apparently unrelated stimuli (mechanical, thermal, osmotic, etc.) could all cause pain. The corollary to the hydrodynamic theory is that anything that can interfere with fluid shifts, can reduce or eliminate dental pain. The variables that are important in fluid movement through small tubules are formalized in the Poiseuille-Hagen equation:

$$V = \frac{\pi \Delta P r^4}{8 \eta l}$$

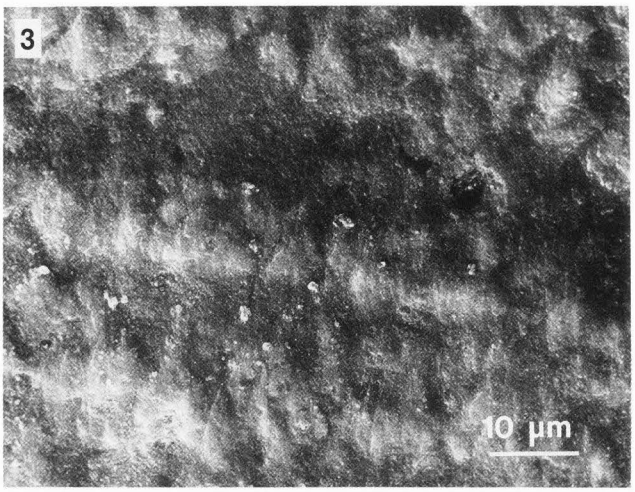
where:

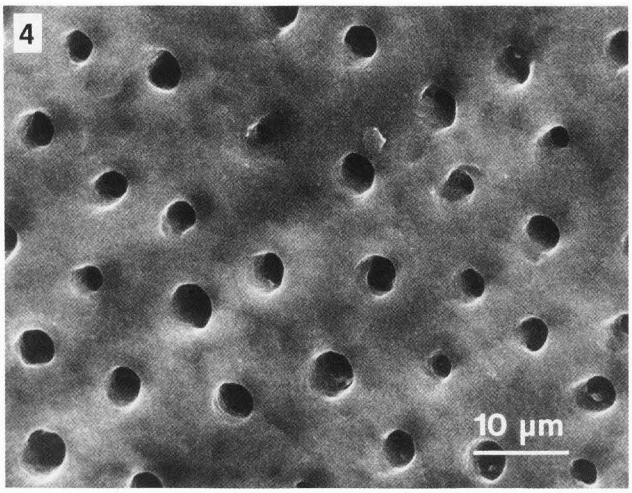
- V = volume flow
- ΔP = hydrostatic pressure difference across dentin
- η = viscocity of fluid
- r = radius of tubule
- l = length of tubule.

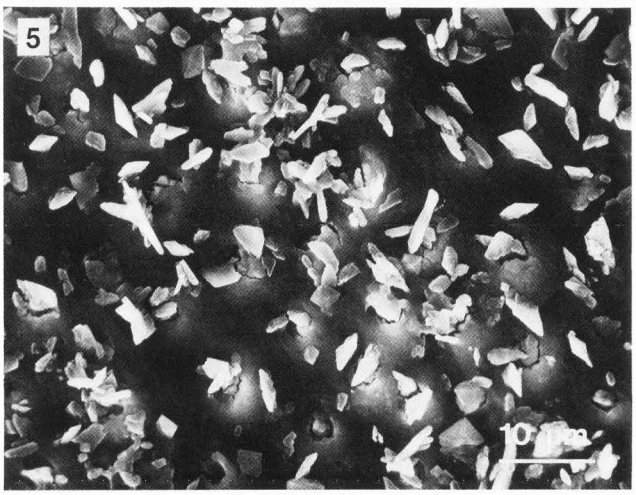
Note that bulk fluid movement varies with the fourth power of the radius rather than with the square of the radius (as is the case of diffusion). The radius to the fourth power is far more important than the length of the tubule (i.e., the thickness of dentin).

This was tested experimentally on freshly extracted teeth (Pashley et al., 1978a). The overall resistance to fluid movement across dentin was calculated as measured before and after creation of a smear layer and before and after removing pulpal soft tissues. The total resistance (R_T, Fig. 2) was calculated as the sum of three resistances arranged in series. The surface resistance (R_S, due to the presence of a smear layer) accounted for 86% of the total resistance to fluid movement across dentin. The resistance offered by pulpal soft tissue (R_P) was only 7% of the total, while the remaining intratubular resistance (R_I) accounted for 6% of the total. In another series of experiments, changes in dentin thickness were also evaluated (Reeder et al., 1978). These experiments clearly demonstrated the importance of the radius of the tubule. Small changes in tubule radius have more profound effects on fluid shifts across dentin than large changes in thickness. Clinically, removal of the smear layer makes dentin much more sensitive than if it is retained (Johnson and Brännström, 1974).

Smear layers are created on root surfaces during tooth cleaning or periodontal therapy (Pashley et al., 1987b). However, the particles which make up







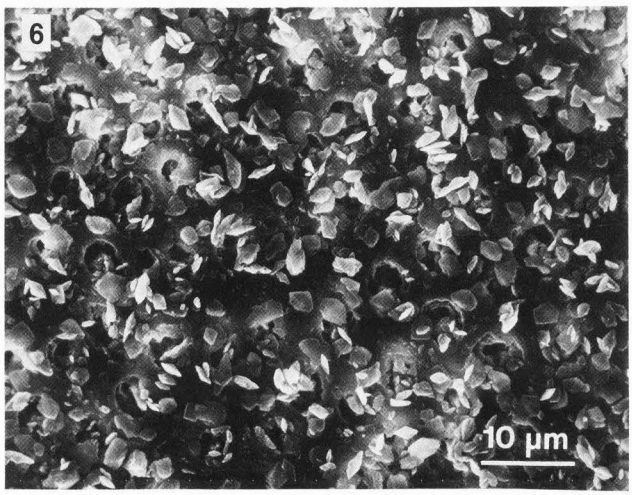


Fig. 3. Typical SEM appearance of the surface of dentin covered with a smear layer. There is little indication of underlying dentinal tubules.

Fig. 4. The appearance of dentin following 2 minutes application of 6% citric acid. The original smear layer shown in Fig. 3 was completely stripped away to reveal tubular structure of dentin.

Fig. 5. Smear layers treated with 30% dipotassium oxalate (pH 7) for 2 minutes and then rinsed with water. The surface exhibited large irregular crystals which appeared to have precipitated over tubule orifices.

Fig. 6. SEM examination of samples, similar to that shown in Fig. 5, but challenged with 6% citric acid for 2 minutes, revealed the loss of all remaining traces of original smear layer and the exposure of more tubule orifices. Even more crystals of calcium oxalate were created.

(Figs. 3 - 6 from Pashley and Galloway, 1985).

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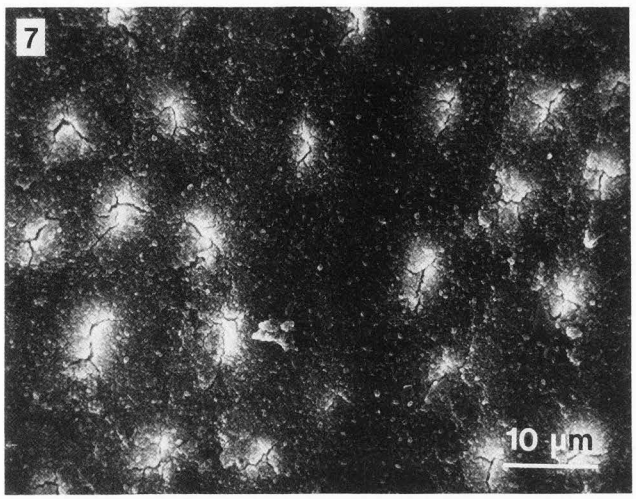
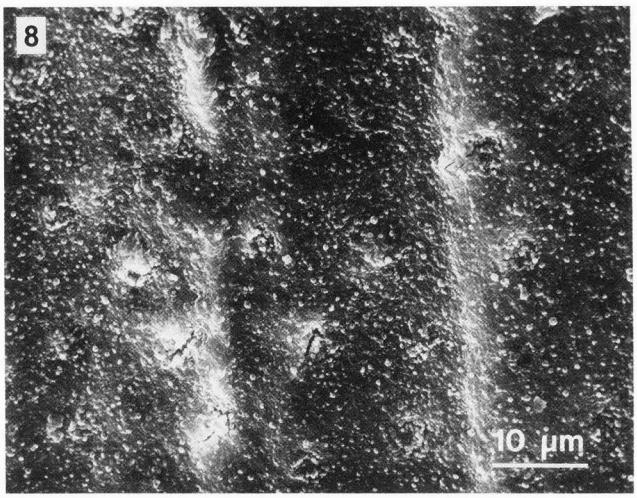
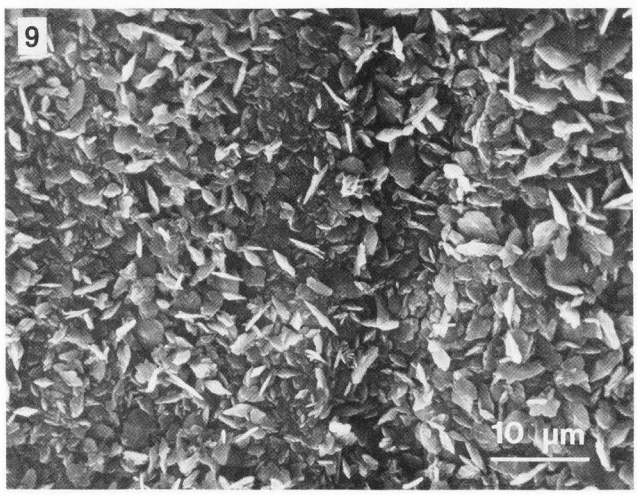
the smear layer are very small and they have an enormous surface area to mass ratio. This makes them more susceptible to dissolution than intact dentin matrix, especially in acidic environment. Microorganisms in dental plaque can produce enough organic acids during glycolysis to cause dissolution of such smear layers. Acids in the diet are also capable of removing the smear layer (Addy et al., 1985; Absi et al., 1987). As the smear layer is lost, the exposed dentin becomes more permeable and more sensitive. The treatment of these sensitive surfaces is aimed at reoccluding the tubules with another smear layer (Pashley et al., 1987b), with resins (Brännström et al., 1979; Nordenvall and Brännström, 1980) or with agents that replace the smear layer with a more acid-resistant structure (Pashley and Galloway, 1985). Examination of a typical smear layer (Fig. 3) reveals an amorphous surface. If such a surface is exposed to a weak acid, such as 6% citric acid for 2 minutes, the smear layer is completely removed and the pores of the underlying dentinal tubules are revealed (Fig. 4). The permeability of such acid-etched dentin increases 5-20 times (Pashley et al., 1981; Pashley, 1984).

Presumably, within the fluid trapped between smear layer constituents, there is some free, ionized calcium since the smear layer consists of pieces of mineralized dentin matrix. When soluble salts of







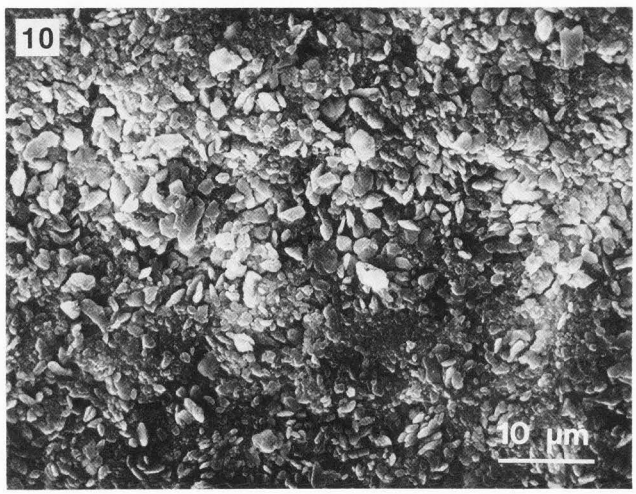


Fig. 7. Treatment of smear layers with 3% half neutralized oxalic acid (pH 2) for 2 min. completely removed the original smear layer and replaced it with a fine, granular surface. This surface was more coarse than the original smear layer. The cracks in the surface over the tubules are artifacts of sample preparation.

Fig. 8. Oxalate-treated surface shown in Fig. 7, but challenged with 6% citric acid for 2 min. No change was evident on SEM examination. The two large ridges were produced by sanding the dentin surface before oxalate treatment.

Fig. 9. Sequential treatment of smear layers with 30% dipotassium oxalate (2 min.) followed by 3% half-neutralized oxalic acid (2 min.) produced a surface that was uniformly covered with calcium oxalate crystals.

Fig. 10. Oxalate-treated surface shown in Fig. 9, but challenged with 6% citric acid for 2 min. There was little change in SEM appearance of the surface.

(Figs. 7 - 10 from Pashley and Galloway, 1985).

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oxalate (30% dipotassium oxalate) are intentionally applied to such surfaces they immediately react with available calcium to form insoluble crystals of calcium oxalate (Fig. 5) which are identified by their bipyramidal or tetragonal shape (Jahn et al., 1980). If this treated surface is challenged with 6% citric acid for 2 minutes, the remaining smear layer material is dissolved and even more crystals of calcium oxalate are formed from the calcium mobilized during dissolution of the smear layer (Fig. 6). These crystals block the tubule orifices as effectively as the original smear layer thereby maintaining the permeability of dentin much lower than would be possible in their absence.

An alternative procedure is to treat smear layer with 3% half-neutralized oxalic acid. This removes the original smear layer and replaces it with a layer of very small crystals of insoluble calcium oxalate (Fig. 7) which do not change their appearance when challenged with citric acid (Fig. 8). When original smear layer was treated in succession with 30% neutral dipotassium oxalate (2 minutes) followed by 3% half-neutralized oxalic acid, the surface became covered with a mixture of large and small crystals (Fig. 9) which also resisted acid attack (Fig. 10). All of these procedures reduced dentin permeability to

levels that were even below those obtained in the presence of smear layers. Such dentin treatments may be useful in future clinical practice.

Sclerotic Dentin

For dentin surfaces that are not covered with a smear layer such as exposed, abraded dentin at the necks of teeth, the intratubular resistance can increase enormously due to the deposition of intratubular deposits, both mineral and organic (Furseth and Mjör, 1972; Mjör, 1985). The interstitial fluids of the pulp chamber are under a small but significant positive pressure (Tonder and Kvinnsland, 1983). This pressure causes fluids to slowly filter from the pulp, through the tubules to the surface. Because the fluids contain both calcium and phosphate, their movement through the exposed tubules to the surface present the tubules with far more mineral than occurs by diffusion when the tubules are closed on their peripheral ends by enamel or cementum. Therefore, the entire volume of fluid in the tubules is replaced many times each day within open, exposed dentin. This leads to the production of intratubular crystals (Mendis and Darling, 1979) that transform the dentin in to what is termed "sclerotic" dentin. Such dentin is both less permeable and less sensitive. It is probably a result of simple physicochemical reactions rather than cellular activity. The production of sclerotic dentin improves the barrier properties of dentin. Similar reactions may take place, albeit much more slowly, in dentin covered with enamel or cementum. However, instead of requiring months, the reactions require many decades (Nalbandian et al., 1960; Jenkins, 1978). Sclerotic dentin can also be produced by the gradual production of more and more peritubular dentin at the expense of the tubule lumen (Mjör, 1985). The crystals produced in this reaction are extremely fine and are probably under cellular control.

Another very important alteration in dentin is the production of "caries crystals" on the advancing front of developing carious lesions in dentin. If the bacteria within the lesion have access to enough fermentable carbohydrates, they will glycolyze them to lactic acid. This acidity causes dissolution of peritubular dentin which is predominately composed of hydroxyapatite. As most of hydroxyapatite is calcium and phosphate, the dissolution of peritubular dentin raises the ionic concentrations of calcium and phosphate in the tubule fluid. Some of these ions diffuse out of the lesion but much of it diffuses down the tubules toward the pulp. As the hydrogen ions of the organic acids are buffered by trivalent phosphate in the walls of the tubules, the calcium and phosphate concentrations in the tubule fluid exceed the solubility product constants for various types of calcium phosphates which precipitate as caries crystals (Daculsi et al., 1979). Studies of the permeability properties of carious dentin indicate that it is much less permeable (and less sensitive) than normal dentin (Miller and Massler, 1962; Sarnat and Massler, 1965). This is a major reason why the carious process does not cause pain. The presence of caries crystals so effectively blocks fluid movement within the tubules that the dentin becomes insensitive even though it may be exposed at its periphery. The production of caries crystals represents a translocation of intrinsic dentin mineral rather than the transport of exogenous mineral into the tubules as often occurs in eroded or attrited dentin. The net result is the same, a large increase in the barrier properties of dentin.

Functional Versus Anatomical Dimensions

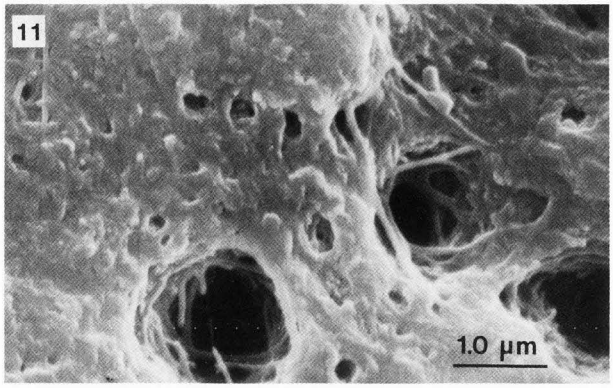
Intratubular deposits restrict diffusive permeation and convective or hydraulic fluid movement across dentin. They restrict the functional diameter of tubules far more than the anatomic diameter (Michelich et al., 1978). It should be remembered that dentinal tubules are extremely long channels. Their length is approximately 2000-3000 times their diameter (1 μm). A crystalline projection or even a collagen bundle extending in to the lumen anywhere along the length of the tubule (Fig. 11) can retard diffusion or bulk fluid movement a great deal. These restrictions easily escape detection using microscopic techniques. The functional diameter of tubules determined using hydrodynamic techniques is only 5-10% that of the anatomic diameter (Michelich et al., 1978). The functional diameter of tubules is less than 0.1 μm when the anatomic diameter is about 1 μm. One important clinical consequence of this is that dentin is very effective at trapping bacteria from bacterial-laden saliva. The fluid that exits the pulpal side of dentin is virtually sterile (Michelich et al., 1980). This may provide considerable protection to pulpal soft tissues.

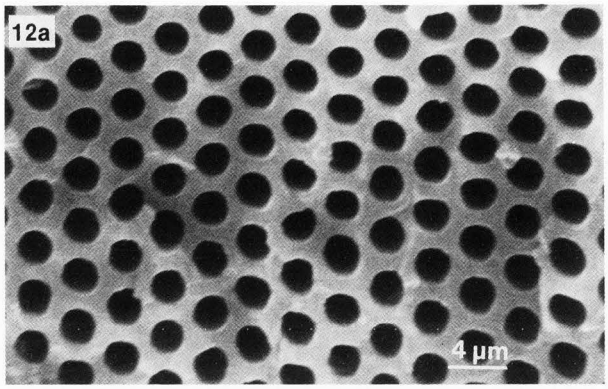
We have used hollow glass tubing arrays to simulate the tubule diameters and densities of dentin (Figs. 12 A & B). Their lumina or bores were absolutely smooth and free of any intratubular material. The hydraulic conductance of such tubules was 538-fold higher than that of smear layer-free dentin indicating how much intratubular material there must be in normal dentin tubules to restrict bulk fluid movement (Michelich et al., 1978).

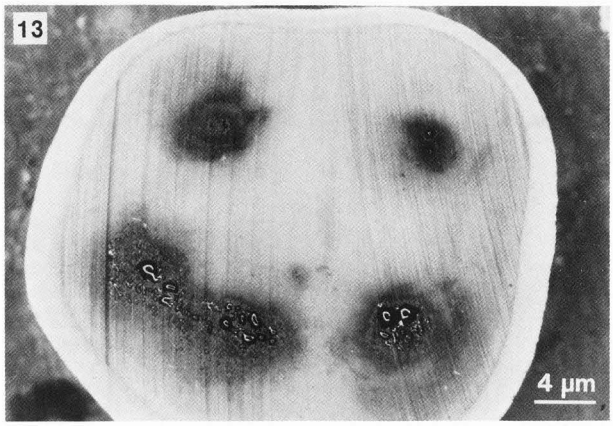
Regional Differences in dentin

The permeability of occlusal coronal dentin is not uniform (Pashley et al., 1987a). It is highest over pulp horn areas and lowest in the center of the occlusal surface (Fig. 13). SEM examination of the permeable and nonpermeable areas revealed the presence of open tubules in both regions. While there were less than half as many tubules in the low permeable region (30,436 ± 3,761 tubular/mm^2) as in the high permeable regions (65,448 ± 4,494 tubules/mm, $\bar{x}$ ± SEM, N = 15), there were no differences in their diameters (1.02 ± 0.02 versus 1.03 ± 0.03 μm, respectively (unpublished observations). Even though nearly all of the tubules in the low permeable area were open, the hydraulic conductance (a quantitative measure of the permeability) of the nonpermeable areas was zero. That is, although the tubules looked like they should be permeable, they were not. This was true even when middle dentin was sampled, which was free of any reparative or irritation dentin on their pulpal ends. Presumably, there is more intratubular material in central dentin than in tubules located over pulp horns. The observations indicate the importance of combining both functional and SEM techniques since either one alone could lead to misinterpretations.

The permeability characteristics of root dentin (Fogel et al., 1988) have recently been examined. Outer root dentin is covered with a thin layer (25-50 μm) of cementum. However, even after removing all of the cementum, the root dentin remained relatively impermeable. Only after 0.3-0.5 mm of external root dentin was removed did the permeability begin to increase. Even the most permeable inner root dentin







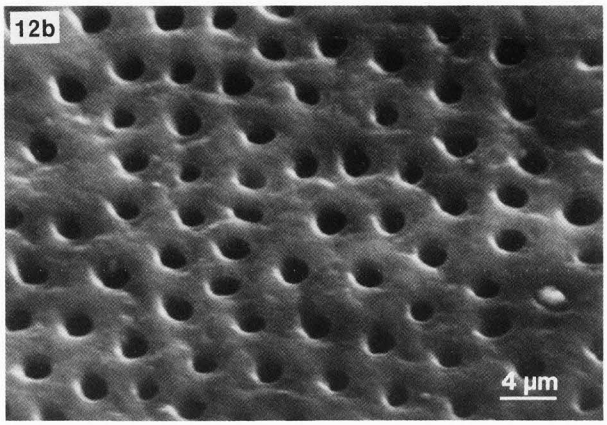


Fig. 11. Dentin surface after removal of the smear layer with 0.5M EDTA. Note the irregular and often fibrous nature of the tubule walls. These are probably collagen fibers. Their presence is very common and may be responsible, in part, for making the functional dimensions of tubules much less than their anatomic dimensions.

Fig. 12 - A. Bundle of glass tubing fused together to simulate the histologic appearance of dentin but not its function. B. Acid-etched human dentin showing typical tubule density and diameters (Michelich et al., 1978).

Fig. 13. Regional differences in dentin permeability. Transverse section of human third molar. The occlusal enamel was removed but remains circumferentially as a white collar. Dye was forced through the dentin to demonstrate that dentin over pulp horns is more permeable than central dentin (Pashley et al., 1987b)

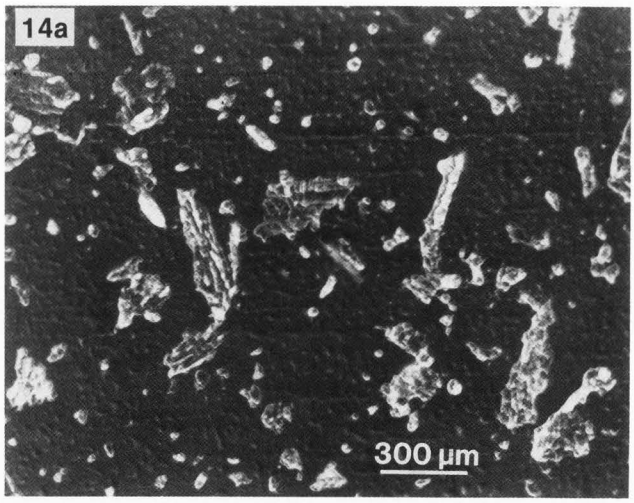
was still only 20% as permeable as coronal dentin (Fogel et al., 1988). Careful examination of published transmission electron micrographs of peripheral root dentin indicates that the tubules do not always extend to the cementum (Furseth, 1974). They also have a smaller diameter than occlusal dentin.

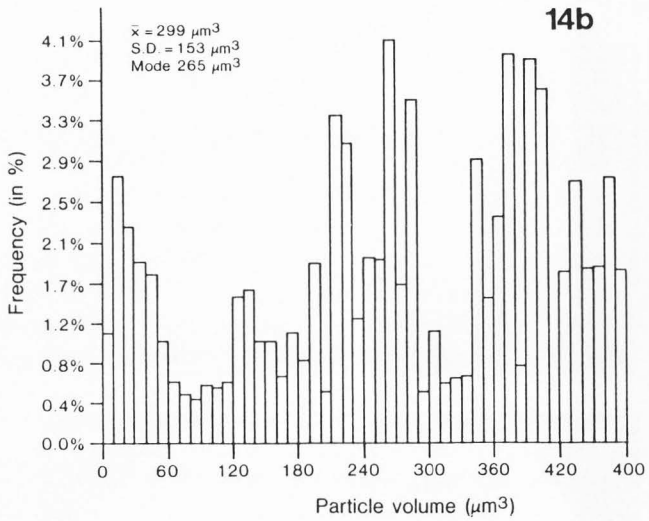
If root dentin were as permeable as occlusal dentin, its manipulation by periodontists and dental hygienists would not be possible without obtaining local anesthesia. Thus, there is considerable evidence that dentin permeability varies a great deal from one region of the teeth to another.

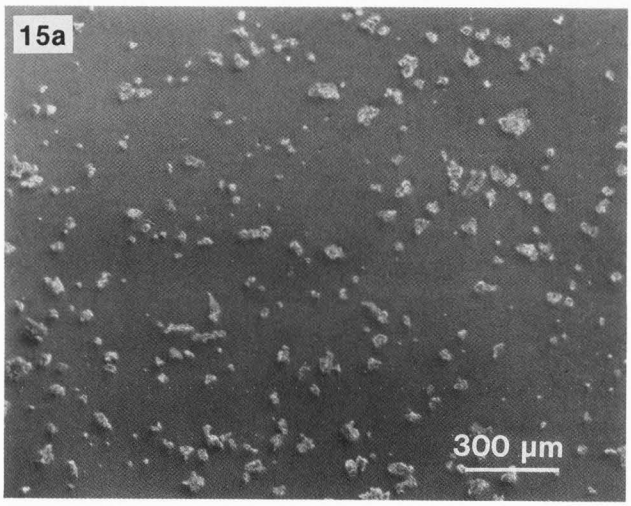
Smear Layer on Cut Dentin

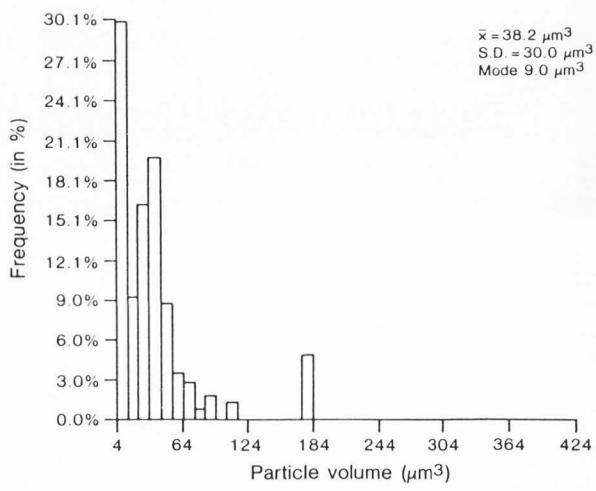
Perhaps the most interesting recent development in the study of dentin has been the discovery of the smear layer. Eick et al (1970) were among the first to describe the presence of the smear layer and to estimate the particle sizes of its constituents components. The presence of the smear layer decreases dentin permeability a great deal (Pashley et al., 1981; Boyer and Savare, 1981; Dippel et al., 1984). It can be regarded as a natural cavity liner that has several advantageous properties (Cotton 1984). It is very thin (ca. 1 µm), well-adapted to the dentin surface, and is hydrophilic. It reduces dentin permeability more than most commercially available cavity liners. Its disadvantages are that it is acid-labile, it may harbor bacteria if created in a septic environment (i.e., carious lesion or saliva contamination) and it prevents the close adaptation of dental materials to the underlying dentin thereby increasing the degree of microleakage of fluids around restorative materials and limiting the bond strength of adhesive resins.

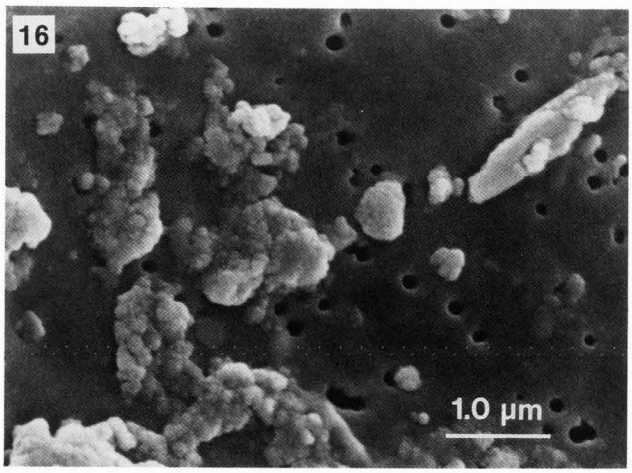
When dentin is cut with hand or rotary instruments, large shear forces are created and heat is produced. The cutting debris consists of a wide range of particle sizes that are different depending upon whether enamel or dentin is being cut. Dentin, which has a higher organic content than enamel, yields large particles of cutting debris (Figs. 14 A & B). Enamel contains little organic matrix and shatters in to relatively small particles (Fig. 15 A & B). The relationship between particle sizes of cutting debris that flow from cut tooth surfaces and the sizes of the particles within the smear layer that stay on the surfaces remains unknown. Smear layers cannot

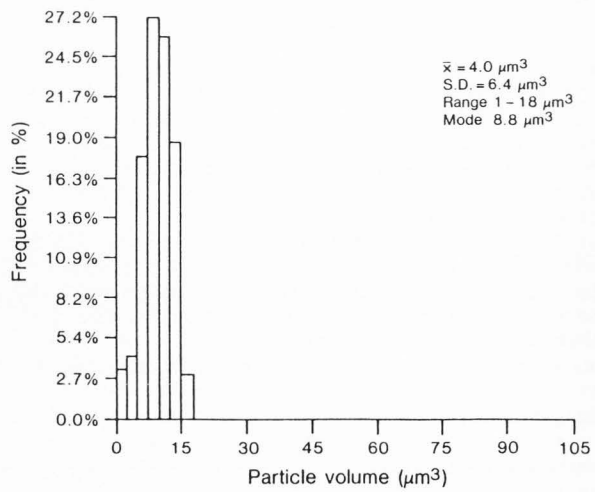

14a
300 µm


14b
x̄ = 299 µm3
S.D. = 153 µm3
Mode 265 µm3
Frequency (in %)
4.1%
3.7%
3.3%
2.9%
2.5%
2.1%
1.7%
1.2%
0.8%
0.4%
0.0%
0
60
120
180
240
300
360
420
400
Particle volume (µm3)


15a
300 µm


15b
x̄ = 38.2 µm3
S.D. = 30.0 µm3
Mode 9.0 µm3
Frequency (in %)
30.1%
27.1%
24.1%
21.1%
18.1%
15.1%
12.1%
9.0%
6.0%
3.0%
0.0%
4
64
124
184
244
304
364
424
Particle volume (µm3)


16
1.0 µm


17
x̄ = 4.0 µm3
S.D. = 6.4 µm3
Range 1 - 18 µm3
Mode 8.8 µm3
Frequency (in %)
27.2%
24.5%
21.7%
19.0%
16.3%
13.6%
10.9%
8.2%
5.4%
2.7%
0.0%
0
15
30
45
60
75
90
105
Particle volume (µm3)

Legends for Figs. 14 - 17 on the facing page

Fig. 14A. Photomicrograph of dentin cutting debris created with a #170 bur operated in a high speed dental handpiece. Fig. 14B. Distribution of dentin particle size (i.e., volumes) in the sample shown in Fig. 14A. Particle diameter is roughly proportional to the cube root of the particle volume.

Fig. 15A. Photomicrograph of enamel cutting debris prepared exactly as in Fig. 14A. Fig. 15B. Distribution of enamel cutting debris particle sizes.

Fig. 16. Scanning electron micrograph of dentin smear layer debris created with #37 inverted cone carbide-steel bur operated in a high speed handpiece with air water spray. The smear layer was removed by sonication and suspended on 0.2 μm Nuclepore filters. The background shows the 0.2 μm pores in the filter. The smear layer particles exhibit a "cobblestone" appearance in which the individual globules have diameters of 0.05 - 0.1 μm.

Fig. 17. Particle size distribution of smear layer debris which was created by polishing with 320 grit SiC sandpaper. This dentin smear layer debris has a much smaller particle size distribution than the dentin cutting debris of Fig. 14B.

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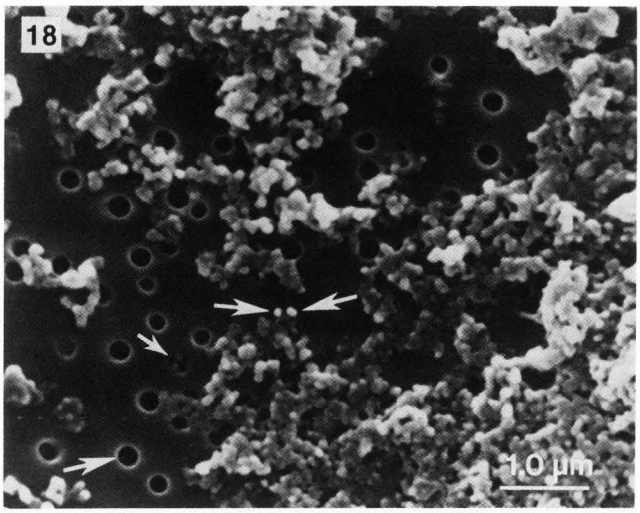
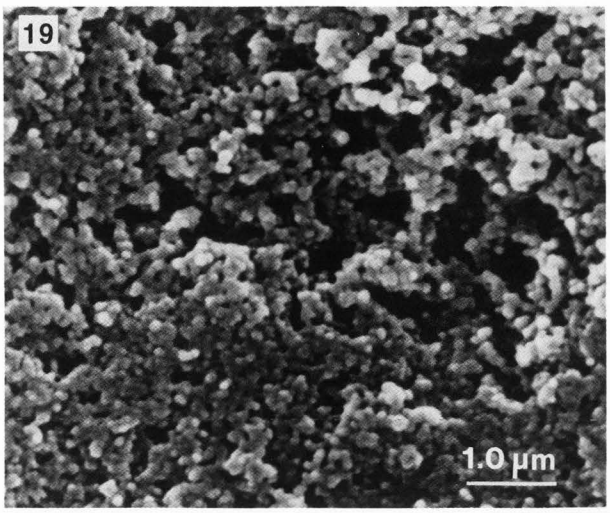
be removed by rinsing or scrubbing with water. The photomicrograph shown in Fig. 14A is of dentin cutting debris created with a high-speed dental bur turning a #170 plain fissure bur. The results of particle size analysis of the same sample are shown in Fig. 14B. When the same procedures were done on enamel rather than dentin, the cutting debris was much smaller. This is reflected in both the photomicrograph (Fig. 15A) and in the particle size analysis (Fig. 15B). Acids and chelating agents (i.e., EDTA) remove the smear layer by dissolving the particles. The only nondestructive method of smear layer removal that we have found is sonication. By measuring the hydraulic conductance of each sample as a function of sonication time, we can determine when the smear layer has been removed. The smear layer and the smear plugs must be removed (Fig. 1) before fluid filtration becomes maximal. During sonication, the smear layer particles that come off are collected and then characterized using an electronic particle size counter (Brinkman Particle Size Analyzer, Model 2010, Brinkman Instruments, Inc., Westbury, NY). It provides the frequency of particle sizes along with reliable statistics about populations of particles (Figs. 14B, 15B). The same smear layer particles are later trapped on a 0.2 μm Nucleopore filter for SEM study of the qualitative nature of the particles. The electronic particle size analyzer permits collection of statistically significant observations in only a few minutes. SEM study of the same samples reveals structural information that is below the resolving power of the particle size analyzer.

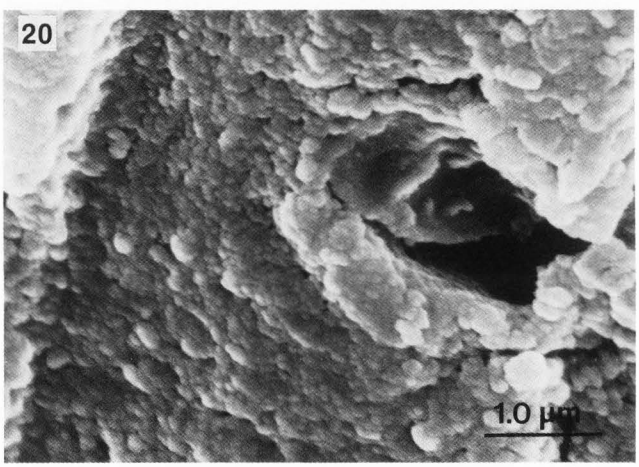
Figure 16 shows the SEM appearance of smear layer particles of dentin that were produced by a #37 inverted cone carbide-steel bur operated in a high-speed dental handpiece. These particles remained on the surface as a smear layer rather than flowing off the surface as cutting debris that was shown in Figs. 14A and 15A. The smear layer was removed by ultrasonication (cuphorn attachment to a 200 watt, Model W-220 sonicator, Heat Systems Ultrasonics, Farmingdale, NY) and the suspended material trapped on 0.2 μm Nucleopore filters. The particles were very irregular and revealed a granular substructure. The surface texture appeared as "cobblestones" of 0.05-0.10 μm diameter. Clinical smear layer production was simulated by polishing the dentin surface with 320-grit SiC sandpaper. When the resulting smear layer was removed by sonication and then analyzed by the electronic particle size analyzer, it also had a relatively small particle size distribution (Fig. 17). When those particles were examined by SEM, they appeared to be aggregates of very small (ca. 0.05 μm) globules (Fig. 18). If the smear layer debris was suspended on a Nucleopore filter to the point where it was many layers thick, it looked like a true smear layer. The resulting layer is called a reconstituted smear layer (Fig. 19). This provides another example of how important it is to analyze particles using both microscopic and electronic techniques. We have suggested that smear layer particles are composed of a globular substructure that represents the smallest dimensions of mineralized collagen fibers which fracture when the mineralized dentin is cut. When smear layers are examined in situ at the same high magnifications, they reveal a similar globular nature (Fig. 20).

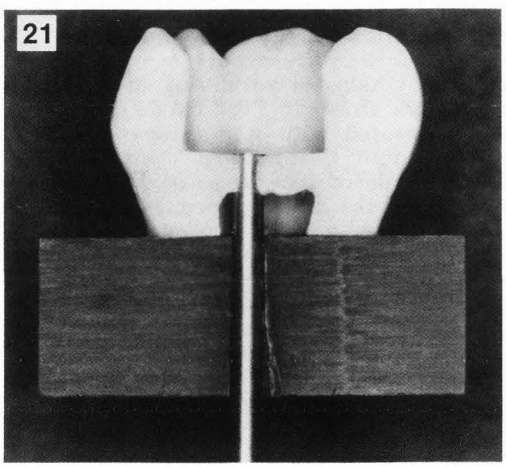
Microleakage Channels on Dentin Surfaces

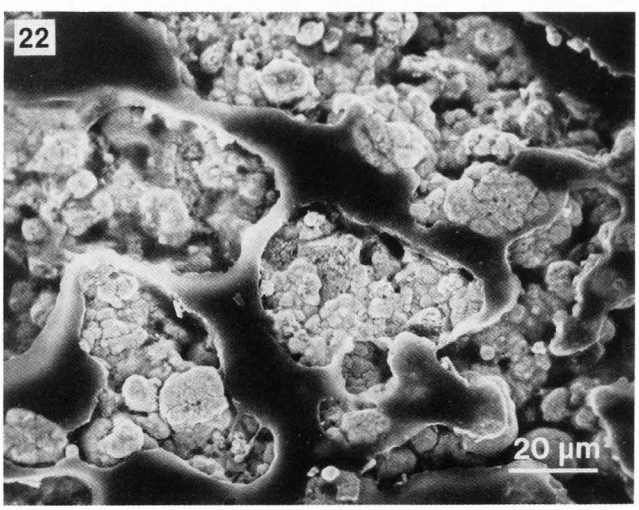
The presence of smear layers seem to increase the microleakage of fluids around restorative materials that are placed on top of the smear layer (Hoppenbrouwers et al., 1974; Jodaikin and Austin, 1981; Pashley and Depew, 1986). Removal of the smear layer decreases microleakage but increases the permeability of the underlying dentin (Pashley and Depew, 1986). It is not clear why dental materials such as amalgam, which do not bond to dentin, show more microleakage when they are placed over smear layers. Perhaps smear layers act like wet sand, deforming under a load. As dental amalgam is condensed against the cavity walls, the smear layer may deform. If the deformation disturbs adjacent regions it may create an irregular interface between the cavity wall and the filling material through which material may leak around amalgam restorations. In an effort to explore the nature of these microleakage channels, we made simple occlusal cavities in extracted human teeth. After cutting off the roots, a hole was drilled through the floor of the cavity and through a piece of plastic which was glued to the tooth (Fig. 21). An 18 gauge stainless steel tube was forced through the hole to serve as an opening to the amalgam-cavity floor interface. The tubing was plugged with a wire to prevent any amalgam particles from entering the tubing. After inserting and carving the amalgam restoration, the wire was removed and the tooth specimen was placed in physiologic salt solution for 24 hours. The next day, a small volume of self-curing methylmethacrylate resin (Concise Enamel Bond, 3M, St. Paul, MN) was injected through the tubing using a 5 cc disposable syringe and hand pressure. The resin could be seen leaking around the upper edges of the amalgam restoration. When several dozen specimens had been collected, they were individually placed in beakers of 2N HNO_3 on a gyratory shaker to dissolve the enamel and dentin material. After several days, the remaining organic matrix was digested using 2N KOH. These solutions had little effect on either control plastic resin or

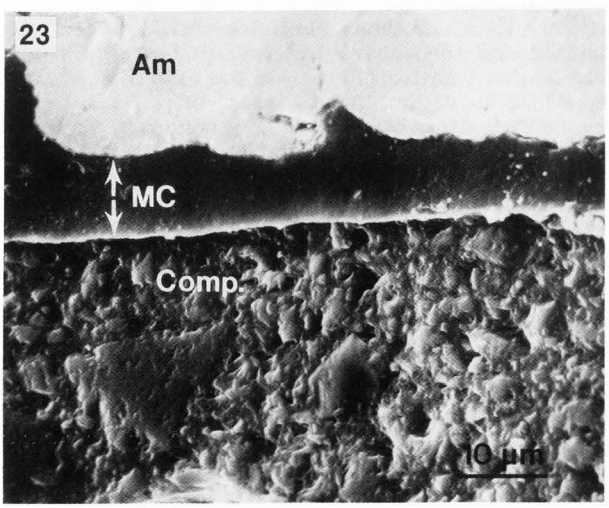

18
1.0 µm


19
1.0 µm


20
1.0 µm


21


22
20 µm


23
Am
MC
Comp.
10 µm

Legends for Figs. 18 - 23 on the facing page

Fig. 18. Suspended smear layer debris, trapped on 0.2 μm Nuclepore filters for SEM examination, revealed that most of the "particles" detected by particle size analyzer were actually aggregates of smaller globular units 0.1 μm in diameter (double arrows). The pores shown in the background (arrow) are 0.2 μm in diameter and represent the intrinsic pores of the microfilter (Pashley et al., 1988b).

Fig. 19. Microfilter overloaded with suspended smear layer material. The surface begis to assume to the appearance of a true smear layer.

Fig. 20. Micrograph of an smear layer created with 320-grit SiC sandpaper. The opening is where a smear plug became dislodged from a tubule orifice. Note the globular nature of the surrounding smear layer and how similar it is to reconsituted smear layer shown in Fig. 19 (Pashley et al., 1988b).

Fig. 21. Cross-section of human tooth cemented to a piece of Plexiglas with cyanoacrylate cement. A deep occlusal cavity preparation was made and then a hole was drilled through the plastic and tooth to accommodate an 18 gauge stainless steel tube. After restoring the cavity with dental amalgam, self-polymerizing liquid resin was introduced through the tubing to fill the space between the cavity walls and the amalgam (Pashley et al., 1989).

Fig. 22. Micrograph of the resin (smooth dark material) covering the silver amalgam (granular material). Note the reticular pattern (Pashley et al., 1989).

Fig. 23. Micrograph of dental amalgam restoration (Am in the light area) covered with resin (dark band) which was embedded in a composite resin (Comp in rough material) to permit preparation of careful cross-sections. The thickness of the original microgap between dental amalgam and the cavity wall (now occupied by rough composite resin) is shown by the thickness of the resin (labeled MC between arrows) which was about 6 μm (Pashley et al., 1989).

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control amalgam specimens. The resulting amalgam fillings were then coated with gold and examined by SEM for the presence of plastic veneers which would represent appearance of microleakage channels between the amalgam filling material and the smear layer. Those amalgams that were placed on smear layers were covered with an extensive, reticular network of plastic (Fig. 22). When these plastic channels (Fig. 23, layer between arrows) were cut in cross-section, they exhibited variable thicknesses of about 6-7 μm. When one compares the size of many oral microorganisms (ca. 0.5-1 μm) with the width of this gap, it is easy to see how bacteria can penetrate the spaces around restorations and colonize these surfaces. This is a common observation in studies of the reaction of the pulp to restoration materials (Browne and Tobias, 1986). When amalgam restorations were placed in dentin devoid of smear layers, much less plastic was found on the amalgam, and it was limited to the bottom of the cavity (not shown) rather than being uniformly distributed around the entire filling, as was commonly found in amalgams placed on smear layers (Pashley et al., 1989).

Clinically, dentists use a cavity varnish to seal the space between the amalgam restorations and the cavity wall. This creates a more hydrophobic surface which may facilitate amalgam wetting during condensation. Amalgam restorations slowly corrode at their surfaces creating particles of corrosion products that tend to occlude the marginal gaps and reduce microleakage.

It seems clear from the above discussion that dental materials such as amalgam adapt rather poorly to teeth. The gaps or channels between these materials and the cavity wall provide the opportunity for bacterial colonization of that space. These colonies can shed bacterial products which permeate via the tubules to the pulpal soft tissue where they can trigger an inflammatory reaction.

Bonding to Dentin

Recent dental materials research has focused on so-called "adhesive resins" that are designed to improve the bond between composite resins and dentin and to eliminate any gap between filling materials and the tooth. Scotchbond® (3M, St. Paul, MN) is an example of a popular dentin adhesive or bonding agent that is to be used on top of smear layers. Several other companies make similar products. The problem with these products is that the strength of the bond of the dental adhesives to the surface of the smear layer is stronger than the cohesive forces holding the smear layer particles together. When such bonds fail, they macroscopically look as though the failure was adhesive in nature. However, upon SEM examination of both sides, smear layer granules can be seen on both sides of the failed bond, (i.e., composite resin and tooth) indicating a cohesive failure of the smear layer (Tao et al., 1988). Such studies emphasize the importance of microscopy in materials research. It is apparent that the limiting value of the bond strength of such bonding systems is the force holding the smear layer particles together. While products could be designed that might strengthen those forces, many investigators are developing bonding methods that consist of a surface treatment which removes the smear layer (i.e., Scotchbond 2, 3M; Gluma, Bayer). After removal of smear layer, a bonding material is directly placed on the dentin. Such treatments increase bond strengths (Munksgaard and Asmussen, 1984; Asmussen and Bowen, 1987) to dentin because they do not suffer from the intrinsic weakness of the smear layer. If, however, the bonding is not complete or if the bond degrades over time, the dentin will be much more permeable to materials leaking into the bonded interface than if a smear layer were left in place. This may lead to pulpal irritation and sensitivity. Recent bonding experiments done under laboratory conditions that simulate in vivo conditions of temperature and pulpal pressure indicate that bond strengths of many adhesive resins are much lower than those measured under usual laboratory conditions (Mitchem et al., 1988; Andreaus et al., 1987).

The bonding of resin to enamel is due primarily to mechanical interlocks rather than chemical bonding. Acid-etching of enamel increases bond strength (Buonocore, 1955; Gwinnett and Smith, 1982) by dissolving surface enamel prisms to create microscopic roughness for mechanical retention. Careful dissolution of the enamel under such bonded specimen reveals hundreds of thousands of resin tags.

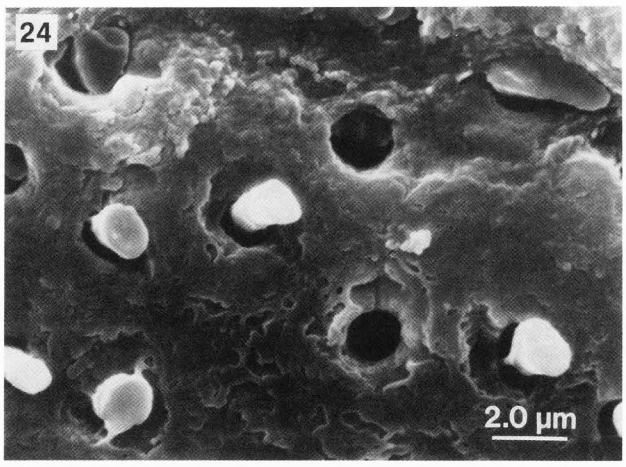

Fig. 24. Micrograph of an acid-etched dentin surface treated with Scotchbond overlaid with composite resin. After debonding the restoration, the dentin surface was examined by SEM. The light, homogeneous material represents resin tags which penetrated into open tubules. They broke off when the bond failed. The material around the tags was bonding agent (i.e., Scotchbond).

It was logical to expect a similar increase in bond strength when smear layers were removed from dentin to expose hundreds of thousands of dentinal tubules. It was thought that penetration of resin into these open tubules would increase bond strengths due to increased mechanical retention. Numerous investigators have reported either no change in dentin bond strengths or decreases in bond strength rather than the expected increases. SEM examination of these surfaces demonstrate that resin does penetrate into the tubules (Fig. 24) but there appears to be little correlation between the presence of resin tags in dentin and resulting bond strength. This is probably due to the fact that the tubules are so wet that the plastics do not actually bond to the tubule surface. As shown in Table 1, the density (i.e., number/area) of dentinal tubules increases as one compares superficial, middle, and deep dentin. If dentin bond strength were due to mechanical retention afforded by resin tags in tubules, one would expect the bond strength of deep dentin to exceed that of superficial dentin. However, exactly the opposite result is found. Deeper dentin demonstrate low bond strengths despite a high density of tags. Superficial dentin produces high bond strengths with few resin tags (Causton, 1984; Stanford et al., 1985; Mitchem and Gronas, 1986). Clearly, the mechanisms responsible for dentin bonding are much different from those producing enamel bonding. The direct communication between dentinal tubules and pulpal soft tissues makes it almost impossible to produce dry dentin surfaces. Removal of the smear layer uncovers the tubule orifices permitting dentinal fluid to wet the surface. New bonding adhesives contain hydrophilic derivatives of methylmethacrylate to improve surface wetting (Munksgaard and Asmussen, 1984).

An alternative approach is to replace the smear layer with a structure that serves the same function (i.e., occlusion of tubule orifices). Bowen et al., (1982) have reported high bond strengths of composite resins to dentin that has been treated with an acidic solution of ferric or aluminum oxalate. These oxalate solutions dissolve the smear layer and provide a sink for the calcium and phosphate ions that are liberated. Thus, the surface becomes covered with insoluble crystals of ferric phosphate (J. David Eick, private communication) and calcium oxalate which occlude the dentinal tubules. Such treatment lowers the permeability of dentin to values that are lower than the original smear layer (Pashley et al., 1988a). The surface is then treated with dentin primers dissolved in acetone. The acetone tends to dehydrate the dentin surface making it more hydrophobic. Such dehydration would not be possible if the tubules were not occluded. Occasional samples show such high bond strengths that, when they are subjected to tensile stress, the dentin shears before the composite resin. Unfortunately, those samples are rare but they do illustrate the ultimate goal. If the bond strengths of composite resins could exceed the cohesive strength of dentin, then clinicians could reinforce teeth that have lost some of their structure due to wear or various therapeutic procedures, to the point that the teeth would be stronger than they were originally.

Over the last decade, a great deal has been learned about the structure and function of dentin and underlying pulp. We are just beginning to appreciate the dynamics of this pulpodentin complex. As our understanding of dentin dynamics increases, we will be able to design materials and procedures for restoring teeth that are based on sound biologic concepts rather than empiricism. The future holds great promise for those interested in this most unusual mineralized tissue.

Acknowledgements

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Discussion with Reviewers

W.R. Cotton: If dentin is effective in trapping bacteria, and thus preventing significant pulpal response, there must be a minimal tubular length at which the dentin is ineffective as a protective barrier. What length do you consider this to be? Why?
Author: We can say with certainty that 1 mm of dentin thickness can trap most bacteria. If dentin is covered with a smear layer, few bacteria can penetrate into dentin because the particles that make up the smear layer are smaller than bacteria and can exclude them. Some bacteria may be capable of dissolving smear layer (Meryon, S.D. et al., Int. Endo. J. 19, 213-230, 1986). Thus, theoretically, dentin as thin as 5-10 μm could exclude bacteria if covered by a smear layer. In the absence of a smear layer, dentin can still trap bacteria because the tubules behave as if they had a function radius of 0.05 μm even though SEM study indicates a radius of 0.5-0.6 μm. I would estimate that 100 μm of dentin without smear layer would be sufficient to block acute bacterial penetration. Once trapped in dentinal tubules, bacteria can, if provided sufficient fermentable carbohydrates, produce organic acids able to dissolve peritubular dentin making the tubules larger. This makes dentin less effective at blocking bacterial penetration.

Although dentin is very effective at excluding bacterial penetration to the pulp, it does permit permeation of bacterial products that are shed from bacterial colonies. These products can induce a pulpal inflammatory response without the actual bacteria themselves reaching the pulp chamber.

W.R. Cotton: Since dentin is an effective protective barrier, are clinicians overly concerned when placing etchants, particularly acid etchants, on dentin?
Author: As was discussed, the barrier properties of superficial dentin are good but they deteriorate in deeper dentin. The presence of a smear layer is critically important in deep dentin because it reduces the permeability of the underlying large tubules packed very close together. Acids remove the smear layer thereby eliminating its masking effect of the underlying permeable tubules. Acids, per se, are not as irritating to the pulpal tissues as the bacterial products. The removal of the smear layer by acid etchants often leads to pulpal inflammation because of the increased permeation of bacterial products that leak around many restorative materials.

W.R. Cotton: According to your data, both amalgam and composite resins are subject to significant microleakage at the restoration-tooth interface, yet clinically, recurrent caries subjacent to amalgams, relative to composite resins, is infrequently observed. How do you explain this inconsistency?
Author: Amalgam has mild antimicrobial properties that do not exist with composite resins. No careful studies have been made of the microbial composition of gaps around amalgam versus composite restorations. If such comparative studies were done, I would predict that there would be both qualitative and quantitative differences in the flora of these spaces. Even if the flora were similar, the metabolic capacities of microbes growing on amalgam versus composites may be quite different due to the effect of heavy metals on bacterial metabolism which is so important in the carious process.

W.R. Cotton: Considering the regional differences of dentin permeability, are there any recommendations that you can offer the clinician for ensuring adequate basing to prevent pulpal irritation?
Author: In occlusal preparations the permeability of the dentin is highest over the pulp horns. If the isthmus of the preparation is wide the chances are good that the dentin over the pulp horns is very thin. We recommend basing the axial-pulpal line angle with light-cured calcium hydroxide containing resin bases or with a polycarboxylate cement. These seal dentin well and have very low solubilities. Placement of the material at the line angle is much more important, in terms of reducing dentin permea-

bility, than is placement in the center of the pulpal floor, an area that usually exhibits low permeability characteristics. Axial walls of proximal cavity preparations are another area of high permeability that should be covered with one of the materials listed above or with a glass ionomer if the remaining dentin thickness is over 0.5 mm.

W.R. Cotton: If the total resistance of fluid movement across dentin is due, in part, to pulpal soft tissue and intratubular resistance, will the in vivo pulpal pressure and presence of odontoblast processes change the percent contribution of each to total resistance?
Author: When we measured the series resistances of dentin in vitro, we used freshly extracted teeth so that we could include the resistances due to the presence of the odontoblast processes and cell bodies. Resistances were measured within minutes after extraction and then at weekly intervals of refrigerator storage under conditions that permitted autolysis of the odontoblast and its process. This change in resistance was then attributed to the odontoblast and its process which we combined to form what we call a "pulpal" resistance. We have recently repeated these studies in dogs in vivo. Fluid filtration across dentin was measured in vivo and then again, in vitro immediately after extraction and then a week later. The calculated resistances (unpublished observations) and their contribution to the total resistance were similar to our previously published data obtained with extracted human teeth. As the dog experiments included normal pulpal pressures, these pressures apparently are too small to influence the overall measurements.

W.R. Cotton: According to your data, root dentin is less permeable than coronal dentin. Clinically, postoperative sensitivity to restorative treatment appears to occur more frequently in the cervical area of the tooth than the coronal area. How do you explain this inconsistency?
Author: Coronal dentin is covered with a thick layer of enamel whereas exposed cervical root dentin is covered with a very thin (10 μm) layer of cementum that is easily abraided by tooth brushing. This places the orifices of exposed dentinal tubules directly in the oral cavity where they are exposed to numerous tactile, thermal, and osmotic stimuli. When cavities are prepared through enamel and in to dentin, the walls of these cavities are often 3-4 mm deep. The enamel portion of the walls is generally 2 mm deep. When the cavity preparation is restored, even if there is some microleakage around the materials, the dentin is at least 2 mm away from the stimulus. Further, under these conditions, there can be no direct tactile stimulation of coronal dentin as their can be of root dentin, because the coronal dentin is deep to the surface of the restorative material. It can be stimulated thermally or osmotically but only indirectly via the microspace or gap around filling materials.

If, for any reason, coronal dentin becomes exposed directly to the oral cavity, as often occurs during tooth fracture, the coronal dentin is often more sensitive than root dentin because it is both more permeable and more highly innervated than root dentin.

When class 5 restorations are placed, there is often some degree of finishing or shaping of the gingival termination of the restoration. This often removes the thin cementum thereby directly exposing sensitive dentinal tubules.

S.C. Bayne: What effect do you think the actual fluid components (ions or macromolecules) have on the adhesive properties of the smear layer particles to themselves and to remaining dentin?
Author: Presumably you are referring to dentinal fluid components. Until we have a much more convenient method of measuring adhesion of smear layer particles to dentin, the question cannot be answered well. We have measured the hydraulic conductance of dentin smear layers as a function of ultrasonication time. The conductance of the sample increases as the smear layer and smear plugs are sonicated from the dentin surface. Pretreatment of the surface with 5% NaOCl for 2 minutes increased the rate of removal of the smear layer and plugs. Presumably, NaOCl dissolved organic material such as gelatinized collagen matrix from particles making the smear layer less cohesive. However, no one has manipulated the ionic or macromolecular structure of dentinal fluid to determine its influence on the adhesive properties of the smear layer.

S.C. Bayne: What is the importance of sealing all the dentin versus only part of the dentin? How much communication is there between intertubule channels that would permit remote leakage sites to undermine otherwise sealed areas?
Author: Ideally, one would like to seal all cut dentin surfaces, especially if they were permeable; that is, if they were open to the pulp. Many dentin surfaces contain dentinal tubules that are not open all the way to the pulp. Some of these may have small (i.e. 0.1-0.2 μm diameter) inter-connecting channels or accessory branches to adjacent tubules. These small, secondary channels offer high resistances to fluid flow and hence are not thought to be implicated in bulk fluid flow or hydrodynamic transduction of stimuli. These small channels are prominent at the junction of enamel and dentin but are found with much less frequency as tubules are examined toward the pulp. Their small size and number suggest that they would not permit much communication between adjacent tubules. This is probably why most carious lesions exhibit well-defined borders in dentin rather than spreading laterally in a diffuse manner.

A. John Gwinnett: Vital teeth show numerous enamel cracks. If this is related to the absence of a dynamic fluid flow across the dentin and enamel, what would be the implications for a tooth with a bonded restoration occupying most of the intracoronal dentin surface area? Would such a bonded interface preclude such fluid flow necessary perhaps for the biomechanical integrity of the tooth?
Author: If enamel cracks extend to the dentinoenamel junction, then fluid should flow through the cracks to the surface. The toughness of dentin from non-vital teeth is less than that of vital teeth. This needs to be tested on teeth which are truly sealed with resins to determine if bonded dentin exhibits altered biomechanical properties.

J. David Eick: How do you know that the crystals in Fig. 5 are calcium oxalate?

Author: We know that calcium oxalate dihydrate forms characteristic bipyramidal crystals which are easily identified (Akbariah M. et al., Scanning Microscopy, 1(3), 1397-1403, 1987).