

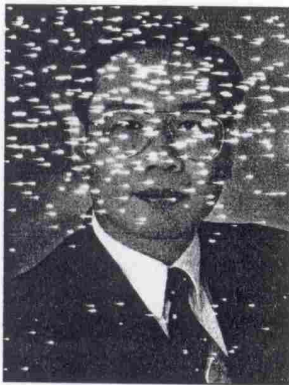
Buonocore Memorial Lecture

Water Treeing in Simplified Dentin Adhesives—Déjà Vu?



Michael Buonocore

FR Tay • DH Pashley • BI Suh
N Hiraishi • CKY Yiu



Franklin R Tay

INTRODUCTION

Over the last decade, the classic concept of three-step bonding to dental tissues has developed rapidly to more user-friendly, simplified adhesive systems. These comprise the two-step etch-and-rinse, two-step self-etch and one-step self-etch adhesives. The last category of adhesives has been further reduced to single-bottle systems for simpler and faster applications.

They carry, along with their simplicity, some characteristics that are detrimental to their efficacy in providing

long-lasting bond stability. Simplified dentin adhesives are similar to resin-modified glass-ionomer cements in their high affinity for water. Such hydrophilicity renders these adhesives very permeable and denies their ability to hermetically seal dentin. Additionally, the water flux across simplified adhesives may compromise bonding in certain circumstances and their durability in the oral environment. This paper presents evidence of water transport across simplified adhesive systems and resin-modified glass-ionomer cements and relates them with clinical implications of the phenomenon.

Adhesive dentistry has come a long way since the development of adhesive monomers by Dr Oskar Hagger (1948), which were first used by McLean and Kramer (1952), and the acid-etching of dental hard tissues by Dr Michael Buonocore (Buonocore, 1955; Buonocore, Wileman & Brudevold, 1956). The availability of dentin adhesives has profoundly improved the quality of conservative work performed in pediatric and geriatric dentistry, prosthodontics, and lately, in endodontics. Today, we salute the commercialization of different classes of etch-and-rinse (total-etch) and self-etch adhesives (Van Meerbeek & others, 2003). Simplified versions of these adhesives have made bonding simpler, faster and more user-friendly (Perdigão, Duarte & Lopes, 2003). The latest one-step self-etch adhesives are now being marketed as single-bottle versions. They represent remarkable scientific and entrepreneurial achievements by research scientists, clinicians and manufacturers.

In all the words spoken and written about dentin adhesives, one theme constantly recurs: how long do these man-made bonds last? This question is becoming

*Franklin R Tay, Pediatric Dentistry and Orthodontics, Faculty of Dentistry, The University of Hong Kong, Hong Kong SAR, China

David H Pashley, PhD, DMD, Department of Oral Biology and Maxillofacial Pathology, School of Dentistry, Medical College of Georgia, Augusta, GA, USA

BI Suh, BISCO Inc, Schaumburg, IL, USA

N Hiraishi, Pediatric Dentistry and Orthodontics, Faculty of Dentistry, The University of Hong Kong, Hong Kong SAR, China and Department of Operative Dentistry, Tokyo Medical and Dental University, Tokyo, Japan

CKY Yiu, Pediatric Dentistry and Orthodontics, Faculty of Dentistry, The University of Hong Kong, Hong Kong SAR, China

*Reprint request: 34 Hospital Road, Hong Kong SAR, China, e-mail: kfctay@netvigator.com

increasingly challenging to answer, as the increasing hydrophilicity and step-reduction in contemporary simplified adhesives render the adhesives very permeable and susceptible to water sorption and movement. These are alarming concepts. But the realities we face should trigger alarms. It is not the intention of this paper to provide an extensive review on the bonding of these adhesives to dentin. Rather, evidence of water movement across resin-dentin interfaces, which are associated with the use of the simplified dentin adhesives, will be illustrated and discussed. Once identified, steps may be taken to minimize the negative impact of water sorption, while maximizing the convenience of self-etching systems.

Permeability of Simplified Dentin Adhesives

Contemporary dentin adhesives are rendered very hydrophilic in order to improve their bonding to intrinsically wet substrates such as dentin. The incorporation of high concentrations of hydrophilic and/or ionic resin monomers in these adhesives increases their osmolalities and attraction of water, leading to increased water sorption (Tay & Pashley, 2003a). As simplified total-etch and self-etch adhesives lack non-solvented resin coatings (Cheong & others, 2003), they behave as permeable membranes (Tay & others, 2002a) that permit rapid, through-and-through water movement across the polymerized adhesives (Tay & others, 2004a). Dentinal fluid transudation across simplified dentin adhesives has been shown to occur when they were bonded to non-carious, deep, vital human dentin (Chersoni & others, 2004; Tay & others, 2004b). This phenomenon could be duplicated *in vitro* using bonded, extracted human teeth (Elgalaid & others, 2004). The entrapment of water droplets that emerged from the adhesive surfaces by slow-polymerizing, chemical-cured resin composites (Tay & others, 2003a; Carvalho & others, 2004) accounts for the apparent incompatibility when these composites were coupled to acidic adhesives in the presence of non-amine-type ternary catalysts (Suh & others, 2003).

The fact that fluid transudation rapidly occurs across polymerized dentin adhesives implies that there are interconnected porosities or channels within the adhesives that are responsible for rapid water movement within these adhesive membranes. This rapid water movement differs from the relatively slower diffusional processes across bulk resin. Unfortunately, these very minute channels cannot be seen with standard electron microscopical techniques, as the water is lost after desiccation of the specimens and the channels collapse when specimens are examined under vacuum. This prompted the immersion of bonded specimens in electron-dense tracers, such as silver nitrate, before they were subsequently prepared for morphologic examination. Once silver nitrate is reduced to metallic silver

grains, it remains trapped in those sites regardless of whether the specimen is dehydrated or not.

Potential Pathways of Fluid Transport in Polymerized Adhesives

Ultrastructurally, water entrapment in simplified dentin adhesives may be visualized as water-filled channels. Using ammoniacal silver nitrate (pH 9.5) as a tracer, Tay and Pashley (2003b) reported the existence of different forms of silver-filled, interconnecting channels within the adhesive layers of some commercial brands of two-step etch-and-rinse and one-step self-etch adhesives. The authors used the term "water trees" to describe these silver-filled water channels within the adhesive layers to distinguish them from the nanoleakage silver deposits (Sano & others, 1995) that were observed within the bulk of the dentin hybrid layers created by these adhesives. These water channels, which were revealed by silver staining, were seen when very thin, undemineralized sections (ca 1/100 mm thick) of the resin-dentin interfaces were examined using transmission electron microscopy (Figure 1A). Similar to nanoleakage, they could not be seen in demineralized sections that were stained with uranyl acetate and lead citrate (Figure 1B). Water trees were predominantly located along the surface of the hybrid layer, extending into the overlying adhesive layers. Other water channels were seen as "reflecting" downward from the junction between the adhesive and the resin composite. Occasionally, through-and-through channels that were oriented perpendicular to the hybrid layers could also be observed spanning the entire thickness of the adhesive layer.

Water trees were initially reported using more technically advanced transmission electron microscopy. However, they can be readily observed when silver-stained resin-dentin interfaces are examined using the backscattered mode of a scanning electron microscope. This is illustrated very nicely by the myriad of "unreported" water trees with resin-dentin interfaces in a recent nanoleakage paper. In this paper, Li, Burrow and Tyas (2003) demonstrated the existence of water trees in simplified self-etch adhesives such as Prompt L-Pop and simplified total-etch adhesives such as Single Bond, although these entities were not mentioned explicitly by the authors. By examining extracted specimens of vital human teeth bonded with a simplified total etch adhesive (Excite DSC), Ferrari and Tay (2003) have shown that water trees are not *in vitro* artifacts and that these potential water channels do exist following *in vivo* bonding procedures (Figure 1C).

Rapid water transport via water channels created within the adhesives is by no means the only way in which water traverses through the resin-dentin interfaces. The transport of ions and small molecules across an amorphous polymer matrix may still occur via dif-

fusion in the absence of physically-detectable water channels, albeit more slowly. Based on the dynamic percolation theory, water and ions can move across a polymer matrix by hopping from one hydrophilic domain to another (Dürr & others, 2002). This kind of ion hopping mechanism has been commonly observed in polymeric ionic conductors in which ionic salts are incorporated in a polar polymer that possesses significant ionic conductivity (Frisch & Stern, 1983). The diffusion of penetrant molecules through a polymer matrix occurs via micro-cavities of different sizes that are formed and destroyed almost instantaneously on an atomic time scale of picoseconds in the polymer (Müller-Plathesup, Laaksonen & van Gunsteren, 1993). The more hydrophilic the polymer, the greater the ease of formation of these micro-cavities, because of the reduced energy required to disrupt the adjacent polymer chains (Soles & Yee, 2000). In the context of dentin adhesives, these hydrophilic domains were seen as very small, discrete silver grains (Figure 1D) that were present in the adhesive after immersion in ammoniacal silver nitrate (Tay, Pashley & Yoshiyama, 2002c).

Historical Perspectives

Tay and Pashley (2003b) did not invent the term "water trees." They borrowed this term from the field of electrical engineering to reflect the similarities of the water channels seen in dentin adhesives with the microscopic tree-like channels that were identified in aged electrical insulation cables after water sorption. This well-known phenomenon was first reported in 1969 at the IEEE-NEMA Electrical Insulation Conference, Boston, MA, USA, in a paper titled "Deterioration of water-immersed polyethylene coating wire by treeing" (Miyashita, 1969). Water treeing is a well-recognized degradation phenomenon in the dielectric insulation cable industry that is responsible for the water-induced deterioration of polymer insulation of electrical cables after aging (Moreau & others, 1993). Water trees in polyethylene-coated cables are submicroscopic, self-propagating, water-filled tracks that are formed electrochemically by the oxidation of the hydrophobic polymer into more hydrophilic moieties, followed by the condensation of moisture within the hydrophilic, electro-oxidized regions (Ross, 1998). Similar to water trees in dentin

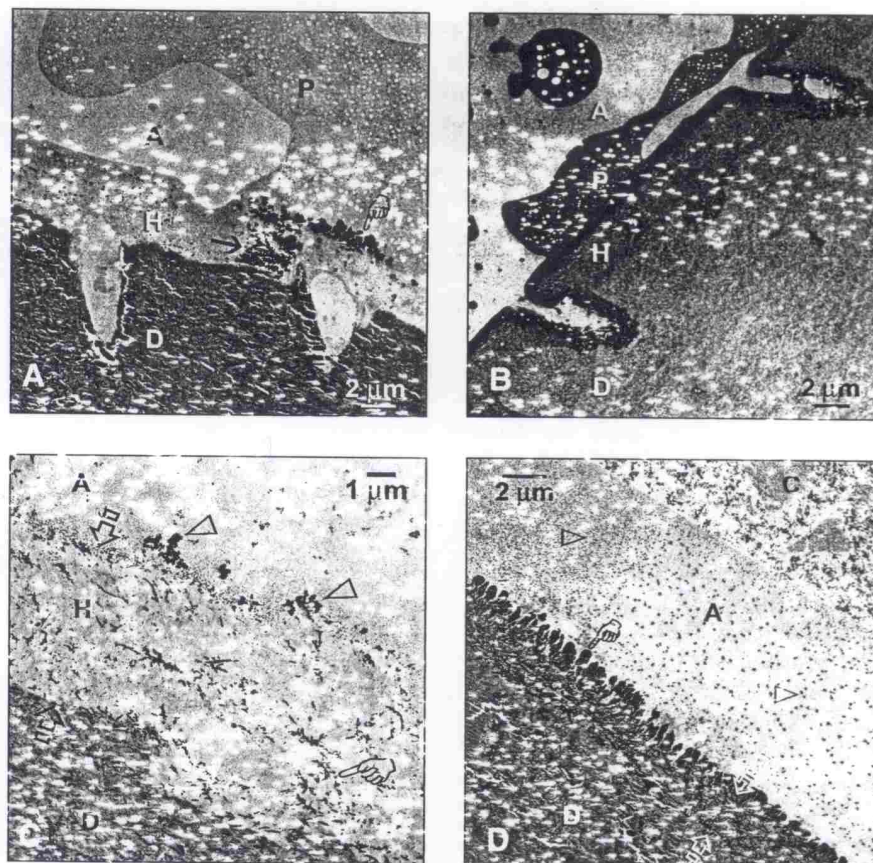


Figure 1A. Differentiation between nanoleakage (arrow) and water trees (pointer) in a two-step etch-and-rinse adhesive containing polyalkenoic acid copolymer (P). B. Both nanoleakage and water trees could not be identified in conventionally stained section of the same adhesive. P: stained polyalkenoic acid copolymer. C. In vivo specimen of another two-step etch-and-rinse adhesive confirming the existence of nanoleakage (pointer) within the unstained hybrid layer (open arrows) and water tree formation (open arrowheads) along the hybrid layer surface. D. Differentiation between discrete silver grains (open arrowheads) that represent water diffusion through an adhesive, and water trees (pointer) on the surface of a hybrid layer with extensive nanoleakage (open arrows) in a one-step self-etch adhesive. A gradient of water diffusion could be seen from the lower right to the upper left of the micrograph along the adhesive layer.

*Labels common to all figures: C: resin composite; A: adhesive; H: hybrid layer; D: dentin. All micrographs are unstained, undemineralized, silver-impregnated sections examined with transmission electron microscopy unless stated otherwise.

adhesives, the original water trees in polymer insulation cables were seen only after immersion of polyethylene in silver nitrate solution (Fan & Yoshimura, 1996; Stepp & others, 1996). They could not be seen in conventional microscopy, as they collapsed after dehydration. Despite the fact that polyethylene is one of the most moisture-resistant polymers available, water has been identified as a contributing factor to this unexpected failure in performance. Water trees, once initiated from microvoids along the surface of the polymer, continue to propagate as hydrophilic tracks in a fractal manner within the hydrophobic polymer (Ding & Xing, 1996). They are converted into electrical trees as a result of lightning surges that result in a rapid deterioration in the cable insulation properties and ultimate

cable failure (Lewis, 1990; Dissado & Fothergill, 1992).

Unlike water trees in polymer-insulated cables that are formed after localized electrochemical transformation of the hydrophobic polymers, hydrophilic resin monomers and water are both present in self-etch and some total-etch dentin adhesives. This led Tay and Pashley (2003b) to hypothesize that water trees in dentin adhesives, together with nanoleakage within the hybrid layers, represent water-rich interfacial regions from which the leaching of hydrophilic resin components may occur readily and expedite the degradation of resin-dentin bonds.

Morphogenesis of Water Trees

Two theories have been proposed on the morphogenesis of water trees within dentin adhesive layers—the “remnant water” theory and the “water flux” theory. It was initially thought that water trees were morphologic expressions of water that was incompletely removed from water contained in simplified dentin adhesives. The rationale for such an assumption was that water trees were almost exclusively identified from resin-dentin interfaces bonded with ethanol-based adhesives and were rarely observed in acetone-based adhesives before aging and water sorption. It is also known that the inclusion of 2-hydroxymethacrylate (HEMA) in these adhesives makes it difficult to remove water completely from these adhesives. Using different HEMA-water concentrations, Pashley and others (1998) observed that the addition of HEMA to water lowered the rate of evaporation of water from the water-HEMA mixtures in a manner proportional to its effect on lowering the vapor pressure of water, making it more difficult to remove the last amounts of water. These ongoing experiments further showed that when experimental self-etch adhesives created with HEMA and acidic monomers were solvated in an ethanol/water mixture, as much as 40% of the solvent mixture remained after 60 seconds of air-blast evaporation. Up to 32% of the solvent mixture still remained upon further evaporation for an additional 60 seconds (a total of 120 seconds) (Yiu & others, unpublished results). It is speculated that this is also due, in part, to hydrogen bonding between ethanol, water and polar resin monomers in the adhesive.

The “remnant water” theory, however, does not account for four aspects of water tree formation:

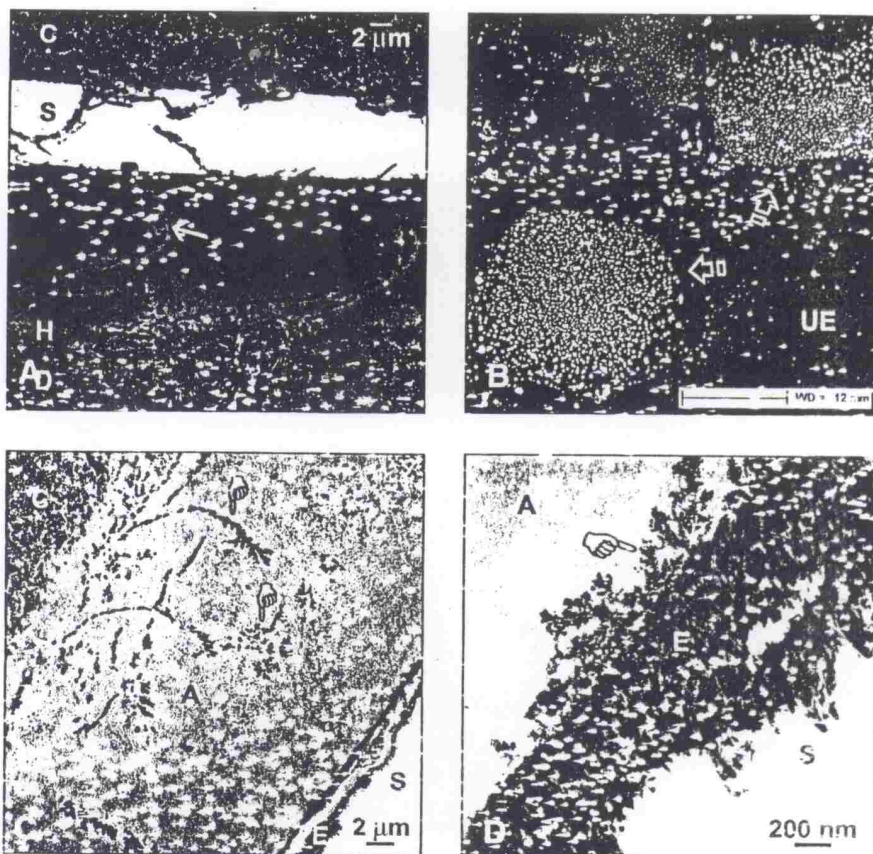


Figure 2A. Through-and-through water tree formation (arrow) from the resin-dentin interface of a one-step self-etch adhesive. S: empty space caused by fracture of the adhesive during sectioning. B. SEM micrograph of a replica of the author's incisor, showing fluid transudation (arrows) along the surface of the uncut enamel (UE). C. Cut enamel (E) bonded with a 4-META containing, single bottle one-step self-etch adhesive showing water trees (pointers) within the adhesive. S: empty space. D. A higher magnification view showing the origination of some water trees (pointer) directly from the enamel surface (E). S: empty space.

1. Uneven Distribution

One would have expected that, by random TEM sectioning of a myriad of resin-bonded dentin specimens conducted over a five-year period, that the distribution of water trees within the adhesive layer would be normal or Gaussian. Interestingly, water trees were seldom identified from the central part of an adhesive layer, and the frequency of observing through-and-through water tree formation (Figure 2A) across the entire cross section of a 5-20 μm thick adhesive layer was less than 10%. Indeed, water trees were predominantly identified from the surface of the hybrid layer. Being mostly vertical in their orientation (perpendicular to the surface of the hybrid layer), they were often interconnected with the horizontal or oblique reticular patterns of nanoleakage that were observed in the hybrid layer (Carvalho & others, 2005).

2. Substrate Dependence

Although water trees were frequently seen in sound dentin bonded with one-step self-etch adhesives, they were not observed when disks of resin composite were used as a bonding substrate if the adhesives were adequately air-dried before bonding (King & others, 2005). Residual water probably exists as water clusters that are attached to the hydrophilic domains of the adhesive via hydrogen bonding. This form of bound water could be seen in the form of discrete silver grains within the polymerized adhesive. As the solvents in the dentin adhesives are evaporated in the same manner when bonding two composite disks together, the absence of water trees when composites are bonded to composites suggested that water trees contribute minimally by residual water derived from the dentin adhesives.

Controversy exists regarding whether the water trees found in bonded enamel are derived from the adhesive. A previous study has shown that there is a slow outward flow of fluid through the enamel of vital human

teeth (Bergman, 1963). This can easily be demonstrated by taking an impression of one's own incisors using a slow-setting polyvinylsiloxane wash (Figure 2A). This phenomenon occurs more readily in young teeth. Although there have been no reports that this fluid flow adversely affects enamel bonding, it is possible that some of this fluid may be trapped by the polymerized adhesive (Figure 2C). One can see from the higher magnification view of the previous TEM micrograph that some of the water trees were originated from the surface of the enamel smear layer (Figure 2D). This suggests that at least some of the water trees that are seen in bonded enamel have been derived from water that is expressed out of the enamel tissues.

3. Reverse Water Trees

Similar to real trees that are found in nature, water trees in dentin adhesives are manifested in different sizes and forms. Some of the water trees are like birch trees with a wide canopy that spreads out in a fractal manner (Figure 3A). Others resemble pine trees and

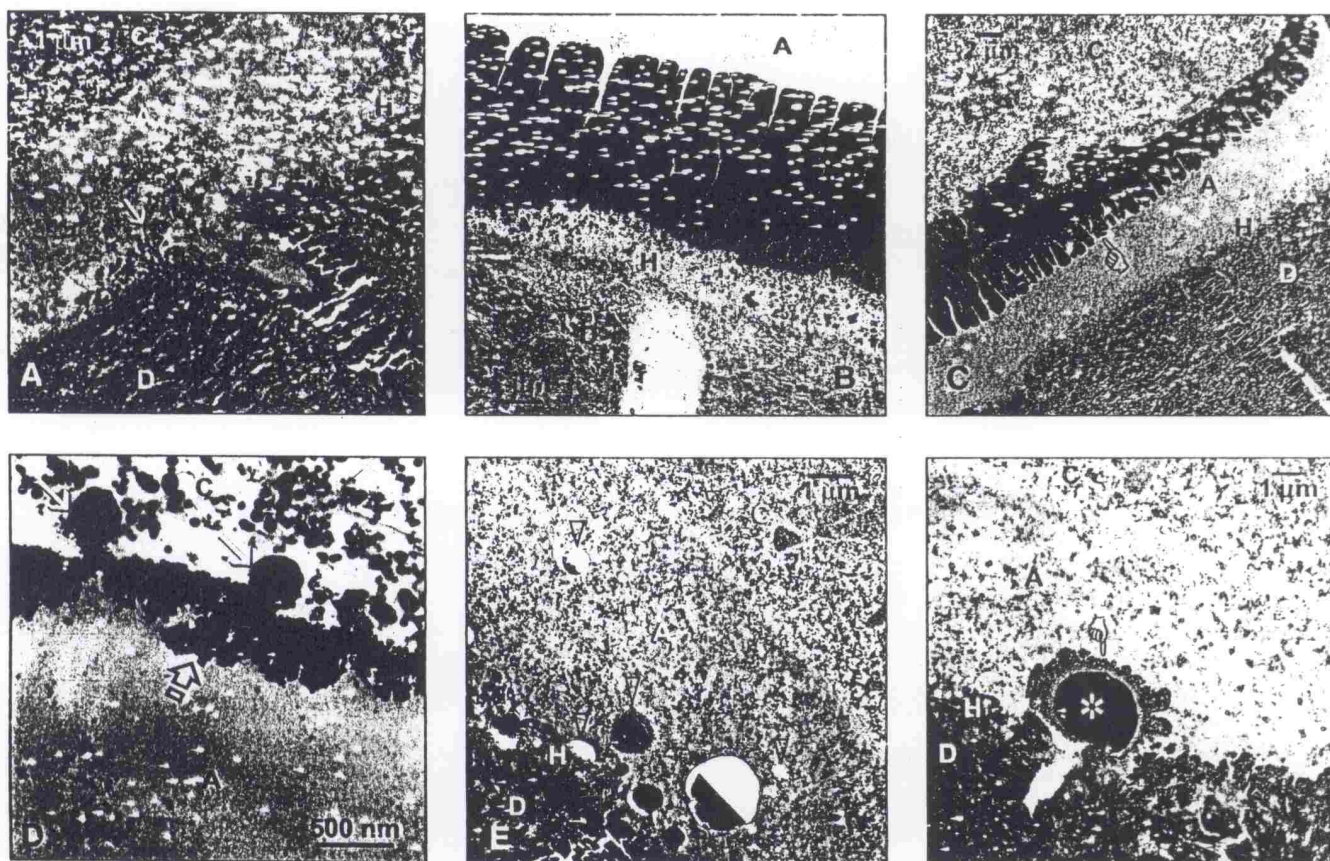


Figure 3A. A canopy-type water tree (pointer) from the resin-dentin interface of a two-bottle one-step self-etch adhesive containing nanofillers. B. Columnar-type water trees from the resin-dentin interface of a single bottle one-step self-etch adhesive. C. Reverse water trees (pointer) that are opposite in direction to those water trees seen in Figures 3A and 3B. D. Reverse water trees (arrow) are frequently connected to water blisters (arrowheads) that are trapped between the adhesive and the composite. E. Water droplets (open arrowheads) formed within the adhesive layer of a fumed silica-filled, single-bottle, non-HEMA containing one-step self-etch adhesive. F. Emanation of a water droplet (asterisk) from a tubular orifice in the adhesive described in Figure 3E. Secondary water tree formation (pointer) along the periphery of the water droplet produced a sun burst effect around the droplet.

are more columnar (Figure 3B). Nevertheless, all exhibit a definitive orientation, in the sense that the location from which they originate can usually be traced. Normally, water trees have points of origin from the surface of dentin, with their branching pointing upward into the adhesive. In the case of reverse water trees, they appear to originate from water trapped at the interface between the adhesive and the overlying resin composite; they spread downward, with their "branches" pointing toward the dentin (Figure 3C). Reverse water trees are frequently affiliated with water blisters that are trapped along the adhesive-composite interface (Figure 3D).

4. Secondary Water Trees Derived From Discrete Water Droplets

The entrapment of water droplets is commonly observed in single-bottle versions of one-step self-etch adhesives such as Brush&Bond, iBond and G-Bond (Tay, Pashley & Peters, 2003; De Munck & others, 2005a) (Figure 3E). These water droplets were surmised to represent phase separation of water from adhesive components after the evaporation of more volatile solvents such as ethanol or acetone (Van Landuyt & others, 2005). When these water droplets were examined without the adjunctive use of a tracer solution, they appeared as clear, circular holes within the adhesive layer. However, when these specimens were previously immersed in a silver nitrate tracer solution prior to laboratory processing, water trees could be seen radiating circumferentially from the periphery of these water droplets, creating a fuzzy sun burst effect around the silver-impregnated droplets (Figure 3F).

Water can exist as free and bound water within a polymer matrix (Klotz & others, 1996). The existence of water trees succinctly indicates the presence of free water within the adhesive matrix. However, it must be stressed that the retention of residual water within a hydrophilic adhesive does not necessarily result in the generation of physical water-filled channels within an adhesive. When a polymer contains hydrophilic or polar functional groups, water molecules can exist as bound water that are weakly attracted to the hydrophilic and/or polar domains of the polymer matrix via hydrogen bonding or van der Waal's forces. With the use of FTIR spectroscopy, three molecular water species have been identified with epoxy resin matrices after water sorption. The S_0 molecular species (Cotugno & others, 2001), or the portion of water that can be frozen at 0°C (Ping & others, 2001), was associated with bulk or free water that occupied the free volume of the polymer matrix. The S_1 species, or the portion of water that can be frozen below 0°C, represent either self-associated dimers or water molecules with weak hydrogen bonding along the secondary hydration shells. Conversely, the S_2 molecular species, or the non-freez-

able portion of absorbed water molecules, is firmly bound to polar sites along the polymer network and exhibit high plasticizing efficiency. Hydrogen bonding between the water molecules and the polar hydroxyl, carboxylate or phosphate groups of polymer networks will disrupt the interchain hydrogen bonding (VanLandingham, Eduljec & Gillespie, 1999), altering the molecular structure and increasing the segmental mobility of the polymer chains (Musto & others, 2002). These changes are reflected by reductions in the mechanical properties and the decline in glass transition temperatures (T_g) of polymerized resins (Nogueira & others, 2001).

The above description applies to a well-polymerized, physically non-porous epoxy resin polymer matrix that is subjected to water sorption. Nevertheless, introduction of the concept of bound water serves to point out that within limits, residual water that is bound to the hydrophilic domains within the dentin adhesive can disrupt interchain hydrogen bonding during polymerization of the adhesive matrix (Lagouvardos & others, 2003). Thus, the different patterns of water tree formation within the adhesive may be regarded as historical traces of free water movement along the relaxed/disrupted regions of a polymer matrix during the initial gelation stage of the adhesive as it is being polymerized.

As water trees seldom exist when water-containing adhesives are used to bond composites to composites, it is unlikely that water tree formation is caused by water that is present within the adhesive formulations. Two questions, which remain to be answered, are: a) where is the water derived from and 2) what triggers the movement of this additional source of water through the disrupted sites of an adhesive matrix during the gelling phase of polymerization. Both of these questions can be reasonably addressed with the "water flux" theory.

The "Water Flux" Theory

Vital dentin, particularly, deep vital dentin, is highly permeable, because of the presence of relatively short and wide dentinal tubules and normal positive pulpal tissue pressure (14 cm H₂O or 10.3 mm Hg; Ciucchi & others, 1995). Three types of fluid movement may occur through dentin: evaporative, osmotic and convective water fluxes. Evaporative water flux may be induced by air blasts (Goodis, Tao & Pashley, 1990), such as those that occur during air-drying of a crown preparation. The presence of the smear layer and smear plugs offers little resistance to evaporative water loss. Dehydrating dentin with an air blast or with absorbent paper will generate capillary forces that induce rapid outward fluid movement from dentinal tubules (Matthews, Showman & Pashley, 1993). Because non-vital dentin also contains water, evaporative water flux may occur irrespective of the tooth's vitality status, even when

smear plugs are retained within the dentinal tubules. Although it is mandatory to remove solvents from solvated adhesives before light-curing, the same air-drying process induces outward evaporative water flux from the smear-layer-covered dentin (Hashimoto & others, 2004a).

Moreover, the high concentrations of water-soluble ionic monomers in the presence of water may also induce osmotic water flux from deep dentin (Panopoulos, Gazelius & Olgart, 1983; Pashley, 1985; Pashley & others, 1996), if the osmolalities of these comonomers exceed the osmolality of dentinal fluid (Pashley, Horner & Brewer, 1992). This is very likely to occur just prior to polymerization. The authors have calculated the concentration of HEMA and other hydrophilic monomers in solvated adhesives as the solvents are evaporated. Just prior to light curing, the concentrations can be between 2.5 and 3.5 moles/L, which is very hypertonic. That is, 2500 to 3500 milliosmoles/kg (mOsm/kg) compared to the osmolality of plasma, which is 290 mOsm/kg. These high solute concentrations (8.6 to 12 times as concentrated as plasma) mean that their water concentration is much lower than the water concentration in dentinal fluid, thus causing osmotic fluid movement from dentinal fluid into these concentrated comonomer films. As soon as the monomers are converted to polymers, the osmotically induced water flux should cease.

Both evaporative and osmotic fluxes may result in the permeation of water along regions within the adhesive where interchain segmental mobility is increased by the hydrogen bonding of retained bound water present

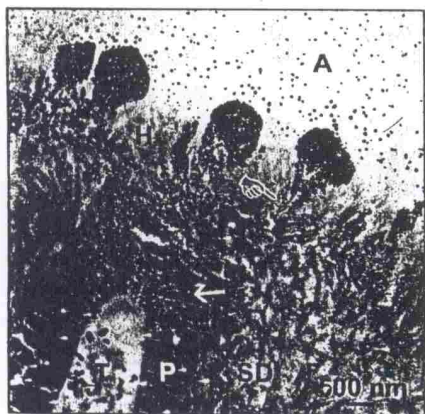


Figure 4A. Water trees and nanoleakage are present when one-step self-etch adhesives are applied to sound dentin (SD). A. Evaporative water flux provides a logical explanation for the observation of water movement from the channels with the least resistance (arrow) in the peritubular dentin (P) into the interfibrillar spaces within the hybrid layer (pointer) that ultimately resulted in water tree formation. T. dentinal tubule.

in the adhesive mixture. This provides a logical explanation for the predilection of normally oriented water trees along the surface of the dentin. In particular, such a theory also accounts for the observation that, in the presence of smear plugs, silver containing tracks are observed around the peritubular dentin (the channels with the least resistance) that are continuous with silver-

containing interfibrillar spaces within the hybrid layers formed by mild self-etching primers, that, in turn, are continuous with the vertically-oriented water trees along the surface of the hybrid layers (Figure 4A). Theoretically, more water trees can be formed with longer periods of air drying or contact of the adhesive with dentin prior to its polymerization. On the other hand, the viscosity of the polymer matrix, cross-linking and the intensity of interchain hydrogen bonding may determine the specific morphology of the water trees created with the polymerized adhesive.

Indirect water flux also provides the *raison d'être* for the observation of reverse water trees. Hashimoto and others (2004a) recently showed that, following the detection of outward evaporative water flux after air drying of the adhesive-coated dentin, additional inward fluid flux was induced by light-activation of the adhesive. It is likely that the inward fluid flux is caused by heat generated during light curing of the adhesive. One can envisage, on a sub-second time scale, that during the light-activation process, some of the water that has reached the top of the adhesive layer cannot escape, as it is trapped by the initially better polymerized surface part of the adhesive. The heat generated by a quartz-tungsten-halogen or a second generation LED light-curing unit (Bouillaguet & others, 2005) could redirect water back into the less well-polymerized subsurface adhesive matrix, forming water tracks that are morphologically identified as reverse water trees.

Convective water flux is considerably more significant when smear plugs are removed by acid etching (Pashley, Michelich & Kehl, 1981). Many researchers have attempted to duplicate these convective water fluxes *in vitro* by bonding to dentin via perfusion at physiological pulpal pressure of about 15–20 cm water (Bouillaguet & others, 2000; Elgalaid & others, 2004; Grégoire & others, 2003; Vaysman, Rajan & Thompson, 2003; Özok & others, 2004). With simplified single-bottle adhesives, convective water movement (Vongsavan, Matthews & Matthews, 2000) has been shown to occur both *in vitro* and *in vivo* during polymerization of the adhesives (Purk & others, 2004). Water droplets trapped between the adhesive and resin composite account for the apparent incompatibility that occurs when acidic versions of these adhesives are used with slow-setting, chemical-cured resin composites. These water droplets not only reduce the bonded surface area, they may act to increase interfacial stress, which may result in the premature dislodging of the resin composites during function (Tay & others, 2003b).

In the presence of smear plugs, only slow convective water flux occurs in vital dentin (Pashley & others, 1981). Even this slow convective water flux is adequate to permit the blebbing of dentinal fluid through single-bottle type, simplified self-etching adhesives that do

not contain HEMA (Tay & others, 2003d). HEMA is miscible with water in all proportions and is often utilized as a transitional polymerizable, slightly volatile solvent for other ionic resin monomers that are sparingly soluble in water. When HEMA is absent, phase separation of the adhesive components occurs (Van Landuyt & others, 2005) on the addition of water to an adhesive in which a more volatile solvent such as ethanol has been evaporated. These phase separations probably account for the observation of discrete droplets of water within the adhesive layers of dentin bonded with the latest non-HEMA-containing, single-bottle type, one-step self-etch adhesives. Further attempts to evaporate the adhesive solvent, and light curing of the adhesive, will generate water fluxes that radiate out from these water droplets as secondary "sun-bursting" type water trees.

Water Trees Are Absent in Bonded Transparent Carious Dentin

When resin bonding is performed on sound dentin with unblocked dentinal tubules, it is difficult to resolve whether the entrapped water that causes the formation of water trees originates from water-containing self-etch adhesives or from hydrated dentin. Substantiation of the "water flux" theory of water tree formation in simplified dentin adhesives really stems from work performed with the application of these adhesives onto transparent carious dentin. It is pertinent to point out that all *in vitro* and *in vivo* studies that reported the existence of water trees were carried out using sound coronal dentin as bonding substrates. For those studies that involved the use of simplified self-etch adhesives, nanoleakage was invariably identified within even very thin hybrid layers, irrespective of the presence or absence of water trees. Ideally, nanoleakage should not occur in hybrid layers created by self-etching primers, as etching and resin infiltration occur concurrently.

Transparent carious dentin is known to be heavily occluded with intratubular mineral deposits (Ogawa & others, 1983; Zheng & others, 2003). Tubular occlusion accounts for the relative impermeability of this type of bonding substrate (Tagami & others, 1992). When convective and evaporative water fluxes are eliminated by bonding to transparent carious dentin (Lee & others, 2003), any water entrapment within the adhesive should be attributed to the retention of water derived from one-step self-etch adhesives. Indeed, the use of this relatively simple experimental design has resulted in a giant step forward in our understanding of the etiology of water tree formation and in resolving the riddle as to why nanoleakage occurs in self-etch adhesives despite the simultaneously occurring processes of etching and resin infiltration.

In this experimental design, sound and carious dentin from the same tooth were bonded under dentin perfu-

sion using an experimental 4-MET containing, single bottle-type, one-step self-etch system in which the all components were known. To prevent complications that may arise from the breakdown of the acidic monomer into non-polymerizable components (Nishiyama & others, 2004), the adhesive was supplied with the water component segregated from the resin monomers. The adhesive formulation was reconstituted immediately before application and applied to human carious teeth in which the soft, caries-infected dentin was removed with a polymer bur, leaving behind hard transparent, caries-affected dentin. The rest of the sound dentin was ground down to the same level as the transparent carious dentin, so that both bonding substrates represented deep dentin derived from the same tooth. The root of each of these teeth was sectioned 2 mm below the cemento-enamel junction and the remaining crown segment was cemented to a Plexiglass slab that was penetrated by a stainless steel tube. This permitted filling of the pulp chamber with water via plastic tubing connected to a syringe barrel to deliver 20 cm of water pressure (Pashley & Depew, 1986). By keeping the syringe barrel at the level of the crown segment or raising it to 20 cm above the crown segment, a convective water flux could either be eliminated or generated. As the pulp chamber was filled with water all the time, air drying of the smear layer-covered dentin would create an evaporative water flux (Sidhu & others, 2004).

When the experimental adhesive was applied to sound dentin under the influence of evaporative water flux only, a layer of columnar-appearing water trees of almost uniform thickness could be identified above the hybrid layer (Figure 4B). In the presence of additional convective water flux, silver-filled water droplets appeared over the dentinal tubular orifices despite their occlusion by smear plugs (Figure 4C). For some sections, more than 50% of the adhesive layers were filled with water trees and droplets that were continuous with the nanoleakage identified within the hybrid layers (Figure 4D). In the design of one-step self-etch adhesives, manufacturers have probably neglected the detrimental effects of convective water flux on these highly permeable adhesives. Indeed, very few bond strength studies of one-step self-etch adhesives, in particular, the single-bottle versions, were conducted under dentin perfusion to simulate what happens in vital, sound dentin. The contribution of convective water fluxes provided the rationale for the observation of water droplets (Figure 4E) over the adhesive surface when these adhesives were applied on deep, vital dentin after crown preparations (Chersoni & others, 2004). The data obtained with the experimental adhesive have also been reproduced by the bonding of commercialized versions of single-bottle one-step self-etch adhesives under dentin perfusion and the use of a light-cured composite that was activated immediately after

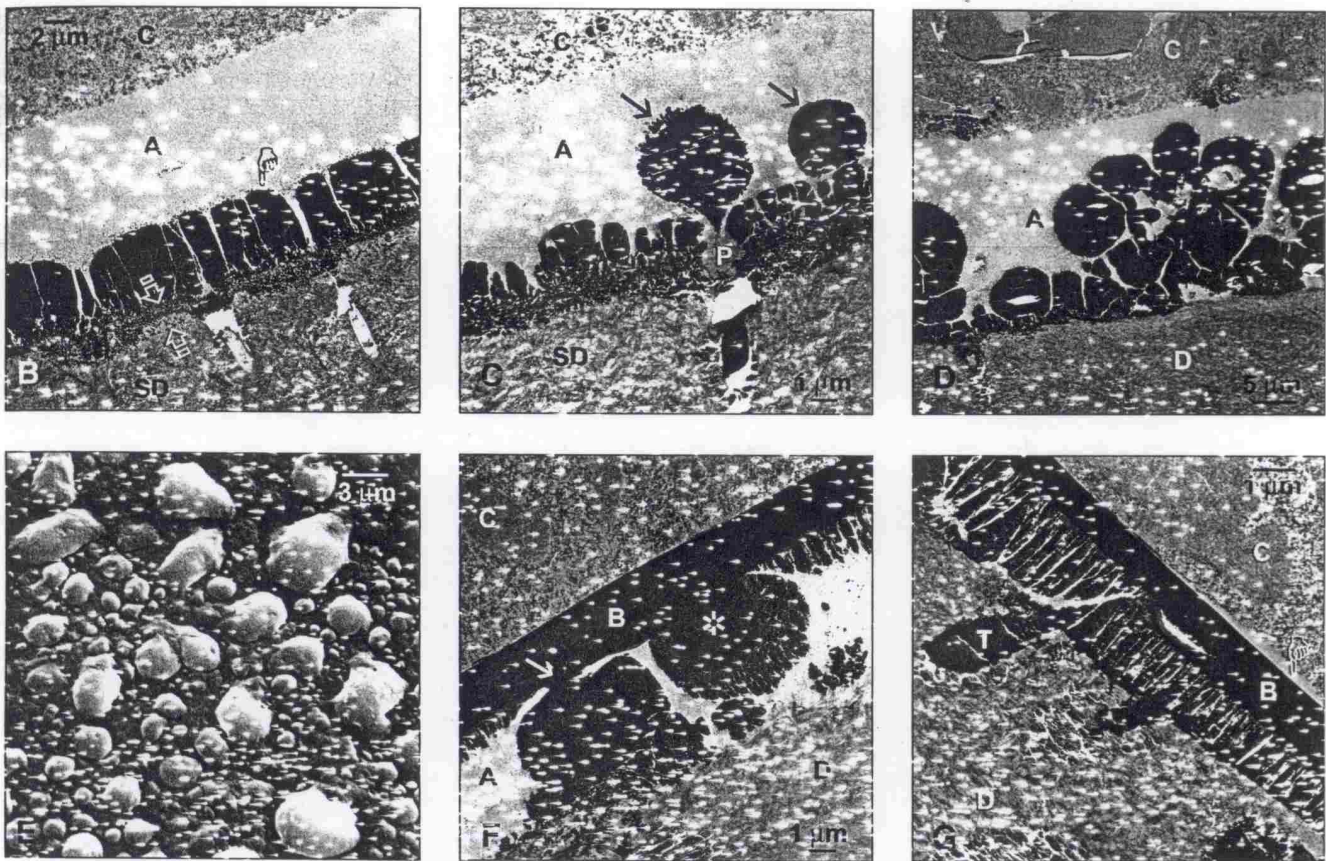


Figure 4B. With the use of an experimental non-HEMA containing, single-bottle one-step self-etch adhesive, evaporative water flux resulted in more aggressive water tree formation (pointer) and extensive nanoleakage within the hybrid layer (open arrows). C. With the same experimental adhesive, the combination of evaporative and convective water flux resulted in the expression of water droplets (arrows) through the smear plugs (P). D. A more severe manifestation of what was depicted in Figure 4C, with silver deposits occupying more than 50% of the adhesive surface area. E. SEM micrograph showing resin replica of fluid droplets that appeared on the surface of vital sound dentin after bonding with a one-step self-etch adhesive. F. The appearance of these *in vivo* fluid droplets could be seen when the same commercial single-bottle one-step self-etch adhesive was bonded to sound dentin *in vitro* under dentin perfusion. In this micrograph, convective water fluxes resulted in the expression of water droplets (asterisks) from the dentinal tubules. These water droplets were connected (arrow) with a large water blister (B, incompletely shown) between the adhesive and the composite. G. In a different commercial single-bottle one-step self-etch adhesive that was bonded under perfusion to deep, sound dentin, through-and-through water trees that completely obscured the entire adhesive layer were connected to a water blister (B, incompletely shown). An artifactual contact gap between the silver deposits and the composite was filled with laboratory embedding epoxy resin during subsequent laboratory processing (pointer). T: dentinal tubules.

placement on the polymerized-adhesive, with equally, if not more dramatically detrimental results (Figures 4F and 4G). These results complemented the concern raised by Özok and others (2004) regarding the pressing need for manufacturers to evaluate the behavior of one-step self-etch adhesives under dentin perfusion prior to the launching of these adhesives. In these two micrographs, the thick band of silver between the adhesive and the composite represented part of a large water droplet trapped by the light-cured composite, corresponding to the free water droplets trapped by a polyvinyl siloxane impression material seen in Figure 4E.

By contrast, both water-treeing and nanoleakage were absent from the hybrid layers of transparent, caries-affected dentin from the same tooth (Figure 5A).

The hybrid layer, created in transparent dentin, was three times as thick as that observed in bonded, sound dentin. It is initially hard to rationalize why the much thicker hybrid layer in the former was devoid of nanoleakage, whereas, the much thinner hybrid layer in the former was completely occupied by nanoleakage. Such a phenomenon contradicts "the monomer diffusion concept of resin infiltration" proposed by Pashley and others (1993), in which a diffusion gradient occurs for resin monomers within a collagen network based on their molecular weight and diffusion capacity. Apparently, air drying of the solvated adhesive and bonding under physiological pulpal pressure resulted in a negligible adverse effect of water movement during bonding to transparent carious dentin. This is because both evaporative and convective water fluxes from

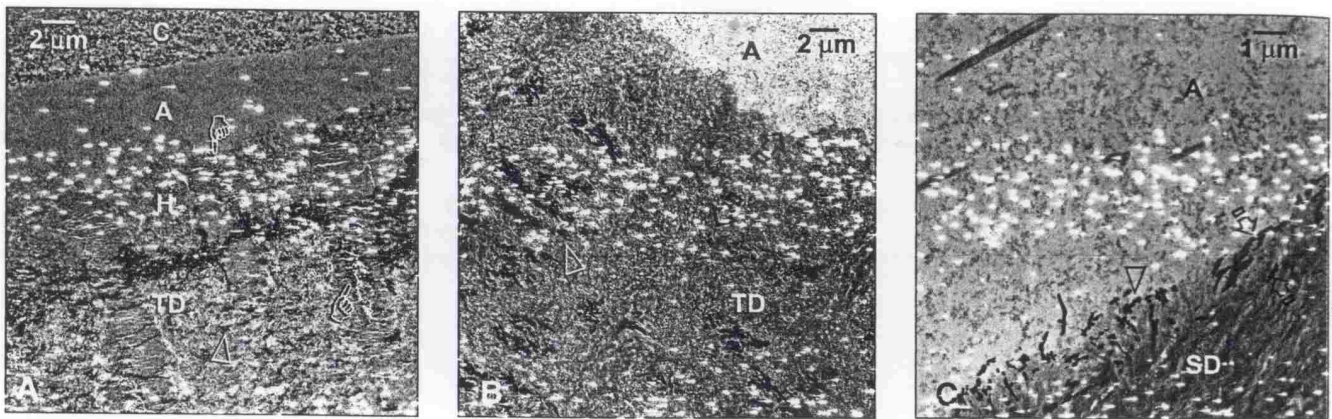


Figure 5. Water trees and nanoleakage are absent when self-etch adhesives are applied to transparent carious dentin (TD). A. Water trees were absent and nanoleakage was minimized within the much thicker hybrid layer when the same experimental one-step self-etch adhesive was applied to transparent carious dentin. Water fluxes were minimized as the dentinal tubules were heavily occluded with mineral deposits (pointers). Severe silver deposits (open arrowhead), however, could be seen in the highly porous substrate. B. When a commercial two-step self-etch adhesive was applied to transparent carious dentin, nanoleakage was completely absent from the hybrid layer (open arrows). Islands of silver deposits within the unbonded transparent carious dentin could similarly be observed (open arrowhead). C. By contrast, nanoleakage (open arrows) and silver deposits (open arrowheads) were observed when the same commercial two-step self-etch adhesive containing fumed silica and NaF crystals was applied to sound dentin (SD).

dentin were blocked by the heavily occluded dentinal tubules. This resulted in an excellent initial resin seal with the complete absence of water-rich interfacial zones above a highly porous bonding substrate that is partially depleted of its mineral content (Angker & others, 2004). Although these hybrid layers were thicker than those created in sound dentin (Yoshiyama & others, 2002), it is impossible for any adhesive to completely diffuse through a zone of partially demineralized carious dentin that may be several hundred micrometers thick (Nakajima & others, 2005). This could be appreciated by the presence of discrete islands of silver deposits directly beneath the silver-free hybrid layer and within the highly porous unbonded transparent carious dentin. At the scanning electron microscopy level, this may give the illusion that interfacial nanoleakage is present when bonding is performed on caries-affected dentin (Kubo & others, 2002).

Unlike one-step self-etch adhesives (Tay & others, 2004c), two-step self-etch adhesives (self-etching primers) rarely exhibit water-treeing prior to aging and water sorption (Donmez & others, 2005). Nevertheless, nanoleakage within the hybrid layer is invariably identified from hybrid layers created in sound dentin bonded by these adhesives (Hashimoto & others, 2004b; Reis & others, 2004). As self-etching primers may contain 30% to 50% water in their formulations, the "remnant water" theory has previously been proposed to explicate the occurrence of nanoleakage in these adhesives (Tay & others, 2002b; Li & others, 2003). Based on the authors' more recent work performed with self-etching primers on transparent carious dentin (Figure 5B) and sound dentin (Figure 5C) derived from the same teeth, nanoleakage was completely absent from the bonded interfaces of the former, despite the occurrence of

islands of silver deposits within the highly porous underlying substrate. In the broadest sense, this feature resembles a much amplified version of an incompletely infiltrated hybrid layer produced by an etch-and-rinse adhesive in sound dentin. There was more nanoleakage in sound dentin bonded with self-etching primers (Figure 5C) than in caries-affected dentin (Figure 5B).

Relationship Between Nanoleakage and Water Trees

Thus, when bonding to sound dentin, the extensive nanoleakage that was seen in the hybrid layers of the latest simplified self-etch adhesives may be attributed to the combined adverse effect of evaporative, osmotic and possibly convective water fluxes that result in an outward fluid movement from both the intertubular dentin and the dentinal tubules. As the inward diffusion of acidic resin monomers demineralize intact dentin and dissolve smear layers and smear plugs, the outward, osmotically-induced water fluxes generated may dilute or even flush out the partially neutralized, but still acidic, resin monomers from the partially demineralized dentin. The presence of dilute water in partially acidic adhesive may retard the polymerization of resin within the already resin-sparse interfibrillar spaces. This is probably the mechanism responsible for the manifestation of heavily silver-impregnated hybrid layers after silver tracer immersion.

Figures 6A through 6C depict "snapshots" of what was probably a continuous series of events that link the expression of nanoleakage in self-etch adhesives with water-tree formation. Water that seeps out of normal hydrated dentin via the paths of least resistance along the smear plugs and peritubular dentin first occupies

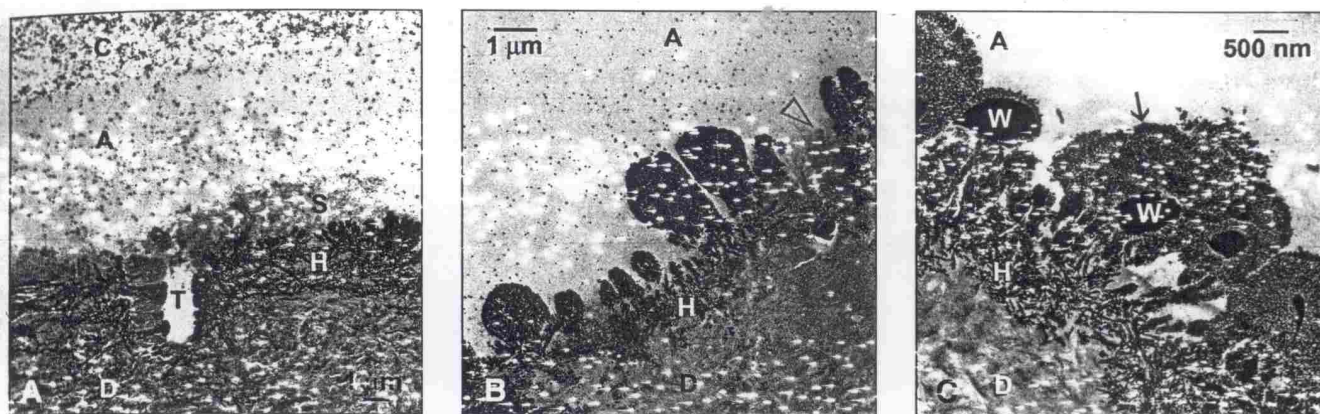


Figure 6. "Snapshots" of what was probably a continuous series of events that link the expression of nanoleakage in a one-step self-etch adhesive with water tree formation. A. Water seeps out of normal hydrated dentin via the paths of least resistance in the peritubular dentin first and occupies the interfibrillar channels of the partially demineralized intertubular dentin beneath the smear layer (S), with the initiation of water trees along the base of the smear layer. B. The water finds its way through the smear layer and is manifested more or less vertically (arrow) as water trees within the adhesive. C. Depending on the chemical composition of the adhesive, phase separation of the bulk water may occur in the form of water droplets (W), from which secondary water trees arise (arrow).

the interfibrillar channels of the partially demineralized intertubular dentin beneath the smear layer, with the initiation of water trees along the base of the smear layer (Figure 6A). The water finds its way through the smear layer and is manifested more or less vertically, as water trees within the adhesive polymer matrix during its gelling phase of polymerization (Figure 6B). Depending on the chemical composition of the adhesive, phase separation of the bulk water may occur in the form of water droplets, from which secondary water trees arise (Figure 6C). According to this concept, nanoleakage in self-etch adhesives, and water trees that are formed along the surface of the hybrid layer of these adhesives, have a common origin. In the case of nanoleakage within the hybrid layer, subsurface water movement occurs preferentially through pre-existing channels (interfibrillar spaces) that are determined by the arrangement of the dentin collagen network. In the case of water treeing, bulk water creates channels through the paths of least resistance within the gelling polymer matrix. These paths are likely to be hydrophilic regions within the polymer matrix in which intermolecular hydrogen bonding has been disrupted by bound residual water molecules that act as plasticizers and increase the segmental mobility of the polymer chains.

Functional Implications of Water Treeing

It has been suggested that the presence of large plasma proteins (Pashley, Galloway & Stewart, 1984) and immunoglobulins (Hahn & Overton, 1997) may reduce outward convective fluid fluxes from deep dentin by adsorbing to intratubular material that reduces the functional radii of dentinal tubules. Coagulation of these dentinal fluid proteins by glutaraldehyde and/or primer components (Bergenholtz & others, 1993;

Nikaido & others, 1995) may further reduce outward convective fluid flow. Clinically, however, the use of a single-bottle, one-step self-etch adhesive containing 4-META and glutaraldehyde did not prevent dentinal fluid transudation in vital, anesthetized human crown preparations covered with the adhesive (Chersoni & others, 2004 [see Figure 4E]). Although coagulation of the plasma proteins by adhesive components may prevent large molecules, such as serum albumin, from traversing the dentinal tubules (Bergenholtz & others, 1993), this probably does not limit the movement of small molecules, such as water, through the proteinaceous hydrogel that is precipitated within the tubules and along the fine crevices that are present between the smear plugs and the walls of peritubular dentin. Both nanoleakage and water treeing are subclinical phenomena. Unlike *in vivo* convective fluid studies with the objective of collecting enough dentinal fluid for analytic purposes (Maita & others, 1991; Knutsson, Jontell & Bergenholtz, 1994), only a very small amount of fluid is necessary to produce nanoleakage and water trees, considering the thickness of the hybrid and adhesive layers.

The absence of interfacial, water-rich zones following the use of self-etch adhesives in transparent carious dentin provides reassurance of the validity of the concept of simultaneous etching and priming. Although tubular occlusion by mineral crystals prevents water treeing and nanoleakage in one-step self-etch adhesives, it is unrealistic that, clinically, one can bond only to transparent carious dentin without involving surrounding sound dentin. A likely scenario is that one may encounter a gradual transition from a strongly bonded but permeable interface to a weakly bonded (Yoshiyama & others, 2003; Yazici & others, 2004) but relatively impermeable interface (Pashley & others,

1991), as the bonding substrate shifts from sound to caries-affected dentin along the cavity floor.

At least from an *in vitro* perspective, improvements in bond strength and reduction in nanoleakage and water tree formation may be achieved when multiple coats of one-step self-etch adhesives were used on sound dentin, instead of the standard number of coats recommended by manufacturers (Hashimoto & others, 2004b; Ito & others, 2005). When one-step self-etch adhesives are applied to sound dentin, water droplets may be deposited between the adhesive-composite interface due to rapid water movement across the adhesive. When a sufficiently thick layer of adhesive is present, the phenomenon of water blistering is of minor clinical consequence, as it only occurs when a slow setting chemical-cured composite is employed or when a light-cured composite is experimentally subjected to delayed light-activation. However, this phenomenon becomes a clinical concern when only a single coat of a one-step self-etch adhesive was applied to sound dentin. (Figure 6D). As the adhesive layer is thin, direct contact of the water trees with the light-cured composite resulted in blister formation even when the composite was light activated immediately.

One-step self-etch adhesives can be rendered less permeable by executing the rationale behind the use of two-step self-etch adhesives—by treating the one-step self-etch adhesive as a primer and covering it with a less hydrophilic resin coating such as those that are employed in conventional etch-and-rinse adhesives (Carvalho & others, 2004). This extra step converts one-step into two-step self-etch adhesives (King & others, 2005) and render them less permeable to water movement (Figure 6E). However, that strategy circumvents the original claims of simpler, faster application and improved user friendliness, which are employed as marketing strategies for these simplified adhesives.

Water Sorption and Degradation of Adhesive Bonds

Water is regarded by many to be an ubiquitous agent in the degradation of adhesive bonds (Kinloch, 1987; Dickstein & others, 1991; Nguyen, Byrd & Bentz, 1995). Bond degradation is preceded by water sorption into a polymer matrix. Water sorption is enhanced by the presence of hydrophilic and ionic resin monomers (Tanaka & others, 1999), which, in turn, facilitate ion movement within a polymerized resin matrix. The supramolecular structure of glassy polymers is normally not in equilibrium. A non-equilibrium structural state may be preserved for an unlimited period of time if the

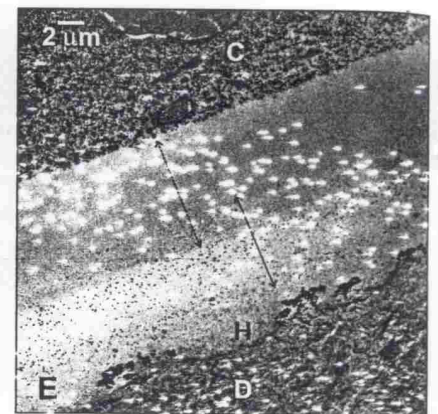
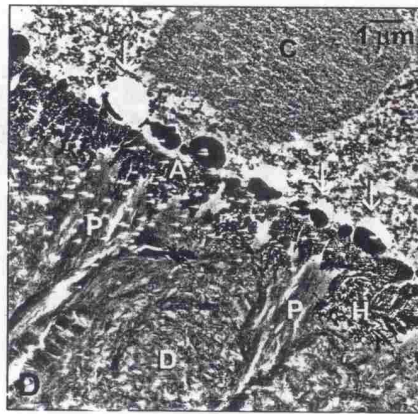


Figure 6D. When only a single coat of a one-step self-etch adhesive was applied to sound dentin, water blisters appeared between the adhesive and the light-cured composite even when the composite was light-cured immediately. This resulted in substantial weakening of the adhesive-composite interface. E. One-step self-etch adhesive can be made less permeable by placing a layer of less hydrophilic bonding resin (solid line) on top of the adhesive (dotted line).

segmental mobility of macromolecules is “frozen,” as exemplified by storing a piece of polymerized adhesive in a dry state below its glass transition temperature (T_g). Such a state is altered on water sorption, as the bound water acts as a plasticizer, reduces the T_g of the polymer, increases the segmental mobility of the polymer chains and “defreezes” the physical process of structural relaxation (Zaikov, Iordanskii & Markin, 1988). Water diffusion in glassy polymer matrices can be divided into three categories (Alfrey, Gurnee & Lloyd, 1996):

- Case I or Fickian diffusion, where the speed of water diffusion is much slower than the speed of polymer segmental relaxation.
- Case II diffusion, where the speed of water diffusion is much faster than with the speed of polymer segmental relaxation.
- Anomalous diffusion or Non-Fickian diffusion, which appears when the speed of water diffusion and polymer segmental relaxation are comparable.

Most water sorption studies with methacrylate resins were performed using neat, non-porous resin monomer blends that were polymerized optimally in the absence of water (Venz & Dickens, 1991; Patel & others, 2001; Hunter & others, 2003; Sideridou, Tserki & Papanastasiou, 2003; Unemori & others, 2003; Mortier & others, 2004). Unlike water transport in non-porous films that is solely controlled by diffusion, water movement may additionally occur through pores, voids and defects in porous films of the polymerized resin matrix of adhesives with hydrophilic and ionic resin monomers. Water transport in porous media is more complicated and can be contributed to simultaneously by a) diffusion, that is driven by a gradient in water concentration, as described by Fick's laws; b) saturated viscous flow, that is driven by a difference in osmotic or

hydrostatic pressure, as described by Darcy's law (Hilfer, 1996) and c) capillary transport, that is driven by gradients in suction stress (Martys & Ferraris, 1997). The latter two components, transport through microcracks and capillarity, have been referred to as damage-dependent mechanisms that enhance water penetration into a polymer or fiber-reinforced composite (Marom, 1985). Thus, a higher permeability and more rapid water movement may be expected in porous films, such as those created by bonding solvated one-step self-etch adhesives on dentin. The water channels present in these resin-dentin bonds may speed up the transport of water and serve as sites for the accumulation of water. It has been shown that moisture can also cause structural damage by inducing micro-cavities or crazing in polymeric materials (Apicella & others, 1979; Diamant, Marom & Broutman, 1981), and this damage can further accelerate moisture diffusion (Brewis, Comyn & Tegg, 1980). Unfortunately, little information is available on the properties of porous dentin adhesive films and how the incorporation of water channels may influence their degradation characteristics; interpretations are often based on bulk properties derived from non-porous neat resins. It is possible that the hydrolytic degradation and leaching of the hydrolyzed components may be expedited in porous dentin adhesive coatings, with the associated increase in permeability creating a vicious cycle that increases the deterioration of mechanical properties by manifold when compared with non-porous coatings produced from the same resin mixtures. These questions may be answered in the future by comparing the impedance properties of porous vs non-porous coatings using electrochemical impedance spectroscopy (Senkevich, 2000; Pradelle-Blasse & others, 2004; Davies, Rich & Drzal, 2004).

Although absorbed fluids may plasticize and induce relaxation in the adhesive polymer and swell the adhesive, leading to a loss of mechanical properties, degradation of the interface is the primary reason for failure of many adhesive joints (Kinloch, 1979). The diffusion of water into adhesive joints created with an epoxy adhesive was studied by comparing the calculated diffusion rates between non-bonded adhesive specimens and bonded adhesive joints (Zanni-Deffarges & Mahanahan, 1995). The authors observed that the diffusion coefficient of the adhesive joint was greater than that of the bulk adhesive and concluded that the diffusion rate at the interface was greater than in the bulk adhesive. Nguyen and others (1997) and Linossier and others (1999) have also compared the diffusion rates of water between bulk specimens and adhesive joints using Fourier transform infrared spectroscopy in the multiple internal reflection mode (FTIR-MIR). They detected significant water diffusion at the interface for poorly adhered adhesive systems where adhesion forces are governed by secondary interactions such as

hydrogen bonding and van der Waals forces. Vine, Cawley and Kinloch (2001) studied the moisture uptake of an epoxy bonded to aluminum adherends with various surface treatments. They observed faster diffusion in three-layer sandwich specimens than predicted, based on mass-uptake experiments performed on diffusion in bulk specimens. They attributed this behavior to the presence of micro-cavities in the adhesive layer and similarly suggested that diffusion at the interface is possibly faster than in bulk.

The studies mentioned above were all conducted on epoxy adhesives bonded to non-degrading metallic adherends. Substrate corrosion had not been taken into account in these studies. Similar to substrate corrosion that accompanies water sorption in some adhesive joints (Stevenson, 1987), bonding to dentin is complicated by degradation of the collagenous components within the hybrid layer, an issue that will be addressed below. Nevertheless, the same principle derived from adhesive joints created with epoxy resins may be extrapolated to thin dentin adhesive films that are bonded to dentin. The degradation of dentin adhesive joints can occur in the absence of water trees, but it is likely that degradation will be expedited in the presence of additional water channels, given the more rapid water sorption and leaching that may occur in their presence. As water trees are predominantly identified from the interface between simplified adhesives and the dentin hybrid layer, it is not unreasonable to expect that such an interface is the most susceptible to degradation (Figure 7A) and that interfacial adhesive failure is the predominant failure mode after aging in an aqueous environment (Bowditch, 1996). Indeed, a recent study reported premature bond failures in almost all specimens bonded with a one-step self-etch adhesive after 15 months of water storage (Armstrong & others, 2003). The unfavorable *in vitro* results associated with this adhesive were also supported by *in vivo* results obtained in a clinical trial (Brackett, Covey & St Germain, 2002).

Post-aging Water Treeing

So far, the discussion has been limited to water trees that were formed immediately during bonding to dentin. As water treeing was hypothesized to be one of the mechanisms that contribute to resin-dentin bond degradation (Tay & Pashley, 2003b), the question remains whether water trees can arise in interfaces that are initially free of water trees during the aging of adhesive interfaces in water. Using an experimental single-bottle etch-and-rinse adhesive, Tay and others (2003c) demonstrated that both the reticular and spot-type of nanoleakage patterns increased in adhesive interfaces after one year of aging in artificial saliva. In particular, interfacial changes occurred between the adhesive and hybrid layer that the authors attributed

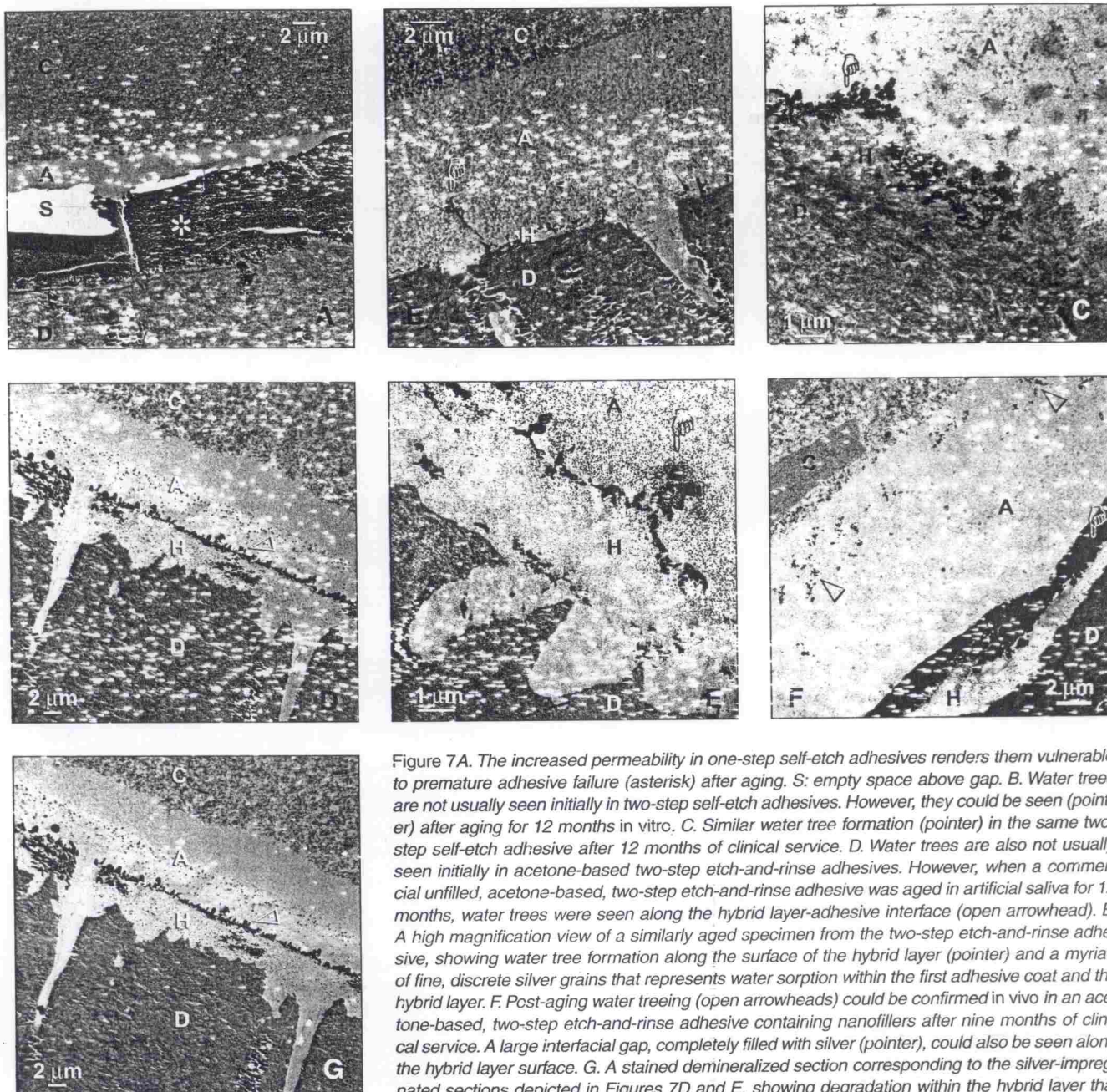


Figure 7A. The increased permeability in one-step self-etch adhesives renders them vulnerable to premature adhesive failure (asterisk) after aging. S: empty space above gap. B. Water trees are not usually seen initially in two-step self-etch adhesives. However, they could be seen (pointer) after aging for 12 months *in vitro*. C. Similar water tree formation (pointer) in the same two-step self-etch adhesive after 12 months of clinical service. D. Water trees are also not usually seen initially in acetone-based two-step etch-and-rinse adhesives. However, when a commercial unfilled, acetone-based, two-step etch-and-rinse adhesive was aged in artificial saliva for 12 months, water trees were seen along the hybrid layer-adhesive interface (open arrowhead). E. A high magnification view of a similarly aged specimen from the two-step etch-and-rinse adhesive, showing water tree formation along the surface of the hybrid layer (pointer) and a myriad of fine, discrete silver grains that represents water sorption within the first adhesive coat and the hybrid layer. F. Post-aging water treeing (open arrowheads) could also be seen *in vivo* in an acetone-based, two-step etch-and-rinse adhesive containing nanofillers after nine months of clinical service. A large interfacial gap, completely filled with silver (pointer), could also be seen along the hybrid layer surface. G. A stained demineralized section corresponding to the silver-impregnated sections depicted in Figures 7D and E, showing degradation within the hybrid layer that was manifested as non-stainable patches (arrows).

as evidence of post-aging water-tree formation. Such a hypothesis has since been confirmed both *in vitro* (Figure 7B) and *in vivo* (Figure 7C) in human teeth bonded with two-step self-etch adhesives that do not demonstrate initial water-tree formation (Donmez & others, 2005). Even more convincing data on post-aging water treeing than that previously reported by Tay and others (2003c) has since been collected by the authors, using an unfilled, two-step etch-and-rinse adhesive (Figure 7D). In this group of specimens, it is significant to point out that only the adhesive coat adjacent to the bonded dentin demonstrated silver

uptake (Figure 7E), while the second coat was not affected. The results could be duplicated *in vivo* with the use of a two-step etch-and-rinse adhesive containing nanofillers after nine months of clinical service (Figure 7F). In this adhesive, only one adhesive coat was applied, and post-aging water-tree formation was apparent on the top part of the adhesive layer, while those that were present along the surface of the hybrid layer were probably obscured by the thick layer of silver that represented frank gap formation. An important lesson that may be learned from these results is that, contrary to the recommendations of some manu-

facturers, simplified adhesives are best utilized in more than one coat (Hashimoto & others, 2004c), with the first coat functioning as a dentin primer, and the second coat as a coupling resin. Even so, substantial post-aging water treeing could be identified along the junction of the hybrid layer and the first adhesive coat.

As mentioned earlier, the degradation of resin-dentin bonds is more complex than non-biological adhesive joints in that hydrolytic degradation of the collagenous component occurs simultaneously, as resin leaches out of these adhesive joints (Hashimoto & others, 2003a,b). Figure 7G represents a stained, demineralized section from the same tooth as Figures 7D and 7E after one year of water storage. In this stained section, water treeing and nanoleakage could not be seen, as the section was not previously subjected to silver tracer penetration, with the first adhesive coat appearing "deceptively normal" with this type of stained sections. However, the section did reveal additional important features that could not be seen in silver-impregnated, unstained, undemineralized sections—the partial disappearance of stained collagen fibrils from the hybrid layer. Such a phenomenon has been reported by De Munck and others (2003) and Armstrong and others (2004). Although not explicitly mentioned by these authors, the absence of stainable fibrillar components within the aged hybrid layers suggested the breakdown of the collagen fibrils into gelatin (Hashimoto & others, 2003b) that is still stainable as individual unraveled strands, and further into peptides that no longer take up these stains. Such a phenomenon is analogous to the appearance of clear bands in Coomassie blue-stained gelatin gels, when Western blots due to the gelatinase activity of matrix metalloproteinases (MMPs), such as MMP-2 or MMP-9 (Gendron & others, 1999; Smith & others, 2004) are used. It is now understood that the breakdown of denuded collagen fibrils within dentin hybrid layers can occur via the release and activation of endogenous matrix metalloproteinases in the demineralized layer or they can be released from the underlying mineralized dentin (Pashley & others, 2004). Using fluorescein-labeled type I collagen and gelatin, low but consistent collagenolytic and gelatinolytic activities have been recently been observed from powdered mineralized human dentin (Pashley & others, 2004).

In its original context, nanoleakage was defined as the manifestation of leakage within a hybrid layer in the absence of interfacial gap formation. However, the lateral communication of bonded interfaces with the external environment (storage solution *in vitro* or saliva *in vivo*) created by post-aging interfacial water treeing may generate an additional dimension wherein leaching of hydrolytic resin and collagen components can rapidly occur. Thus, in the broadest sense, the development of post-aging water treeing along surface asperities of the

resin-dentin interface may be viewed upon as an antecedent that slowly creates gap formation upon fatigue and loading stresses (Fernando, Harjoprayitno & Kinloch, 1996) and ultimate catastrophic failure of the dentin-adhesive joint.

CONCLUSION

All these examples of water treeing and their potential consequences depicted in simplified adhesives, especially the latest versions of single-bottle one-step self-etch adhesives, may be regarded as fantasy by some, or discomforting to others. Then again, they may be not—for there are antecedents, as such concerns have been well articulated in other branches of adhesive sciences such as the paint industry, photoresists and more. In the most recent review on the durability of adhesion to tooth tissues, De Munck and others (2005b) remarked that of all the classes of dentin adhesives available, the three-step etch-and-rinse adhesives remain the gold standard in terms of durability, and that only the two-step self-etch adhesives approach this gold standard. Water treeing is a morphologic phenomenon that may help us understand the initial problems associated with the bonding of simplified adhesive systems and the underlying causes of their relative lack of durability. As we approach the Golden Jubilee of enamel etching in 2005, let us double our efforts to improving adhesive dentistry to continue the legacy of the late Dr Michael Buonocore by not simply producing faster and more user-friendly adhesives, but towards improving the quality of resin bonds created in dentin.

Acknowledgements

This work was supported by RCG CERG grant 10204604/07840/08004/324/01, Faculty of Dentistry, The University of Hong Kong and by grants DE 014911 and DE 015306 from the National Institute of Dental and Craniofacial Research. The authors thank Michelle Barnes for secretarial support.

References

- Alfrey TJ, Gurnee EF & Lloyd WG (1996) Diffusion in glassy polymers *Journal of Polymer Science, Part C Polymer Letters* **12** 249-261.
- Angker L, Nockolds C, Swain MV & Kilpatrick N (2004) Correlating the mechanical properties to the mineral content of carious dentine—a comparative study using an ultra-micro indentation system (UMIS) and SEM-BSE signals *Archives of Oral Biology* **49**(5) 369-378.
- Apicella A, Nicolais L, Astarita G & Drioli E (1979) Effect of thermal history on water sorption, elastic properties and the glass transition of epoxy resins *Polymer* **20**(9) 1143-1148.
- Armstrong SR, Vargas MA, Chung I, Pashley DH, Campbell JA, Laffoon JE & Qian F (2004) Resin-dentin interfacial ultra-structure and microtensile dentin bond strength after five-year water storage *Operative Dentistry* **29**(6) 705-712.

- Armstrong SR, Vargas MA, Fang Q & Laffoon JE (2003) Microtensile bond strength of a total-etch 3-step, total-etch 2-step, self-etch 2-step, and a self-etch 1-step dentin bonding system through 15-month water storage *Journal of Adhesive Dentistry* **5**(1) 47-56.
- Bergenholtz G, Jontell M, Tuttle A & Knutsson G (1993) Inhibition of serum albumin flux across exposed dentine following conditioning with GLUMA primer, glutaraldehyde or potassium oxalates *Journal of Dentistry* **21**(4) 220-227.
- Bergman G (1963) Microscopic demonstration of liquid flow through human dental enamel *Archives of Oral Biology* **8**(3-4) 233-234.
- Borisova B & Kressler J (2003) Environmental stress-cracking resistance of LDPE/EVA blends *Macromolecular Materials and Engineering* **288**(6) 509-515.
- Bouillaguet S, Caillot G, Forchelet J, Cattani-Lorente M, Wataha JC & Krejci I (2005) Thermal risks from LED- and high-intensity QTH-curing units during polymerization of dental resins *Journal of Biomedical Materials Research. Part B Applied Biomaterials* **72B**(2) 260-267.
- Bouillaguet S, Duroux B, Ciucchi B & Sano H (2000) Ability of adhesive systems to seal dentin surfaces: An *in vitro* study *Journal of Adhesive Dentistry* **2**(3) 201-203.
- Bowditch MR (1996) The durability of adhesive joints in the presence of water *International Journal of Adhesion and Adhesives* **16**(2) 73-79.
- Brackett WW, Covey DA & St Germain HA Jr (2002) One-year clinical performance of a self-etching adhesive in Class V resin composites cured by two methods *Operative Dentistry* **27**(3) 218-222.
- Brewis DM, Comyn J & Tegg JL (1980) The uptake of water vapour by an epoxide adhesive formed from the diglycidyl ether of bisphenol-A and di-(1-aminopropyl-3-ethoxy) ether *Polymer* **21**(2) 134-138.
- Buonocore MG (1955) A simple method of increasing the adhesion of acrylic filling materials to enamel surfaces *Journal of Dental Research* **34**(6) 849-853.
- Buonocore MG, Wileman W & Brudevold F (1956) A report on a resin composition capable of bonding to human dentin surfaces *Journal of Dental Research* **35**(6) 846-851.
- Carvalho RM, Chersoni S, Frankenberger R, Pashley DH, Prati C & Tay FR (2005) A challenge to the conventional wisdom that simultaneous etching and resin infiltration always occurs in self-etch adhesives *Biomaterials* **26**(9) 1035-1042.
- Carvalho RM, Pegoraro TA, Tay FR, Pegoraro LF, Silva NR & Pashley DH (2004) Adhesive permeability affects coupling of resin cements that utilise self-etching primers to dentine *Journal of Dentistry* **32**(1) 55-65.
- Cheong C, King NM, Pashley DH, Ferrari M, Toledano M & Tay FR (2003) Incompatibility of self-etch adhesives with chemical/dual-cured composites: Two-step vs one-step systems *Operative Dentistry* **28**(6) 747-755.
- Chersoni S, Suppa P, Grandini S, Goracci C, Monticelli F, Yiu C, Huang C, Prati C, Breschi L, Ferrari M, Pashley DH & Tay FR (2004) *In vivo* and *in vitro* permeability of one-step self-etch adhesives *Journal of Dental Research* **83**(6) 459-464.
- Ciucchi B, Bouillaguet S, Holz J & Pashley DH (1995) Dentinal fluid dynamics in human teeth, *in vivo* *Journal of Endodontics* **21**(4) 191-194.
- Cotugno S, Larobina D, Mensitieri G, Musto P & Ragosta G (2001) A novel spectroscopic approach to investigate transport processes in polymers: The case of water-epoxy system *Polymer* **42**(15) 6431-6438.
- Davies GD, Rich MJ & Drzal LT (2004) Monitoring moisture uptake and delamination in CFRP-reinforced concrete structures with electrochemical impedance sensors *Journal of Non-destructive Evaluation* **23**(1) 1-9.
- De Munck J, Van Landuyt K, Coutinho E, Poitevin A, Peumans M, Lambrechts P & Van Meerbeek B (2005a) Micro-tensile bond strength of adhesives bonded to Class-I cavity-bottom dentin after thermo-cycling *Dental Materials* (in press).
- De Munck J, Van Landuyt K, Peumans M, Poitevin A, Lambrechts P, Braem M & Van Meerbeek B (2005b) A critical review of the durability of adhesion to tooth tissue: Methods and results *Journal of Dental Research* **84**(2) 118-132.
- De Munck J, Van Meerbeek B, Yoshida Y, Inoue S, Vargas M, Suzuki K, Lambrechts P & Vanherle G (2003) Four-year water degradation of total-etch adhesives bonded to dentin *Journal of Dental Research* **82**(2) 136-140.
- Diamant Y, Marom G & Broutman LJ (1981) The effect of network structure on moisture absorption of epoxy resins *Journal of Applied Polymer Science* **26**(9) 3015-3025.
- Dickstein PA, Spelt JK, Sinclair AN & Bushlin Y (1991) Investigation of non-destructive monitoring of the environmental degradation of structural adhesive joints *Materials Evaluation* **49**(12) 1498-1505.
- Ding H-Z & Xing X-S (1996) Non-equilibrium statistical theory of water treeing in polymeric cable insulators *Journal of Physics D: Applied Physics* **29**(10) 2682-2688.
- Dissado LA & Fothergill JC (1992) Electrical degradation and breakdown in polymers. Chapter 4. Water treeing degradation *IEEE Material and Devices Series 9* London Peregrinus 75-116.
- Donmez N, Belli S, Pashley DH & Tay FR (2005) Ultrastructural correlates of *in vivo/in vitro* bond degradation in self-etch adhesives *Journal of Dental Research* (in print).
- Dürr O, Volz T, Dieterich W & Nitzan A (2002) Dynamic percolation theory for particle diffusion in a polymer network *Journal of Chemical Physics* **117**(1) 441-447.
- Elgalaid TO, Youngson CC, McHugh S, Hall AF, Creanor SL & Foye RH (2004) *In vitro* dentine permeability: The relative effect of a dentine bonding agent on crown preparation *Journal of Dentistry* **32**(5) 413-421.
- Fan Z-H & Yoshimura N (1996) Silver tree *The Institute of Electrical and Electronic Engineers—Transactions on Dielectrics and Electrical Insulation* **3**(1) 131-135.
- Fernando M, Harjopravito WW & Kinloch AJ (1996) A fracture mechanics study of the influence of moisture on the fatigue behaviour of adhesively bonded aluminium-alloy joint *International Journal of Adhesion and Adhesives* **16**(2) 113-119.
- Ferrari M & Tay FR (2003) Technique sensitivity in bonding to vital acid-etched dentin *Operative Dentistry* **28**(1) 3-8.
- Frisch FL & Stern SA (1983) Diffusion of small molecules in polymers *CRC Critical Reviews in Solid State and Material Science* **11** 123-147.
- Gendron R, Grenier D, Sorsa T & Mayrand D (1999) Inhibition of the activities of matrix metalloproteinases 2, 8, and 9 by chlorhexidine *Clinical and Diagnostic Laboratory Immunology* **6**(3) 437-439.

- odis HE, Tao L & Pashley DH (1990) Evaporative water loss from human dentine *in vitro* *Archives of Oral Biology* **35**(7) 523-527.
- égoire G, Joniot S, Guignes P & Millas A (2003) Dentin permeability: Self-etching and one-bottle dentin bonding systems *Journal of Prosthetic Dentistry* **90**(1) 42-49.
- gger O (1949) [Neue katalysatoren zur polymerisation der ethene bei raumtemperatur] New catalyst for polymerization of ethylene at room temperature *Helvetica Chemical Acta* **XXXI** 1624-1631.
- ohn CL & Overton B (1997) The effects of immunoglobulins on the convective permeability of human dentine *in vitro* *Archives of Oral Biology* **42**(12) 835-843.
- ashimoto M, De Munck J, Ito S, Sano H, Kaga M, Oguchi H, van Meerbeek B & Pashley DH (2004b) *In vitro* effect of nanoleakage expression on resin-dentin bond strengths analyzed by microtensile bond test, SEM/EDX and TEM *Biomaterials* **25**(25) 5565-5574.
- ashimoto M, Ito S, Tay FR, Svizero NR, Sano H, Kaga M & Pashley DH (2004a) Fluid movement across resin-dentin interface during and after bonding *Journal of Dental Research* **83**(11) 843-848.
- ashimoto M, Ohno H, Sano H, Kaga M & Oguchi H (2003a) *In vitro* degradation of resin-dentin bonds analyzed by microtensile bond test, scanning and transmission electron microscopy *Biomaterials* **24**(21) 375-383.
- ashimoto M, Sano H, Yoshida E, Hori M, Kaga M, Oguchi H & Pashley DH (2004c) Effects of multiple adhesive coatings on dentin bonding *Operative Dentistry* **29**(4) 416-423.
- ashimoto M, Tay FR, Ohno H, Sano H, Kaga M, Yiu C, Sumagai H, Kudou Y, Kubota M & Oguchi H (2003b) SEM and TEM analysis of water degradation of human dentinal collagen *Journal of Biomedical Materials Research Part B: Applied Biomaterials* **66**(1) 287-298.
- fer R (1996) Transport and relaxation phenomena in porous media in Prigogine I, Rice SA eds *Advances in Chemical Physics*, Volume 92 John Wiley & Sons Inc New York 299-424.
- nter G, Lane DM, Scrimgeour SN, McDonald PJ & Lloyd CH (2003) Measurement of the diffusion of liquids into dental restorative resins by stray-field nuclear magnetic resonance imaging (STRAFI) *Dental Materials* **19**(7) 632-638.
- S, Tay FR, Hashimoto M, Yoshiyama M, Saito T, Brackett VW, Waller L & Pashley DH (2005) Effect of multiple coatings of two all-in-one adhesives on dentin bonding *Journal of Adhesive Dentistry* (in press).
- ng NM, Tay FR, Pashley DH, Hashimoto M, Ito S, Brackett VW, García-Godoy F & Sunico M (2005) Conversion of one-step to two-step self-etch adhesives for improved efficacy and extended application *American Journal of Dentistry* (in press).
- loch AJ (1979) Interfacial fracture mechanical aspects of adhesive bonded joints a review *Journal of Adhesion* **10**(2) 93-219.
- loch AJ (1987) *Adhesion and Adhesives: Science and Technology* Chapman and Hall New York.
- tz J, Brostow W, Hess M & Veeman WS (1996) Epoxy and glass composites in water studied with ³H-NMR *Polymer Engineering and Science* **36**(8) 1129-1133.
- Knutsson G, Jontell M & Bergenholtz G (1994) Determination of plasma proteins in dentinal fluid from cavities prepared in healthy young human teeth *Archives of Oral Biology* **39**(3) 185-190.
- Kube S, Li H, Burrow MF & Tyas MJ (2002) Nanoleakage of dentin adhesive systems bonded to Carisolv-treated dentin *Operative Dentistry* **27**(4) 387-395.
- Lagouvardos PE, Pissis P, Kyritsis A & Daoukaki D (2003) Water sorption and water-induced molecular mobility in dental composite resins *Journal of Materials Science: Materials in Medicine* **14**(9) 753-759.
- Lee KW, Son HH, Yoshiyama M, Tay FR, Carvalho RM & Pashley DH (2003) Sealing properties of a self-etching primer system to normal caries-affected and caries-infected dentin *American Journal of Dentistry* **16**(Special No) 68A-72A.
- Lewis TJ (1990) Charge transport, charge injection and breakdown in polymeric insulators *Journal of Physics D: Applied Physics* **23**(12) 1469-1478.
- Li H, Burrow MF & Tyas MJ (2003) The effect of concentration and pH of silver nitrate solution on nanoleakage *Journal of Adhesive Dentistry* **5**(1) 19-25.
- Linossier I, Gaillard F, Romand M & Nguyen T (1999) A spectroscopique technique for studies of water transport along the interface and hydrolytic stability of polymer/substrate system *Journal of Adhesion* **70**(3) 221-239.
- Maita E, Simpson MD, Tao L & Pashley DH (1991) Fluid and protein flux across the pulpodentine complex of the dog *in vivo* *Archives of Oral Biology* **36**(2) 103-110.
- Marom G (1985) The role of water transport in composite materials Chapter 9 *Polymer Permeability*, Comyn J, Editor. Elsevier Applied Science United Kingdom 341-374.
- Martys NS & Ferraris CF (1997) Capillary transport in mortars and concrete *Cement and Concrete Research* **27**(5) 747-760.
- Matthews WG, Showman CD & Pashley DH (1993) Air blast-induced evaporative water loss from human dentine, *in vitro* *Archives of Oral Biology* **38**(6) 517-523.
- McLean JW & Kramer IRH (1952) A clinical and pathologic evaluation of a sulfinic acid activated resin for use in restorative dentistry *British Dental Journal* **93** 255-269.
- Miyashita T (1969) Deterioration of water-immersed polyethylene coating wire by treeing Proceedings of the 1969 IEEE-NEMA Electrical Insulation Conference, Boston, September 1969 131-135.
- Moreau E, Mayoux C, Laurent C & Boudet A (1993) The structure characteristics of water trees in power cables and laboratory specimens *The Institute of Electrical and Electronic Engineers—Transactions on Dielectrics and Electrical Insulation* **28**(1) 54-64.
- Mortier E, Gerdolle A, Jacquot B & Panighi MM (2004) Importance of water sorption and solubility studies for couple bonding agent—resin-based filling material *Operative Dentistry* **29**(6) 669-676.
- Müller-Plathesup F, Laaksonen L & van Gunsteren WF (1993) Cooperative effects in the transport of small molecules through an amorphous polymer matrix *Journal of Molecular Graphics* **11**(2) 118-136.

- Musto P, Ragosta G, Scarinza G & Mascia L (2002) Probing the molecular interactions in the diffusion of water through epoxy and epoxy-bismaleimide networks *Journal of Polymer Science Part B: Polymer Physics* **40(10)** 922-938.
- Nakajima M, Kitasako Y, Okuda M, Foxton RM & Tagami J (2005) Elemental distributions and microtensile bond strength of the adhesive interface to normal and caries-affected dentin *Journal of Biomedical Materials Research Part B: Applied Biomaterials* **72B(2)** 268-275.
- Nguyen T, Byrd E & Bentz D (1995) Quantifying water at the organic film/hydroxylated substrate interface *Journal of Adhesion* **48** 169-194.
- Nguyen T, Byrd WE, Bentz DP & Lin C (1997) *In-situ* measurement of water at the organic coating/substrate interface *Progress in Organic Coatings* **27(1-4)** 181-193.
- Nikaido T, Burrow MF, Tagami J & Takatsu T (1995) Effect of pulpal pressure on adhesion of resin composite to dentin: Bovine serum versus saline *Quintessence International* **26(3)** 221-226.
- Nishiyama N, Suzuki K, Yoshida H, Teshima H & Nemoto K (2004) Hydrolytic stability of methacrylamide in acidic aqueous solution *Biomaterials* **25(6)** 965-969.
- Nogueira P, Ramirez C, Torres A, Abad J, Cano J, López J, López-Bueno I & Barral L (2001) Effect of water sorption on the structure and mechanical properties of an epoxy resin system *Journal of Applied Polymer Science* **80(1)** 71-80.
- Ogawa K, Yamashita Y, Ichijo T & Fusayama T (1983) The ultrastructure and hardness of the transparent layer of human carious dentin *Journal of Dental Research* **62(1)** 7-10.
- Özok AR, Wu M-K, de Gee A & Wesselink PR (2004) Effect of dentin perfusion on the sealing ability and microtensile bond strength of a total-etch versus an all-in-one adhesive *Dental Materials* **20(5)** 479-486.
- Panopoulos P, Gazelius B & Olgart L (1983) Responses of feline intradental sensory nerves to hyperosmotic stimulation of dentin *Acta Odontologica Scandinavica* **41(6)** 369-375.
- Pashley DH (1985) Dentin-predentin complex and its permeability: Physiologic overview *Journal of Dental Research* **64(Special No)** 613-620.
- Pashley DH, Ciucchi B, Sano H & Horner JA (1993) Permeability of dentin to adhesive agents *Quintessence International* **24(9)** 618-631.
- Pashley DH & Depew DD (1986) Effects of the smear layer, Copalite and oxalate on microleakage *Operative Dentistry* **11(3)** 95-102.
- Pashley DH, Galloway SE & Stewart F (1984) Effects of fibrinogen *in vivo* on dentine permeability in the dog *Archives of Oral Biology* **29(9)** 725-728.
- Pashley DH, Horner JA & Brewer PD (1992) Interactions of conditioners on the dentin surface *Operative Dentistry Supplement* **5** 137-150.
- Pashley DH, Matthews WG, Zhang Y & Johnson M (1996) Fluid shifts across human dentine *in vitro* in response to hydrodynamic stimuli *Archives of Oral Biology* **41(11)** 1065-1072.
- Pashley DH, Michelich V & Kehl T (1981) Dentin permeability: Effects of smear layer removal *Journal of Prosthetic Dentistry* **46(5)** 531-537.
- Pashley DH, Tay FR, Hashimoto M, Breschi L, Carvalho RM & Ito S (2004) Degradation of dentin collagen by host-derived enzymes during aging *Journal of Dental Research* **83(3)** 216-221.
- Pashley EL, Talman R, Horner JA & Pashley DH (1991) Permeability of normal versus carious dentin *Endodontics and Dental Traumatology* **7(5)** 207-211.
- Pashley EL, Zhang Y, Lockwood PE, Rueggeberg FA & Pashley DH (1998) Effects of HEMA on water evaporation from water-HEMA mixtures *Dental Materials* **14(1)** 6-10.
- Patel MP, Johnstone MB, Hughes FJ & Braden M (2001) The effect of two hydrophilic monomers on the water uptake of a heterocyclic methacrylate system *Biomaterials* **22(1)** 81-86.
- Perdigão J, Duarte S Jr & Lopes MM (2003) Advances in dentin adhesion *Compendium of Continuing Education in General Dentistry* **24(8 Supplement)** 10-16.
- Ping ZH, Nguyen QT, Chen SM, Zhou JQ & Ding YD (2001) States of water in different hydrophilic polymers—DSC and FTIR studies *Polymer* **42(20)** 8461-8467.
- Pradelle-Plasse N, Colon P, Wenger F & Picard B (2004) Quantitative evaluation of self-etching primer action on dentin permeability: A correlation between impedance measurements and acidity *American Journal of Dentistry* **17(2)** 131-136.
- Purk JH, Dusevich V, Glaros A, Spencer P & Eick JD (2004) *In vivo* versus *in vitro* microtensile bond strength of axial versus gingival cavity preparation walls in Class II resin-based composite restorations *Journal of the American Dental Association* **135(2)** 185-193.
- Reis AF, Arrais CA, Novaes PD, Carvalho RM, De Goes MF & Giannini M (2004) Ultramorphological analysis of resin-dentin interfaces produced with water-based single-step and two-step adhesives: Nanoleakage expression *Journal of Biomedical Materials Research* **71B(1)** 90-98.
- Ross R (1998) Inception and propagation mechanisms of water treeing *The Institute of Electrical and Electronic Engineers—Transactions on Dielectrics and Electrical Insulation* **5(5)** 660-680.
- Sano H, Yoshiyama M, Ebisu S, Burrow MF, Takatsu T, Ciucchi B, Carvalho R & Pashley DH (1995) Comparative SEM and TEM observations of nanoleakage within the hybrid layer *Operative Dentistry* **20(4)** 160-167.
- Senkevich JJ (2000) Degradation of an alkyd polymer coating characterized by AC impedance *Journal of Materials Science* **35(6)** 1359-1364.
- Sideridou I, Tserki I & Papanastasiou G (2003) Study of water sorption, solubility and modulus of elasticity of light-cured dimethacrylate-based dental resins *Biomaterials* **24(4)** 655-665.
- Sidhu SK, Agee KA, Waller JL & Pashley DH (2004) *In vitro* evaporative vs convective water flux across human dentin before and after conditioning and placement of glass-ionomer cements *American Journal of Dentistry* **17(3)** 211-215.
- Smith PC, Munoz VC, Collados L & Oyarzún AD (2004) *In situ* detection of matrix metalloproteinase-9 (MMP-9) in gingival epithelium in human periodontal disease *Journal of Periodontal Research* **39(2)** 87-92.
- Soles CL & Yee AF (2000) A discussion of the molecular mechanisms of moisture transport in epoxy resins *Journal of Polymer Science and Polymer Physics* **38(5)** 792-802.

- Stepp D, King JA, Worrall J, Thompson A & Cooper DE (1996) High-resolution study of water trees grown in silver nitrate solution. The Institute of Electrical and Electronic Engineers *Transactions on Dielectrics and Electrical Insulation* **3(3)** 392-398.
- Stevenson A (1987) The effect of electrochemical potentials on the durability of rubber/metal bonds in sea water *Journal of Adhesion* **21(4)** 313-327.
- Suh BI, Feng L, Pashley DH & Tay FR (2003) Factors contributing to the incompatibility between simplified-step adhesives and chemically-cured or dual-cured composites. Part III. Effect of acidic resin monomers *Journal of Adhesive Dentistry* **5(4)** 267-282.
- Tagami J, Hosoda H, Burrow MF & Nakajima M (1992) Effect of aging and caries on dentin permeability *Proceedings of the Finnish Dental Society* **88(Supplement 1)** 149-154.
- Tanaka J, Ishikawa K, Yatani H, Yamashita A & Suzuki K (1999) Correlation of dentin bond durability with water absorption of bonding layer *Dental Materials Journal* **18(1)** 11-18.
- Tay FR, Frankenberger R, Krejci I, Bouillaguet S, Pashley DH, Carvalho RM & Lai CN (2004b) Single-bottle adhesives behave as permeable membranes after polymerization. I. *In vivo* evidence *Journal of Dentistry* **32(8)** 611-621.
- Tay FR, Hashimoto M, Pashley DH, Peters MC, Lai SC, Yiu CK & Cheong C (2003c) Aging affects two modes of nanoleakage expression in bonded dentin *Journal of Dental Research* **82(7)** 537-541.
- Tay FR, King NM, Chan KM & Pashley DH (2002b) How can nanoleakage occur in self-etching adhesive systems that demineralize and infiltrate simultaneously? *Journal of Adhesive Dentistry* **4(4)** 255-269.
- Tay FR & Pashley DH (2003a) Dentin adhesives: Have they become too hydrophilic? *Journal of the Canadian Dental Association* **69(11)** 724-731.
- Tay FR & Pashley DH (2003b) Water treeing—a potential mechanism for degradation of dentin adhesives *American Journal of Dentistry* **16(1)** 6-12.
- Tay FR, Pashley DH, García-Godoy F & Yiu CK (2004c) Single-step, self-etch adhesives behave as permeable membranes after polymerization. Part II. Silver tracer penetration evidence *American Journal of Dentistry* **17(5)** 315-322.
- Tay FR, Pashley DH & Peters MC (2003d) Adhesive permeability affects composite coupling to dentin treated with a self-etch adhesive *Operative Dentistry* **28(5)** 610-621.
- Tay FR, Pashley DH, Suh BI, Carvalho RM & Itthagarun A (2002a) Single-step adhesives are permeable membranes *Journal of Dentistry* **30(7-8)** 371-382.
- Tay FR, Pashley DH, Suh B, Carvalho RM & Miller M (2004a) Single-step, self-etch adhesives behave as permeable membranes after polymerization. Part I. Bond strength and morphologic evidence *American Journal of Dentistry* **17(4)** 271-278.
- Tay FR, Pashley DH, Yiu CK, Sanares AM & Wei SH (2003a) Factors contributing to the incompatibility between simplified-step adhesives and chemically-cured or dual-cured composites. Part I. Single-step self-etching adhesive *Journal of Adhesive Dentistry* **5(1)** 27-40.
- Tay FR, Pashley DH & Yoshiyama M (2002c) Two modes of nanoleakage expression in single-step adhesives *Journal of Dental Research* **81(7)** 472-476.
- Tay FR, Suh BI, Pashley DH, Prati C, Chuang SF & Li F (2003b) Factors contributing to the incompatibility between simplified-step adhesives and self-cured or dual-cured composites. Part II. Single-bottle, total-etch adhesive *Journal of Adhesive Dentistry* **5(2)** 91-105.
- Unemori M, Matsuya Y, Matsuya S, Akashi A & Akamine A (2003) Water absorption of poly(methyl methacrylate) containing 4-methacryloxyethyl trimellitic anhydride *Biomaterials* **24(8)** 1381-1387.
- VanLandingham MR, Eduljee RF & Gillespie JW (1999) Moisture diffusion in epoxy systems *Journal of Applied Polymer Science* **71(5)** 787-798.
- Van Landuyt K, De Munck J, Snauwaert J, Coutinho E, Poitevin A, Yoshida Y, Inoue S, Peumans M, Suzuki K, Lambrechts P & Van Meerbeek B (2005) Monomer-solvent phase-separation in one-step self-etch adhesives *Journal of Dental Research* (in press).
- Van Meerbeek B, De Munck J, Yoshida Y, Inoue S, Vargas M, Vijay P, Van Landuyt K, Lambrechts P & Vanherle G (2003) Buonocore Memorial Lecture Adhesion to enamel and dentin: Current status and future challenges *Operative Dentistry* **28(3)** 215-235.
- Vaysman T, Rajan N & Thompson VP (2003) Effect of bur cutting patterns and dentin bonding agents on dentin permeability in a fluid flow model *Operative Dentistry* **28(5)** 522-528.
- Venz S & Dickens B (1991) NIR-spectroscopic investigation of water sorption characteristics of dental resins and composites *Journal of Biomedical Materials Research* **25(10)** 1231-1248.
- Vine K, Cawley P & Kinloch AJ (2001) Correlation of non-destructive measurements and toughness changes in adhesive joints during environmental attack *Journal of Adhesion* **77(2)** 125-161.
- Vongsavan N, Matthews RW & Matthews B (2000) The permeability of human dentine *in vitro* and *in vivo* *Archives of Oral Biology* **45(11)** 931-935.
- Yazici AR, Akca T, Ozgunaltay G & Dayangac B (2004) Bond strength of a self-etching adhesive system to caries-affected dentin *Operative Dentistry* **29(2)** 76-181.
- Yoshiyama M, Tay FR, Doi J, Nishitani Y, Yamada T, Itou K, Carvalho RM, Nakajima M & Pashley DH (2002) Bonding of self-etch and total-etch adhesives to carious dentin *Journal of Dental Research* **81(8)** 556-560.
- Yoshiyama M, Tay FR, Torii Y, Nishitani Y, Doi J, Itou K, Ciucchi B & Pashley DH (2003) Resin adhesion to carious dentin *American Journal of Dentistry* **16(1)** 47-52.
- Zaikov GE, Iordanskii AL & Markin VS (1988) *Diffusion of Electrolytes in Polymers* VSP BV (formerly VNU Science Press BV): Utrecht, The Netherlands, 48-70.
- Zanni-Deffarges MP & Shanahan MER (1995) Diffusion of water into an epoxy adhesive: Comparison between bulk behaviour and adhesive joints *International Journal of Adhesion and Adhesives* **15(3)** 137-142.
- Zheng L, Hilton JF, Habelitz S, Marshall SJ & Marshall GW (2003) Dentin caries activity status related to hardness and elasticity *European Journal of Oral Science* **111(3)** 243-252.