THE DENTAL CLINICS OF NORTH AMERICA

Dent Clin N Am 46 (2002) 211–245

The effects of dentin permeability on restorative dentistry

David H. Pashley, DMD, PhD[a,*],
Edna L. Pashley, DMD, MEd[b],
Ricardo M. Carvalho, DDS, PhD[c],
Franklin R. Tay, BDS, PhD[d]

[a]*Department of Oral Biology and Maxillofacial Pathology, School of Dentistry, Medical College of Georgia, Augusta, GA 30912-1129, USA*
[b]*Department of Oral Diagnosis and Patient Services, School of Dentistry, Medical College of Georgia, Augusta, GA 30192, USA*
[c]*Departmnet of Operative Dentistry, Endodontics and Dental Materials at Bauru, School of Dentistry, University of São Paulo, Bauru, Brazil*
[d]*Department of Restorative Dentistry, Prince Philip Dental Hospital, The University of Hong Kong, Hong Kong, China*

In his classic thesis work on pulpal tissue reactions to restorative procedures and materials, Langeland [1] drew a number of important conclusions. Among them was that "dentin exposed by preparation of cavities or abutments should be covered immediately with a non-irritating material that does not permit leakage." That is, he recommended that dentin be sealed. At that time, there was no knowledge of smear layers or adhesion of materials to dentin. Most restorative materials in use then did not adapt perfectly to cavity walls and left gaps between the walls and the materials, permitting oral bacteria to colonize the gaps and to shed bacterial products to the pulp, where they could cause pulpal inflammation. Pulpal inflammation can be induced simply by leaving dentin cavities open to the oral environment [2]. Brännström and Nyborg [3,4] proposed that the presence of microorganisms in gaps between cavity walls and restorative materials was the major cause of pulpal inflammation after restorative treatment. Bergenholtz and Lindhe [5] and Bergenholtz [6,7] demonstrated that cell-free bacterial products could induce pulpal inflammation through freshly prepared dentin,

This work was supported, in part, by grant no. DE06427 from the National Institute of Dental and Craniofacial Research.

* Corresponding author.
E-mail address: dpashley@mail.mcg.edu (D.H. Pashley).

PII: S0011-8532(01)00009-X

but not after dentin had been treated with calcium hydroxide [8]. Mjör's [9] research during this same time led him to conclude that "dentin permeability is probably the most important factor determining pulp reactions to caries, operative procedures and other localized lesions affecting it. If the dentin is impermeable, either because of intratubular mineralizations or due to a lack of tubular communication between primary and secondary dentin, it will be able to prevent pulp damage even if toxic substances from caries or restorative materials are present. The reason being that the substances would never reach the pulp to elicit an inflammatory response. In fact, this particular reaction pattern may be the basis for successful operative dentistry." In Fig. 1, the original or primary dentin is shown in the upper portion as having open tubules that originated at the enamel and extended to the pulp. Because of disturbances in the odontoblasts caused by caries or exposed dentin, however, the odontoblasts sometimes die and are replaced by new odontoblasts (tertiary dentin formation). Several changes can occur that profoundly change dentin permeability. If the new odontoblasts do not line up properly with the original tubules in primary dentin, the new tubules may not be continuous with the old, leading to reduced permeability (Fig. 1A). Alternatively, the new odontoblast-like cells often do not have odontoblast processes; the early matrix that they secrete is, therefore, atubular. Once mineralized, even a thin layer of atubular dentin can seal off primary dentin, thereby lowering the permeability to near zero (Fig. 1B) even though the tubules of the underlying reparative dentin were larger than normal [10]. Even if these odontoblasts develop a process and begin to form tubular dentin, it remains sealed by the atubular zone (Fig. 1B). Another common reaction of exposed dentin is the deposition

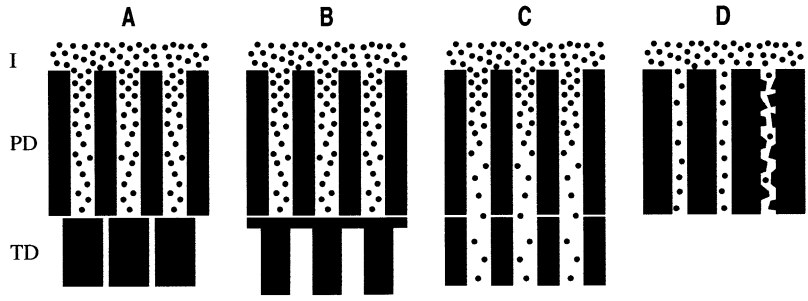

Fig. 1. Schematic illustrating how primary dentin (**PD**) can be modified by formation of tertiary dentin (**TD**; A–C) or by sclerosis (D). If original odontoblasts are destroyed, they can be replaced by newly differentiated odontoblast-like cells to form tertiary tubular dentin in which the tubules are not continuous (A). This would lower the permeability of dentin (shown as black dots) almost as effectively as if these new odontoblasts made a thin one of atubular dentin (B) prior to differentiating enough of an odotoblast process to make tubular dentin. Note that the tubules may be fewer in number and either smaller or larger in diameter in tertiary dentin. (*Adapted from* Tziafas D, Smith AJ, Lesot H. Designing new treatment strategies in vital pulp therapy. J Dent 2000;28:77–92; with permission.)

of mineral crystals in the tubule lumen (e.g., sclerotic dentin) that lowers permeability (Fig. 1D). Sometimes these crystals can completely obliterate the tubules, making the dentin impermeable (Fig. 1D). If the odontoblasts have been irritated but not destroyed, they can increase their rate of secretion and mineralization of dentin matrix. These tubules would remain in continuity with the overlying dentin because the same odontoblasts are making the matrix (Fig. 1C). In each panel of Fig. 1, the dots indicate the concentration of a diffusable substance in the dentinal fluid. Note that there is no permeation of dots in panels A and B, and fewer in panel C because the dentin has been made thicker. Less material can diffuse across sclerotic dentin (Fig. 1D) because the tubules have smaller diameters. Mjör's work stimulated the authors of this article to develop methods for quantitating the permeability properties of normal and abnormal dentin (see reviews [11,12]). This article covers the literature on the effects of dentin permeability on restorative dentistry that has occurred since 1992.

Dentin as a porous barrier

Dentin is the living, vital, sensitive portion of teeth that has been described as a biologic composite made up of a collagen matrix filled with nanofillers of apatite crystallites (Fig. 2) [13]. This matrix is penetrated by hollow tubules lined by (Fig. 2A) mineral-rich, collagen-poor, intratubular dentin. If that dentin is demineralized with ethylenediamine tetra-acetic acid or acids, the peritubular or intratubular matrix is removed, thereby enlarging the tubule diameters and revealing the fibrillar nature of the collagen matrix (Fig. 2B). The tubules are filled with dentinal fluid that is saturated in calcium and phosphate ions such as other extracellular fluids [14–16]. The fluid is under a slight positive pressure (15 cm H_2O) [17,18]. No outward fluid movement can occur so long as the tubules are sealed peripherally by enamel and cementum. If these external seals are lost, then dentinal fluid can slowly seep outward, especially if the tubules are open. Under such conditions, the tubules represent a fluid-filled continuum from the oral surface to the pulp chamber [19]. Substances dissolved in oral fluids or pulpal fluids can diffuse inward or outward, respectively, through these microscopic channels to modify the structure of dentin and the state of the pulp. Because dentinal tubules are only 1 μm in diameter but about 3 mm (or 3000 μm) long, these long diffusion distances dissipate the concentration of noxious materials 100- to 1000-fold, so that by the time they reach the pulp, their concentrations are often very low [20–23]. This tends to prevent initiation of inflammatory reactions to bacterial products [6,21,24–26], but also prevents local anesthetics, growth factors, and other possible therapeutic agents from achieving therapeutic pulpal concentrations [23,27]. As dentin is made thinner by cavity preparation or abrasion, these diffusion distances are shortened, and the dentin becomes more permeable [28]. Details on how dentin permeability can be measured were reviewed by Pashley [29].

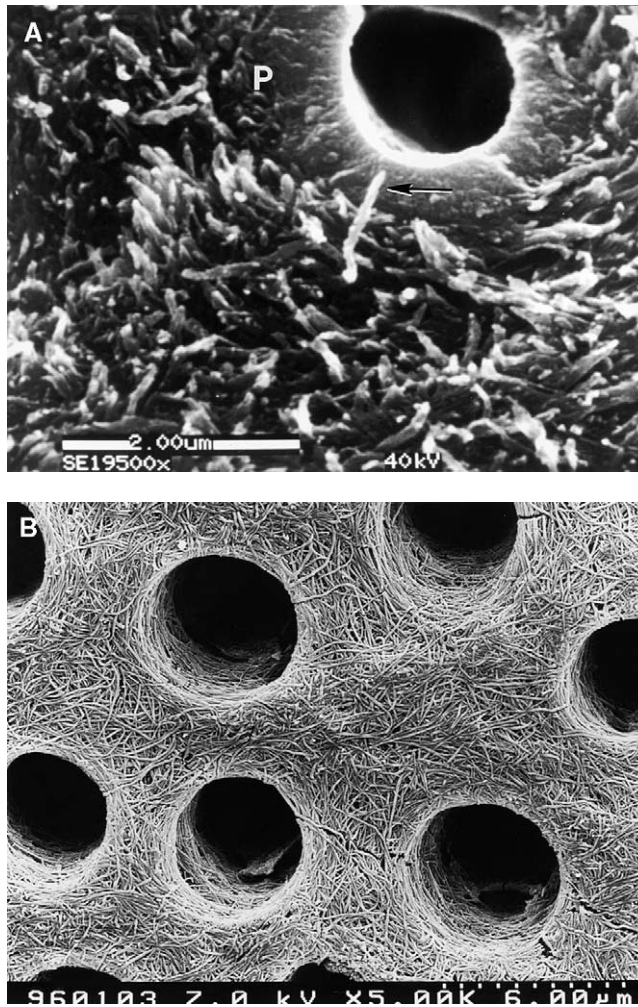

Fig. 2. Scanning electron micrographs of fractured mineralized dentin (A) and dentin following acid etching to expose the underlying collagen fibrils of the matrix (B). (A) Fractured dentin surface showing the cuff of mineral-rich, collagen-poor peritubular (**P**) (or intratubular) matrix surrounding the lumen of a dentinal tubule. The original tubule diameter was about 2.5–3.0 μm but became restricted to 1.3–1.5 μm in diameter by the deposition of peritubular dentin. Surrounding each tubule is a collagen-rich, less-mineralized intertubular dentin matrix made up of mineralized collagen fibrils about 0.1 μm wide. Note a single mineralized fibril extending from the intertubular matrix (arrow) superimposed on the peritubular matrix. (*From* Pashley DH. Dynamics of the pulpodentin complex. Crit Rev Oral Biol Med 1996;7:104–133; with permission.) (B) After acid-etching, the mineral phase of the matrix is removed from the surface along with the peritubular matrix to a depth of about 5 μm, exposing the collagen fibrils that make up the matrix. (Courtesy of Dr. Jorge Perdigão, University of Minnesota.)

Effects of dentin thickness on permeability

One convenient method of quantitating dentin permeability is to measure its hydraulic conductance or the ease with which fluid can flow through the tubules. The hydraulic conductance of dentinal tubules is related inversely to their length and directly related to the fourth power of their radius [30,31]. According to the hydrodynamic theory of dentin sensitivity [32,33], fluid shifts through dentin activate pulpal Aδ fibers to cause pain. As dentin is made thinner during cavity or crown preparations, the tubules become shorter and hyperconductive relative to thick dentin. This makes thin dentin intrinsically more sensitive than thick dentin; however, dentin can be shown to have three resistances to fluid movement arranged in series (Fig. 3). In thick dentin, the surface resistance due to the presence of the smear layer/smear plug combination accounted for 86% of the total resistance to fluid movement [34]. Well-hybridized resin tags in bonded dentin provide about the same resistance to fluid flow as did the smear layer/smear plug unit [35], but they are not acid-labile, as are smear layers. The pulpal resistance, caused by the presence of odontoblasts and their processes, only accounts for approximately 7.5% of the total resistance. The intratubular resistance, due to the presence of collagen fibers [36], and mineral constrictions only accounted for 6% of the total resistance. In acid-etched dentin or dentin that has lost smear layer/smear plugs, there is no surface resistance. In this case, the only resistances are the intratubular and pulpal resistances, each contributing about half of the total resistance. Both of these resistances can increase because of intratubular mineral or new dentin formation. In deep cavities (i.e., small remaining dentin thicknesses), the short tubules offer even less intratubular resistance to fluid flow. This increases the potential for dentin sensitivity and the need to seal the thin dentin with adhesive resin. As dentin is

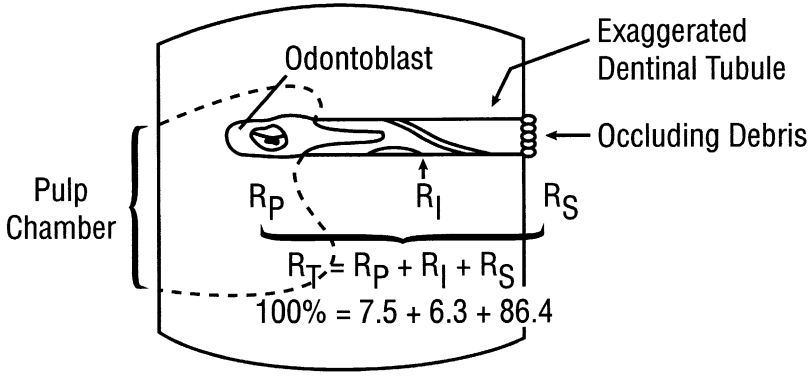

Fig. 3. Schematic representation of a crown segment, exaggerated dentinal tubule, and three series resistances contributing to the total resistance to fluid movement across dentin. (*From* Pashley DH, Livingston MJ, Greenhill JD. Regional resistances to fluid flow in human dentine, *in vitro*. Arch Oral Biol 1978;23:807–10; with permission.)

made thinner, the surface resistance, whether owing to smear plugs or resin tags, dominates.

Regional differences in permeability

The permeability of dentin is not uniform throughout teeth, because the number of tubules/mm^2 is not uniform (Fig. 4). Dentin located just beneath the dentoenamel junction has approximately 1500 to 1900 tubules/mm^2 that are about 0.8 μm in diameter, whereas the dentin near the pulp contains 4500 tubules/mm^2 that are about 2.5 μm in diameter [11]. When the surface area of each tubule is calculated and then the sum of all of the tubules is calculated at the dentoenamel junction versus near the pulp, the area occupied by water-filled tubules is only 1% versus 22%, respectively [39]. That also means that the water content of superficial dentin is only 1%, whereas it is 22% near the pulp. Superficial dentin is therefore very different from deep dentin. Hybrid layers in superficial dentin are almost free of resin tags [37,38], whereas approximately 50% of the hybrid layers in deep dentin (Fig. 5) are composed of hybridized resin tags. When creating post spaces in root canals, one is shaping the very deepest dentin. In such nonvital dentin, the water content of the dentin can be controlled, whereas in deep vital hyperconductive dentin, it is difficult to control the outward seepage of dentinal fluid [38] that sometimes interferes with resin bonding.

Dentin near pulp horns is more permeable (Fig. 6) than dentin further away because the density and diameter of tubules are highest near pulp horns [40]. Axial dentin is more permeable than the pulpal floors of class II cavities [31,41,42]. Root dentin is less permeable than coronal dentin be-

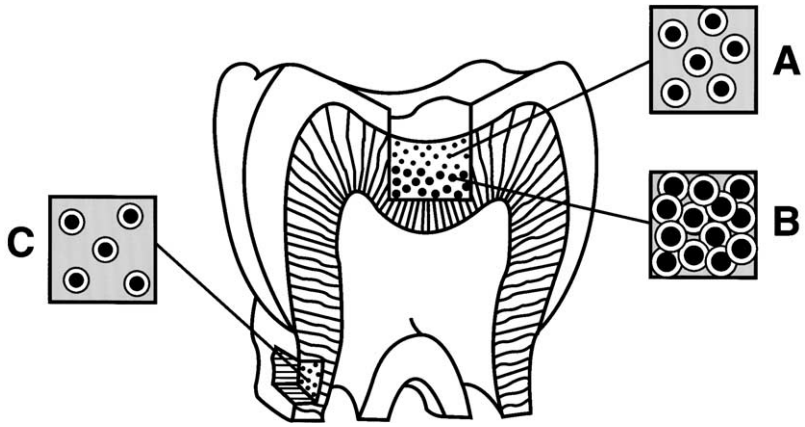

Fig. 4. Schematic showing that there are fewer tubules/mm^2 in superficial dentin than in deep dentin, and still fewer tubules per unit area in root dentin. Both the water content and the permeability of dentin follow the size and tubule number. (*Adapted from* Trowbridge; with permission.)

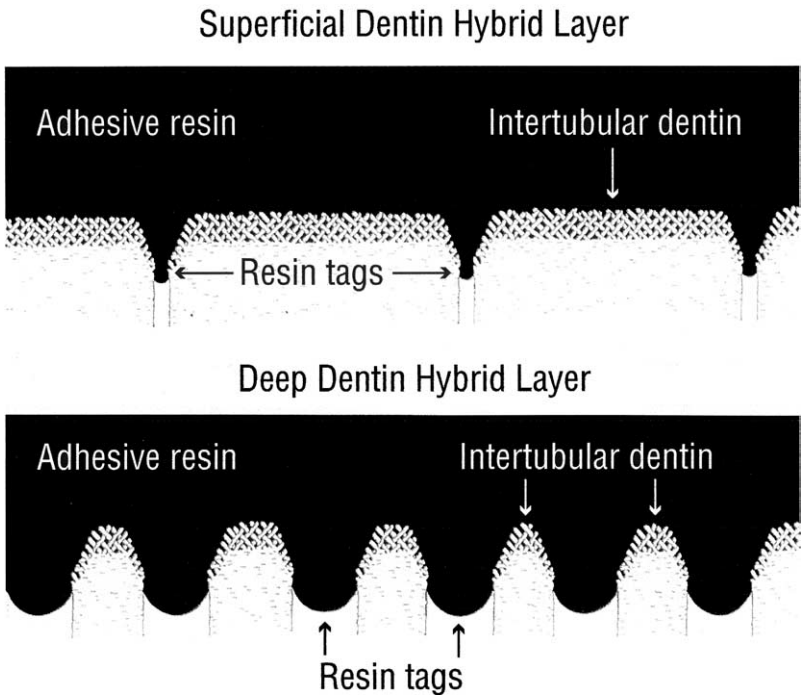

Fig. 5. Schematic diagram of acid-etched superficial versus deep dentin that has been infiltrated with resin (black) to form a hybrid layer. Cross-hatched structures depict collagen fibrils. Note that hybrid layers in deep dentin are composed mainly of resin tags, while superficial hybrid layers are composed primarily of resin-infiltrated collagen fibrils. (*From* Pashley DH, Sano H, Yoshiyama M, et al. Dentin, a dynamic bonding substrate: the effects of dentin variables on resin adhesion. In: Shimono M, Maeda T, Suda H, Takahashi K, editors. Proc Int Conf on Dentin/Pulp Complex. Tokyo: Quintessence Publishing Co.; 1996. p. 11–21; with permission.)

cause there are fewer tubules per square millimeter (Table 1) [43]. The dentin beneath carious lesions (the so-called caries-affected or sclerotic dentin) is much less permeable than normal dentin because the tubules of caries-affected dentin are filled with mineral crystals [44–46], and the tubules of caries-infected dentin are filled with bacteria [47]. These abnormal forms of dentin offer so much resistance to fluid movement that they are insensitive to masticatory, thermal, and osmotic stimuli. This insensitivity of carious dentin permits slow progression of the lesion toward the pulp without patients being aware of the potential danger.

Most postoperative sensitivity is caused by fluid shifts [48,49] that occur across normal dentin that is either too thin or poorly sealed or both. Clinicians are unaware of how porous and permeable deep dentin is because they have given patients injections of local anesthetics containing vasoconstrictors. This causes such intense pulpal vasoconstriction that the pulpal tissue pressure falls to zero and there is no further outward fluid flow [50]. In

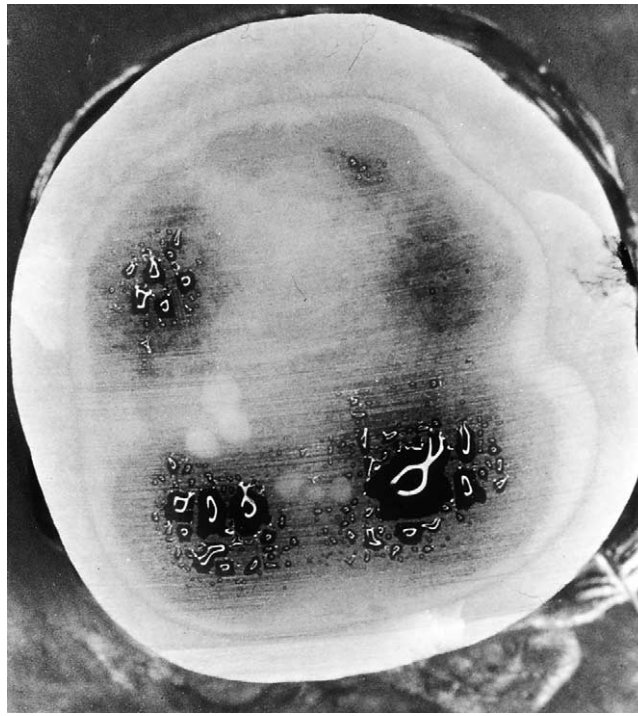

Fig. 6. Dentin disk positioned over a dye-filled chamber under a slight positive pressure to force fluid through the most permeable dentin located over the pulp horns. Even though the dentin thickness of the disk was uniform, the tubule diameters and densities are higher over the region of the pulp horns. (*From* Pashley DH, Andringa HJ, Derkson GD, et al. Regional variability in the permeability of human dentin. Arch Oral Biol 1987;32:519–523; with permission.)

the absence of such vasoconstriction, dentinal fluid seeps across dentin but is lost by evaporation [51,52] as fast as it seeps across, so that it never accumulates enough to create a reflective surface. Several investigators have shown that this dentinal fluid can accumulate beneath impression material before it sets, creating microscopic bubbles (Fig. 7), "hot dogs," and other structures [53,54].

Table 1
Comparison of coronal versus radicular hydraulic conductance of dentin

	Lp ($\mu L\ cm^{-2}\ min^{-1}\ cm\ H_2O^{-1}$)	
Thickness (mm)	Coronal	Radicular
0.9	0.1210	0.0005
0.7	0.1850	0.0204
0.6	0.390	0.0304
0.2	—	0.0701
0.1	1.500	0.0801

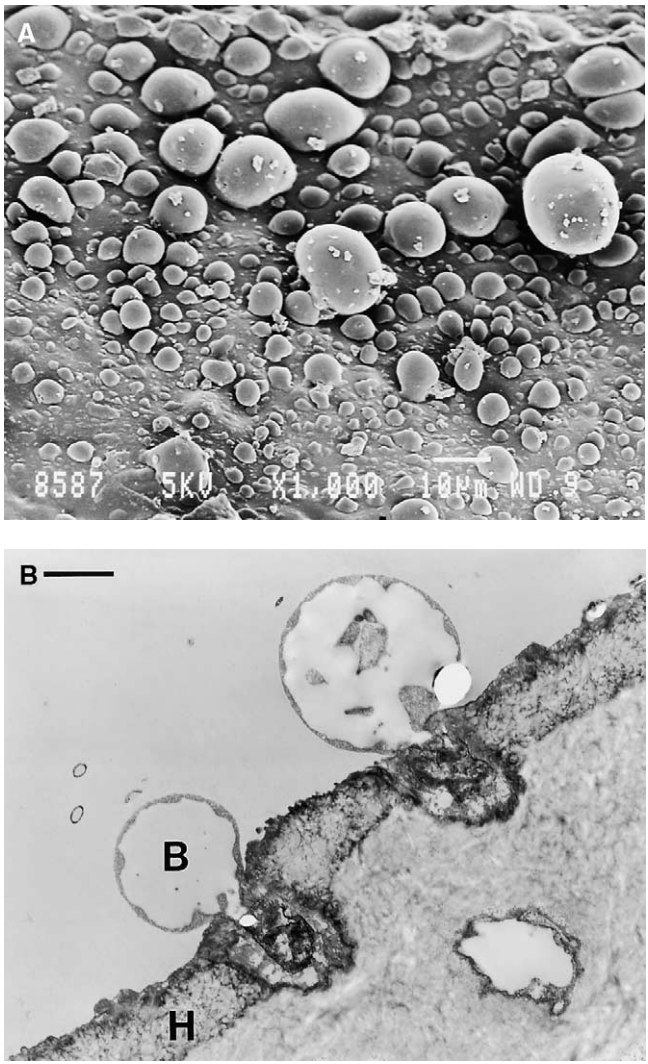

Fig. 7. (A) Scanning electron micrograph of surface of impression made of the pulpal floor of a freshly prepared deep cavity made *in vivo* without the use of vasoconstrictor in the local anesthetic. The bubble-like structures represent dentinal fluid that accumulated on the surface during the setting time of the impression material. (Courtesy of Dr. Franklin Tay, University of Hong Kong.) (B) Transmission electron micrograph of dentin bonded *in vivo* under local anesthesia without vasoconstrictor. Dentinal fluid accumulated in the self-curing adhesive layer as small blister-like (**B**) structures that prevented resin tag information in the tubules as they passed though the hybrid (**H**) layer. (Courtesy of Dr. Franklin Tay, University of Hong Kong.)

Restoration of peripheral dentin seal

One of the goals of restorative dentistry should be to restore the peripheral seal of dentin that originally existed because of the presence of enamel on coronal dentin and cementum on radicular dentin. This restoration should be done immediately after completion of cavity or crown preparations, before any impressions are taken or provisional materials are inserted. The ideal sealing material is an adhesive resin [55,56]. The choice of whether to use a multiple-step (i.e., separate acid etch, rinse, prime, or one-bottle adhesive), a two-step (i.e., self-etching primer and adhesive), or an all-in-one adhesive is left up to the clinician, although the authors prefer the use of self-etching primers. These systems are designed to be used on smear layers, and they often leave residual smear plug material hybridized by resin in the tubules, thereby preventing dilution of monomers by dentinal fluid [57,58]. Some adhesive systems are sensitive to varying degrees of intrinsic wetness [59]. This sensitivity is usually evaluated by comparing resin bond strengths in the presence or absence of pulpal pressure [60–69]. In general, these studies showed that most resin adhesive systems that use a separate acidic conditioner develop lower bond strengths when bonded under a simulated pulpal pressure. Glass ionomers and self-etching primer systems that do not require a separate etching step were insensitive to the presence or absence of pulpal pressure. Obviously, these would be the types of products that would be best suited for sealing dentin, especially deep dentin. The ideal adhesive is one that bonds equally well to superficial or deep dentin in the presence or absence of pulpal pressure.

Restoration of internal dentin seal

In addition to the hard tissue seal at the periphery of dentin, there is a soft tissue seal of dentin at the pulpal-predentin junction. Adjacent odontoblasts are joined by junctional complexes that include tight, desmosome-like, and gap junctions. They do not seem to form the classic arrangement of junctional complexes in epithelia, however. The gap junctions contain connexins [70] that permit intercellular communicating and may permit the odontoblast layer to secrete a collagenous matrix and mineralize it in a synchronized manner [71]. The junctional complexes between odontoblasts are sufficient to serve as a barrier to lanthanum [72] or horseradish peroxidase [73]. It therefore is likely that there is a functional barrier between the distal segments of pulpal odontoblast cell bodies that prevents the passage of macromolecules from the pulp into predentin and dentin. The barrier is perturbed after routine restorative procedures [74,75] that remove the peripheral hard tissue seal of dentin, however. This perturbation allows a rapid outward movement of pulpal/dentinal fluid containing plasma proteins, including fibrinogen [76,77], that may be responsible for subsequent reductions in dentin permeability that tend to protect the pulp from

additional irritation [23]. After reactionary or reparative tertiary dentin formation takes place [78], this soft tissue barrier is thought to be restored.

Cytotoxicity of dental materials

There is considerable interest about the cytotoxicity of materials. Some authors have argued that adhesive resins are cytotoxic [79–83]. Presumably, the resins leach unpolymerized monomers that permeate through dentin to reach the pulp. For this to occur, the monomers must be water-soluble because the tubules are full of water. Hanks et al. [22] have shown that many adhesive monomers such as bisphenyl-A-gly cidyl methacrylate (Bis-GMA) and urethane dimethacrylate are not sufficiently water soluble to reach a cytotoxic concentration unless placed directly on the pulp, where their cytotoxic action is due to their lipid solubility in cell membranes [84]. Under those conditions, they are cytotoxic. Hydroxyethyl methacrylate (HEMA) and other water-soluble monomers achieve much higher concentrations in dentinal fluid and can be cytotoxic, however, depending on the remaining dentin thickness and permeability [79,80,83,85–87]. A number of so-called all-in-one adhesives contain high concentrations of hydrophilic carboxylic and phosphoric acid derivatives of methacrylate that may also be cytotoxic because of their relatively high water solubility and HEMA content. In-vitro cytotoxicity studies tend to exaggerate the toxicity of materials, however, because the cells are exposed to relatively high concentrations of monomers that remain constant. In vivo, monomers diffuse into dentin as a concentration pulse. That is, they begin diffusing into dentinal fluid (to the extent that they are soluble in that fluid) for 20 to 30 seconds, as recommended by the manufacturer. They are then light cured, which converts most of the liquid monomers to solid polymers. Some additional monomers leach out of the polymer for several days, but this falls off over time [88–91]. Pulpal blood flow is efficient at clearing or removing exogenous substances [92] that diffuse across dentin to reach pulpal soft tissues [93], so it is unlikely that odontoblasts and nearby mesenchymal cells are exposed to high concentrations of adhesive monomers for long periods. Resin monomers are known to alter the immune response of the pulp, however, and may sensitize pulpal cells, making them more vulnerable to additional insults (as reviewed by Bergenholtz) [94].

When adhesive resins are placed on dentin, the liquid monomers rapidly diffuse down open tubules toward the pulp. The depth of penetration is less in vivo than in vitro because of the presence of dentinal fluid. In acetone-based systems applied to deep dentin in vivo, some resin formed microscopic resin globules as the acetone diffused into the water in the dentinal tubules (Fig. 8) [95]. Some of these globules reached the pulp chamber and were partially cleared by macrophages. Some resin tags seem to form by the coalescence of these submicron globules (Fig. 8). These tags can contribute to resin bond strengths if they are hybridized to the surrounding collagen fibril

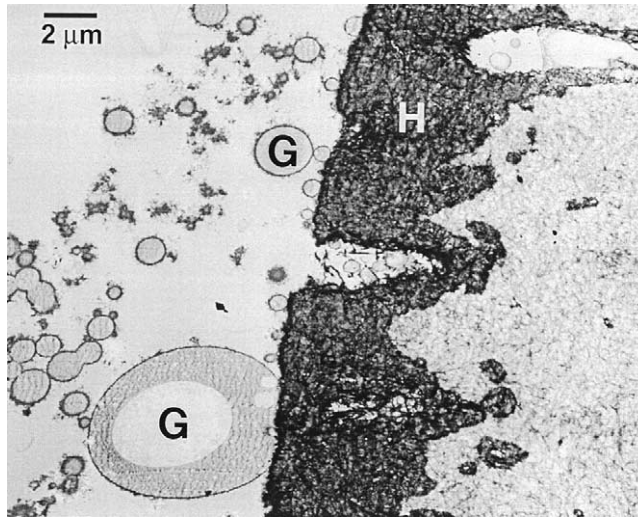

Fig. 8. Transmission electron micrograph of Prime & Bond applied to over-wet dentin, causing phase separations of the adhesive into electron dense resin globules (**G**). Note, however, that the resin hybridized (**H**) the acid-etched dentin surface well, although many tubules remain open and permeable. (Courtesy of Dr. Franklin Tay, University of Hong Kong.)

network (Fig. 9). The relative contribution of resin tags to bond strength depends on the tubule density, its diameter, and the strength of the resin [96]. These tags also can seal the tubules and greatly lower their permeability.

Problems associated with sealing dentin with resins

In the past, too many bonding studies that were used to evaluate the sealing ability of resins were flawed. They used shear bond tests that do not really apply pure shear forces [96–99]. Because of nonuniform stress application, local stress concentrations higher than 80 to 100 megapascals (MPa) develop that open up cracks in dentin at some distance from the bonded interface. This catastrophic failure is often misinterpreted. Even though the resin-dentin bond did not break, investigators divide the load at failure by the surface area of the bond (that did not actually fail) and obtain an apparent "bond strength" of 20 to 25 MPa. They interpret this result as indicating that this "bond strength" of 25 MPa is stronger than the cohesive strength of dentin, which is not true because the cohesive strength of dentin varies from about 50 to 100 MPa [100]. To avoid nonuniform stressing, the authors have developed the microtensile bond test [101–103]. Using this test, the failures are almost always adhesive or mixed and seldom involve cohesive failures in dentin when the cross-sectional areas are approximately 1 mm^2. The second problem with most bond tests is that they create flat dentin surfaces using abrasive papers of various grits at relatively low speeds instead of using complex cavity designs that are routinely made clinically.

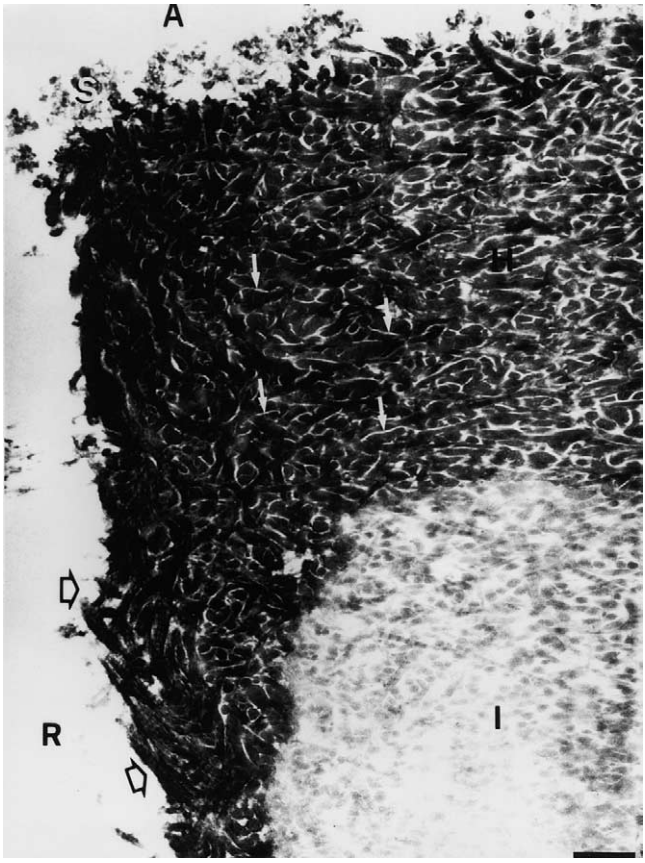

Fig. 9. Transmission electron micrograph of one side of a hybridized resin (**R**) tag. For resin tags to seal tubules, the peritubular matrix must be removed to expose the surrounding intertubular dentin matrix. Then liquid comonomers must flow down the lumen and diffuse radially into the 20–30 nm wide interfibrillar spaces that exist between collagen fibrils. When this resin polymerizes it forms a continuum between the resin tag and the surrounding hybridized dentin. The clear zone on the left is the lumen of a dentinal tubule that is filled with an electron lucent resin tag (**R**). **A** is the adhesive resin on the dentin surface. The regions that are dark are where the resin containing phosphate-derivations of methacrylate (Optibond, Kerr) have interacted with collagen fibrils and then with heavy metal stains. The laboratory demineralized (**I**) underlying dentin infiltrated with epoxy resin did not stain as darkly. Vertical arrows indicate 20 nm wide interfibrillar spaces filled with electron-lucent resin. (*From* Eick et al. Crit Rev Oral Biol Med 1997;8:306–335; with permission.)

When composite resins are bonded to opposing walls, the volumetric shrinkage that occurs in polymerization creates stresses as high as 17 to 20 MPa on the bonded walls in box-like cavities or in thin parallel-walled spaces such as between dentin and the walls of inlays or crowns. The geometrically determined contraction stress has been described as the *C-factor* (configuration factor) by Davidson et al. [104,105]. Box-like class I cavities in which

the walls have equal dimensions would have a C-factor of 5, whereas a flat surface would have a C-factor of 1 (Fig. 10). High C-factors can lead to debonding from one wall during light curing, which may lead to dentin sensitivity due to fluid shifts across unsealed dentin. The lower the C-factor, the less likely that polymerization shrinkage can stress the bonded interface. Laboratory studies done at C-factors of 1 tend to overestimate the bonding performance compared with complex cavity preparations with high C-factors [106–108]. The other problem with such studies is the use of abrasive paper rather than burs. A recent in-vitro study of self-etching primers bonded to dentin made flat with abrasive paper versus fissure burs at low speed and versus diamond burs at high speed showed much lower bond strengths obtained on diamond bur-ground dentin compared with abrasive paper [109]. The authors showed that the self-etching primers could not completely open the tubule orifices of diamond bur-ground dentin, unlike their good etching effects on dentin surfaces ground with abrasive paper.

Problems associated with sealing abnormal forms of dentin

Indeed, another disadvantage of adhesive system product development is the exclusive use of normal dentin. Clinically, resin bonds must be made to complex cavity preparations that contain sclerotic [110] and normal dentin.

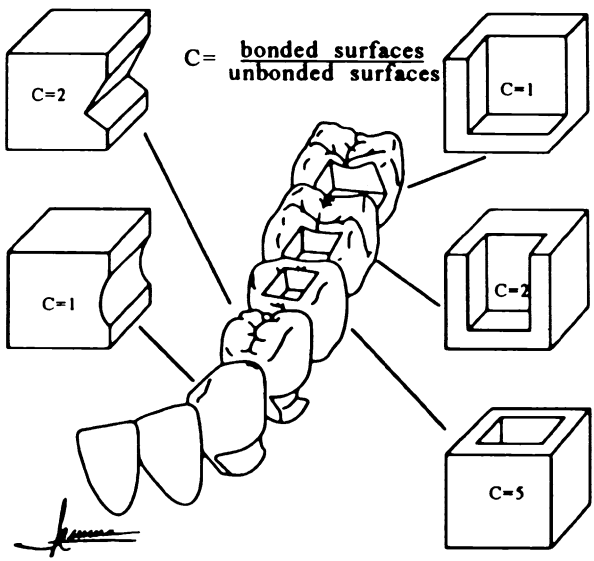

Fig. 10. Schematic summarizing the C-factor concept. The stress-generating potential is proportional to the bonded/unbonded surface area. The higher the C-factor the more the competition between the strength of the dentin bond and the forces of polymerization contraction, that can lead to debonding somewhere in the cavity. (*From* Carvalho RM, Pereira JC, Yoshiyama M, et al. A review of polymerization contraction: the influence of stress development vs stress relief. Oper Dent 1996;21:17–24; with permission.)

The dentinal tubules in sclerotic dentin are occluded with acid-resistant whitlockite crystals (Fig. 11). If resin cannot penetrate into tubules, the contribution of resin tags to the total bond strength is lost [37], leading to lower bond strengths [111–113] in middle to deep dentin. Recent research has shown that it is possible to obtain high bond strengths to caries-affected sclerotic dentin using total-etch, single-bottle systems if the moist bonding technique is used [114,115]. There is concern that the same acid-resistant mineral in the tubules of sclerotic dentin also may be formed in intertubular dentin, making it more resistant to acid conditioners used in adhesive dentistry.

Intertubular versus tubular permeability

This brings up the issue of intertubular dentin permeability. Up to this point, the use of the term *dentin permeability* was limited to tubular permeability or transdentin permeability (Fig. 12). This term is used to express the diffusion of substances through dentinal tubules to the pulp. It is also responsible for the movement of fluids within tubules and is responsible

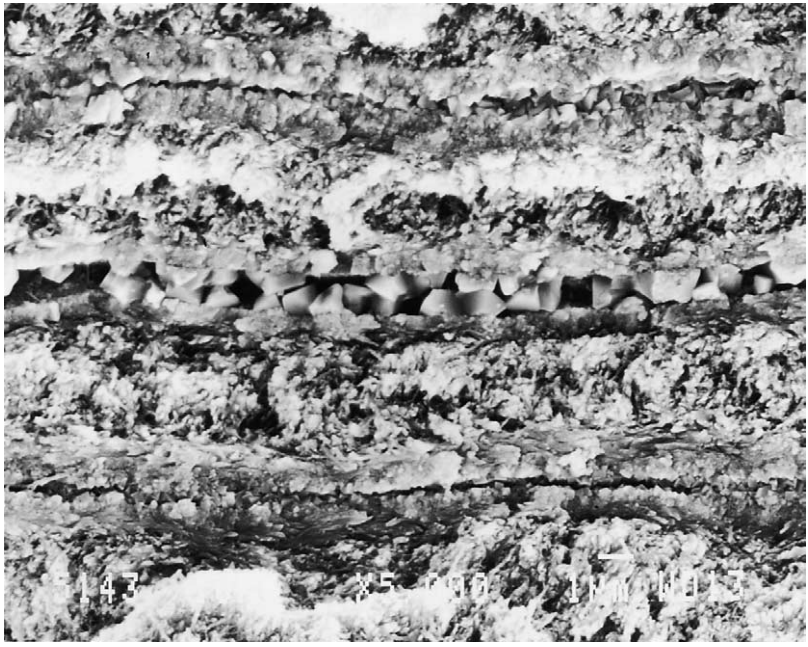

Fig. 11. Scanning electron microscopy of sclerotic dentin that has been fractured. There are three tubules running horizontally across the micrograph. In the top and bottom tubules, the peritubular dentin has grown thicker thereby restricting the size of the lumen that has almost disappeared. In the middle tubule the peritubular dentin matrix was less thick but the lumen became occluded with large rhombohedral crystals of whitlockite, a magnesium containing tricalcium phosphate. Such dentin is nearly impermeable and insensitive. (Courtesy of Dr. Franklin Tay, University of Hong Kong.)

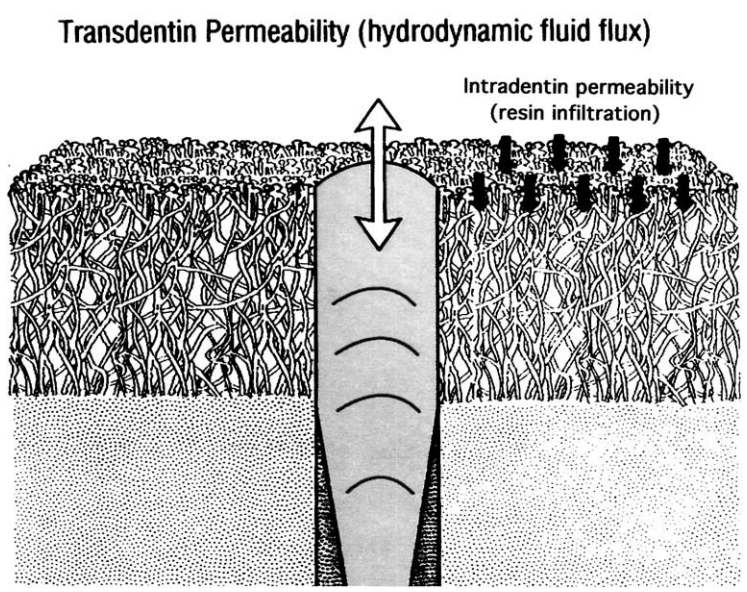

Fig. 12. Schematic of acid-etched dentin showing the fibrillar nature of the intertubular matrix and a single-etched dentinal tubule. The large white arrow in the lumen designates transdentin or tubular dentin permeability. This is the permeability that is responsible for dentin sensitivity and pulpal irritation. The smaller black arrows indicate intradentin permeability that is important in resin infiltration of the matrix to create a hybrid layer. Resin flow into the lumen and its radial diffusion into the surrounding fibrils effectively seals the tubules. (*From* Pashley et al. Crit Rev Oral Biol Med 1996;7:104–133; with permission.)

for dentin sensitivity. There is another kind of dentin permeability that is responsible for the diffusion of adhesive monomers into the dentin matrix between dentinal tubules or for monomer diffusion from etched tubule lumens into the surrounding collagen to hybridize the resin tags to the walls of the tubules, however. This has been called *intradentin permeability* or the *permeability properties of intertubular dentin*. It depends on the creation of spaces between collagen fibrils when the apatitic crystallites are removed by acidic conditioners (Fig. 13). If separate acid conditioners are used, the solubilized mineral phase of dentin is extracted by the acid and rinsed away by water, leaving the collagen fibrils floating in water. Many studies have shown that air drying such a surface, for as little as 5 seconds, causes it to collapse because the water between the collagen fibrils is lost (see Fig. 12) [116–119], leading to much lower bond strengths [120–125]. Many adhesive systems contain mixtures of monomers and polymers. For instance, Scotchbond Multi-Purpose primer (3M ESPE, St. Paul, MN) contains approximately 40% HEMA (molecular weight 130), 13% polyalkenoic acid (molecular weight 25,000 to 35,000), and the rest water. When this primer is applied to acid-etched dentin, the HEMA easily diffuses into interfibrillar spaces [126,127], but the larger polyalkenoic acid polymers largely remain

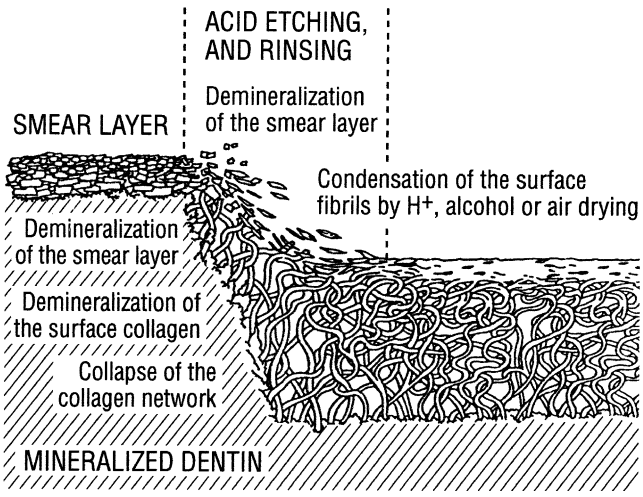

Fig. 13. Schematic showing the smear layer on the left; in the middle panel the dentin was acid-etched to remove the smear layer and demineralize the underlying 5 μm of dentin to expose the collagen fibrils. Acids, the solvents in some adhesives and brief air drying can cause collapse of the fibrillar network, decreasing its porosity for resin uptake. (*Adapted from* Pashley DH, Ciucchi B, Sano H. Dentin as a bonding substrate. Dtsch Zahn Z 1994;49:760–63; with permission.)

on the surface (Fig. 14) because they are too large to diffuse into the interfibrillar spaces that are only 20 nm wide [57,58,128–134]. Similar molecular sieving occurs with methylmethacrylate (molecular weight 100) and polymethyl methacrylate (molecular weight 300,000). It is not known how much sieving, if any, occurs between monomethacrylates and dimethacrylates (e.g., HEMA versus Bis-GMA). It is likely, however, that the composition of resin at the bottom of thick hybrid layers is different from the applied concentration (Fig. 15). This may alter the mechanical properties of these hybrid layers. Many manufacturers have begun adding nanometer-sized silica fillers to their adhesives to increase their viscosity. This makes the layer thicker, a desirable goal [135], and prevents clinicians from overthinning the adhesive layer before light curing. Some manufacturers have claimed that such silica particles are nanofillers that enter interfibrillar spaces in the hybrid layer, thereby reinforcing it; however, Tay et al. [136] were unable to find any silica particles in the interfibrillar spaces of hybrid layers. If the demineralized dentin matrix can act as a molecular sieve for relatively large polymers, can it also limit the diffusion of adhesive monomers? Is there any evidence for restriction of diffusion of adhesive monomers into demineralized dentin? Would the mechanical properties of the hybrid layer be different than if no such molecular sieving occurred? Only further research can answer these questions.

The influence of shrinkage of interfibrillar spaces on resin infiltration can be illustrated by comparing the micromorphology of completely resin-

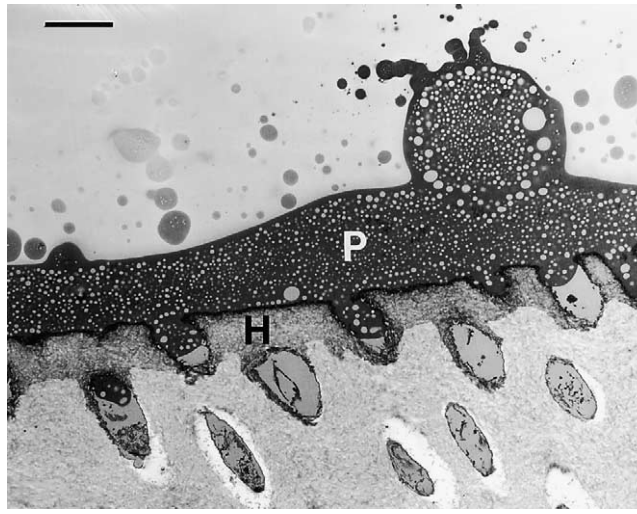

Fig. 14. TEM micrograph of Single Bond bonded to visibly moist acid-etched dentin. The adhesive layer (**P**) is electron dense because of the many carboxylic acid groups in the polyalkenoic acid that bind heavy metal stains. The small electronlucent droplets within the black layer may be water or HEMA. Note the electronlucent globules of varying size within the tubules. Note that the dark polyalkenoic acid can penetrate tubules but not the hybrid (**H**) layer. (Courtesy of Dr. Franklin Tay, University of Hong Kong.)

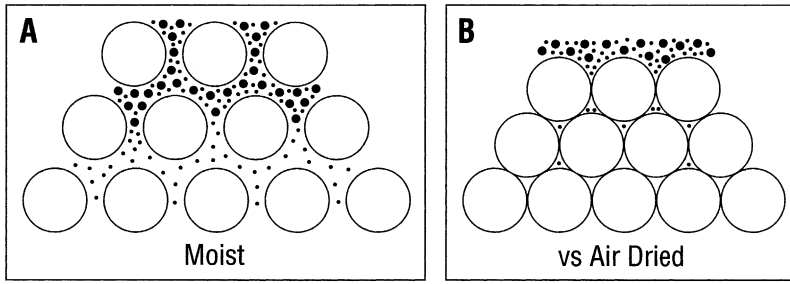

Fig. 15. Schematic illustrating potential molecular sieving of large from small molecular weight components in demineralized dentin. The large circles depict collagen fibrils cut in cross-section. They are 100 nm in diameter, the interfibrillar spaces are 20–30 nm wide. Large dots signify polymers and small dots, small monomers such as HEMA. (A) In moist dentin, the fibrils are well separated. (B) In air-dried demineralized dentin the interfibrillar spaces almost disappear. (*Adapted from* Pashley DH, Ciucchi B, Sano H, et al. Permeability of dentin to adhesive agents. Quintessence Int 1993;24:618–31; with permission.)

infiltrated dentin matrix with that of incompletely infiltrated dentin. Well-infiltrated, moist dentin forms thick hybrid layers (Fig. 16A) and resin tags that are hybridized to the walls of the tubules. If acid-etched dentin is over-dried before application of an adhesive system [56,57,128,130], the dentin matrix rapidly collapses [117,118]. When water-free adhesive is added to the dry dentin, the monomers have difficulty diffusing into the surface of

the collapsed network, although they can diffuse down tubules to form resin tags (Fig. 16B) [128,129]. When the tensile bond strengths of such ''dry dentin'' bonds are compared with those of optimal moist dentin bonds, they are only half as great [120–125,129]. In specimens that were bonded under moist conditions, the water separated the collagen fibrils, allowing the resin to infiltrate the collagen network uniformly (Fig. 17A), thereby offering the collagen fibrils support during bond strength testing and yielding bond strengths that were twice as high as air-dried collapsed dentin (Fig. 17B). If dentin is etched more deeply than it can be infiltrated with resin, zones of naked collagen fibrils are created in the bottom half of the hybrid layers [137,138]. Such procedures result in lower resin-dentin bond strengths [129,139,140].

Nanoleakage

Because it is difficult to detect the absence of resin in interfibrillar spaces in transmission electron microscope (TEM) studies owing to the electron-lucency of resin, several groups of investigators have used an indirect

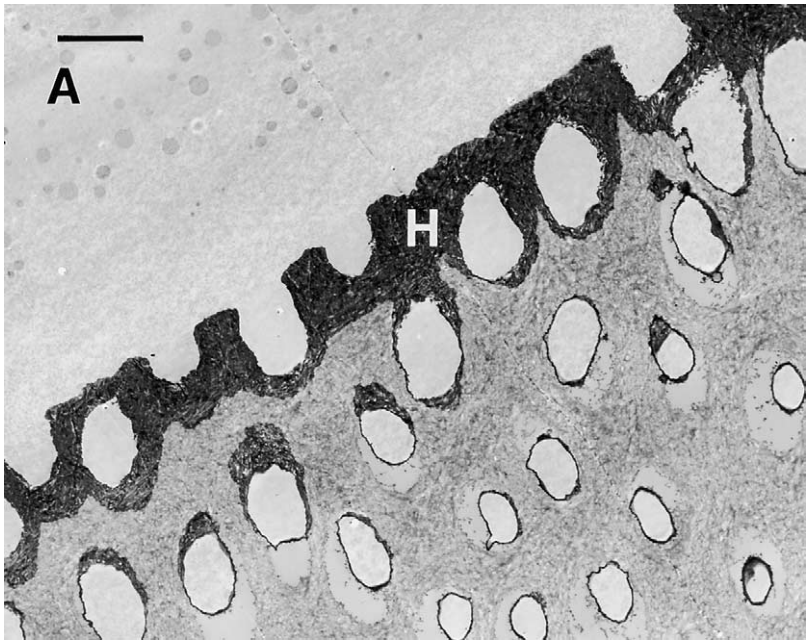

Fig. 16. Transmission electron micrographs of acid-etched dentin bonded with Prime & Bond under moist (A) and dry (B) conditions. (A) Moist dentin. Note that the adhesive uniformly infiltrated the dentin surface and the walls of subsurface tubules to form a thick electron-dense hybrid (**H**) layer. The tubules are filled with electronlucent resin. (B) Acid-etched dentin air-dried for 10 seconds prior to bonding. Note the limited penetration of resin into the dentin surface. Bar = 2 μm. (Courtesy of Dr. Franklin Tay, University of Hong Kong.)

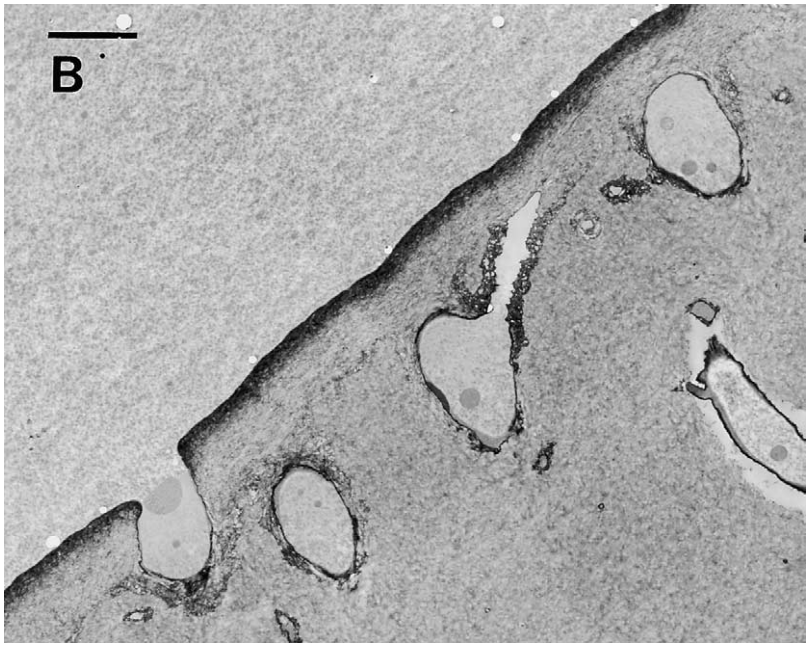

Fig. 16 (*continued*)

approach to solve that problem. They have soaked resin-bonded dentin in 50% silver nitrate solutions for varying lengths of time to permit the silver ions to fill any interfibrillar spaces that were not occupied by resin. This technique has been called *nanoleakage* to distinguish it from more traditional microleakage studies. Microleakage is the movement of bacteria, dyes, or other substances within gaps created between tooth structure and restorative materials. These gaps are often 20 to 50 μm wide and are due to the lack of adaptation and adhesion of many dental materials (see Hilton [141] for review). Nanoleakage, in contrast, occurs in the absence of gaps through nanometer-sized spaces (ca. 20-nm interfibrillar spaces or 0.02 μm). Although resin-enamel bonds do not exhibit much nanoleakage, all resin-dentin bonds that have been tested show nanoleakage to some degree (Fig. 18A), although the location and distribution of it vary with each material [142–146]. These nanometer-sized spaces are too small to permit penetration by bacteria, but bacterial enzymes, mineral salts, and water could easily permeate such spaces. Concerns that water in these spaces may weaken the naked collagen fibers by hydrolysis may not be valid in view of the lack of 4 years of storage of demineralized matrices on the mechanical properties of the matrix [147].

The delicate linear distribution of silver deposits in resin bonds in which nanoleakage has been examined by TEM is probably caused by silver ions diffusing within water-filled interfibrillar spaces (Fig. 18B). This distribution

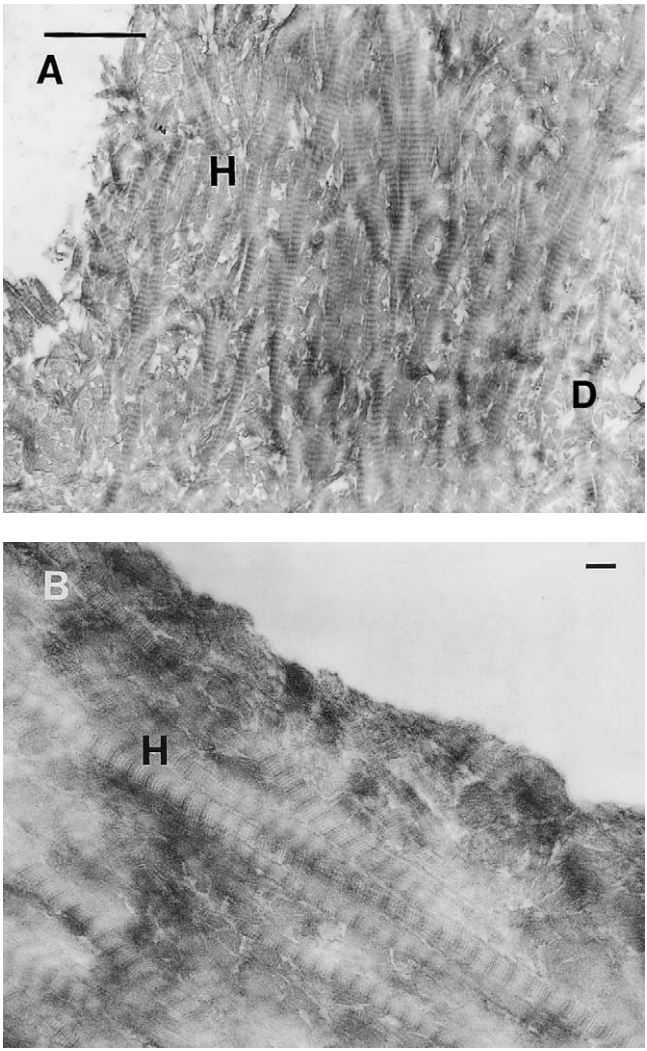

Fig. 17. High magnification transmission electron micrographs of acid-etched dentin bonded with All Bond 2 under moist (A) and dry (B) conditions. (A) Under moist conditions, the collagen fibrils of the hybrid layer (**H**) are well separated, allowing sufficient space between the fibrils for resin uptake. The adhesive (**A**) is radiolucent both above the hybrid layer and within the hybrid layer. These sections were treated with phosphotungstic acid and uranyl acetate to enhance the substructure of the collagen fibrils. **D** is the laboratory demineralized dentin. (B) Acid-etched dentin that was air-dried for 5 seconds prior to bonding with All Bond 2. Note the absence of separation between the collagen fibrils within the hybrid (**H**) layer. (Courtesy of Dr. Franklin Tay, University of Hong Kong.)

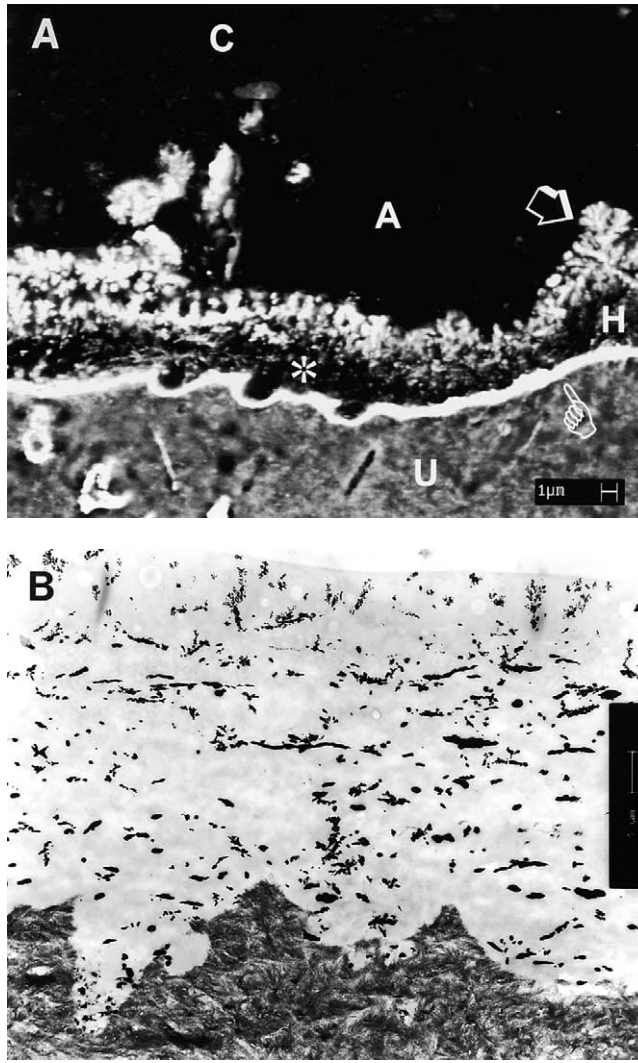

Fig. 18. (A) Backscatter electron image of dentin-resin interface of an air-dried specimen bonded with Single Bond that was immersed in 50% silver nitrate. The white areas indicate regions where electron dense silver has accumulated during 24 hour immersion in silver nitrate. Note the presence of silver deposits within the adhesive layer (**A**) especially the delicate branches (arrow). **C** = composite resin; **U** = undemineralized, underlying intact dentin. Note how dense the silver is along the base of the hybrid layer (pointer) and how little was in the middle of the hybrid layer (asterisk). (B) Transmission electron micrograph of the region of the hybrid layer of a specimen that was not air-dried before immersion in silver nitrate showing silver particles scattered throughout the hybrid layer but no large accumulation of silver at the base of the hybrid layers. (Courtesy of Dr. Franklin Tay, University of Hong Kong.)

may be exaggerated in specimens that have been air dried before application of nail varnish, so the clinical implications of nanoleakage remain to be determined.

Although the authors had speculated that anything that caused collapse of the demineralized dentin matrix would reduce resin uptake [119], there was no direct support for that idea. Recently, however, that hypothesis was proved correct using a simple in-vitro approach [148]. Two-millimeter cubes of mineralized dentin were incubated in 100% HEMA for 1000 minutes to permit maximum uptake of the monomer. After measuring the slight uptake by mineralized dentin, the cubes were demineralized to simulate acid-etched dentin, and the incubation in HEMA was repeated. The uptake of HEMA increased eight-fold. When these demineralized dentin cubes were air-dried, they shrank in volume, and the HEMA uptake was only about 10% that of the moist expanded dentin [38]. This study also demonstrated that exposing the already demineralized dentin to 37% phosphoric acid for 10 minutes had no effect on HEMA uptake. Concerns that overetching might denature collagen and interfere with resin penetration therefore seem unwarranted.

The acid resistance of whitlockite crystals in dentinal tubules of sclerotic dentin remains a significant problem for the uptake of resins in intertubular dentin, especially in wedge-shaped cervical lesions. These crystals resist the action of both self-etching primers [110] and phosphoric acid etching [111], leading the authors of that article to follow the recommendations of others—-that such dentin be mechanically roughened, if possible, to remove this material. On some, but not all of the dentin surfaces of wedge-shaped lesions, a hypermineralized, nonporous layer forms (Fig. 19). This layer resists even 37% phosphoric acid and prevents hybrid layer formation [111]. It is more enamel-like and permits good resin adhesion and relatively high bond strengths [111]. Sometimes, especially at the apex of the wedge-shaped lesions where toothbrush bristles may not reach, the hypermineralized layer is colonized by plaque microorganisms. They can invade the hypermineralized layer (Fig. 20), or they can mineralize their surrounding matrix. During dentin bonding, these bacteria can become incorporated into the bonded interface (Fig. 21). Similarly, when bonding to caries-affected dentin, it is not unusual to see occasional bacteria trapped within resin tags (Fig. 22).

Permeability of dentin contaminated with bacteria

This topic raises the issue of whether adhesive resins may seal bacteria beneath restorations where they can continue to progress to the pulp. This issue was first raised after Buonocore [149] demonstrated that acid etching of enamel increased the strength of resin-enamel bonds. This led to development of pit-and-fissure sealants [150]. Many practitioners were reluctant to use sealants because of the fear that if they covered carious lesions in enamel or dentin, the "hidden" caries would progress undetected. Handel-

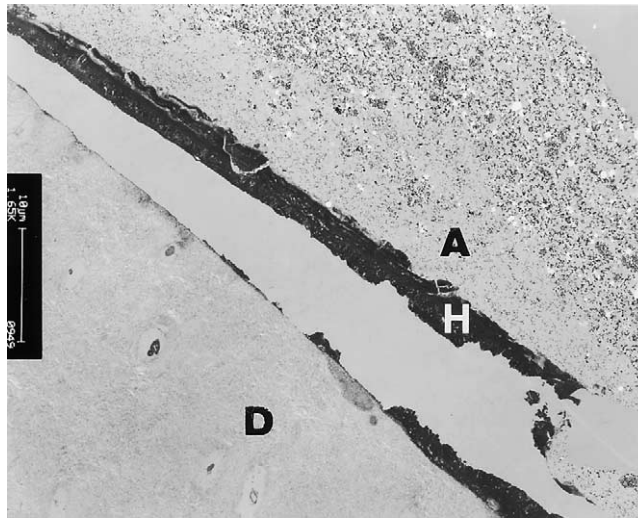

Fig. 19. TEM of the hypermineralized layer on top of sclerotic cervical dentin that was bonded with Clearfil SE Bond and then stressed to failure. Note that the bond between the resin and the hypermineralized layer (diagonal black structure) was stronger than the "bond" between the hypermineralized layer (**H**) and the underlying dentin which split off the dentin (**D**). **A** = adhesive. Bar = 1 μm. (Courtesy of Dr. Franklin Tay, University of Hong Kong.)

man et al. [151] and Jensen and Handelman [152] began a series of now-classic studies to determine what happens to bacteria in carious lesions that are sealed with resins. They showed a large decrease in viable bacteria after acid etching and resin sealing that continued to fall over time, indicating that the sealants isolated the bacteria from their source of nutrition. Similar results were obtained by Mertz-Fairhurst et al. [153] in a 10-year clinical trial of the use of sealants plus a posterior composite on nonexcavated carious lesions extending no more than halfway to the pulp. None of these lesions progressed radiographically, and few viable microorganisms could be found when biopsies were performed on the resin-sealed lesions, yet there was further progression of the lesions in untreated control teeth. These results were similar to the results of Magnusson and Sundell [154], who proposed a two-step approach to the treatment of deep carious lesions in which there is the danger of mechanical pulp exposure. They first excavated most of the carious lesions, taking care to avoid removing the residual carious material near pulp horns. They then covered the remaining soft, wet, demineralized dentin with $Ca(OH)_2$ as an indirect pulp cap, followed by placement of a temporary restoration. It is now known that placement of $Ca(OH)_2$ on dentin lowers the intrinsic permeability of the dentin both in vitro [155,156] and in vivo [7]. The removal of most of the carious material, together with the alkalinity of the $Ca(OH)_2$, converts the acidiogenic microenvironment to a neutral or basic environment that promotes

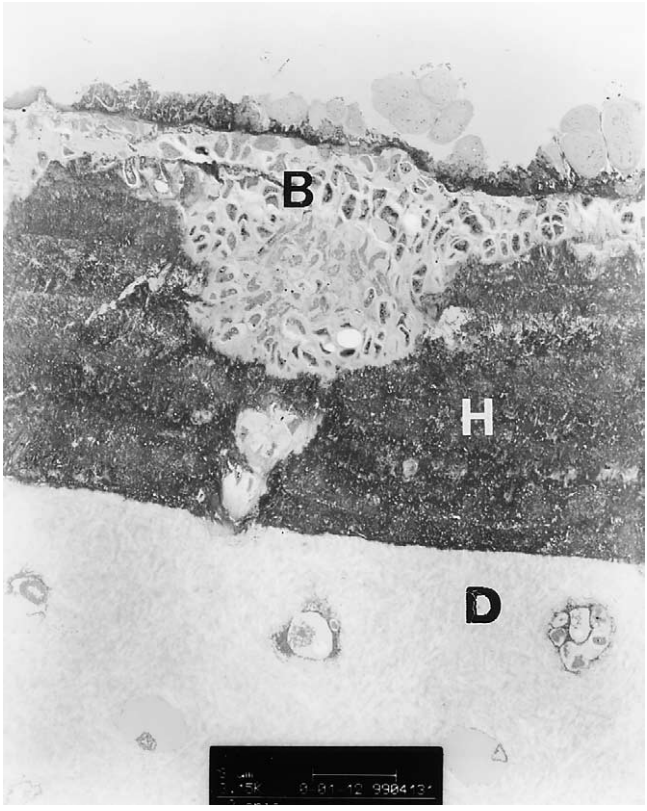

Fig. 20. TEM showing bacteria (**B**) eroding into the hypermineralized layer (**H**) that formed on dentin (**D**). Both layers were covered by a self-etching primer while bonding a cervical wedge-shaped lesion. Bar = 10 μm. (Courtesy of Dr. Franklin Tay, University of Hong Kong.)

remineralization. When these lesions were reopened 6 to 9 months later, the residual carious material was dry, darker, and harder than before. Similar results were obtained more recently by Bjorndal et al. [157]. Apparently, the $Ca(OH)_2$ seal of the surface dentin and the deeper sclerosis of the affected tubules by mineral crystals isolate any residual bacteria from the oral source of their nutrition, and they become dormant. Any treatment of dentin that lowers its permeability to fermentable carbohydrates therefore should arrest the progression of carious lesions. The presence of bacteria in deep dentin can be irritating to pulpal soft tissues, however [6,8,75], because of the shedding of bacterial products that can permeate to the pulp [24,26]. Here they can induce pulpal inflammation, which, together with the bacterial products, may injure or destroy odontoblasts that occupy the terminus of the tubules. This may be countered by the presence of immunoglobulins in dentinal fluid [158].

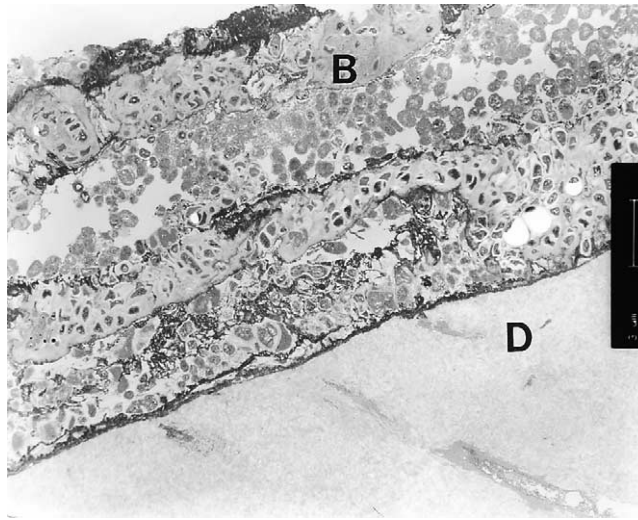

Fig. 21. TEM micrograph of layers of bacteria (**B**) infiltrated with adhesive resin in a wedge-shaped cervical lesion. **D** = underlying dentin. Bar = 10 μm. (Courtesy of Dr. Franklin Tay, University of Hong Kong.)

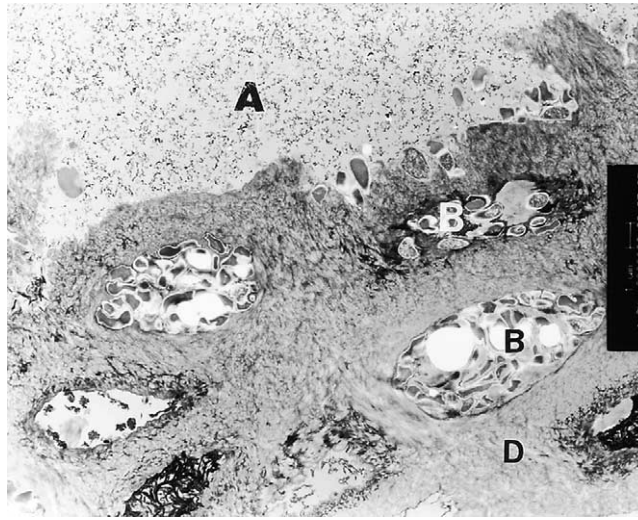

Fig. 22. TEM micrograph of bacteria in resin-bonded caries-affected dentin. Some, but not all, intratubular bacteria (**B**) were embedded by the adhesion resin (**A**) dentin matrix (**D**). Bar = 1 μm. (Courtesy of Dr. Franklin Tay, University of Hong Kong.)

The effects of growth factors

During the demineralization of dentin by invading bacteria, the hypermineralized cuff of peritubular dentin is dissolved, thereby liberating considerable amounts of calcium and phosphate. Although some of the salts diffuse

outward to the oral cavity, there is enough inward diffusion toward the pulp to permit precipitation of these salts as various types of calcium phosphates that sclerose the tubules. The demineralization also spreads radially from the tubule lumen into adjacent intertubular dentin, which also becomes demineralized. The presence of organic acids (lactate, citrate, and so forth) causes both demineralization of apatite and perhaps chelation of additional mineral. Both processes can liberate a host of noncollagenous proteins from their bound condition on collagen [159]. Some of those proteins are potent growth factors such as transforming growth factor-β and insulin-like growth factors-I, II [78]. These growth factors were synthesized and secreted by the odontoblasts along with collagen when the primary dentin matrix was formed. They were made virtually insoluble when the matrix became mineralized. These trapped growth factors are released whenever the matrix becomes demineralized either by therapeutically applied acids [160], exogenous organic acids [161] produced by bacteria or by endogenous acids produced during pulpal inflammation. In addition to growth factors that promote differentiation of odontoblasts, the matrix of dentin also contains angiogenic growth factors that may facilitate capillary growth. Thus, according to Tziafas et al. [10] "the dentine matrix should not be considered as an inert dental hard tissue, but rather as a potential tissue store of a cocktail of bio-active molecules (particularly growth factors) waiting to be released if appropriate tissue conditions prevail."

If the released growth factors are within 400 μm of the pulp [78,162], they can trigger chemotaxis of nearby mesenchymal cells to migrate to the site of injury and induce these cells to become transformed into primitive odontoblast-like cells. This mechanism is thought to be responsible for the formation of reparative dentin beneath carious lesions [78]. These primitive odontoblasts are cuboidal and lack an odontoblast process. The dentin matrix that they form is often atubular. When mineralized, this atubular dentin can cause a large reduction in dentin permeability, thereby protecting the pulp from noxious material (see Fig. 1). Although the current emphasis is the stimulation of cytodifferentiation by growth factors [78,163], sometime in the future it might be therapeutically useful to bioactive molecules that would cause either apoptosis of odontoblasts or dedifferentiation of mature odontoblasts to more primitive forms lacking odontoblast processes, so that they would form atubular dentin that would seal off peripheral tubular dentin from the pulp. Such a reaction would remove the diffusional stimulus for continued dedifferentiation, thereby allowing the odontoblasts to differentiate to their normal mature forms. Transdental delivery (e.g., diffusion of growth factors down fluid-filled tubules after topical application to cavity dentin) of growth factors is not simple, however. The diffusion coefficient of molecules is related inversely to the cube root of the molecular weight. Therefore, glycoproteins such as transforming growth factor-β (monomeric molecular weight 19,000; dimer 30,000) [164] diffuse more slowly than smaller molecules. Molecules as large as fibrinogen (molecular weight 360,000),

however, have been shown to diffuse across dentin in vitro [22], where they reduce dentin permeability [76]. Growth factors are generally used in μg quantities if the diffusion is across dentin, although the effective concentrations in vitro are measured in nanograms [163]. Dentin is known to bind many molecules that pass through it [158], which may be why the remaining dentin thickness must be less than 400 μm in monkeys [163] or 100 μm in ferrets [162] to obtain significant pulpal responses to growth factors. Similarly, even calcium hydroxide applications fail to produce pulp responses unless the remaining dentin thickness is less than 400 μm [163]. If dentin is sacrificed to make it thin enough for optimal diffusional transport of growth factors to the pulp, it becomes hyperconductive, allowing even slight pulpal hydrostatic pressures to permit sufficient outward fluid flow that may tend to rinse away inwardly diffusing growth factors [165,166]. The solution will be to bind the growth factors to a suitable carrier molecule, such as albumin, to prevent binding to dentin, thereby allowing it to diffuse across 1 to 1.5 mm of dentin.

Summary

It should be clear that the permeability properties of dentin [79,80,83,87] can have a significant influence on how teeth respond to restorative procedures [1,9,167–171] and to materials [79–83,85–91,172]. As further advances in tissue engineering are made, it is likely that a number of growth factors and other therapeutic agents will be applied to intact dentin to elicit a specific pulpal response. This may be followed by creating a surface seal with adhesive resins to prevent back-diffusion of these agents and to prevent any intratubular fluid shifts that might irritate differentiating or dedifferentiating cells and the pulpodentin border. The more we understand the permeability characteristics of dentin, the more we can manipulate it for therapeutic advantage.

Acknowledgments

The authors are grateful to Michelle Barnes for secretarial support, and to the National Institute of Dental and Craniofacial Research for support (DE06427).

References

[1] Langeland K. Tissue changes in the dental pulp: an experimental study [PhD thesis]. Oslo: Oslo University Press; 1957.
[2] Lundy T, Stanley HR. Correlation of pulp histopathology and clinical symptoms in human teeth subjected to experimental irritation. Oral Surg 1969;27:187–201.

[3] Brännström M, Nyborg H. The presence of bacteria in cavities filled with silicate cement and composite resin materials. Swed Dent J 1971;64:149–55.
[4] Brännström M, Nyborg H. Cavity treatment with a microbicidal fluoride solution: growth of bacteria and effect on the pulp. J Prosth Dent 1963;30:303–13.
[5] Bergenholtz G, Lindhe J. Effects of soluble plaque factors on inflammatory reactions in the dental pulp. Scand J Dent Res 1975;83:155–8.
[6] Bergenholtz G. Effect of bacterial products on inflammatory reactions in the dental pulp. Scand J Dent Res 1977;85:122–9.
[7] Bergenholtz G. Inflammatory response to the dental pulp to bacterial irritation. J Endodont 1981;7:100–4.
[8] Bergenholtz G, Reit C. Reactions of the dental pulp to microbial provocation of calcium hydroxide treated dentin. Scand J Dent Res 1980;88:187–92.
[9] Mjör IA. Dentin and pulp: reaction patterns in human teeth. Boca Raton: CRC Press; 1983.
[10] Tziafas D, Smith AJ, Lesot H. Designing new treatment strategies in vital pulp therapy. J Dent 2000;28:77–92.
[11] Pashley DH. Clinical correlations of dentin structure and function. J Prosth Dent 1991; 66:777–81.
[12] Pashley DH, Pashley EL. Dentin permeability and restorative dentistry: a status report for the *American Journal of Dentistry.* Am J Dent 1991;4:5–9.
[13] Marshall GW, Marshall SJ, Kinney JH, et al. The dentin substrate: structure and properties related to bonding. J Dent 1997;25:441–50.
[14] Larsson PA, Howell DS, Pita JC, et al. Aspiration and characterization of predentin fluid in developing rat teeth by means of a micropuncture and micro-analytical technique. J Dent Res 1988;67:870–75.
[15] Lundgren T, Nannmark U, Linde A. Calcium ion activity and pH in the odontoblast-predentin region: ion-selective microelectrode measurements. Calcif Tiss Int 1992;50: 134–6.
[16] Rasmussen P. The concentration of calcium, inorganic phosphate and protein in the interstitial fluid of rats. Calcif Tiss Res 1970;6:197–203.
[17] Ciucchi B, Bouillaguet S, Holz J, et al. Dentinal fluid dynamics in human teeth, *in vitro.* J Endodont 1995;21:191–4.
[18] Vongsavan N, Matthews B. Fluid flow through cat dentine *in vivo.* Arch Oral Biol 1992; 37:175–85.
[19] Pashley DH. Consideration of dentin permeability in cytotoxicity testing. Int Endod J 1988;21:143–54.
[20] Hanks CT, Diehl ML, Craig RG, et al. Characterization of the "*in vitro* pulp chamber" using the cytotoxicity of phenol. J Oral Path Med 1989;18:97–107.
[21] Hanks CT, Syed SA, Craig RG, et al. Modeling bacterial damage to pulpal cells *in vitro.* J Endodont 1991;17:21–5.
[22] Hanks CT, Wataha JC, Parsell RR, et al. Permeability of biological and synthetic molecules through dentine. J Oral Rehab 1994;21:475–87.
[23] Pashley DH. Dentin-predentin complex and its permeability: physiological overview [special issue]. J Dent Res 1985;64:613–20.
[24] Nissan R, Segal H, Stevens RH, et al. The ability of bacterial endotoxin to diffuse through human dentin. J Endodont 1995;21:62–4.
[25] Pissiotis E, Spangberg L. Dentin permeability to bacterial proteins *in vitro.* J Endodont 1994;20:118–22.
[26] Pissiotis E, Spangberg L. Dentin as inhibitor of bacterial toxicity on pulpal cells *in vitro.* J Endodon 1992;18:166–71.
[27] Ciarlone AE, Pashley DH. Medication of the dental pulp: a review and proposals. Endod Dent Traumatol 1992;8:1–5.
[28] Trowbridge HO. Pathogenesis of pulpitis resulting from dental caries. J Endodont 1981; 7:52–60.

[29] Pashley DH. Dentin permeability: theory and practice. In: Spangberg L, editor. Experimental endodontics. Boca Raton: CRC Press; 1990. p. 19–49.
[30] Merchant VA, Livingston MJ, Pashley DH. Dentin permeability: comparison of diffusion with filtration. J Dent Res 1977;56:1161–64.
[31] Richardson D, Tao L, Pashley DH. Dentin permeability: effects of crown preparation. Int J Prosthod 1991;4:219–25.
[32] Brännstrom M. The cause of post restorative sensitivity and its prevention. J Endod 1986;12:475–81.
[33] Brännstrom M. Etiology of dentin sensitivity. Proc Finn Dent Soc 1992;88(Suppl 1):1–7.
[34] Pashley DH, Livingston MJ, Greenhill JD. Regional resistances to fluid flow in human dentine, *in vitro.* Arch Oral Biol 1978;23:807–10.
[35] Bouillaguet S, Duroux B, Ciucchi B, Sano H. Ability of adhesive systems to seal dentin surfaces: an *in vitro* study. J Adhes Dent 2000;2:201–8.
[36] Dai XF, Ten Cate AR, Limeback H. The extent and distribution of intratubular collagen fibrils in human dentine. Arch Oral Biol 1991;36:775–9.
[37] Pashley DH, Ciucchi B, Sano H, et al. Bond strength versus dentine structure: a modeling approach. Arch Oral Biol 1995;40:1109–18.
[38] Pashley DH, Sano H, Yoshiyama M, et al. Dentin, a dynamic bonding substrate: the effects of dentin variables on resin adhesion. In: Shimono M, Maeda T, Suda H, Takahashi K, editors. Proceedings of the International Conference on the Dentin/Pulp Complex. Tokyo: Quintessence Publishing Co.; 1996.
[39] Trowbridge HO. Dentistry today. Dent 1982;82:22–9.
[40] Pashley DH, Andringa HJ, Derkson GD, et al. Regional variability in the permeability of human dentine. Arch Oral Biol 1987;32:519–23.
[41] Maroli S, Khea SC, Krell KV. Regional variation in permeability of young dentin. Oper Dent 1992;17:93–100.
[42] Rauschenbeger CR. Dentin permeability: the clinical ramifications. Dent Clin North Am 1992;36:527–42.
[43] Fogel HM, Marshall FJ, Pashley DH. Effects of distance from the pulp and thickness on the hydraulic conductance of human radicular detin. J Dent Res 1988;67:1381–85.
[44] Pashley EL, Talmaan R, Horner JA, et al. Permeability of normal versus carious dentine. Endod Dent Traumatol 1991;7:207–11.
[45] Tagami J, Hosoda H, Burrow MF, et al. Effects of aging and caries on dentin permeability. Proc Finn Dent Soc 1992;88(Suppl 1):149–54.
[46] Prati C, Chersoni S, Mongiorgi R, et al. Thickness and morphology of resin-infiltrated dentin layer in young, old and sclerotic dentin. Oper Dent 1999;24:66–72.
[47] Pashley DH, Nelson R, Kepler EE. The effects of plasma and salivary constituents on dentin permeability. J Dent Res 1982;61:978–81.
[48] Hirata K, Nakashima M, Sekine I, Mukouyama Y, Kimura K. Dentinal fluid movement associated with loading of restorations. J Dent Res 1991;70:975–987.
[49] Paphangkorakit J, Osborn JW. The effect of normal occlusal forces on fluid movement through human dentine, in vitro. Arch Oral Biol 2000;45:1033–41.
[50] Matthews B, Vongsavan N. Interactions between neural and hydrodynamic mechanisms in dentin and pulp. Arch Oral Biol 1994;39(Suppl):875–955.
[51] Goodis HE, Tao L, Pashley DH. Evaporative water loss from human dentine, *in vitro.* Arch Oral Biol 1990;35:523–28.
[52] Matthews WG, Showman CD, Pashley DH. Air blast-induced evaporative water loss from human dentine, *in vitro.* Arch Oral Biol 1993;38:517–23.
[53] Itthagarum A, Tay FR. Self-contamination of deep dentin by dentinal fluid. Am J Dent 2000;13:195–200.
[54] Sasazaki H, Okuda R. Effect of etching on the exudation of internal fluids. In: Shimono M, Maeda T, Suda H, Takahashi K, editors. Proceedings of the International Symposium on Dentin/Pulp Complex. Tokyo: Quintessence Publishers; 1996. p. 280–82.

[55] Pashley EL, Comer RW, Simpson MD, et al. Dentin permeability: sealing the dentin in crown preparations. Oper Dent 1992;17:13–20.
[56] Tay FR, Gwinnett AJ, Pang KM, et al. Structural evidence of a sealed tissue interface with a total-etch wet-bonding technique *in vivo*. J Dent Res 1994;73:629–36.
[57] Tay FR, Gwinnett AJ, Pang KM, et al. Resin permeation into acid-conditioned, moist, and dry dentin: a paradigm using water-free adhesive primers. J Dent Res 1996;75: 1034–44.
[58] Tay FR, Gwinnett AJ, Wei SHY. Relation between water content in acetone/alcohol-based primer and interfacial ultrastructure. J Dent 1998;26:147–56.
[59] Moll K, Haller B. Effect of intrinsic and extrinsic moisture on bond strength to dentine. J Oral Rehabil 2000;27:149–64.
[60] Mitchem JC, Terkla LG, Gronas DG. Bonding of resin dentin adhesives under simulated physiological conditions. Dent Mater 1988;4:351–3.
[61] Tao L, Pashley DH. Dentin perfusion effects on the shear bond strengths of bonding agents to dentin. Dent Mater 1989;5:181–4.
[62] Mitchem JC, Gronas DG. Adhesion to dentin with and without smear layer under varying degrees of wetness. J Prosthet Dent 1991;66:619–23.
[63] Prati C, Pashley DH, Montanari G. Hydrostatic intrapulpal pressure and bond strength of bonding systems. Dent Mater 1991;7:54–8.
[64] Davidson CL, Abdalla AI, DeGee AJ. An investigation into the quality of dentine bonding systems for accomplishing a durable bond. J Oral Rehabil 1993;20:291–300.
[65] Elhabashy A, Swift EJ, Boyer DB, et al. Effects of dentin permeability and hydration on the bond strengths of dentin bonding agents. Am J Dent 1993;6:123–6.
[66] Prati C, Pashley DH. Dentin wetness, permeability and thickness and bond strength of adhesive systems. Am J Dent 1992;5:33–7.
[67] Paul S, Schärer P. Intrapulpal pressure and thermal cycling: effect on shear bond strength of eleven modern dentin bonding agents. J Esth Dent 1993;5:179–84.
[68] Pereira PNR, Okuda M, Sano H, et al. Effect of region and dentin perfusion on bond strengths of resin-modified glass ionomer cements. J Dent 2000;28:347–54.
[69] Zheng L, Pereira PNR, Somphone P, et al. Effect of hydrostatic pressure on regional bond strengths of compomers to dentine. J Dent 2000;28:501–8.
[70] George CH, Kendall JM, Evans WH. Intracellular trafficking pathways on assembly of connexins into gap junctions. J Biol Chem 1999;274:8678–85.
[71] Sasaki T, Nakagawa K, Higashi S. Ultrastructure of odontoblasts in kitten tooth germs as revealed by freeze-fracture. Arch Oral Biol 1982;27:897–904.
[72] Bishop MA, Yoshida S. A permeability barrier to lanthanum and the presence of collagen between odontoblasts in pig molars. J Anat 1992;181:29–35.
[73] Turner D. Immediate physiological response of odontoblasts. Proc Finn Dent Soc 1992; 88(Suppl 1):55–63.
[74] Turner D, Marfurt C, Sattelburg C. Demonstration of physiological barrier between pulpal odontoblasts and its perturbation following routine restorative procedures: a horseradish peroxidase tracing study in the rat. J Dent Res 1989;68:1262–68.
[75] Kitamura C, Kimura K, Nakayama T, Toyoshima K, Terashita M. Primary and secondary induction of apoptosis in odontoblasts after cavity preparation of rat molars. J Dent Res 2001;80:1530–34.
[76] Pashley DH, Galloway SE, Stewart F. Effects of fibrinogen in vivo on dentine permeability in the dog. Arch Oral Biol 1984;29:725–28.
[77] Chiego DJ. An ultrastructural and autoradiographic analysis of primary and replacement odontoblasts following cavity preparation and wound healing in the rat molar. Proc Finn Dent Soc 1992;88(Suppl 1):243–56.
[78] Smith AJ, Sloan AJ, Matthews JB, Murray PE, Lumley P. Reparative processes in dentine and pulp. In: Addy M, Emberg G, Edger WM, Richardson RO, editors. Tooth wear and sensitivity. London: Marin-Dunitz, Ltd.; 2000. p. 53–66.

[79] Bouillaguet S, Wataha JC, Hanks CT, et al. *In vitro* cytotoxicity and dentine permeability of HEMA. J Endodont 1996;22:244–8.
[80] Bouillaguet S, Virgillito M, Wataha J, et al. The influence of dentine permeability on cytotoxicity of four dentine bonding systems, *in vitro.* J Oral Rehab 1998;25:45–51.
[81] Hanks CT, Craig RG, Diehl ML, et al. Cytotoxicity of dental composites and other materials in a new *in vitro* device. J Oral Path 1988;17:396–403.
[82] Hanks CT, Anderson M, Craig RG. Cytotoxic effects of dental cements on two cell culture systems. J Oral Path 1981;10:101–6.
[83] Hasheih IA, Franquin JC, Cosset A, et al. Relationship between dentine hydraulic conductance and the cytotoxicity of four dentine bonding resin *in vitro.* J Dent 1998;26:473–7.
[84] Sikkema J, DeBont JAM, Poolman B. Interaction of cyclic hydrocarbons with biologic membranes. J Biol Chem 1994;269:8022–28.
[85] Hamid A, Sutton W, Hume WR. Variation in phosphoric acid concentration and treatment time and HEMA diffusion through dentin. Am J Dent 1996;9:211–4.
[86] Hamid A, Hume WR. The effect of dentine thickness on diffusion of resin monomers *in vitro.* J Oral Rehabil 1997;24:20–5.
[87] Hashieh IA, Camps J, Dejou J, et al. Eugenol diffusion through dentin related to dentin hydraulic conductance. Dent Mater 1998;14:229–36.
[88] Roulet J-F. Degradation of dental polymers. Basel: Karger; 1987.
[89] Ferracane JL. Elution of leachable components from composites. J Oral Rehabil 1994; 21:441–52.
[90] Gerzina TM, Hume WR. Diffusion of monomers from bonding resin-resin composite combinations through dentine *in vitro.* J Dent 1996;24:125–8.
[91] Lygre H, Hal PJ, Solheim M, et al. Organic leachables from polymer-based dental filling materials. Eur J Oral Sci 1999;107:378–83.
[92] Pashley DH. The influence of dentine permeability and pulpal blood flow on pulpal solute concentrations. J Endodont 1979;5:335–61.
[93] Fitzergerald M, Hanks CT. *In vivo* study of resin diffusion through intact vital human dentin. In: Program Abstracts of the 75th Session of the International Association for Dental Research. Orlando: International Association for Dental Research; 1997. p. 305.
[94] Bergenholtz G. Evidence for bacterial causation of adverse pulpal responses in resin-based dental restorations. Crit Rev Oral Biol Med 2000;11:467–80.
[95] Gwinnett AJ, Tay FR. Early and intermediate time response to the dental pulpal to an acid etch technique *in vivo.* Am J Dent 1997;10:535–44.
[96] Van Noort R, Noroozi S, Howard IC, et al. A critique of bond strength measurements. J Dent 1989;17:61–7.
[97] Van Noort R, Cardew GE, Howard IC, Noroozi S. The effect of local interfacial geometry on the measurements of the tensile bond strength to dentin. J Dent Res 1991;70:889–93.
[98] Versluis A, Tantbirojn D, Douglas WH. Why do shear bond tests pull out dentin? J Dent Res 1997;76:1298–307.
[99] Sudsangiam S, Van Noort R. Do dentin bond strength tests serve a useful purpose? J Adhes Dent 1999;1:57–67.
[100] Pashley DH. Dynamics of the pulpodentin complex. Crit Rev Oral Biol Med 1996;7:104–33.
[101] Sano H, Shono T, Sonoda H, et al. Relationship between surface area for adhesion and tensile bond strength: evaluation of a micro-tensile bond test. Dent Mater 1994;10:236–40.
[102] Pashley DH, Sano H, Ciucchi B, et al. Adhesion testing of dentin bonding agents: a review. Dent Mater 1995;11:117–25.
[103] Pashley DH, Carvalho RM, Sano H, et al. The microtensile bond test: a review. J Adhes Dent 1999;1:299–310.
[104] Davidson CL, de Gee AJ, Feilzer A. The competition between the composite-dentin bond strength and the polymerization contraction stress. J Dent Res 1984;63:1396–99.
[105] Feilzer AJ, de Gee AJ, Davidson CL. Setting stress in composite resin in relation to configuration of the restoration. J Dent Res 1987;66:1636–39.

[106] Carvalho RM, Pereira JC, Yoshiyama M, et al. A review of polymerization contraction: the influence of stress development vs stress relief. Oper Dent 1996;21:17–24.
[107] Bouillaguet S, Ciucchi B, Jacoby T, et al. Bonding characteristics to dentin walls of class II cavities, *in vitro*. Dent Mater 2001;17:316–21.
[108] Yoshikawa T, Sano H, Burrow MF, et al. Effects of dentin depth and cavity configuration on bond strength. J Dent Res 1999;78:898–905.
[109] Ogata H, Harada N, Yamaguchi S, et al. Effect of burs on dentin bond strength of self-etching primer bond systems. Operative Dent 2001;26:375–82.
[110] Kwong S-M, Tay FR, Yip H-K, et al. An ultrastructural study of the application of dentine adhesives to acid-conditioned sclerotic dentin. J Dent 2000;28:515–28.
[111] Kwong S-M, Cheung GSP, Kei L-H, et al. Microtensile bond strength of sclerotic dentin using self-etching and a total-etch technique. Dent Mater 2002;(in press).
[112] Nakajima M, Sano H, Burrow MF, et al. Tensile bond strength and SEM evaluation of caries-affected dentin using dentin adhesives. J Dent Res 1995;74:1679–88.
[113] Yoshiyama M, Sano H, Ebisu S, et al. Regional strengths of bonding agents to cervical-sclerotic root dentin. J Dent Res 1996;75:1851–58.
[114] Nakajima M, Sano H, Zheng L, et al. Effect of moist vs dry bonding on normal vs caries-affected dentin with Scotchbond Multi-Purpose Plus. J Dent Res 1999;78:1298–303.
[115] Nakajima M, Sano H, Urabe I, et al. Bond strengths of single-bottle dentin adhesives to caries-affected dentin. Oper Dent 2000;25:2–10.
[116] Pashley DH, Ciucchi B, Sano H. Dentin as a bonding substrate. Dtsch Zahn Z 1994; 49:760–3.
[117] Marshall GW, Saeki K, Gansky SA, et al. AFM study of citric acid-ferric chloride etching characteristics of dentin. Am J Dent 1999;12:271–6.
[118] Nakaoki Y, Nikaido T, Pereira PNR, et al. Dimensional changes of demineralized dentin treated with HEMA primers. Dent Mater 2000;16:441–6.
[119] Pashley DH, Ciucchi B, Sano H, et al. Permeability of dentin to adhesive agents. Quintessence Int 1993;24:618–31.
[120] Gwinnett AJ. Moist versus dry dentin. Its effects on shear bond strengths. Am J Dent 1992;5:127–9.
[121] Gwinnett AJ. Dentin bond strength after air drying and rewetting. Am J Dent 1994;7:144–8.
[122] Kanca J. Improved bond strength through acid-etching of dentin and bonding to wet dentin surfaces. J Am Dent Assoc 1992;123:35–43.
[123] Kanca J. Wet bonding: effect of drying time and distance. Am J Dent 1996;9:273–6.
[124] Perdigâo J, Swift EJ, Heymann HO, et al. Effect of a re-wetting agent on the performance of acetone-based dentin adhesives. Am J Dent 1998;11:207–13.
[125] Perdigâo J, Van Meerbeek B, Lopes MM, et al. The effect of a re-wetting agent on dentin bonding. Dent Mater 1999;15:282–95.
[126] Eick JD, Robinson SJ, Byerly TJ, et al. Scanning transmission electron microscopy/energy dispersive spectroscopy analysis of the dentin adhesive interface using a labeled 2-hydroxyethyl methacrylate analog. J Dent Res 1995;74:1246–52.
[127] Eick JD, Miller RG, Robinson SJ, et al. Quantitative analysis of the dentin adhesive interface by Anger spectroscopy. J Dent Res 1996;75:1027–33.
[128] Tay FR, Gwinnett AJ, Wei SHY. Micromorphological spectrum from over-drying to overwetting acid-conditioned dentin in water-free, acetone-based, single-bottle primer/adhesives. Dent Mater 1996;12:236–44.
[129] Tay FR, Carvalho RM, Yiu CKK, et al. Mechanical disruption of dentin collagen fibrils during resin-dentin bond testing. J Adhes Dent 2000;2:175–92.
[130] Tay FR, Sano H, Carvalho RM, Pashley EL, Pashley DH. An ultrastructural study of the influence of acidity of self-etching primers and smear layer thickness on bonding to intact dentin. J Adhes Dent 2000;2:83–98.
[131] Tay FR, Carvalho RM, Sano H, Pashley DH. Effect of smear layers on the bonding of a self-etching primer to dentin. J Adhes Dent 2000;2:98–116.

[132] Van Meerbeek B, Dhem A, Goret-Nicaise M, Braem M, Lambrechts P, Vanherle G. Comparative SEM and TEM examination of the ultrastructure of the resin-dentin interdiffusion zone. J Dent Res 1993;72:495–501.
[133] Van Meerbeek B, Yoshida Y, Lambrechts P, et al. A TEM study of two water-based adhesive systems bonded to dry and wet dentin. J Dent Res 1998;77:50–9.
[134] Van Meerbeek B, Conn LJ, Duke ES, et al. Correlative transmission electron microscopy examination on nondemineralized and demineralized resin-dentin interfaces formed by two dentin adhesive systems. J Dent Res 1996;75:879–88.
[135] Choi KK, Condon JR, Ferracane JL. The effects of adhesive thickness on polymerization contraction stress of composite. J Dent Res 2000;79:812–7.
[136] Tay FR, Moulding KM, Pashley DH. Distribution of nanofillers from a simplified-step adhesive in acid-conditioned dentin. J Adhes Dent 1999;2:103–17.
[137] Spencer P, Swafford JR. Unprotected protein at the dentin-adhesive interface. Quintessence Int 1999;30:501–7.
[138] Spencer P, Wang Y, Walker MP, et al. Interfacial chemistry of the dentin/adhesive bond. J Dent Res 2000;79:1458–63.
[139] Hashimoto M, Ohno H, Endo K, et al. The effect of hybrid layer thickness on bond strength: demineralized dentin zone of the hybrid layer. Dent Mater 2000;16:406–11.
[140] Paul SJ, Welter DA, Ghazi M, et al. Nanoleakage at the dentin adhesive interface vs μ-tensile bond strength. Oper Dent 1999;24:181–8.
[141] Hilton T. Can modern restorative procedures and materials reliably seal cavities? *In vitro* observations. Trans Acad Dent Mater 1998;12:21–71.
[142] Sano H, Shono T, Takatsu T, et al. Microporous dentin zone beneath resin-impregnated layer. Oper Dent 1994;19:59–64.
[143] Sano H, Takatsu T, Ciucchi B, et al. Nanoleakage: leakage within the hybrid layer. Oper Dent 1995;20:18–25.
[144] Sano H, Yoshiyama M, Ebisu S, et al. Comparative SEM and TEM observations of nanoleakage within the hybrid layer. Oper Dent 1995;20:160–70.
[145] Griffith BM, Watson TF, Sherriff M. The influence of dentine bonding systems and their handling characteristics on the morphology and micropermeability of the dentine adhesive interface. J Dent 1999;27:63–71.
[146] Li H, Burrow MF, Tyas MJ. Nanoleakage patterns of four dentin bonding systems. Dent Mater 2000;16:48–56.
[147] Carvalho RM, Tay FR, Sano H, et al. Long-term mechanical properties of EDTA-demineralized dentin matrix. J Adhes Dent 2000;2:193–200.
[148] Pashley DH, Zhang Y, Agee KA, Rouse CJ, Carvalho RM, Russell CM. Permeability of demineralized dentin to HEMA. Dent Mater 2000;16:7–14.
[149] Buonocore MG. A simple method of increasing the adhesion of acrylic filling materials to enamel surfaces. J Dent Res 1955;34:846–51.
[150] Buonocore MG, Wileman W, Brudevold F. A report on a resin composition capable of bonding to human dentin surfaces. J Dent Res 1956;35:846–51.
[151] Handelman SL, Buonocore MG, Schonte PC. Progress report on the effect of a fissure sealant on bacteria in dental caries. J Am Dent Assoc 1973;87:1189–91.
[152] Jensen OE, Handelman SL. Effect of autopolymerizing sealants on the viability of microflora in occlusal dental caries. Scand J Dent Res 1980;88:338–82.
[153] Mertz-Fairhurst EJ, Curtis JW, Ergle JW, et al. Ultraconservative and cariostatic sealed restorations: results at year 10. J Am Dent Assoc 1998;129:55–66.
[154] Magnusson BO, Sundell SO. Stepwise excavation of deep carious lesions in primary molars. J Int Assoc Dent Child 1977;8:36–40.
[155] Pashley DH, Kalathoor S, Burnham D. The effect of calcium hydroxide on dentin permeability. J Dent Res 1986;65:417–20.
[156] Tao L, Reynolds JM, Pashley DH. Effect of Dycal on dentin permeability. Endod Dent Traumatol 1988;4:16–8.

[157] Bjorndal L, Larsen T, Thylstrup A. A clinical and microbiological study of deep carious lesions during stepwise excavation using long treatment intervals. Caries Res 1997;31:411–7.
[158] Hahn C-L, Overton B. The effects of immunoglobulins on the convective permeability of human dentine *in vitro.* Arch Oral Biol 1997;42:835–43.
[159] Dahl T, Sabsay B, Veis A. Type I collagen-phosphophoryn interactions: specificity of the monomer-momoner binding. J Struct Biol 1998;123:162–8.
[160] Smith AJ, Smith G. Solubilization of TGF-β1 by dentin conditioning agents. In: Program Abstracts of the 27th Session of the American Association for Dental Research, Minneapolis. Alexandra, VA: IADR; 1988. p. 1034.
[161] Bjorndal L, Darvann T. A light microscopic study of odontoblastic and nonodontoblastic cells involved in tertiary dentinogenesis in well-defined cavitated carious lesions. Caries Res 1999;33:50–60.
[162] Smith AJ, Tobias RS, Cassidy N, et al. Odontoblast stimulation in ferrets by dentine matrix components. Arch Oral Biol 1994;39:13–22.
[163] Rutherford RB, Spangberg L, Tucker M, et al. Transdentinal stimulation of reparative dentine formation by osteogenic protein-1 in monkeys. Arch Oral Biol 1995;40:681–3.
[164] Lindholm TS. Bone morphogenetic proteins: biology, biochemistry and reconstructive surgery. Austin, TX: Academic Press; 1996. p. 9–11.
[165] Vongsavan N, Matthews B. The permeability of cat dentine *in vitro* and *in vivo.* Arch Oral Biol 1991;36:641–6.
[166] Pashley DH, Matthews WG. The effects of outward forced convective flow on inward diffusion in human dentine *in vitro.* Arch Oral Biol 1993;38:577–88.
[167] Stanley HR. Human pulp response to restorative dental procedures. Gainesville, FL: Storter Printing Company; 1981.
[168] Camps J, Dejou J, Remusat M, et al. Factors influencing pulpal response to cavity preparation. Dent Mater 2000;16:432–40.
[169] Langeland K. Tissue response to dental caries. Endod Dent Traumatol 1987;3:149–71.
[170] Mjör IA. Dentin-predentin complex and its permeability: pathology and treatment overview. J Dent Res 1985;64:621–7.
[171] Stanley HR. Pulpal considerations of adhesive materials. Oper Dent 1992;17(Suppl 15):151–64.
[172] Melin M, Joffre-Romeas A, Farges J-C, et al. Effects of TGF-β1 on dental pulp cells in cultured human tooth slices. J Dent Res 2000;79:1689–96.