

Fluid and adhesive dentistry

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Trend in dentistry

In the last decade, several trends have developed in the formulation and marketing of current dentin bonding agents in an attempt to simplify their application procedure and reduce the amount of time required to do it. One of the earliest identified trend was the development of combined enamel/dentin conditioners (capable of etching enamel and dentin simultaneously). One of the first product that used this method for enamel and dentin treatment was Scotchbond Multi-Purpose Adhesive (which used maleic acid). Scotchbond MPA and most other current-generation products nowadays use the same acid to etch dentin and enamel (phosphoric acid that range from 32 to 40%). This method of etching dentin with phosphoric acid is called the total-etch technique and was first discussed by Fusayama in 1977. Today, the total etch procedure is accepted by clinicians and researchers and is the standard for current dentin bonding agents. Another trend was to reduce the number of components or bottles that constitute the bonding agent. Components were adjoined combining the conditioner and the primer into one solution. These systems consist of two bottles (one is the acidified primer or self-etching primer and the other is the resin adhesive). From the two-bottle products, however, we have progressed to the one-bottle products. These products are actually two-component or two-steps products because they still require total etch. Finally, today we have the single-solution products called All in ONE that use one comprehensive solution for etching, priming, and bonding. These One-step-self-etch require mixing two separate components prior to use. Recently single component that were ready for use were market. Semplification trend was steered by the market and the advertising “simpler and faster” increased the customers affection. Unfortunately any improvement in the direction of clinical simplification of bonding procedures can bring us far from the required characteristics of an ideal bonding system” (Kugel G. Ferrari M. JADA, Vol. 131, June 2000). Bond durability of “simplified bonding System” is not well documented. In other word time saving can compromise time durability.

Sealing

One approach to preserve the health of the pulpal-dentinal complex consists in sealing the exposed dentin by using resin bonding systems The studies on nanoleakage by means of silver nitrate demonstrated the presence of areas that have a large amount of water and/or hydrophilic
monomers in the hybrid layer and in the bonding resin layer. These areas were shown by Transmission Electron Microscopy and defined as water trees (Tay FR, Pashley DH. *Am J Dent* 2003; 16: 6-123) Water trees are real miniature channels that are considered responsible for the passage of fluids from the underlying hydrated dentin, through the bonding layer all the way to the interface with the composite(Tay FR, et al. *J Dent Res* 2002: 472-476.). The extent of nanoleakage increases with time in relation to water absorption (Tay FR, et al. *J Dent Res* 2003: 537-541).

Conventional thought is that a perfect seal of the resin–dentine interface can be established once the dentinal tubules and spaces within the demineralised collagen matrices are completely infiltrated by adhesive resins. Such a notion is based on the assumption that polymerised resins used for bonding are nonporous and impermeable to fluid movement. In view of the increased adhesive permeability associated with simplified-step adhesives, it is not known whether a hermetic seal can be achieved with the use of single-bottle total etch adhesives or All in One self etch adhesives Of particular concern is whether these adhesives can be effectively employed for eliminating dentinal fluid movement in freshly exposed, vital deep dentine close to pulp horns. (Tay FR et al Journal of Dentistry 2004 32, 611–621)

Replica technique

The aim of this thesis is to evaluate *in vitro* and *in vivo* the role of fluid on dental adhesion. When hydrophobic unfilled resins and composite restorations were placed on vital acid-etched dentine, expression of dentinal fluid droplets during the polymerization of the resin composite resulted in the manifestation of pits and blisters along the intaglio surface of the restorations, being negative impressions of the transudated dentinal fluid. (Nordenvall KJ. Swedish Dental Journal 1978;2:141–50)

Dentinal tubules rendered patent by acid-etching and rinsing permit transudation of dentinal fluid from deep dentine that are close to vital pulps. (Itthagarun A, Tay FR. American Journal of Dentistry 2000;13: 195–200.)

The replica technique has been selected in order to detect the presence of small quantities of fluid. Hydrophobic polyvinyl siloxane impression material was applied on observational area and after 4 min, the polymerized impression material was removed and degassed for 24 hrs. Positive replicas were then fabricated with a polyether impression material. Since there is no chemical reaction between polyether and polyvinyl siloxane, this replica technique has been shown to be effective in replicating water exudation. The polyvinyl siloxane during setting time is able to recall water by osmotic gradient. Polyvinyl siloxane type 3 (ISO4823), low consistency (addition-type) contains wetting agent that allow incorporations of small droplets of fluid.

Setting reaction of polyvinyl siloxane increases viscosity that block fluid in other word droplets of water that have no time to be incorporated into polivinilsiloxane remain at the observation interface. (Fig. 1)

Replicas showed the presence of droplets that are morphological manifestations of passage of fluids at interface that were trapped by hydrophobic vinyl polyxiloxane material.
Presence and transudation of fluid during bonding procedure are able to influence dental adhesion. Fluid effect could be of clinical relevance especially in adhesives technique that require prolonged setting time. Fluid can moreover reduct durability of adhesion.

**Chapter 1**

**Water movement in the hybrid layer after different dentin treatments**

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Summary The aim of this study was to examine the morphology of the hybrid layer (HL) of bonded water-stored specimens after different chemical pre-treatments of dentin. Materials and methods. Twenty-seven recently extracted human molars were selected. Fifty-four dentin disks in middle/deep dentin were obtained with a slow speed saw with a diamond-impregnated disk under water lubrication. Smear layers were created with 180 grit silicon carbide under running water for 1 min. Different pretreatments of dentin were: Group (1) no treatment; Group (2) 35% H3PO4 etch for 15 s followed by 10% glutaraldehyde for 120 s; Group (3) 37% H3PO4 etch for 15 s followed by 5% NaOCl for 120 s. Three dentin bonding agents (DBAs), Prime and Bond NT (P and B), AdmiraBond (AB), and Clearfil SE Bond (SEB) were applied in association with a resin composite following the manufacturers’ directions. Each specimen was then longitudinally sectioned and polished with wet SiC papers (up to #4000 grit). Impressions of the polished dentin were immediately taken with a silicone impression material. Bonded specimens were then stored for 3 or 12 h in deionized water. Further impressions of stored specimens were taken after air-drying of specimens for 10 s. Positive replicas were obtained using a polyether impression material. All the replicas and the original specimens were inspected by SEM. Results. A line of droplets (0.5–4 mm in diameter) was observed along the region of the adhesive–HL junction in all replicas of specimens stored in water, except in group 3, when P and B and AB were used. When SEB was used in each group the droplet were found in all groups except the zone of droplets was thinner. No differences in droplets dimensions were seen between 3 or 12 h water storage, or between the different dentin treatments.

Introduction The ability of dentin bonding agents (DBAs) to seal dentin is one of the most important requirements for the durability of adhesive restorations in vivo, when dentin margins are exposed to the oral cavity. Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) morphological studies have evaluated the relationships between adhesive agents and dentin. DBAs bond to dentin surfaces by creating a so-called ‘hybrid layer’ (HL) formed by the infiltration of the adhesive monomer into demineralized dentin. To overcome the problems related to intrinsically wet dentin, adhesive monomers have been
modified to contain both hydrophilic and hydrophobic moieties. By virtue of these modifications, DBAs have an increased potential to absorb water. The absorbed water may plasticize polymers and degrade the resin–dentin interface and the related swelling may induce interfacial stress within adhesives and resin composite. As certain components of resins are leached into water, minute water-filled voids are produced within the polymer matrix and this may have an adverse effect on the longevity of bonded restorations. Recently, Tay et al. reported evidence of relatively high water permeability of single-step adhesives that adversely affect the bond with chemical-cured or light-cured composites when the polymerization was delayed. The authors suggested that water can diffuse from hydrated dentin through HL to reach the adhesive–composite interface. In its extreme, this behavior leads to a lack of coupling between adhesive and composite that was manifested as a continuous line of silver- filled water droplets at the adhesive–composite interface in TEM studies. Many studies have already showed that small ions or molecules can diffuse into the HL in the absence of detectable interfacial gap formation. This phenomenon is called ‘nanoleakage’ and has been defined as the passage of a tracer (e.g. silvernitrate) throughout the HL. Reticular silver deposits, oriented perpendicularly to the surface of the HL have been described. Small ions or molecules are also able to penetrate into the HL in linear reticular patterns. These delicate, branching channels of connected nanovoids have been described as ‘water trees’, a phenomenon probably responsible for water-induced deterioration of polymers after aging. The initial description of nanoleakage was in specimens treated with the total-etch bonding technique, and was thought to be due to the incomplete infiltration of resins into acid etched dentin. More recent studies demonstrated the phenomenon of nanoleakage in many self-etching adhesive systems in which there should be no discrepancy between the depth of etch and the depth of resin infiltration. The presence of tracers inside the HL is evidence of its porosity. Recently, Itthagarun and Tay described a technique able to replicate the dentin surface and any fluid exudation on its surface by using a slow setting polyvinyl siloxane (PVS) impression material that incorporates the exudation in droplet-like formations. Epoxy resin replicas were produced from these impressions and observed under SEM. With a modified replica technique that replaces the epoxy resin with a polyether impression material that does not stick to the original PVS impressions, we investigated the morphology of HL after water storage of resin-bonded dentin specimens. Moreover, we evaluated the role of dentin matrix by observation of specimens in which the organic matrix was modified or removed. This was done by examining replicas of the resin – dentin interfaces created by different DBAs applied on untreated dentin, on etched and glutaraldehyde-treated dentin, and on etched and collagen-depleted (by NaOCl) dentin to evaluate their effects on water sorption. Thus, the objective of this study was to test the hypothesis that water exudation can be detected by impressions of water-stored specimens, and that dentin pre-treatment can affect this phenomenon.

Materials and methods

Specimen preparation
Twenty-seven recently extracted (1 month) human third molars stored in physiological saline at 4 °C were selected. Fifty-four 2 mm thick dentin disks of middle/deep dentin were obtained using a slow-speed saw (Remet, Casalecchio di Reno, Italy) with a diamond-impregnated disk under water cooling. A standardized smear layer was created with 180 grit SiC paper under running water for 1 min. The flat dentin surfaces were rinsed with water for 2 min. Dentin pre-treatment procedures Specimens were randomly divided into three treatment groups (nine teeth each): Group (1) dentin surface was etched with 35% H3PO4 [PA] (Scotchbond Etchant 3M-ESPE, St Paul, MN, USA) for 15 s (except for six specimens in which a self-etching DBA was used); Group (2) dentin surface was etched with 35% PA for 15 s, washed for 20 s and treated with buffered 10% glutaraldehyde (pH 7.4) for 120 s; Group (3) dentin surface was etched with 35% PA for 15 s, washed for 20 s, and treated with 5% NaOCl (Ogna, Maggio`, Italy) for 120 s. The specimens in each group were rinsed with water for 20 s. Bonding procedures Each treatment group was randomly divided into three DBA groups of three teeth each: (A) Prime and Bond NT [P and B] (Dentsply DeTrey, Konstanz, Germany); (B) AdmiraBond [AB] (Voco, Cuxhaven, Germany); (C) Clearfil SE Bond [SEB] (Kuraray Medical Inc., Osaka, Japan). DBAs were used following manufacturers’ directions. (A) Prime and Bond NT (P and B) (Total etch, wet bonding technique): after dentin pre-treatment procedures, the specimens were gently air-dried for 2 s. Adhesive was applied with a microbrush and gently spread with air syringe to remove excess adhesive and to evaporate the solvent. The adhesive was light-cured for 20 s at 400 mW/cm² (Visilux Command II, 3M ESPE). A second layer of adhesive was applied and similarly treated. (B) AdmiraBond (AB) (Total etch, wet bonding technique): after dentin pre-treatment procedures, the specimens were gently air-dried for 2 s. Adhesive was applied with a microbrush and gently spread to form a thin film. The adhesive was light cured for 20 s. A second layer of adhesive was applied and similarly treated. (C) Clearfil SE Bond (SEB) (self-etching technique): after dentin pre-treatment procedures, specimens were gently dried with air for 2 s; Clearfil SE Bond primer was applied with a microbrush for 20 s and gently air-dried to evaporate the solvent. A layer of Clearfil SE Bond adhesive was applied with a microbrush, spread with air and light-cured for 20 s. The flat bonded surface of all samples was covered with a 2 mm-thick layer of Clearfil APX resin composite (Kuray Medical Inc.) and light-cured for 40 s. Each specimen was then transversally sectioned into equal halves in order to expose two dentin-DBA interfaces. The cut surfaces were polished with increasingly finer grit SiC papers (#600, #1000, #1200, #2400, #4000 grit) under copious water irrigation. All specimens were then stored at room temperature in individually labeled 20 ml test tubes filled with deionized water in order to expose interfaces for either 3 h (six specimens per treatment group per each DBA) or 12 h (six specimens per treatment group per each DBA). Preparation of replicas—SEM examination Before water storage, an impression of the polished interface was immediately made using a PVS impression material (President Jet light Colte`ne- Whaledent, Alsta¨tten, Switzerland). After water storage (3 or 12 h), specimens were carefully air-dried for 10 s and a second impression was taken of each specimen. After separation of the impression material from the bonded interface, positive replicas were then obtained using a polyester impression material (Permadyne Garant, 3M ESPE). All replicas (before and after water storage) were gold-sputtered and inspected by a SEM (JEOL, Model 5400, Tokyo, Japan). The original specimens were also prepared for SEM
observation, in order to evaluate the HL morphology and to exclude preparation artifacts during the impression procedures. The original specimens were additionally fixed in 2.5% glutaraldehyde (pH 7.2) in 0.2 M cacodylate buffer for 4 h at room temperature, then rinsed in cacodylate buffer. After dehydration in ascending ethanol series, the specimens were HMDS-dried. After gold coating, the specimens were analyzed by SEM.

Results

Original samples
P and B and AB. All specimens showed the formation of HL (Figs. 1(a) and 2(a)) except for the specimens treated with NaOCl (Figs. 1(d) and 2(d)). The HL S. Chersoni et al. 798 thickness ranged from 4 to 10 mm. No obvious voids and porosities were observed in the HL. Resin tags were visible that completely filled the dentinal tubules. SEB. Each specimen showed the formation of a thin HL. The HL thickness range from 0.2 to 1 mm (Fig. 3(a)) except for group 2, in which the etching procedure increased the thickness to 5–7 mm (data not shown to reduce number of figures).

Replicas before water storage
SEM analysis of the replicas obtained before water storage revealed morphological features that were very similar to the original samples, confirming the validity of the replica procedure (data not shown).

Replicas after water storage
Treatment group 1. Large droplets (2–6 mm in diameter) were visible along the adhesive–HL region, while small droplets (0.2–2.0 mm in diameter) were observed throughout the entire HL and sometimes on the adjacent dentin (Figs. 1(b), 2(b) and 3(b)). Droplet diameter decreased from the top to the base of this zone. There was a gradient in droplet dimensions observed along the entire zone (Figs. 1(b) and 2(b)). Specimens bonded with SEB showed a thin line of single droplets in accordance with the thinness of the HL (Fig. 3(b)). All the DBA groups showed a strong relationship between HL thickness and the diameter/number of droplets. Superficial dentin exhibit a thinner HL with fewer droplets than middle to deep dentin (data not shown to reduce number of droplets were observed on the top of the resin composite. Treatment group 2. When acid-etched dentin was treated with 10% glutaraldehyde for 120 s, the samples revealed an HL morphology similar to those obtained without glutaraldehyde. Droplets were still present (Figs. 1(c), 2(c), and 3(c)) that were similar in number and dimension to those droplets observed over the HL in Group 1. Treatment group 3. When acid-etched resinbonded dentin was treated with 5% NaOCl for 120 s, P and B and AB specimens showed a complete absence of HL. No droplets were observed in replicas obtained either before or after water immersion (Figs. 1(d) and 2(d)). In contrast, in specimens bonded with SEB, the HL was still present even after acid-etching and NaOCl pre-treatment, and its thickness ranged from 0.2 to 1 mm. Droplet morphology was very similar to those observed in specimens in treatment group 1 (Fig. 3(d)). For all the treatments groups, replicas obtained after immersion of the polished resin–dentin interfaces in water for 3 h looked identical to those obtained after immersion for 12 h.
Discussion

This study demonstrated the formation of droplets along the exposed adhesive–HL zone after the immersion of resin-bonded dentin specimens in water. Since the droplets were observable only on the replica sample, and not during the observation of the original sample, which was dehydrated prior to SEM observation, the authors believe that the droplets were formed by water that flowed out of the adhesive–HL region during the setting of the impression material. However, no droplets were detected in replicas obtained before water immersion of the resin-bonded dentin specimens. These findings suggest that the droplets are caused by water that was absorbed into the adhesive–HL region during water storage, a process that must be time-dependent. Moreover, observations revealed a direct relation between thickness of HL and number of droplets, as demonstrated by SEM images. The droplets observed on replicas are thought to be microscopic water droplets that flowed out from this region during the setting time of the impression material. Interestingly, the droplets were only detected along the sectioned adhesive–HL and not in the underlying sound dentin surfaces, thus suggesting that during water storage, these structures took up more water than sound dentin or resin composite.

The existence of many nano-channels within the HL was recently demonstrated by Tay et al.17 They described the presence of reticular patterns of silver within the HL that they believe represented the retention of bulk water among the demineralized collagen fibrils. The present observations are consistent with those observations and also reveal water release from the HL, suggesting that the HL could behave as a permeable membrane. This water movement implies that the HL is a permeable structure that allows water movement in both directions. Others have speculated on water sorption by hydrophilic resins within HL since water sorption has been measured in hydrophilic resins.7 This may be responsible for the degradation of resin–dentin bond strength and for the rapid deterioration of mechanical properties of the interface.27 – 30 External agents (lactic acid, salivary esterases, proteolytic enzymes etc.) may cross the HL and penetrate along the previously described nano-channels and contribute to the degradation of collagen fibrils or their bond with resins. There may be some steric restriction of the diffusion of such molecules, especially larger molecules. Water in these nanochannels may contribute to the direct degradation of resins by extracting unpolymerized monomers or small oligomers over time.28 Our observations revealed that in acid-etched specimens that were treated with NaOCl to remove most of the collagen fibrils, droplets did not form at any location. It is widely known that the collagen fibril meshwork is the main substrate for the HL formation, and that the removal of most of the collagen fibrils on the top of the dentin surface does not allow the formation of HL.29 Furthermore, subsequent treatment of the self-etching primer (SEB) was able to demineralize the underlying matrix, expose new collagen fibrils and form a new HL on dentin previously treated with NaOCl. In group 3 specimens that were acid-etched, treated with NaOCl and bonded with single bond DBAs, the absence of HL resulted in replicas of resin-bonded dentin specimens that were free of droplets. This finding further confirms and supports the strong relationship between HL formation, by infiltration of resin around collagen fibrils, and subsequent droplet formation on exposed HL. The water movements within the HL may be explained by the hydrogel-like behavior of adhesive resins in the HL that swell during water storage and release the water when removed from the storage.
solution. The author speculate that the swelling distends the network and is counteracted and balanced by the elastic strain of the stretched polymer network. When specimens are removed from the water, this tension could be responsible for the outflow of the water trapped inside the HL and could explain the droplets in replicas seen by SEM. Water sorption into water trees (i.e. channels occupied by water) in the adhesive layer may also contribute to the manifestation of the droplets along the resin–dentin interface, since the thickness of the zone of water droplets (ca. 25 mm) was greater than the thickness of the hybrid layer (compare Fig. 1(a) and (b)). The hybrid layer consists of two types of polymers: collagen fibrils contain helical chains of peptides that are relatively hydrophilic; the collagen fibril meshwork is filled with hydrophilic resins that interpenetrate into the collagen biopolymer to create a new biopolymer, the hybrid layer, that apparently can take up or release water. Collagen peptides contain high numbers of carboxyl and amino groups with fixed charges that are associated with monovalent counter-ions that may produce osmotic swelling according to the Donnan effect. In addition, recent immunochemical analysis of the collagen-associated proteoglycans revealed the presence of these molecules after 15 s etching along the exposed collagen fibrils. Due to the strong affinity of these molecules for water, the authors hypothesize that they also have a functional role in acting as a reservoir of absorbed water. The ability of the HL to absorb water and probably other small molecules indicates that HL is much more complex than previously thought. The fact that larger water droplets were localized in the upper half of the HL while smaller ones were seen on the lower half is puzzling. MicroRaman studies have shown higher concentrations of resin in the top half of HL compared to the lower half. The presence of larger droplets at the top of the adhesive–HL zone suggests that more water absorption occurs into resin- infiltrated dentin than into less well infiltrated dentin. In order to evaluate the effect of a chemical agent that potentially allows stabilization (cross-linking) of collagen fibrils, this study used glutaraldehyde as a pre-treatment in group 2 specimens. The effect of glutaraldehyde cross-linking on the collagen matrix is only partially known. It was previously shown that the more collagenous material is cross-linked, the less it can bind water. A recent report demonstrated that glutaraldehyde cross-linked gelatin films showed a significant reduction in their swelling in water. Cross-linking of collagenous materials with glutaraldehyde involves the reaction of free amino groups of lysine or hydroxylsine amino acid residues of the polypeptide chains with the aldehyde groups of glutaraldehyde. The use of glutaraldehyde to fix collagen fibrils exposed by the etching agents did not affect water droplet formation in the present study, as droplets were still present along the HL. The number and dimension of the droplets remained the same, thus in HL, cross-linking of collagen apparently has little effect on water uptake or release. In conclusion, the present study demonstrated that HL seems to behave in a sponge-like manner, adsorbing and releasing water. The formation of water droplets may depend on the chemical composition of HL, since removal of the collagen fibrils by NaOCl solution was shown to prevent water droplets formation. However, the clinical and biological implications of water movement within the HL remain unclear. Future research on this topic should be done on resin-bonded specimens that have been aged for many months.
Figure 1 (P and B): (a) SEM micrograph (X3500) of the HL (between open arrows) formed by the P and B observed in the original specimen. The thickness of the HL ranged from 4 to 7 mm. The small cracks (pointer) are artifacts of shrinkage in the high vacuum of the SEM. (b) SEM micrograph (X 500) of the resin–dentin interface observed in a replica specimen. The specimen was stored in water for 3 h. A single line of large droplets (4–5 mm in diameter—pointer) was observed at the top of the adhesive–HL region. Smaller droplets (0.5–2 mm in diameter—open arrowhead) were observed throughout the lower region, with gradually decreasing size from the surface towards the deep parts of HL. The line of droplets was perpendicular to the orientation of the tubuli. Note that the region containing droplets was about 25 mm deep, while the hybrid layer was 5 mm thick. (c) SEM micrograph (X500) of the HL observed in a replica specimen. The dentin was acid-etched and glutaraldehyde-treated. No difference in droplet arrangement or morphology was observed between specimens in this group vs group 1. Note that the zone containing droplets was approximately 20 mm thick, while the hybrid layer in Fig. 2(a) was only 5 mm thick. (d) SEM micrograph (X 750) of HL observed in replica specimen. The specimen was stored in water for 3 h. Dentin (D) was etched and NaOCl treated to remove the demineralized matrix. The removal of the collagen layer prevented the formation of HL and water droplet formation. Pointer: resin tag.
Figure 2 (AB): (a) SEM micrograph (×3500) of HL formed by AB and observed in the original specimen. The thickness of the HL (between open arrows) was about 10 μm. No defects were present. At the junction of the tubuli with the HL, the tubules (T) exhibited the typical funnel appearance. C: composite; A: adhesive; D: dentin. (b) SEM micrograph (×350) of the replica of a specimen after water storage. Droplets arrangement and dimension (pointer) were similar to that observed with P and B. D: dentin. (c) SEM micrograph (×500) of a replica obtained after water storage of specimen. The dentin was etched and glutaraldehyde-treated. A zone of water droplets could similarly be identified. (d) SEM micrograph (×750) of replica obtained after water storage of specimen. Dentin (D) was etched and NaOCl-treated. No droplets were observed in the absence (between open arrows) of a HL. Pointer: resin tag.
Figure 3 (SEB): (a) SEM micrograph (£ 350) of the HL (H) formed by SEB in the original specimen. The thickness of HL is appreciably lower than that obtained by total-etch adhesive. It ranged from 0.2 to 1 mm. D: dentin; T: dentinal tubule. (b) SEM micrograph (£ 350) of a replica obtained after water storage of specimen. A thin, single line of water droplets was identified (pointer), corresponding with the thin HL. Droplets diameter ranged from 2 to 6 mm. D: dentin. (c) SEM micrograph (£ 1000) of a replica after water storage of the specimen. Dentin was etched and treated with glutaraldehyde. No difference in blister arrangement and morphology were observed between the other treatment groups. (d) A high magnification SEM micrograph (£ 2000) of a replica of a specimen that was stored in water. Dentin was treated with NaOCl. Droplets were still present. At this magnification, it is possible to observe some secondary droplets of smaller dimensions (open arrowheads).

References
Chapter 2

Permeability of marginal hybrid layers in composite restorations
Abstract The goal of adhesive dentistry is to restore the peripheral seal of dentin lost from removal of enamel. Unfortunately, the hybrid layer (HL) that is used to create that seal is permeable to small ions or molecules, even in the absence of detectable, interfacial gap formation via nanoleakage. This nanoleakage results from several mechanisms including incomplete infiltration of adhesive monomers into demineralized collagen matrix, presence of hydrophilic monomers, and insufficient removal of solvent or water that remains trapped inside the HL. These mechanisms lead to a porous interface with nanometersized channels that increase the permeability of the HL. The null hypothesis tested in this study was that water and acidic solution storage are able to alter in vitro the resindentin interface, further increasing the marginal hybrid layer (MHL) permeability. Class II cavities were made in vitro. The specimens were stored in water for 1 week and in lactic acid solution for 3 days. Polyvinyl siloxane impressions of restoration margins were taken before and after storage in water and lactic acid solution. Polyether replicas were obtained using the silicon impressions as molds. Replicas and original samples were observed under scanning electron microscopy. Lines of water droplets were detected on MHLs and overlying adhesive only after storage. Replicas obtained after acidic solution storage showed great numbers of irregularities such as gaps, voids, and degradation of the dentin-restoration surface margin, but also a great number of droplets. Dentin-restoration resin interfaces absorb water and are damaged by storage in dilute lactic acid. The presence of water droplets probably indicates water that flows out of the interface during the setting time of the impression and thus represents an index of marginal HL water permeability.

Introduction

Conventional thought is that a perfect seal along the resindentin interface can be established within demineralized collagen matrix when it is completely infiltrated by adhesive resins in permanent and primary teeth [8, 9, 16]. This notion is based on the assumption that the polymerized resins used for bonding are nonporous and impermeable to fluids. However, small ions or molecules can permeate the hybrid layer (HL) even in the absence of detectable interfacial gap formation. This phenomenon is called “nanoleakage” and results from several mechanisms that include the incomplete infiltration of adhesive monomers into a hydroxyapatite-depleted collagen network [24, 25] and retention of residual solvent or water that remains trapped inside the HL, creating porosities or nanochannels that increase the HL’s permeability [28]. The functional groups of many adhesive monomers may have only weak chemical affinity for demineralized collagen, leaving much water bound to collagen [31]. Adhesive resins contain hydrophilic monomers that, after polymerization, may behave as a hydrogel, creating a three-dimensional copolymer network that can attract water and swell, similar to a sponge [1, 2, 17, 18, 30]. Water and other oral plaque constituents (lactic acids, salivary esterases, proteolytic enzymes, etc.) may cross the HL, even in the presence of optimally hybridized dentin, and contribute to the leaching of adhesives or to the degradation of collagen fibrils and their bond with resins [10].

The importance of water uptake in the long-term durability of restorations is still under question, but it may be related to water tree formation as described by Tay and Pashley [28]. A recent study [5] reported that water storage leads to degradation of HLs if they are directly
exposed to water for 4 years. Dentinal nanoleakage phenomena increased when bonded specimens were stored in water but not in oil. This morphological alteration may be a consequence of the hydrolytic degradation of polymers and exposed collagen network over time [15]. For these reasons, the HLs formed in most peripheral dentin sites that have no enamel or cementum are potentially highly vulnerable to nano- and microleakage and deterioration [5, 10, 15]. The HLs located at these sites are called marginal hybrid layers (MHLs), since they are commonly found in cavosurface margins ending in dentin [21]. MHL exposed to the oral environment has several features that render it less effective than internal HL in preserving marginal sealing. For instance, MHL exposed to the oral environment is very thin [21]. This may be due to a higher acid resistance of dentin in this zone than deeper dentin areas and to the unfavorable orientation of tubules that limits the penetration of monomers [26]. Recently, a replica technique was used to demonstrate water uptake and release from dentin HL. During the setting of impression material, absorbed water flowed back to the outer surface of the HL and was trapped by the setting impression, producing a line of blisters that represented a replica of water droplets [2, 14]. These investigations support the hypothesis that there can be bidirectional water movement within the adhesive-HL complex [2, 3, 4]. In the present study, the morphology of MHL along the external margins of restorations was evaluated with the same technique, after storage in water and in a lactic acid solution, to simulate the effects of exposure to the oral environment. The hypotheses tested by this study were: (1) water droplets are detected along the marginal HL after storage in water of restoration samples and (2) lactic acid storage increases the number of droplets along marginal HL.

Materials and methods

Sample preparation
Thirty erupted third molars obtained from young patients (age range 25–40 years, mean 28.2), stored at 4°C in saline solution for no more than 1 month, were selected for the study. Nonretentive, standardized, class II cavities (3.5 mm width and 3.0 mm depth) were prepared with the proximal box extended to just below the cementumenamel junction on the distal surface. This permitted examination of resin-dentin bonds was made under clinically relevant conditions. Medium- and fine-grit diamond burs were used with a high-speed, water-cooled handpiece (Castellini, Bologna, Italy). Different restorative systems were used: (1) Quadrant UniBond/Quadrant Universal LC (Cavex, Haarlem, The Netherlands), (2) Clearfil SE Bond/Clearfil APX (Kuraray, Osaka, Japan), (3) iBond + Venus (Haereus Kulzer, USA), (4) Quadrant Uni-1-bond + Universal LC (Cavex), and (5) Scotchbond 1+Z250 (3M-ESPE, St. Paul, Minn., USA). Dentin pretreatment procedures The specimens were randomly divided into the five groups of six teeth each: the dentin surface of groups 1, 4, and 5 was etched with 37% H3PO4 (Scotchbond etchant) (3MESPE) for 15 s. The specimens in each group were then rinsed with water for 20 s.

Bonding procedures
Resin composites were applied using a stainless spatula with 1-mm-thick increments and light-cured for 40 s. All bonding agents were used following the manufacturers’ directions.
Quadrant UniBond (total etch with bonding technique): after dentin pretreatment procedures, the specimens were gently air-dried for 2 s. Adhesive was applied with a microbrush and gently spread with an air syringe to remove excess adhesive and evaporate the solvent. The adhesive was light-cured for 20 s at 400 mW/cm² with a previously tested unit (Visilux Command 2) (3M).

2. Clearfil SE Bond (self-etching technique): the bond primer was applied with a microbrush and gently air-dried for 6–8 s to evaporate the solvent. A layer of Clearfil SE Bond adhesive was applied with a microbrush, spread with air, and light-cured for 20 s.

3. iBond (self-etching technique): adhesive was applied with a microbrush for 30 s and gently spread with air for 6 s to evaporate the solvent. The adhesive layer was light-cured for 20 s.

4. Quadrant Uni-1-Bond (total etch with bonding technique): after dentin pretreatment procedures, the specimens were gently air-dried for 2 s. Adhesive was applied with a microbrush and gently spread with an air syringe to remove excess adhesive and evaporate the solvent. The adhesive was light-cured for 20 s.

5. Scotchbond 1 (total etch with bonding technique): after dentin pretreatment procedures, the specimens were gently air-dried for 2 s. Adhesive was applied with a microbrush and gently spread with an air syringe to remove excess adhesive and evaporate the solvent. The adhesive was light-cured for 20 s.

Replica preparation

After finishing with the Sof-Lex PopOn system (3M), each restoration was immediately polished along the margins with wet silicon carbide abrasive papers (nos. 600, 800, 1000, 1200, 2400, and 4000). The specimens were dried with gentle air blast for 10 s, and the first replica of each restoration was immediately made using a polyvinyl-siloxane impression material (President Jet light) (Coltene, Alstatten, Switzerland), applying the material to the restoration surface without any pressure. After 6 min, the impression material was separated from the tooth surface to obtain a negative replica of each sample. Positive replicas were then made using a polyether impression material (Permadyne Garant) (3M) using the silicon negative replica as a mold. Each restored tooth was stored in deionized water (pH 6.4) at 37°C for 1 week. Then each sample was removed from the water and gently dried with an air syringe for 10 s, and a second impression was taken as previously described.

Demineralization procedures

All restored teeth that had been stored in water for 1 week were then immersed in a demineralizing lactic acid solution (pH 4.4, 0.1 M, 37°C) for 3 days. The solution was changed every 8 h. After storage in this solution, each restored tooth was removed, washed under tap water for 2 min, and gently air-dried for 10 s, and a third replica was obtained.

Scanning electron microscope examination

Replicas (before and after water storage and after storage in lactic acid solution) were gold-coated and prepared for scanning electron microscope (SEM) observation (JEOL, Tokyo, Japan). The restored teeth were then fixed in 4% glutaraldehyde (pH 7.4) in 0.2 M cacodylate buffer for 24 h at room temperature, rinsed in cacodylate buffer solution, dried, gold-coated, and analyzed by SEM in order to evaluate directly the HL morphology and exclude any preparation artefacts during the impression procedures. The length of marginal gaps was measured as percentage of margin alteration respect to the total length of the margin. Positions of voids, droplets and blister-like structures, gaps, and fractures were recorded and inspected under SEM at ×1000 magnification.
Results

The dentin-restoration interface was easily observed on SEM images of the replicas. Interfacial gaps 1–5 µm wide (Fig. 1a, b) were occasionally seen in restorations of all groups of materials (Table 1, Table 2). Replication of samples obtained before water immersion resulted completely free of water droplet replicas (Fig. 1a, b). No droplets were observed on dentin smear layer surfaces or enamel surface replicas.

Scanning electron microscope examination of the specimen replicas stored in water revealed small, scattered droplets (0.5–3 µm in diameter) only along the dentin margins of composite restorations associated with the MHL. All materials showed droplets, as illustrated in Fig. 2a–c. No droplets were observed on dentin smear layer surfaces. Twenty droplets were detected for approximately every 100 µm of MHL. Composite and enamel surface replication also resulted free of droplets. Lines of bigger water droplet replicas (0.5–3.5 µm) were seen along the entire length of the MHL in replicas (Fig. 3a, b) after storage in lactic acid solution. Open dentinal tubules were well observed on the dentin surface, suggesting that the acid solution was able to remove the smear layer produced during the polishing procedure. The diameter of tubules ranged from 0.5 µm (partially opened) to 2.0 µm (completely opened) (Fig. 4). No water droplets were detected on the tops of open dentinal tubules or on dentin surfaces.

Enamel surfaces appeared altered after storage in lactic acid. In many specimens, it was possible to observe an etching pattern, with loss of intraprismatic structure or roughening of the interprismatic enamel (Fig. 5a). The etching of enamel was most clearly visible close to the restoration margins, while the enamel surfaces distant from margins was relatively acid-resistant. All resin-enamel margins appeared free of the droplets seen on resin-dentin margins (Fig. 5b). Obviously, the original samples resulted free of water droplets and blister-like structures. The morphology of original dentin surfaces and marginal gaps was similar to that observed in the replicas. Several gaps and fractures were observed but were free of droplets.

Discussion

The replica method for detecting margin alterations has been widely used in the past [7, 14]. In this study, such a technique was used to analyze evidence for water released from the MHL before and after immersion of teeth samples in water and lactic acid solution. Interestingly, in this study, all replicas of restored teeth stored in water showed lines of droplets located only along or close to the resin-dentin margin. As revealed by the SEM images, this line was evident approximately 1 µm above the interface between resin and dentin. Since the droplets were visible only on the replicas of samples stored in water and not on the original specimens that were dehydrated prior to SEM observation, we believe that they were formed by water flowing out of the adhesive-MHL region during setting of the impression material [3, 4, 29]. Droplets were not present on sound dentin, resin composite, or marginal resin-enamel interfaces but only along the HL and at the bottom part of adhesives, suggesting that, during water storage, these structures took up and released more water than the other structures and tissues. We believe that these droplets are not artefacts produced by moisture condensation during impression taking, as they were absent when specimens were not preincubated in water.
Moisture transfer in organic coatings on porous materials has been studied extensively in nondental fields [11, 13, 20, 27]. It may occur via different mechanisms. While the demonstration of interconnecting water channels (water trees) in dentin adhesives [28] is an example of capillary fluid movement, in the absence of waterconducting pathways, water movement across polymer coatings may still occur via nanopores in the polymer that are created by the segmental mobility of the polymer chains, according to the free volume theory of Cohen and Turnbull [11]. Water molecules and small ions can move through these nanopores via a hopping mechanism that occurs in the range of picoseconds during the beta relaxation of these polymer chains [11]. Water diffusivity in polymer networks is probably enhanced by the incorporation of hydrophilic groups in the adhesive copolymers [27]. Permeability of water through these adhesives is caused not only by a loss of integrity between the adhesive and dentin but by the nanoporosity of the HL-adhesive complex. Most adhesive formulations include hydroxyethylmethacrylate and acidic monomers [6, 31, 32] that may
cause swelling pressures commonly seen in hydrogels [18, 33]. We speculate that hydrophilic resins in restorations absorb water during water storage, causing minute swelling in these structures that stretches the cross-linked polymer network [33]. When the specimens are removed from water, briefly dried, and then covered with impression material, the absorbed water is squeezed out by recoil of the polymer network at the free surface. Interestingly, we found increased numbers and dimensions of droplets after acidic storage. We speculate that this was due either to a degradation effect of the acidic solution on the resin or that the low pH caused more water sorption [18, 21]. The presence of a wide marginal gap may increase the uptake and release of water.

Sound dentin is also a porous tissue. It contains approximately 10%/weight of aqueous fluids, most of which is in dentinal tubules. When dentin was removed from water and gently dried for 5–7 s, no water droplets were seen in the replicated dentin surface. Presumably, the mineralized dentin matrix is too stiff to stretch at a molecular level during water sorption, so the matrix cannot recoil when it is removed from water. The results of the present study also revealed that there is no obvious direct relation between marginal integrity and number/dimensions of droplets on resin-bond dentin. Thus, we speculate that water absorbed by the HL was “stored” in small voids, porosities, or channels within the hybrid and adhesive layers and subsequently released, independently of any marginal breakdown [28]. This work indicates that both HLs and at least some contemporary adhesives absorb water. This water can come out of free surfaces within 3–6 min. Whether a similar bidirectional movement of water can occur under occlusal loading remains to be determined. The development of marginal defects and secondary caries around restoration margins may be considered one of the major reasons for restoration failures and replacements [5, 12]. Little information is available on the mechanisms of marginal deterioration in the gap-free interfaces. Enamel surface resulted rich in porosities and alterations, especially close to the margin of restoration [22] but free of water droplets. The permeability characteristics of polymers have important implications in polymer degradation and have been extensively investigated in other fields [23, 34]. In MHL, we may suppose that the relatively high permeability of this area vulnerable to water may be responsible for the degradation of resin-dentin bond strength [19] and for the rapid, long-term hydrolytic deterioration of structural properties of the interface [10].
Fig. 1a, b Scanning electron micrographic images of dentin-restoration interface (×1000). a Margins of a resin composite restoration immediately after polishing and finishing procedures. Image shows the replica of a specimen obtained before its immersion in water. The visible composite (C) and the dentin surface (D) are easily identified. E enamel, b Image of a marginal gap obtained immediately after polishing and finishing and before water immersion. No water droplets are visible.
Fig. 2a–c Replicas of restoration specimens after immersion in water. C composite, D dentin. a Scattered droplets (0.5–2 µm in diameter) were detected at margins of restoration associated with the MHL and overlying adhesive (×1000). b Dentin-composite interface after immersion of the sample in water. Dentin is well visible. Water droplets are detected only along the hybrid layer and in its proximity (×1000). c Replica of specimen stored in water. Scattered droplets are visible along the MHL. A gap is well visible (×500)
Fig. 3a, b Replicas of specimens stored in water and lactic acid solution (∗1000). a A line of small and large droplets (0.5–5 µm in diameter) is observed along the MHL and the adhesive layer. B Another specimen. C composite, E enamel

Fig. 4 Marginal dentin at the cervical portion of a cavity restoration (∗1000). Dentinal tubules appear open and free of a residual smear layer. The tubule diameter ranges from 0.5 µm to 2 µm. Interesting is that the presence of open dentinal tubules is not responsible for the formation of water droplets in the replica, suggesting that dentinal fluids from tubules are not able to create any droplet-like structures. Droplets are visible only along the MHL
Fig. 5a, b Images by SEM of the enamel-composite margin (×1000). a Deterioration of an enamel surface after lactic acid immersion. The erosion of enamel is most clearly visible close to the margin of the restoration. E enamel, C composite. b Typical enamel alterations along the margin of the restoration

References
Chapter 3

SUMMARY

Objectives: Dentin adhesives have been proposed as desensitizing agents to seal exposed root dentin surfaces. Simplified “one-step” dentin adhesives are highly permeable to water. We hypothesized that lactic acid challenge may increase permeability of simplified adhesives and may induce a fast degradation of bonding. This phenomenon adversely affects their durability as long term desensitizing agents. The aim of this in vitro study was to evaluate the ability of four dentin adhesives to seal root dentin surfaces that were exposed to water and lactic acid challenges.

Methods: Four commercially-available dentin adhesives were applied to the root dentin of extracted human molars as desensitizing agents with a small sponge. Impression replicas of the adhesive-covered root dentin were fabricated after water immersion, as a control, and after lactic acid challenge. The replicas were examined with SEM for quantitative comparison of
fluid droplet formation on the surfaces. The bonded specimens were also examined using reflected light confocal microscopy.

**Results:** Replicas of water droplets were observed by SEM on the adhesive surfaces, which corresponded with direct confocal observation of blisters and voids from the surface of the bonded specimens. There were significantly more water droplets from samples that were subjected to lactic acid challenge than to only water immersion.

**Significance:** Although the dentin adhesives examined were able to cover exposed root dentin, they all exhibited fluid transudation through the polymerized adhesives. Dentin adhesives were also susceptible to surface degradation after lactic acid challenge. As simplified self-etch adhesives were highly water permeable and exhibited the most extensive surface damage, they may not be the best adhesives to be used for long-term dentin desensitization. These preliminary in vitro findings warrant validation in vivo.

**INTRODUCTION**

Dentin adhesives have been employed for the treatment of dentin sensitivity [1-3]. The efficacy of dentin adhesives as desensitizers is attributed to their ability to wet exposed dentin surfaces, occlude patent dentinal tubules and coagulate plasma proteins from dentinal fluids [4-7]. This helps to reduce evaporative water fluxes derived from air-blasts [8], and convective water fluxes derived from vital dental pulps [9].
Despite these encouraging results, recent in vitro [10], and in vivo studies [11-12], have shown that simplified dentine adhesives are highly permeable to water. This is probably caused by the incorporation of hydrophilic resin components into adhesive mixtures [13-14], that lack additional hydrophobic resin coatings. Subsequent water uptake via hydrogen bonding of water molecules to the polar domains of hydrophilic polymers may result in plasticizing the adhesives [15], adversely affecting their durability as desensitizing agents. As resin-dentin interfaces created by these simplified adhesives permit water movement, it is further anticipated that oral plaque constituents such as lactic acid and salivary/bacterial enzymes may diffuse through potential water-filled channels within the hybrid layers and adhesives [16-17]. This may further expedite the hydrolysis of the collagenous or resinous component of the bonded interfaces [18].

Water movement across adhesive-bonded dentin could be demonstrated by using a double polyvinyl siloxane/polyether impression technique to create impression replicas of the bonded dentin surfaces for scanning electron microscopy (SEM) examination [19]. Likewise, confocal microscopy has been employed for non-destructive examination of fluid permeability in resin-modified glass-ionomer cements [20]. The latter is an important instrument for qualitative confirmation of fluid movement in non-dehydrated adhesive-bonded dentin. Thus, the aim of this in vitro study was to evaluate, with the use of SEM and reflected light confocal microscopy, the ability of several simplified adhesives to seal exposed root dentin that were exposed to water and lactic acid.

The null hypotheses tested were that permeability of bonded root dentin, as manifested by water movement through the polymerized adhesives, was neither affected by the adhesive type,
nor by simulated lactic acid challenge.

MATERIALS AND METHODS

Sample Preparation

Forty-eight human maxillary canines that were extracted for periodontal reasons were used in this study. All teeth were extracted after an informed consent was obtained from each patient and in according to individual treatment protocol approved by the Review Board the Department of Dental Sciences of the University of Bologna, Italy. The teeth were stored at 4°C in physiological saline for no more than one month. The teeth were divided equally and randomly into two groups, according to the method of specimen immersion (control – water immersion only vs experimental – lactic acid challenge). Each group was further divided in four subgroups (N=6) based on the adhesives examined. The teeth were sectioned 1-mm beneath the cemento-enamel junction, using a low speed water-cooled diamond saw (Labcut, Agar Scientific, Stanstead, UK) to create flat root dentin surfaces. The coronal 4 mm of the root segments were hand-polished with 400-grit, wet silicon carbide paper for 30 sec to remove the cementum and to create smear layers along the curvature of the root surfaces. This procedure was performed to simulate dentin that was exposed after root planning. The remaining part of each root was covered with wax, to prevent demineralization of the dental hard tissues by the lactic acid employed in the experimental protocol. (Diagram 1)

Experimental Design

Commento [T2]: Dear P Watson,
We added two tables including DBS composition and application’s mode as the referees required.
We also added a new diagram figure1 and a diagram which shows the sample preparation the referees required.
Four dentin bonding systems were examined in this study. They included a two-step total-etch system (Scotchbond 1, 3M/ESPE, St. Paul, MN, USA), a two-step self-etch system (Clearfil SE Bond, Kuraray Medical Inc., Tokyo, Japan) and two one step self-etch systems (I Bond, Heraeus-Kulzer, Hanau Germany; Xeno III, Dentsply DeTrey, Konstanz, Germany (Table 1). These adhesives were applied to the root dentin according to the manufacturers’ instructions (Table 2). As these adhesives were used in the context of dentin desensitizers, without additional coupling of resin composites, a second coat of each adhesive was applied to circumvent the effect of oxygen inhibition. Each adhesive was polymerized as per manufacturer's instruction with a halogen light-curing unit XL-2500 (XL, 3M ESPE, St. Paul, MN, USA) at a light intensity of 600 mW/cm² light intensity with a standardized distance of 5 mm from the dentin. The sticky, un-polymerized oxygen inhibition layer was removed from each bonded root dentin surface with a cotton pellet, leaving behind a glassy layer of adhesive in order to avoid replicating of oxygen inhibition droplets. Prior to the start of the experiments, reference impressions of these bonded surfaces were taken with a low viscosity, slow-setting (4 - 5 min setting time), polyvinyl siloxane impression material (President Light Body; Colténe AG, Altstätten, Switzerland) for subsequent fabrication of replicas for SEM examination [21].

Control and Experimental Groups

In the control group, the 24 bonded specimens were immersed in de-ionized water at 37°C for 48 h. In the experimental group, the 24 bonded specimens were subjected to lactic acid challenge. These specimens were immersed in a temperature-controlled perfusion tank
positive impression replicas were fabricated with a polyether impression material (Permadyne Garant, 3M/ESPE), using the polyvinyl siloxane negative replicas as molds, according to the polyether replica technique reported by Chersoni et al. [19]. As there is no chemical reaction between polyether and polyvinyl siloxane, this replica technique has been shown to be effective in replicating water transudation from dentin hybrid layers. The polyether replicas
were coated with gold and examined using a SEM (Model 5400, JEOL, Tokyo, Japan) at 5-10 kV. Ten secondary electron images were taken randomly from different areas of each replica. The number of “droplets formation” /50 µm² that corresponded with the manifestations of water droplets was recorded by two co-authors who did not participate in bonding and were unaware of the group designations.

As the numerical data were not normally distributed (Kolmogorov-Smirnov test), they precluded statistical analysis using the originally planned two-way ANOVA design to examine the contribution of adhesive type and lactic acid challenge on adhesive permeability. The data were subsequently analyzed with non-parametric statistical methods. To examine the effect of different adhesives on fluid expression, the results from the control subgroups or the experimental subgroups were separately analyzed with Kruskal-Wallis ANOVA on ranks and Dunn’s multiple comparison tests. To evaluate the effect of lactic acid challenge on fluid expression, data from the water immersion subgroup and the lactic acid challenge subgroup of each adhesive were analyzed using the Mann-Whitney rank sum test. Statistical significances were set in advance at α=0.05.

Confocal microscopic examination

After taking the first reference impression, each bonded specimen was re-immersed in de-ionized water for 10 min before examination with the confocal microscope (Tandem Scanning Microscope – TSM; Noran Instruments, Middleton, WI, USA) using a X40/0.55 numerical aperture, extra long working distance dry objective (Nikon, Japan), to collect reference images of the surface of the sample. Samples were illuminated with a mercury arc lamp filtered at
546nm wavelength (green) and images captured using a CCD camera (COHU, CA, USA) and reconstructed with a computer software program (Kinetic AQM 2002, Kinetic Imaging, Nottingham, UK)

RESULTS

SEM examination of impression replicas revealed predominantly smooth and flat surfaces from specimens bonded with the four adhesives before water immersion (Figures 2A and 2C). After 48 h of water immersion, profuse globular elevations, being manifestations of water entrapment by the polyvinyl siloxane impression material [21], could be identified from all the replica surfaces of the two simplified self-etch adhesives I Bond (not shown) and Xeno III (Figure 2B). Similar, but sparser globular elevations were observed in Scotchbond 1 (Figure 2D), the simplified total-etch adhesive, and Clearfil SE Bond, the conventional self-etch adhesive (not shown).

In addition to globular elevations, extensive deterioration of the adhesive surface could be identified from the impression replicas after the adhesive surfaces were subjected to lactic acid challenge. This feature was characteristic of all the adhesives examined. Figures 3A and 3B are representative examples from specimens bonded with I Bond and Clearfil SE Bond.

Statistical analysis of the manifestation of water droplets in the eight subgroups are shown in the Table. The control and experimental groups exhibited the same trend, in that the severity of water droplet formation was similar in the two simplified self-etch adhesives (P>0.05), and was significantly higher than the simplified total-etch adhesive (P<0.05). The latter, in turn, was significantly higher than the conventional self-etch adhesive (P<0.05). When each adhesive
was individually analysed, significantly more water droplets were unanimously observed from the experimental subgroups that were subjected to lactic acid challenge. Differences between the control and experimental subgroups were highly significant (P<0.001) for I Bond, Xeno III and Scotchbond 1.

Fluid transudation from the adhesive surfaces was further confirmed with TSM examination. Prior to water immersion, the adhesive surfaces appeared flat and smooth (Figure 4A) or slightly porous (Figure 4C). However, after 48 h of water immersion, adhesive surfaces from the I Bond (Figure 4B) and Xeno III specimens were swollen and extensively covered with water blisters. Void formation could also be observed from the flat, non-swollen adhesive surfaces in Scotchbond 1 (not shown) and Clearfil SE Bond (Figure 4D).

**DISCUSSION**

Application of the adhesives as de-sensitizing agents on root dentin resulted in the formation of relatively homogenous resin films that were water permeable and susceptible to surface deterioration after immersion in water. The characteristic of water uptake with these thin resin films and their susceptibility to damage were highest for the two simplified one-step self-etch adhesives, moderate in the simplified two-step total-etch adhesive and comparatively mild for the conventional two-step self-etch adhesive. The inability of these adhesives to completely eliminate dentin-water movement in bonded sound dentin was similar to the results reported in previous fluid filtration studies [10, 22-24]. Although the use of water instead of serum in dentin perfusion studies has been challenged in that it may over-estimate the adverse effect of dentin permeability [5], fluid movement was not completely eliminated when a glutaraldehyde-
containing simplified self-etch adhesive was used to seal exposed vital crown preparations [11]. The differences in water permeability through the various adhesives may be attributed to the increased concentrations of hydrophilic resin components in the simplified adhesives [25, 26], and their lack of non-solvented hydrophobic protective resin coatings [27]. Thus, resin films fabricated from simplified adhesives are able to absorb water from the oral environment. The water may be incorporated as either free water within potential flaws or water channels within the adhesives [28, 17], or as bound water within the hydrophilic domains of the adhesives [29, 15]. Unlike in vivo studies that demonstrated fluid movement from vital crown preparations bonded with simplified self-etch [11], and total-etch adhesives [12], bonding was not performed under simulated physiological water pressure in this study. Thus, water movement through the adhesives could not be attributed to convective water fluxes derived from vital dental pulps [30]. Nevertheless, it is apparent that the water could be released from the free water that was trapped within the resin films or via diffusion from the underlying dentin when the specimens were removed from water. The release of water droplets from longitudinal sections of hybrid layers and adhesive layers has previously been demonstrated using a similar double impression technique [19].

Previous reports on the manifestation of fluid droplets over the surfaces of polymerized dentin adhesives have been based on indirect observations of the entrapment of these droplets by a slow setting polyvinyl-siloxane impression material. The fluid droplets were perceived as globular elevations on the surface of either epoxy resin or polyether replicas. With the use of polyether replicas, it is debatable whether these globular elevations were caused by swelling of the impression material. A significant contribution of this study is the direct confirmation of
water movement from the bonded root dentin using confocal microscopy. Originally, we anticipated that water movement could be better demonstrated by covering the adhesive with a layer of oil, so that water that came through the adhesive surface would be trapped as water bubbles within the oil layer. However, such a technique was unsuccessful and only voids, collapsed or burst blisters could be identified using the dry objective. This is probably because our experiments were conducted in the absence of dentin perfusion, so that water movement through the adhesives could only be achieved by evaporative water fluxes [31], through the permeable adhesives. In the absence of dentin perfusion, placement of a layer of oil over the permeable adhesive would be analogous to the elimination of evaporative water flux when a coat of varnish was applied to glass-ionomer-based materials [32]. This necessitates rejection of the first null hypothesis that water movement through polymerised adhesives is not affected by adhesive type. Notwithstanding these limitations, direct and indirect observations of the same specimens confirmed that the simplified adhesives examined were highly permeable to water movement when they were bonded and polymerized in thin films, in the manner that dentin desensitizers are usually applied to exposed root dentin.

Lactic acid solutions or gels with acidity within or below the critical pH range [33] have often been utilised to simulate the interaction of dental plaque with dentin or restorative materials [34-37]. We understand that the use of a continuous lactic acid flux creates a taxing environment in which the interaction between dental plaque and adhesive-covered root dentin may be embellished, as the buffering capacity of saliva and the protective function of salivary pellicles were absent in the in vitro model [38, 39]. On the other hand, as the fatigue behavior of the adhesives was not considered, under-estimation of these interactions may also result
In spite of these compromises, the increase in adhesive permeability after lactic acid challenge paralleled the results of a recent report that single or double applications of dentin adhesives were ineffective in preventing bonded dentin from laboratory-induced lactic acid demineralization [41]. As the manifestation of water droplets increased significantly after lactic acid challenge, we have to reject the second null hypothesis that adhesive permeability was not affected by simulated lactic acid challenge. It could not be determined, however, based on the use of SEM examination, whether the surface deterioration and erosion of the adhesive surfaces after lactic acid challenge was also accompanied by bulk degradation of the adhesive. As lactic acid is a small molecule with a molecular weight of 90, we speculate that diffusion of the lactic acid into the bulk of the adhesive may have occurred via voids and water channels within the simplified adhesives. Bulk degradation, in turn may account for the highly significant increase in the manifestation of fluid droplets after lactic acid challenge. The lower water permeability of the conventional two-step self-etch adhesive [27] could have resulted in less extensive lactic acid penetration and consequently, a less highly significant increase in water droplet formation.

Within the limits of this study, it may be concluded that when bonded to non-caries root dentin, all the adhesives examined were unable to eliminate completely fluid transudation that occurred through the polymerized adhesives and were susceptible to lactic acid attack.

Conclusions:
The efficacy of dentin adhesives as de-sensitizing agents is attributed to their ability to wet exposed root dentin surfaces and to occlude open dentinal tubules. This study has shown that
simplified all-in-one dentin adhesives are more susceptible to water uptake and lactic acid challenge than a two-steps self etching adhesive resulting in deterioration of the adhesives. This renders them unsuitable to be used by themselves as long-term dentin desensitisers. Thus, clinicians should prefer two-steps self etching adhesive for the treatment of root sensitivity and they should repeat the treatment preferably with the same dentin adhesive.

REFERENCES


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[34] Nicholson JW, Czarnecka B, Limanowska-Shaw H. The long-term interaction of dental


TABLE. Quantitative comparison of the appearance of water droplets from adhesive-covered root dentin surfaces based on SEM examination of positive impression replicas.

<table>
<thead>
<tr>
<th>Adhesive category</th>
<th>Dentin adhesive</th>
<th>Number of water droplets/50μm² (water immersion only)</th>
<th>Number of water droplets/50μm² (after lactic acid challenge)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simplified (1-step) self-etch adhesive</td>
<td>iBond</td>
<td>*20.2 ± 3.1 A₁</td>
<td>*27.2 ± 4.1 a₁</td>
</tr>
<tr>
<td></td>
<td>Xeno III</td>
<td>19.2 ± 3.2 A₁</td>
<td>25.2 ± 4.1 a₁</td>
</tr>
<tr>
<td>Simplified (2-step) total-etch adhesive</td>
<td>Scotchbond 1</td>
<td>6.8 ± 1.7 B₁</td>
<td>8.8 ± 2.5 b₁</td>
</tr>
<tr>
<td>Conventional (2-step) self-etch adhesive</td>
<td>Clearfil SE Bond</td>
<td>3.2 ± 1.6 C₁</td>
<td>4.8 ± 2.5 c₁</td>
</tr>
</tbody>
</table>

* Values are means ± standard deviations, based on the analysis of ten micrographs taken from each of the six specimens in each subgroup (N=60). As the data were not normally distributed, they were analyzed using non-parametric statistical analyses.

§ For the water immersion column, subgroups with the same upper case letter superscripts are not statistically significant (Kruskal-Wallis ANOVA on ranks and Dunn's test; P>0.05).

¶ For the lactic acid challenge column, subgroups with the same lower case letter superscripts are not statistically significant (Kruskal-Wallis ANOVA on ranks and Dunn's test; P>0.05).

* For each adhesive row, subgroups with different numerical superscripts are statistically significant (Mann-Whitney rank sum tests; P<0.001 for iBond, Xeno III and Scotchbond 1; P<0.05 for Clearfil SE Bond)

Diagram 1. Schematic Illustration of Sample Preparation
Extracted teeth

Division in two principal groups

Control-group

Water immersion

Experimental-group

Lactic acid

Division of each principal group into 4 subgroups (N=6) based on the adhesives tested

Sample preparation

Crown preparation with diamond saw

Buccal aspect

Smear layer formation with SiC-paper

Bonding agents application

Application of wax as protection on the rest parts of the tooth

Application of wax as protection

Completed sample for water immersion or for acid lactic challenge as per experimental design

buccal aspect other aspects
Fig.1 An overall view of the laboratory setup for delivering a continuous flux of lactic acid at controlled temperature to the bonded root dentine specimens.
Fig.2 Representative SEM micrographs of polyether positive impression replicas of the surfaces of the bonded root dentine before and after water immersion. 

A. Root dentine bonded with Xeno III, a simplified self-etch adhesive before water immersion. The impression surface appeared flat and predominantly smooth. 

B. The same Xeno III specimen after water immersion. Globular elevations could be identified from the impression surface. These elevations represented water droplets that transudated the adhesive surface, and were trapped by the low viscosity, slow-setting polyvinyl siloxane impression material. 

C. Root dentine bonded with Scotchbond 1, a simplified total-etch adhesive, showing a flat and predominantly smooth surface before water immersion. 

D. The same Scotchbond 1 specimen after water immersion, showing the presence of similar, but sparser globular elevations.
**Figure 3**

Representative SEM micrographs of polyether positive impression replicas of the surfaces of the bonded root dentine after lactic acid challenge. **A.** A specimen bonded with I Bond, a simplified self-etch adhesive. In addition to the dense arrangement of globular elevations, extensive surface erosion and crack formation could be identified from the adhesive surface. **B.** A specimen bonded with Clearfil SE Bond, a conventional two-step self-etch adhesive. In localised regions where globular elevations were detected, deterioration of the adhesive surface could also be observed after lactic acid challenge.
Figure 4

Fig.4  Representative TSM images of the surface of the bonded root dentin before and after water immersion. A. An I-Bond specimen before water immersion showing a smooth but slightly porous adhesive surface. B. The same I-Bond specimen after water immersion,
showing the presence of innumerable collapsed blisters over the adhesive surface. Most of these blisters have burst, resulting in a volcano-like appearance (pointer). C. A Clearfil SE Bond specimen before water immersion, showing a smooth and predominantly void-free surface. D. The same Clearfil SE Bond specimen after water immersion, showing the appearance of voids along the flat adhesive surface.

Chapter 4

In vivo and in vitro Permeabilità of One-step Self-etch Adhesives

ABSTRACT
Adhesive dentistry should effectively restore the peripheral seal of dentin after enamel removal. We hypothesize that non-rinsing, simplified, one-step self-etch adhesives are effective for minimizing dentin permeability after tooth preparation procedures. Crown preparations in vital human teeth were sealed with Adper Prompt, Xeno III, iBond, or One-Up Bond F. Epoxy resin replicas were produced from polyvinyl siloxane impressions for SEM examination. Dentin surfaces from extracted human teeth were bonded with these adhesives and connected to a fluid transport model for permeability measurements and TEM examination. Dentinal fluid droplets were observed from adhesive surfaces in resin replicas of in vivo specimens. In vitro fluid conductance of dentin bonded with one-step self-etch adhesives was either similar to or greater than that of smear-layer-covered dentin. TEM revealed water trees within the adhesives that facilitate water movement across the polymerized, highly permeable adhesives. Both in vitro and in vivo results did not support the proposed hypothesis.

INTRODUCTION
An important goal in conservative dentistry is to restore the peripheral seal of dentin that originally exists prior to the removal of enamel (Pashley et al., 2002). For crown preparations of vital teeth that involve a considerable sacrifice of sound tooth structures, the use of provisional cements may permit more microleakage of bacteria and their products than the final restorations (Baldissara et al., 1998). For preservation of the health of the pulpodentinal complex, an alternative approach is for the exposed dentin to be sealed with resin-based
adhesives prior to the taking of impressions (Pashley et al. 1992; Lam and Wilson, 1999; Jayasooriya et al., 2003).

Being non-rinsing, the milder versions of self-etch adhesives preserve smear plugs and prevent the dilution of resin monomers with dentinal fluid (Perdigão, 2002). For the more aggressive self-etch adhesives that completely dissolve smear plugs, coagulation of plasma proteins by primer components may contribute to a reduction in dentin permeability during the processes of simultaneous etching and priming (Nikaido et al., 1995). Although the complete absence of leakage is not a realistic expectation with the use of these adhesives (Tay et al., 2002a), the recent introduction of one-step self-etch adhesives represents a further reduction in working steps that eliminates some of the technique sensitivity and practitioner variability that are associated with the use of total-etch adhesives (Finger and Balkenhol, 1999; Peschke et al., 2000).

Since dentin adhesives are effective in reducing cervical hypersensitivity (Prati et al., 2001), it is prudent to determine if one-step self-etch adhesives can be used for sealing vital teeth following crown preparations. Thus, the objective of this study was to test the hypothesis that one-step self-etch adhesives are effective in reducing dentin permeability under in vivo and in vitro conditions.

MATERIALS & METHODS

For the in vivo part of the study, 24 vital posterior teeth (maxillary and mandibular premolars and molars from 17 subjects) that required crown preparations for fixed prosthodontics were selected. The age of the subjects ranged from 23 to 42 yrs. Informed consent of the subjects was obtained under an in vivo protocol reviewed and approved by an ethics committee from the University of Bologna.

For the in vitro part of the study, 35 recently extracted human third molars were collected after the patients' informed consent had been obtained under a protocol reviewed and approved by the institutional review board from the Medical College of Georgia. These teeth were stored in a 1% chloramine T solution at 4°C and used within 1 mo following extraction. We prepared each tooth by first removing the occlusal enamel using a slow-speed saw (Isomet, Buehler Ltd., Lake Bluff, IL, USA) under copious water-cooling. We used 180-grit silicon carbide (SiC) paper to create a smear layer on the exposed dentin surface.

Experimental Design

Four one-step self-etch adhesives were examined. They included 3 two-component systems (Adper Prompt, 3M ESPE, St. Paul, MN, USA; Xeno III, Dentsply DeTrey, Konstanz, Germany; One-Up Bond F, Tokuyama Corp., Tokyo, Japan) and 1 single-component system (iBond, Heraeus Kulzer, Hanau, Germany). They were used according to the manufacturers' instructions. A two-step selfetch adhesive (UniFil Bond, GC Corp., Tokyo, Japan) was used as the control. The chemical compositions of these adhesives are shown in Appendix 1.

Scanning Electron Microscopy (SEM)

For the in vivo part of the study, 4 teeth were randomly assigned to each adhesive group. Following crown preparations under local analgesia (mepivacaine hydrochloride 2% with adrenaline 1/100,000), they were sealed with the respective adhesive. The oxygen inhibition
layer was gently removed with a cotton pledget soaked in 50% ethanol. Since it has been shown that impression taking does not affect the integrity of the bonded adhesive (Nahon et al., 2001), a low-viscosity polyvinyl siloxane impression material (Affinis LightBody; Coltène AG, Altstätten, Switzerland) with an intra-oral setting time of 3.5 min was used for taking impressions of these crown preparations. After the research impressions were taken, working impressions were then produced for the construction of the fixed prostheses. Research impressions were also prepared for the remaining 4 crown preparations, in which the smear-layer-covered dentin was not bonded with any adhesive. Epoxy resin replicas were produced from these impressions, according to the protocol reported by Itthagarun and Tay (2000). They were sputter-coated with gold/palladium and examined with a SEM (Cambridge Stereoscan 360, Cambridge, United Kingdom) operating at 20 kV.

Fluid Conductance Measurements
We used an \textit{in vitro} fluid-transport model to measure the fluid conductance through adhesives, following the protocol for hydraulic conductance evaluation reported by Pashley and Depew (1986). The roots were removed from each tooth at 3 mm below the cemento-enamel junction, by means of the Isomet saw. We gently removed pulpal tissue with a small spoon excavator so as not to touch the predentin. The dentin surface was further abraded until a remaining dentin thickness of 1 mm was achieved from at least one region of the ground surface to the highest pulp horn, as measured with a pair of Iwonson calipers. The crown segment was cemented to a piece of Plexiglass by means of C&B Metabond (Sun Medical, Shiga, Japan). The Plexiglass was penetrated by a piece of 18-gauge stainless steel tubing that ended flush with the top. This tubing permitted the pulp chamber to be filled with water and to be connected to a waterfilled syringe for measurement of the fluid movement across the dentin surface under 15 cm of H2O pressure (Vongsavan \textit{et al.}, 2000).

We measured fluid conductance (mL/min-1) by following the displacement of an air bubble in a micropipette with a constant barrel (Appendix 2). Five teeth were selected at random for each adhesive. For each tooth, fluid conductance was measured three times (Bouillaguet \textit{et al.}, 2000): (a) after dentin was acid-etched for the determination of maximum baseline conductance, (b) after the creation of smear-layer-covered dentin by abrasion of the same tooth with 180-grit SiC paper, and (c) after the dentin was sealed with the respective one-step selfetch adhesive under perfusion at 15 cm of H2O pressure. For each dentin surface, fluid flow (mL/min-1) across the smear-layer-covered dentin and bonded dentin was expressed as a percentage of that of acid-etched dentin, which was assigned a value of 100% flow rate. This allowed each specimen to serve as its own control by expressing each of the 3 procedures as a percent of the maximum value, and circumvented the use of surface area for the calculation of hydraulic conductance. Fluid flow for acid-etched dentin was measured for 10 min, and those of smear-layer-covered dentin and bonded dentin for 20 min, with all values corrected to per min. The results were statistically analyzed by two-way analysis of variance [adhesive type and substrate type (\textit{i.e.}, smear-layer-covered dentin vs. bonded dentin)] and Tukey's multiple-comparison tests with statistical significance set at $a = 0.05$.

Transmission Electron Microscopy (TEM)
The remaining 10 teeth were used for the second *in vitro* part of this study, with a resin composite used as an "impression material". Two teeth were selected at random for each adhesive. Each crown segment was similarly connected to the fluid-transport assembly and bonded with the respective adhesive under 15 cm of H2O pressure. A 2-mm-thick layer of microfilled composite (EPICTMPT, Parkell Inc., Farmington, NY, USA) was placed over the cured adhesive under water perfusion. The composite was left in the dark for 3.5 min to simulate the intra-oral setting time of the impression material. The tooth, coupled with the light-cured composite, was sectioned longitudinally into 1-mm-thick slabs and immersed in a 50 wt% ammoniacal silver nitrate tracer solution, following the nanoleakage protocol reported by Tay et al. (2002c).

Following the reduction of the diamine silver ions into metallic silver, undemineralized, epoxy-resin-embedded, 90-nm-thick sections were prepared and examined with the use of a TEM (Philips EM208S, Philips, Eindhoven, The Netherlands) operated at 80 kV.

**RESULTS**

Resin replicas of *in vivo* crown preparations revealed sporadic regions along the surfaces of the adhesive-coated dentin in which there was swelling of the adhesive. For the one-step selfetch adhesives, transudation of dentinal fluid droplets could be universally identified from the surfaces of all resin replicas examined. Adper Prompt exhibited fairly profuse transudation (Fig. 1A), with coalescence of multiple fluid droplets into large water bundles (Fig. 1B). Fluid transudation appeared in localized areas that were close to pulp horns in Xeno III (Fig. 1C), with the presence of myriad small submicron droplets among the larger droplets (Fig. 1D). The extent of dentinal fluid transudation in iBond and One-Up Bond F was comparable with that from the unbonded smearlayer-covered dentin (Fig. 2A). Fluid transudation was not evident in the two-step self-etch adhesive UniFil Bond (Fig. 2B).

Fluid conductance measurements are summarized in the Table. The presence of a smear layer resulted in a reduction of fluid conductance that was only 12-18% of those recorded for acid-etched dentin. The *in vitro* fluid conductance of dentin bonded with the 4 one-step self-etch adhesives was similar to or greater than that of the corresponding smear-layercovered dentin. Conversely, fluid conductance of dentin bonded with the control two-step self-etch adhesive was significantly less than that of the corresponding smear-layercovered dentin (P < 0.001). Two-way ANOVA revealed a significant difference for the factor "substrate type" when the two types of adhesives were pooled for analysis (P = 0.015). When the two types of substrates for fluid conductance evaluation were pooled, a highly significant difference was noted for different adhesives (P < 0.001). A significant interaction between "substrate type" and "adhesive type" (P < 0.001) was also observed. Results of the multiple-comparison tests are represented in the Table. For Adper Prompt and Xeno III, separation of sections along the composite-adhesive interfaces occurred during ultramicrotomy, and no intact section could be retrieved. TEM micrographs of One-Up Bond F and iBond bonded under perfusion revealed the presence of water blisters along the composite-adhesive interface (Figs. 3A, 3C), without the loss of integrity between the hybrid layer and the adhesive. Apart from nanoleakage within the hybrid layer, two modes of silver deposition could be identified within the adhesive. Water trees (*i.e.*, silver-filled water channels) extended from the surface of the hybrid layer into the adhesive, and could be observed either adjacent to the basic glass filler clusters (Fig. 3B) or in
the unfilled adhesive (Fig. 3D). Fine, isolated silver grains were also present in the adhesive layers (Figs. 3B, 3D). Water blisters were not observed along the adhesive-composite interface in the control two-step self-etch adhesive.

**DISCUSSION**

The smear layer and smear plugs account for 86% of the total resistance to fluid movement in deep dentin (Pashley et al., 1978). Both the *in vivo* and *in vitro* results of this study showed that when bonded under dentin perfusion, none of the one-step self-etch adhesives examined was any more effective at sealing dentin than the original smear layer (Gilliam et al., 1997). Thus, the hypothesis that one-step self-etch adhesives are effective in reducing dentin permeability must be rejected.

The dentinal fluid droplets that were observed *in vivo* along the surface of adhesive-bonded dentin were not artifacts produced by moisture condensation during impression-taking, since they were absent when vital dentin was bonded with the control two-step self-etch adhesive. Transudation of dentinal fluid was found to be non-uniform and localized to specific regions, reflecting the variation in permeability from different regions of a crown preparation (Richardson et al., 1991). Moreover, these droplets were absent from epoxy resin replicas of dehydrated dentin bonded *in vitro* with one-step self-etch adhesives (Chersoni, unpublished results), or from the adhesive-composite interfaces when dentin was replaced with processed composite as a bonding substrate (Tay et al., 2003). TEM results further showed that the permeability associated with these adhesives is not caused by a loss of integrity between the adhesive and dentin, but by the presence of water channels (*i.e.*, water trees) that probably expedite such water movement *via* capillary fluid flow (Tay and Pashley, 2003). Furthermore, the isolated silver grains that were detected throughout the adhesive layer may provide an additional diffusion mechanism for the movement of ions and small molecules across an amorphous polymer matrix based on the free volume theory—*via* a process known as jump

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**Table. Fluid Conductance across Dentin during Different Stages of Application of Self-etch Adhesives**

<table>
<thead>
<tr>
<th>Self-etch Adhesive (N = 5)</th>
<th>% Fluid Flow Induced by 15 cm H$_2$O of Hydrostatic Pressure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Smear-layer-covered Dentin</td>
</tr>
<tr>
<td>One-step</td>
<td></td>
</tr>
<tr>
<td>Adper Prompt</td>
<td>17.3 ± 4.5$^A$1</td>
</tr>
<tr>
<td>Xeno III</td>
<td>12.4 ± 6.2$^A$1</td>
</tr>
<tr>
<td>iBond</td>
<td>15.1 ± 5.6$^A$1</td>
</tr>
<tr>
<td>One-Up Bond F</td>
<td>14.0 ± 3.1$^A$1</td>
</tr>
<tr>
<td>Two-step (control)</td>
<td></td>
</tr>
<tr>
<td>UniFil Bond (control)</td>
<td>18.2 ± 5.0$^A$1</td>
</tr>
</tbody>
</table>

* Values are means ± standard deviation. Results of post hoc multiple-comparison tests are indicated by the superscripts. For each column, groups labeled with the same letter superscripts are not significantly different (P > 0.05). The differences between smear-layer-covered dentin and bonded dentin for each adhesive are indicated by the row results. For each row, groups labeled with the same numeric superscripts are not significantly different (P > 0.05).
diffusion or ion hopping (Dürr et al., 2002). This study confirms the in vitro model, previously proposed by Tay et al. (2002b), that one-step self-etch adhesive behaves as a permeable membrane after polymerization. For the 2 less-permeable adhesives, iBond and One-Up Bond F, in vitro fluid conductance was comparable with that of smear-layer-covered dentin. This may be due to their less aggressive etching effects, that preserve rather than dissolve smear plugs. It is pertinent to note that transudation of dentinal fluid was also observed in vivo for iBond, since this adhesive contains Gluma desensitizer, which is supposed to coagulate plasma proteins (Schüpbach et al., 1997) and form partitions within the dentinal tubules to reduce the dentinal fluid flow (Bergenholtz et al., 1993). The inclusion of cubical/spherical glass fillers in One-Up Bond F or fumed silica fillers in Xeno III did not completely block the paths of water migration through the adhesive, as predicted by the "tortuous path theory" of Nielson (1967). Clinically, since water movement through the polymerized adhesive layer involves slow diffusion rather than rapid fluid transport through the dentinal tubules (Mjör and Ferrari, 2002), it is unlikely that their capability for reducing post-operative sensitivity will be affected. However, the results indicate that the new simplified adhesives do not seal dentin very well. If water and small ions can move across the adhesives, one wonders how large molecules must be before their diffusion is restricted. The potential detrimental effect of increased adhesive permeability associated with one-step self-etch adhesives can be seen in low-viscosity self-etching resin cements that contain activator components to render them compatible with acidic adhesives. For those resin cements that utilize one-step self-etching adhesive components, fluid transudation through the adhesive may result in emulsion polymerization of the resin cement to form resin globules under the influence of water (Mak et al., 2002). Adhesive permeability accounts for the compromised bond strength observed when such resin cements were used for bonding to dentin (Carvalho et al., 2003). Conversely, bonding of indirect restorations was improved when dentin was first bonded with a two-step self-etch adhesive prior to impression-taking (Jayasooriya et al., 2003).

REFERENCES


Figure 1. SEM micrographs of epoxy resin replicas of crown preparations of vital human teeth after being bonded with one-step self-etch adhesives Adper Prompt (A-B) and Xeno III (C-D). (A) A low-magnification view of the surface of the adhesive-coated dentin after being sealed with 2 coats of Adper Prompt according to the manufacturer's instructions. The bulk of the dentin surface is covered with adhesive (A), and there are only small areas in which exposed dentinal tubules are observed (open arrowheads). Transudation of dentinal fluid is not evident from the exposed dentinal tubules. However, in areas coated with the adhesive, swelling of the adhesive can be observed (pointer), with transudation of dentinal fluid droplets from the adhesive surface. (B) Pooling of multiple droplets resulted in the appearance of large water bundles (asterisks) over the adhesive surface. Small discrete dentinal fluid droplets can also be found (arrow). (C) A low-magnification view of an epoxy resin replica of the crown preparation of a vital tooth sealed with Xeno III. The dentin surface is completely coated with adhesive (A), and no exposed dentinal tubules are observed. In isolated regions of the crown preparation that probably correspond with areas of deep dentin, swelling of the adhesive layer can be observed (pointers), together with transudation of dentinal fluid over the surface of the adhesive. (D) A high-magnification view of Fig. 1C showing the presence of dentinal fluid droplets over the adhesive surface. No exposed dentinal tubules can be seen. A large number of small, submicron fluid droplets (arrow) can be seen among the larger droplets.
Figure 2. SEM micrographs of epoxy resin replicas of crown preparations of vital human teeth with intact smear layers (A) and after being bonded with the control two-step self-etch adhesive UniFil Bond (B). (A) Unbondsmed smear-layer-covered dentin (S) showing the transudation of sparse, dentinal fluid droplets trapped by the impression material. (B) An irregular adhesive surface texture is observed after the oxygen inhibition layer was removed in vital deep dentin bonded with the control two-step self-etch adhesive. No transudation of dentinal fluid droplets can be identified.

Figure 3. TEM micrographs of unstained, undemineralized resin-dentin interfaces bonded in vitro with One-Up Bond F (A-C) and iBond (D) under a hydrostatic pressure of 15 cm H2O and further coupled to a resin composite under the same pressure in the dark for 3.5 min before light-activation (to simulate the intra-oral setting time of the impression material). (A) Entrapment of water blisters (pointers) between the adhesive (A) and composite (C) in One-Up Bond F. Silver remnants can be seen along the periphery of some blisters (solid arrowheads), but the majority of the blisters are filled with epoxy resin. A 1-μm-thick, partially demineralized hybrid layer can be seen along the adhesive-dentin interface. D = intertubular dentin. (B) A high-magnification view of the adhesive layer in One-Up Bond F, showing the existence of a water tree (arrow) among the basic glass filler clusters (open arrows). Very fine, isolated silver grains (open arrowhead) are dispersed throughout the entire adhesive layer. (C) The resin-dentin interface in iBond showing the presence of water blisters (pointers) between the adhesive (A) and the composite (C). Remnant silver deposits (open arrowhead) can be identified with the water blisters. Between open arrows = hybrid layer; arrows = water trees; D = intertubular dentin. (D) A high-magnification view of the adhesive-composite interface in iBond, showing the presence of additional water trees (arrows) and isolated silver grains (open arrowhead) within the bulk of the adhesive (A). Water blisters (B) can be found within the microfilled composite (C).
## APPENDIX 1

Compositions and Application Protocols of the Self-etch Adhesives Used in This Study

<table>
<thead>
<tr>
<th>Adhesive</th>
<th>Component</th>
<th>Composition</th>
<th>Application Protocol</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adper Prompt Bider A</td>
<td>Methacrylated phosphoric acid ester, photo-initiator (camphorquinone), stabilizer</td>
<td>Mix bider A and B. Subm. continuously for 1.5 sec and re-apply until glossy surface appears. Dry thoroughly. Re-apply a second coat (no waiting time). Dry thoroughly and light cure.</td>
<td>3M ESPE, St. Paul, MN, USA</td>
<td></td>
</tr>
<tr>
<td>Bider B</td>
<td>Water, complexed fluoride, stabilizer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Xeno III Universal</td>
<td>HEMA*, excess R-947 (terminated resin), BHT (stabilizer), ethanol, water</td>
<td>Mix liquids A and B. Apply mixed adhesive and leave undisturbed for 20 sec.</td>
<td>Dentply DeTrey, Konstanz, Germany</td>
<td></td>
</tr>
<tr>
<td>Catalyst</td>
<td>Pyro-EA-SK, PEMA, UDMA, BHT, camphorquinone, trimethyl-3-me ethyl benzocyclooctene (co-initiator)</td>
<td>Spread adhesive gently for 2 sec until no more flow of the adhesive occurs and light-cure for 10 sec.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>One-Up Bond F Liquid A</td>
<td>Water, HEMA, methyl methacrylate, coumarin dye, Methacryloxyethyl acid phosphate, MAC-10</td>
<td>Mix liquids A and B. Apply mixed adhesive within 1.5 min after mixing. Leave the mixed adhesive on dentin for at least 20 sec, briefly air-dry and light-cure for 10 sec.</td>
<td>Tokuyama Corp., Tokyo, Japan</td>
<td></td>
</tr>
<tr>
<td>Liquid B</td>
<td>Multifunctional methacrylic monomer, Fluorolink moninate glass, Photoinitiator (aryl borate catalyst)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bond</td>
<td>Single bottle, no-mix system</td>
<td>Acetone, water, glutaraldehyde, 4-META</td>
<td>Apply a minimum of 3 consecutive coats of adhesive with no drying in between. Agitate for 30 sec, gently air-dry, and light-cure for 20 sec.</td>
<td>Heraeus Kulzer, Hanau, Germany</td>
</tr>
<tr>
<td>Until Bond Self-etching primer</td>
<td>Water, ethanol, 4-MET, HEMA, UDMA, photoinitiator</td>
<td>Apply primer, leave undisturbed for 20 sec. Apply Bond, light-cure for 10 sec.</td>
<td>GC Corp., Tokyo, Japan</td>
<td></td>
</tr>
<tr>
<td>Bonding resin</td>
<td>HEMA, UDMA, TEGEMA, sterilized fumed silica</td>
<td></td>
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</tbody>
</table>

* Abbreviations: 4-META, 4-methacryloyloxyethyltrimellitic anhydride, 4-MET, 4-methacryloyloxyethyltrimellitic acid, BHT, 2,6-di-tert-butyl-p-cresol, Bio-GMA, (1-methylethylidene)bis(4,1-phenyleneoxymethylene)-2-hydroxy-3,1-propanediol bismethacrylate, HEMA, 2-hydroxyethyl methacrylate, MAC-10, Methacryloyloxydecyl diacrylate acid, PEMA, Penta methacryloyloxyethyl phosphoryl monofluoroborate, Pyro-DMA SK, Tetra methacryloyloxyethyl phosphophosphate, and UDMA, urethane dimethacrylate 1, 4-dimethyl(6-methacryloyloxyethylphosphoryl)monofluoroborate.
Chapter 5

The protection of vital abutments in prosthetic preparations. An in vivo study
Stefano Chersoni, Giovanni Luca Acquaviva, Pietro Suppa, Marco Ferrari, Paolo Ferrieri, Franklin R. Tay and Carlo Prati

SUMMARY
Aim The aim of this study was to evaluate if bonding system use enables to protect in vivo prosthetic crown preparations. This procedure is potentially able to seal exposed dentin so that irritating agents can’t reach the pulpal tissue. Materials and methods 18 teeth without carious lesions that needed prosthetic treatment were selected for this study. Two bonding agents were used: a self-etching adhesive (Clearfil SE BOND) and a total-etching adhesive (Prime & Bond NT). An evaluation was carried out with a replica system: polyvinylsiloxane
impressions for prosthetic crown preparation were taken after the application of the bonding agent and at one and six months after the first application. Polyether replicas were obtained using the silicon impression as a mould. Replicas were then observed under the SEM.

**Results** Fluid movement through the adhesive layer was morphologically put in evidence by bubble formation on crown preparation surface. Time tends to increase this phenomenon.

**Conclusions** The application of the bonding agent can’t protect exposed dentin and irritating agents can reach the pulpal tissue because of a permeability phenomenon. Permeability can be responsible for dentinal post-surgical sensitivity. In addition fluid movement through bonding agents is able to prevent adhesive cementation procedures.

**KEY WORDS**

bonding systems, permeability

An important objective in prosthetic dentistry is to obtain marginal closure guaranteed by the presence of enamel. The use of temporary cements, especially in teeth having a reduced amount of hard tissues, could lead to a microleakage of bacteria and related products1. An alternative approach to preserve the health of the pulpal-dentinal complex consists in sealing the exposed dentin by using resin bonding systems before taking impressions2. The studies on nanoleakage by means of silver nitrate demonstrated the presence of areas that have a large amount of water and/or hydrophilic monomers in the hybrid layer and in the bonding resin layer. These areas were shown by Transmission Electron Microscopy and defined as water trees3. Water trees are real miniature channels that are considered responsible for the passage of fluids from the underlying hydrated dentin, through the bonding layer all the way to the interface with the composite3-5. The extent of nanoleakage increases with time in relation to water absorption6. There are up to now no studies in literature that evaluate, *in vivo* and over time, permeability of dentinal bonding systems applied to exposed dentin. The aim of the present study is to evaluate the passage of fluids through bonding systems that have been applied to protect prosthetic abutment preparations at three different times: immediately after application and after 1 and 6 months.

**MATERIALS AND METHODS**

18 vital teeth without carious lesions or fractures needing prosthetic treatment were selected for the study. The patients had an average age of 48 years. To avoid any modification of pulp pressure an anaesthetic without vasoconstrictor was used: carbocaine 3% (Astra, Sweden). The teeth were carefully isolated by means of a rubber dam and preparatory work was carried out by means of a turbo drill and medium grit Intensiv diamond burs, in order to obtain prosthetic abutments. The teeth were divided in 3 groups: I, II and III. To the teeth belonging to Group I, the Clearfil SE BOND (Kuraray, Japan) adhesive was applied. To the teeth belonging to Group II, the Prime & Bond NT (Dentsply, Germany) adhesive was applied. The remaining teeth were included in control Group III, and did not receive any type of treatment. Each group was
made up of 6 teeth. All the material utilized was applied according to manufacturers’ instructions. A unit that had been previously tested was used to cure the adhesive, with an intensity not lower than 600 mW/cm² (Visilux Command 2, 3M, MN, USA). The adhesive layer, inhibited by oxygen, was removed by means of cotton pellets dipped in acetone. The surface of the abutments was thoroughly dried with compressed air for 10 seconds. We took the first impression immediately after application of the adhesive. The samples were covered with impression material, a polyvinylsiloxane-based material (Affinis light body, COLTENE, Switzerland). After six minutes the material was removed from the teeth. The temporary crowns were temporarily cemented with Temp Bond NE (Kerr, USA). The operating procedure did not permit to perform direct relining of the temporaries. One month later, after being isolated with a rubber dam, the abutments were thoroughly cleansed with cotton pellets and acetone and a second impression was taken. Six months later, before finally cementing the crowns, a further impression was taken following the same procedure as before. Replicas were obtained by taking an impression with a polyether material (Permadyne Garant, 3M ESPE, USA) of the impressions that had been taken previously. The polyvinylsiloxane-based impressions were poured after at least 48 hours to avoid the release of gas by the material. The replicas were cast with gold and observed at SEM (JEOL, Model 5400, Japan) with enlargements of X1000 and X2000. The replica procedure agrees with the findings described in a previous study.

RESULTS

SEM analysis of the replicas showed the presence of bubbles in all the samples observed. Bubbles represent a morphological expression of the presence of fluid that is imprinted by hydrophobic silicone. The abutments that had just been prepared and covered by a smear layer showed the presence of some bubbles of fluid (figure 1). The presence of dentinal fluids is extremely evident in the replica made one month after tooth preparation (figure 2) and has a similar morphology after six months (figure 3). The replicas of the preparations of the teeth belonging to Group I displayed the presence of fluids and therefore, of bubbles covering vast areas of the bonding surface observed (figure 4). Some areas were perfectly sealed and did not present bubbles. The replicas that were made after one and six months showed a progressive increase in the number and dimensions of the bubbles, as set out in figures 5 and 6. The replicas of the preparations of the teeth belonging to Group II displayed a greater initial permeability (figure 7) than the teeth in Group I. Moreover, replicas made after one (figure 8) and six months (figure 9) were being completely covered with bubbles.

DISCUSSION

Recent studies showed that bonding systems are able to guarantee a partial seal at dentinal level. The present study proves in vivo that the dentinal bonding systems that were tested are permeable to fluids and that this permeability progressively tends to increase over time. The study confirms in vivo that the smear layer is only able to maintain complete dentin permeability for a limited period of time. Smear layers and smear plugs are able to increase resistance to the passage of fluids by 86% of the maximum permeability found in deep dentin. The modern bonding systems that were considered useful to protect exposed dentin cannot provide an appropriate seal; on the contrary, they demonstrate permeability that
progressively increases with time. Recent studies have demonstrated that bonding systems behave as semi-permeable membranes. This permeability is potentially able to interfere with the hydrophobic cement and the bonding itself, creating an interface with reduced bonding retention capacity. Resin cements applied on a substratum that allows the passage of fluids may be subject to an emulsion phenomenon during polymerisation procedures, with formation of resin globules due to the presence of water. Self-curing cements with a long polymerisation time are undoubtedly more susceptible to this problem. The formation of micro-areas in which adhesion is not obtained potentially leads to create areas that can be colonised by bacteria, which are in contact with the underlying dentin that is not protected by a covering layer of adhesive. This situation could determine direct communication between oral environment and dentin through the resin in the bonding system. Bonding system permeability allows water absorption by resins and, furthermore, it facilitates the spread of hydrolytic enzymes. All this is potentially able to determine a progressive deterioration of the bonding system, which is well known in literature. Bonding system permeability can be correlated to the presence of post-surgical tooth sensitivity, in accordance with the hydrodynamic theory. The self-etching system has demonstrated less immediate permeability and this is in accordance with a study that assumes an incomplete removal of the smear plugs and of the hydroxyapatite that lines the collagen fibres, which results from the use of the system. Self-etching systems having a less aggressive etching action seem able to provide reduced initial permeability, which in any event tends to increase progressively with time.

The present study provides morphological evidence showing, in vivo, the presence of a phenomenon that is well known in vitro. This type of method is not able to provide a quantitative assessment, but has the advantage of allowing repeated evaluations on the same substratum and in standardisable conditions. The evolution of new bonding systems must address the reduction of the permeability problem, to provide the clinician with bonding systems that are reliable and long lasting.

CONCLUSIONS
The results of the present study enable to state that bonding systems do not allow to re-establish a complete seal of the exposed dentin. Furthermore, bonding system permeability tends to increase progressively with time. Clinical bonding procedures with self-curing or dual-curing systems, in absence of light curing, must be considered situations with a very high risk of failure.
1. Scanning electron microscopy image of the replica of a tooth prepared with diamond burrs; the presence can be noted of some bubbles of dentinal fluid that are due to the permeability connected with the smear layer.

2. Scanning electron microscopy image of the replica of a tooth prepared with diamond burrs after one month. Notice that the entire surface is covered with bubbles of dentinal fluid.
3. Scanning electron microscopy image of the replica of a tooth prepared with diamond burrs after six months, where the surface is completely covered with dentinal fluid.

4. Scanning electron microscopy image of the replica of a tooth prepared with diamond burrs and protected with the self-etch bonding system Clearfil SE Bond. Notice the presence of some bubbles on the surface.
5. Scanning electron microscopy image of the replica of a tooth prepared with diamond burs and protected with the self-etch bonding system Clearfil SE Bond. One month after application, the presence of fluids increases.

6. Scanning electron microscopy image of the replica of a tooth prepared with diamond burs and protected with a self-etch bonding system Clearfil SE Bond at six months from the application. It may be clearly seen that a large part of the surface is thickly covered with bubbles.
7. Scanning electron microscopy image of the replica of a tooth prepared with diamond burrs and protected with a total-etch bonding system Prime & Bond NT. Notice the presence of large bubbles of dentinal fluid.

8. Scanning electron microscopy image of the replica of a tooth prepared with diamond burrs and protected with a total-etch bonding system Prime & Bond NT. After one month it is clear that a large part of the surface is permeable to dentinal fluid.
Chapter 6

In vivo Fluid Movement through Dentin Adhesives in Endodontically Treated Teeth


ABSTRACT

Fluid transudation through simplified dentin adhesives can occur in bonded vital crown dentin, since these adhesives behave as permeable membranes after polymerization. The effect of adhesive permeability in endodontically treated teeth is unknown. This study examined the hypothesis that in vivo fluid movement through simplified adhesives occurs when they are applied to root canals. Dowel spaces were prepared in endodontically treated teeth with single root canals. Six adhesives were applied to the intraradicular dentin of canal walls. Impressions were obtained with polyvinyl siloxane, and replicas were fabricated with the use of polyether impression material. Replica hemisections were gold-coated for SEM examination. Fluid transudation was evident on the adhesive surfaces of all simplified total-etch and self-etch adhesives. Conversely, most of the specimens bonded with the control three-step total-etch adhesive were devoid of fluid droplets. Permeability of simplified adhesives results in water movement, even in root-treated dentin. This may adversely affect the coupling of auto-/dual-cured resin cements.
INTRODUCTION

The development of dentin adhesives has reached a point where a genuine technological breakthrough cannot be expected without the input of paradigms from other scientific disciplines (Tay and Pashley, 2002). As existing bonding strategies are modified to create adhesives that are simpler and faster (Perdigão et al., 2003), compromises have to be made when these adhesives are formulated with a reduced number of bonding steps (Van Meerbeek et al., 2003). An immediate consequence of adhesive simplification is a sacrifice of the universality of the multi-bottle adhesives, with most of the simplified versions capable of bonding only to light-cured composites (Sanares et al., 2001; Pfeifer et al., 2003). Although the adhesion of auto-/dual-cured composites may be improved with the adjunctive use of tertiary catalysts that offset the acid-base incompatibility between acidic methacrylate monomers and tertiary amines (Suh et al., 2003), the bonding efficacy of both simplified total-etch (Tay et al., 2003b) and simplified self-etch adhesives (Tay et al., 2003a) to auto-/dual-cured composites/resin cements is hampered by the intrinsic permeability of these adhesives to the water that results from their increase in hydrophilicity (Carvalho et al., 2004; Chersoni et al., 2004a). Transudation of dentin fluid through simplified dentin adhesives has been shown to occur in vivo in bonded vital crown dentin (Chersoni et al., 2004b; Tay et al., 2004), since these adhesives behave as permeable membranes after polymerization (Tay et al., 2002a). This may result in the entrapment of water blisters between the adhesive surface and slow-setting resin composites/cements. The water blisters may act as stress raisers and account for the apparent incompatibility of these adhesives with the bonding of indirect restorations, even in the absence of the true adhesive incompatibility derived from adverse acid-base reactions (Tay et al., 2003b). Although a positive pulpal pressure is absent in endodontically treated teeth, an increase in radicular permeability may follow reduction in root dentin thickness and removal of sealers that penetrated the dentinal tubules during the preparation of spaces for the cementation of endodontic posts (Fogel et al., 1988; Guignes et al., 1996). This is particularly so when total-etch adhesives and the aggressive type of self-etch adhesive (Tay and Pashley, 2001), that completely remove or dissolve the smear layers, are used for bonding to root canals (Tao et al., 1991). Since vital teeth and endodontically treated teeth do not differ significantly in their moisture content (Papa et al., 1994), the effect of adhesive permeability on bonding to root canals with conventional or simplified total-etch and self-etch adhesives is unknown. Thus, the objective of this in vivo study was to examine, by a novel impression replica technique (Chersoni et al., 2004), the permeability of adhesive-bonded intra-radicular dentin to fluid movement. The null hypothesis tested was that there is no difference among the 4 different classes of dentin adhesives (i.e., three-step total-etch, two-step total-etch, two-step self-etch, and one-step self-etch) in preventing fluid movement across bonded intra-radicular dentin.

MATERIALS & METHODS

Twenty-eight endodontically treated teeth with single root canals that required dowel retention as part of the restorative treatment plan were selected for the study. The root canals were previously obturated with vertically compacted warm gutta-percha and a root canal sealer (CRCS, Coltén AG, Altstätten, Switzerland). The patients were between 26 and 47 yrs of age,
with a mean age of 36. The subjects' informed consent was obtained under an *in vivo* protocol reviewed and approved by an ethics committee from the University of Bologna. Preparation of dowel spaces and the subsequent bonding procedures were performed with the use of rubber dam isolation and an endodontic microscope (Karl Kaps, base model, Asslar, Wetzlar, Germany). The coronal part of each root filling was removed, with a root canal filling of 5-6 mm remaining, and a post space was created for the insertion of a size 2 DT Light-Post (RTD, Grenoble, France), a radiopaque glass fiber post, by use of the corresponding drills. The residual thickness of remaining dentin around the dowel space of each tooth was evaluated radiographically to be at least 1.5 mm. Debridement was performed with distilled water under the microscope to ensure that the post space was free of cutting debris, and that the coronal portion of the remaining gutta-percha could be identified from the base of the post space. Prior to the bonding procedure, each post space was air-dried with a triple syringe equipped with a Stropko irrigator (SybronEndo, Orange, CA, USA) and further dried with 4 paper points to ensure that the post space was free from residual moisture (Hosoya *et al.*, 2000).

**Experimental Design**

Six dentin adhesives were randomly selected for application to 4 teeth in each experimental or control group. They included: All- Bond 2 (Bisco Inc., Schaumburg, IL, USA), a conventional three-step total-etch adhesive that was used as the control; Single Bond (3M ESPE, St. Paul, MN, USA) and One-Step Plus (Bisco), as representatives of simplified two-step total-etch adhesives; Tyrian SPE/One-Step Plus (Bisco), a two-step self-etch adhesive; and Xeno III (Dentsply DeTrey, Konstanz, Germany) and One-Up Bond F (Tokuyama Corp., Tokyo, Japan), examples of simplified one-step self-etch adhesives. Since the experimental protocol required a polymerized adhesive surface for impression-taking, the uncured Pre-Bond resin in All-Bond 2, that was to be placed over the primed dentin, was replaced with a mixture of Pre-Bond and D/E resin, so that a curable adhesive layer could be obtained (B.I. Suh, personal communication). For the 3 total-etch adhesives, the post spaces were blot-dried with paper points after the phosphoric acid was rinsed, to provide a moist substrate for the wet-bonding technique. Although the one-step self-etch adhesives were not recommended for the coupling of auto-cured composites, they were used with the understanding that the incompatibilities associated with these 2 adhesives were apparent in nature, and were attributed to the permeability of the adhesives instead of to adverse acid-base interactions (Tay *et al.*, 2003a). To ensure that subsequent restorative treatments were performed in the best interest of the subjects, we placed an additional thin coat of Scotchbond Multi- Purpose bonding resin (3M ESPE) over these adhesives and lightcured them after the experimental impression-taking protocol. This additional step converts these adhesives from one-step to two-step self-etch adhesives, making certain that they are compatible with auto-/dual-cured resin cements. To ensure optimal curing, we applied each adhesive to the post spaces with a microbrush, and light-cured it via a slightly undersized, non-bonding, light-transmitting plastic post (Luminex, Dentatus AB, Hägersten, Sweden), that was inserted into the post space and transilluminated from the top by means of a LED lightcuring unit (L.E. Demetron 1, Sybron-Kerr, Orange, CA, USA) for 40 sec at an output of 800 mW/cm2. Since the uncured adhesive caused by oxygen inhibition must be removed prior to impressiontaking, a second coat of the adhesive was applied. Removal of the adhesive oxygen inhibition layer was performed by means of acetone
and a microbrush. The bonded post space was air-dried for 10 sec by insertion of the Stropko irrigator into the root canal. An impression was immediately taken with a polyvinyl siloxane impression material (Affinis light-body, Colténe AG). The material was injected into the post space and distributed by means of a spiral, followed by the insertion of the previously used plastic post as an anchor. After 4 min, the polymerized impression material was removed and degassed for 24 hrs. Positive replicas were then fabricated with a polyether impression material (Permadyne Garant, 3M ESPE), with the polyvinyl siloxane negative replica used as a mold, according to the polyether replica technique reported by Chersoni et al. (2004a). Since there is no chemical reaction between polyether and polyvinyl siloxane, this replica technique has been shown to be effective in replicating water exudation from dentin hybrid layers. The polyether replicas were cut into hemisections by means of a sharp razor blade, coated with gold/palladium, and examined under a scanning electron microscope (SEM; Model 5400, JEOL, Tokyo, Japan) at 5-10 kV. Five SEM images of each of the 4 post spaces in each group were taken randomly from different areas of the replicas (N = 20). The number of fluid droplets/1000 μm² of polymerized adhesive was recorded by two co-authors who did not participate in bonding and were unaware of the group designations. Data from the 6 groups were statistically analyzed by Kruskal-Wallis ANOVA and Dunn's multiple comparison tests at α = 0.05.

Controls
The remaining 4 dowel spaces were not bonded with adhesives and were used as controls. For 2 of these dowel spaces, impressions were taken of the smear-layer-covered intra-radicular dentin after the cutting debris was rinsed off and the dentin dried with paper points. For the other 2 dowel spaces, impressions were taken of the intra-radicular dentin after being acid-etched with 32% phosphoric acid (Uni-etch, Bisco) for 15 sec, then rinsed and dried with paper points. Polyvinyl siloxane replicas were prepared and examined as previously described.

RESULTS
Fluid droplets could be identified from the control unbonded dowel spaces with intact smear layers (Fig. 1A) and after acidetching (Fig. 1B). All-Bond 2, the control three-step total-etch adhesive, exhibited no fluid transudation across the polymerized adhesive in 3 of the specimens examined (Fig. 1C), and minimal fluid droplets were observed only in the fourth specimen (Fig. 1D). By contrast, ample fluid droplets were seen along the polymerized adhesive surfaces in the simplified two-step total-etch adhesives Single Bond (Fig. 2A) and One-Step Plus (Fig. 2B). Fluid droplets along the adhesive surface were also observed in the intra-radicular dentin bonded with the non-rinsing, two-step self-etch adhesive Tyrian SPE/One-Step Plus (Fig. 3A). Similarly, fluid droplets were seen in specimens bonded with the simplified onestep self-etch adhesives Xeno III (Fig. 3B) and One-Up Bond F (Fig. 3C). Based on morphologic examination, the sizes of the fluid droplets were smaller in the selfetch adhesives. Data on the distribution of fluid droplets are summarized in the Table. All Bond 2 exhibited significantly fewer fluid droplets than the other 5 experimental groups (P < 0.05). There were no differences in the numbers of fluid droplets/1000 μm² among the other 5 groups (Fig. 3D).

DISCUSSION
The results of this in vivo study warrant a rejection of the null hypothesis that there is no difference among the different classes of dentin adhesives in preventing fluid movement across bonded intra-radicular dentin. The observation of fluid droplets in bonded root-treated dentin was similar to our previous observations of dentinal fluid transudation from vital bonded deep dentin (Chersoni et al., 2004b; Tay et al., 2004), although, in the latter, there was a propensity for more profuse fluid droplets to be formed. Since root canal sealers can penetrate only 35-80 mm into the dentinal tubules, even with complete smear layer removal (Kouvas et al., 1998), residual dentinal tubules are likely to be devoid of sealers when dentin thickness is reduced after post space preparation. Rinsing with water during the course of a totaletch technique probably results in the retention of substantial amounts of water within the widened tubular entrance created by acid-etching, that may not be completely removed via the use of paper points. This may account for the occurrence of larger fluid droplets in the simplified total-etch adhesives (Figs.2A, 2B). With the use of self-etch adhesives, we speculated that water could have been partially derived from the residual water that is retained in these water-containing adhesives (Tay et al., 2002b), and partially from the unbound water that is present in pulpless teeth (Helfer et al., 1972; Papa et al., 1994). Since the smear plugs were not removed with the use of these self-etch adhesives (Tay and Pashley, 2001), this could have resulted in the appearance of smaller fluid droplets (Figs. 3C, 3D). In this study, we have refrained from recording the actual dimensions of these fluid droplets, due to the inaccuracy involved with interpretation of micrographs that were taken at different specimen tilts. The volume of fluid that permeates these adhesive in intra-radicular dentin should be quantified in the future by means of fluid filtration protocols (Fogel et al., 1988; Abramovitz et al., 2001) or electrical impedance methods (Momoi et al., 2003). Previous fluid transport studies reported the presence of an inferior seal along the coronal two-thirds of the root-filled canals after post space preparation, when compared with intact root fillings (Abramovitz et al., 2001; Wu et al., 2003). Presumably, the use of dentin adhesives would improve the seal of this critical region, minimize coronal leakage, and improve the prognosis of endodontic therapy. However, the in vivo observation of fluid transudation through simplified adhesives in endodontically treated teeth suggests that a hermetic seal of the coronal third of root canals is unlikely to be achieved with the use of either simplified total-etch or self-etch adhesives. Theoretically, the use of a two-step self-etch adhesive should minimize water movement through the adhesive (Carvalho et al., 2004). However, the system we examined utilizes a fairly permeable adhesive (One-Step Plus; Fig. 2B) over the intraradicular dentin that was treated with the self-priming etchant Tyrian SPE. Nguyen et al. (1996) investigated the permeation of water through multiple layers of organic resin coatings. They observed that increasing the number of coats of a hydrophilic resin affected only the time required for water to move from the outside to the inside of the coatings, but did not reduce the permeability of the coatings. It is only when a polymer exhibits a notable change in diffusion coefficient that the permeability of the coatings can be substantially reduced. This could have accounted for the permeability observed for Tyrian SPE/One-Step Plus. The permeability of other two-step self-etch adhesives in endodontically treated teeth should be further examined. Although the use of bonded posts has been clinically successful, a recent SEM study of endodontically treated teeth extracted after clinical service reported that subclinical gap formation and adhesive failures occurred along the resindentin
interfaces, in spite of the absence of clinical failure (Mannocci et al., 2003). Unfavorable cavity configuration factors in post spaces have been cited as the major reason for low bond strengths and marginal gap formation (Morris et al., 2001; Bouillaguet et al., 2003). Slow-setting resin cements have been recommended to offset the development of high polymerization shrinkage stresses along root canal walls (Goracci et al., 2004). The extended setting time for these resin cements, which is perceived to be beneficial in reducing shrinkage stresses, may be offset by the use of simplified total-etch or self-etch adhesives, since this prolongs the time for water diffusion through the adhesives, creating fluid blisters along the adhesive-cement interface that act as stress-raisers and precipitate bond failure (Carvalho et al., 2004). In the event that coronal leakage occurs during functional stresses, leaching of resin components from these highly hydrophilic adhesives may further contribute to the degradation of the bonds between the adhesive and intra-radicular dentin. Although simplified acidic adhesives may be rendered compatible with auto-/dual-cured resin cements via the use of ternary catalysts (Suh et al., 2003), they must be used with caution for bonding to root canals, in light of the fluid movement that occurs through these adhesives under in vivo conditions. The use of conventional, less permeable adhesives appears to be a more rational alternative.

REFERENCES


TABLE  Number of fluid droplets that appeared on the surface of the bonded intra-radicular dentin of the root canals in the six adhesives

<table>
<thead>
<tr>
<th>Dentin adhesive</th>
<th>Classification</th>
<th>Number of fluid droplets per 1000 µm² of adhesive surface (N=20) *</th>
</tr>
</thead>
<tbody>
<tr>
<td>All-Bond 2</td>
<td>3-step total-etch</td>
<td>1.1 ± 2.2 ^a</td>
</tr>
<tr>
<td>Single Bond</td>
<td>2-step total-etch</td>
<td>238.0 ± 92.5 ^b</td>
</tr>
<tr>
<td>One-Step Plus</td>
<td>2-step total-etch</td>
<td>176.4 ± 98.6 ^b</td>
</tr>
<tr>
<td>Tyrian SPE/One-Step Plus</td>
<td>2-step self-etch</td>
<td>196.7 ± 146.3 ^b</td>
</tr>
<tr>
<td>Xeno III</td>
<td>1-step self-etch</td>
<td>225.8 ± 197.6 ^b</td>
</tr>
<tr>
<td>One-Up Bond F</td>
<td>1-step self-etch</td>
<td>240.4 ± 293.0 ^b</td>
</tr>
</tbody>
</table>

* Values are means ± standard deviation. Groups with the same letter superscript are not statistically significant (P>0.05)
Figure 1. SEM micrographs of replicas of post spaces in endodontically treated teeth. (A) An unbonded post space with smear layer intact, showing the transudation of water droplets through the smear layer (open arrowheads). (B) An unbonded post space after acid-etching, showing the presence of water droplets over the tubular orifices (arrows). (C,D) Post spaces bonded with All-Bond 2, a three-step total etch adhesive, in preparation for cementation of fiber posts. (C) A representative example of the bonded root dentin that was completely devoid of fluid movement through the adhesive. (D) A representative example of the root dentin in which there was minimal fluid movement through the adhesive, resulting in the presence of discrete fluid droplets (pointers) along the adhesive surface. These water droplets were trapped during the setting of the polyvinyl siloxane impression material.
In Vivo Reduction of Permeability by oxalate desensitizer

Introduction

Simplified dentin adhesives were previously reported to be permeable to fluid movement after application and polymerization on dentin. Such a phenomenon has been attributed to the
increased hydrophilicity of these adhesives and to the lack of a more hydrophobic adhesive coating. This problem may affect the coupling efficacy of composites especially for auto-care composites because a lot of water may flow at the interface before complete polymerization. In post cementation procedures the phenomenon of water flowing is emphasized for the incomplete polymerization of the adhesive. This study tested the hypothesis that BisBlock (BISCO) application can reduce permeability of simplified dentin adhesives during the in vivo application of the adhesive for composite restoration and post cementation procedures.

Materials and Methods

20 cavities were prepared on vital human teeth under local anaesthesia (Mepivacaine hydrochloride 2% with adrenaline 1/100,000) and rubber dam isolation. 5 cavities were bonded with either Scotchbond 1 (3M-ESPE) or One Step Plus (BISCO) following manufacturers' instructions (Group A). In the remaining 10 cavities, Bisblock, a potassium oxalate gel, was applied to the acid-etched dentin for 30 s before adhesive placement (Group B). Additional post spaces were prepared in vivo on endodontically-treated teeth with single root canal. Half of teeth (Group C) were bonded with One-step Plus (Bisco) and the others (group D) received Bisblock application before adhesive. After removal of the oxygen inhibited layer and air-drying of the bonded root/coronal dentin, an impression was immediately obtained by polyvinyl-siloxane (Affinis light, Coltene) following manufacturer’s instructions. Replicas were obtained by polyether impression material (Permadyne Garant, 3M-ESPE). After 24h, replicas were gold coated and observed under SEM.

Results

Replica specimens of both simplified adhesives surface showed fluid droplets formation in composite cavities (Figs. 1b, 2b, 3b) and post spaces (Fig. 4b). When oxalate gel was applied on composite cavities, replica specimens of adhesive surfaces were devoid of dentinal fluid droplets. Small droplets were detected only in deep dentin. Water flowing reduction was observed also in root dentin but any zones were covered by small droplets.

Conclusions

Droplets are morphological evidence of the presence of fluid impressed during the setting time of the hydrophobic polyvinyl siloxane. 30 s application of BisBlock before the use of these simplified adhesive systems reduces fluid movements across the bonded vital and non-vital dentin reducing the morphological evidence of water droplets. Water flowing probably affect also long term stability of interfaces. Dentin permeability reduction obtain by oxalate salts application may be the main reason for the observed
Chapter 8

**Why do we use 35% phosphoric acid on dentin?**

Objective: This study aims to correlate the etching pH with dentin permeability, microtensile bond strength values and nanoleakage pattern of a total etch adhesive. Methods: Twenty-six extracted teeth were transversally sectioned in order to expose superficial/middle dentin. Each specimen was divided in two symmetric halves (A & B) to obtain similar bonding substrate. Specimens were divided in two groups and submitted to two different etching procedures. Group 1: dentin surface (A) (N=8) was etched with 35% (pH 0,65) phosphoric acid (PA) for 15 s; Group 2: dentin surface (B) (N=8) was etched with 0,32% PA (pH 1,8) for 15s. Both halves were bonded with One Step (BISCO). Specimens were submitted to standard procedures for microtensile bond strength evaluation. Six teeth were used for bonded dentin fluid conductance evaluation. Epoxy resin replicas were obtained from bonded dentin surface during the permeability evaluation and observed under SEM. Four teeth were used for the FEI-SEM nanoleakege evaluation of bonded dentin obtained with the different etching procedures. Results: Means of the microtensile bond strength values (group 1: 51,8± 10,4 MPa and group 2: 48,4± 9,7 MPa) did not show statistically significant differences between groups. The fluid conductance of adhesive-bonded dentin was statistically higher in dentin bonded after strong etching [22,8±2,3 vs 35,86±2,3 (P<0.001)]. Water droplets were observed in epoxy resin replicas of boot groups but they were reduced in number and dimension with mild etching. FEI-SEM nanoleakage evaluation revealed less silver depositions in mild etched dentin. Conclusion: The results of this study suggest that the bonding efficacy of the tested adhesive is not negatively influenced by reduction of etching pH. Probably the reduced dentin permeability counterbalances the reduction of monomers penetration. The lower collagen network exposure,
that determines better infiltration and lower matrix degradation, could also improve the long term performances of the restoration.

**Summary general discussion,**

It was possible to valuate permeability in dentistry through mould technique. In particular in vivo information was possible because this technique is not invasive and has no contraindications. This type of method is not able to provide a quantitative assessment, but has the advantage of allowing repeated evaluations on the same substratum and in standardisable conditions. The first part of the present thesis demonstrated that hybrid layer seems to behave in a sponge-like manner, adsorbing and releasing water. The formation of water droplets may depend on the chemical composition of hybrid layer, since removal of the collagen fibrils by NaOCl solution was shown to prevent water droplets formation.

Permeability is evident also on marginal hybrid layer and through bonding systems applied on root dentin surfaces.

Clinically, the passage of water and its diffusion can justify post-operative sensitivity, marginal discoloration and hybrid layer degradation. The marginal permeability is a window for uptake of saliva, acid solutions, bacteria enzymes.

However, the main problem is the possible degradation of the collagen that is infiltrated but not protected by resin. The results indicate that new simplified hydrophilic adhesives are the most permeable compared with hydrophobic bonding resin systems.

Permeability was observed not only in the hybrid layer thickness but also through cured bonding agent. Simplified and hydrophilic bonding systems are not able to retain dentinal fluid. This systems have to be used with particular attention in deep dentin where dentinal fluid permeability is higher (as in buccal deep dentin, the most permeable area of each cavity (Pashley 1990 J. Endod). This fact is particularly important when high setting time restoration materials are used. In fact cement or composite osmotically attract fluid until luting reaction is not complete. Even a photo curing material not activated in the right time may recall a sufficient quantity of fluid to interfere with adhesion process.

Dentinal fluid flow through cured adhesive systems was also demonstrated in vivo in endodontically treated teeth. So during adhesive procedures of cementation of posts, dentinal fluid may interfere with adhesion, in particular when the system is not photo cured.

Finally fluid flow reduction by oxalates application was evaluated. This process is able to reduce dentinal fluid movement after etchant and before adhesive system application if used in combination with adhesive systems with no fluoride in their composition (yu et al 2005).

In addition pH reduction of etchant is able to reduce fluid flow problem because dentinal permeability is not increased like in a traditional total-etch technique.

**Clinical conclusions:**

Simplified adhesive systems have very high micro-mechanic dentinal adhesion values but cannot guarantee a perfect seal because of their permeability to fluid, even if cured.

Fluid flow is present even when adhesive system is applied in endodontically treated teeth (so in absence of pulpal pressure).

Fluid flow is influenced by osmotic pressure of restoration or cementation materials applied on adhesive system. A fundamental fact in this process is curing time. Auto-cured systems are highly exposed to fluid blister formation risk in adhesive interface (between adhesive system and auto-curing cement). Even adhesive systems with an hydrophobic bonding that are able to prevent fluid flow present
permeability phenomenon in hybrid layer. Oxalates application after etching procedure is able to reduce permeability of compatible adhesive systems. Mild self etching two steps adhesive systems present the lowest permeability because low acid primer are not able to increase dentinal permeability as with total etch technique or with primer with a pH lower than 1.4. Simplification of adhesive systems has increased dentinal permeability that is potentially able to affect short and medium term clinical result. In other word “simpler and faster” in adhesion is not a reality at the moment. Surely the best treatment for enamel includes orthophosphoric acid etchant at high concentration. On the contrary in vital teeth dentin without carious lesions, correct sealing without oxalate application is impossible with a total-etch technique. A good alternative is represented by mild-self-etch two-steps systems that present a good agreement on the enamel and permit an adequate seal in the dentin because of the low increase of dentinal permeability. Recently a paper demonstrated improved durability in vitro when EDTA was applied instead of phosphoric acid (Osorio R. et al. J. Dent. Res 84:736-40.2005) Futermore a recent revieuw of clinical trials (Peumans M. et al Dent. Mater. 2005 sep;21(9):864-81) concluded that there is a tendency towards adhesives with simplified application procedures. Simplification so far appears to induce loss of effectiveness.

Future directions

Adhesive systems development has to keep in consideration the possible clinical life and so a perfect seal between dental structure and polymer has to be searched. Optimal balance can be achieved by avoiding to increase dentinal permeability or by reducing it like with the application of oxalates. Adhesive system has to complete the seal and so it cannot contain high quantity of hydrophilic monomers in its composition. Monomers that change hydrophilic properties after curing are very interesting. Finally a further development consists in fixing hybrid layer that is subjected at cycling load in humid environmental.

Sommario, Discussione generale

Mediante la tecnica a replica è stato possibile valutare la permeabilità in campo odontoiatrico ed in particolare è stato possibile ottenere informazioni oltre che in vitro anche in vivo invero la tecnica non è invasiva ed è priva di controindicazioni ed effetti collaterali. Tale metodica non è in grado di fornire una valutazione quantitativa ma ha il vantaggio di poter effettuare ripetute stime sullo stesso substrato ed in condizioni standardizzabili. La prima parte della tesi ha dimostrato che lo strato ibrido sembra comportarsi come una spugna assorbendo e rilasciando acqua. La formazione delle gocce d’acqua dipende dalla composizione chimica dello strato ibrido, in quanto la rimozione delle fibre collagene mediante NaOCl è in grado di prevenire la presenza delle gocce d’acqua sulle repliche.

La Permeabilità è evidente anche a livello dello strato ibrido marginale ed attraverso sistemi adesivi applicati sulla dentina radicolare. Clinicamente il passaggio di acqua e la diffusione può giustificare la sensibilità post operatoria, disicolorazione marginale e il degrado dello strato ibrido.
La permeabilità marginale è la porta di ingresso per l’entrata di saliva, acidi, batteri ed enzimi. Comunque il problema principale è rappresentato dal possibile degrado del collagene infiltrato dalla resina ma non protetto da quest’ultima.

I risultati indicano che i nuovi sistemi adesivi semplificati ad elevata idrofilicità sono molto più permeabili se confrontati con resine costituite da bonding idrofobici.

La permeabilità è stata osservata non solo nello spessore dello strato ibrido ma anche attraverso il sistema adesivo polimerizzato. I sistemi adesivi semplificati ed idrofili non sono in grado di trattenere il fluido proveniente dalla dentina sottostante. I sistemi semplificati devono essere utilizzati con particolare attenzione in dentina profonda dove la permeabilità del fluido dentinale è particolarmente elevata. Come nella dentina vestibolare profonda, l’area più permeabile di ogni cavità (Pashley 1990, j. Endod). Tutto ciò interferisce specialmente quando si posizionano sistemi autopolimerizzanti con setting time elevato. Infatti il cemento o il composito richiamano osmoticamente fluido fintanto che la reazione di polimerizzazione non è avvenuta. Pertanto anche un sistema fotopolimerizzante applicato e non fotoattivato in tempi adeguati può richiamare sufficiente fluido da interferire progressivamente con l’adesione fino ad annullarla completamente.

Il passaggio di fluido dentinale attraverso i sistemi adesivi polimerizzati è stato dimostrato in vivo anche in denti trattati endodonticamente e quindi plausibile che durante il posizionamento di perni cementati con tecniche adesive il fluido possa interferire con l’adesione specialmente quando il sistema adesivo non viene fotopolimerizzato ma richiede una autoattivazione.

Infine è stato valutato il grado di riduzione del passaggio di fluido mediante applicazione di ossalati dopo la mordenzatura e prima dell’applicazione del sistema adesivo tale procedura è in grado di ridurre in gran parte il movimento di fluido dentinale se usato in combinazione con sistemi adesivi che non contengono fluoro nella loro composizione (yu et al 2005).

Infine la riduzione del pH del mordenzante è sicuramente in grado di ridurre il problema legato al passaggio di fluido in quanto non aumenta la permeabilità dentinale come un mordenzante total etch tradizionale.

**Conclusioni Cliniche**

I sistemi adesivi semplificati pur fornendo alti valori di adesione micromeccanica alla dentina non sono in grado di garantire un perfetto sigillo in quanto anche polimerizzati sono permeabili ai fluidi. Il passaggio di fluido è presente anche quando il sistema adesivo viene applicato in denti trattati endodonticamente e quindi in assenza di pressione pulpare. Il passaggio di fluido è influenzato dalla pressione osmotica esercitata dai materiali da ricostruzione o cementazione applicati sopra al sistema adesivo. Un fattore fondamentale che influenza il passaggio di fluidi è il tempo di polimerizzazione pertanto i sistemi autopolimerizzanti che richiedono tempi lunghi di polimerizzazione sono maggiormente esposti al rischio di formazione di blister di fluido all’interfaccia adesiva (tra sistema adesivo e cemento auto). Anche sistemi adesivi che prevedono un bonding idrofobico in grado di ostacolare quasi completamente il passaggio di fluidi sono soggetti ai fenomeni di permeabilità a livello dello strato ibrido.

L’applicazione di ossalati dopo la mordenzatura è in grado di ridurre la permeabilità dei sistemi adesivi compatibili.
I sistemi adesivi mild self etching two step sono i meno permeabili in quanto i primer a ridottaacidità non sono in grado di aumentare la permeabilità dentinale come con la total etch tecnique o con primer a pH inferiore di 1.4.

La semplificazione dei sistemi adesivi ha aumentato il grado di permeabilità che potenzialmente è in grado di inficiare il risultato clinico immediato ed a breve-medio termine. In altre parole semplice e veloce nel campo dell’adesione non sono una realtà al momento. Sicuramente il miglior trattamento per lo smalto è a base di acido ortofosforico ad alta concentrazione di contro la dentina di denti vitali e privi di lesioni cariose se trattati con total etch tecnique è praticamente impossibile da sigillare correttamente senza applicare gliossalati.

Una valida alternativa è rappresentata dai sistemi mild-self-etch two-step che forniscono un buon compromesso a livello dello smalto e consentono un adeguato sigillo in dentina in virtù del ridotto aumento di permeabilità dentinale.


Inoltre una recente review relativa a studi clinici (Peumans M. et al Dent. Mater. 2005 sep;21(9):864-81) conclude che c’è una tendenza alla semplificazione delle procedure di applicazione degli adesivi. La semplificazione sembra finora indurre la perdita di efficacia.

**DirezioniFuture**

Lo sviluppo dei sistemi adesivi deve necessariamente tenere in considerazione la possibile durata clinica ed in quest’ottica è indispensabile creare un perfetto sigillo tra struttura dentaria e polimero.

L’equilibrio ottimale si può ottenere evitando di aumentare la permeabilità dentinale o riducendola come ad esempio applicando ossalati. L’adesivo deve completare il sigillo e per fare ciò non può contenere eccessive quantità di monomeri idrofili. Molto interessanti sono i monomeri che cambiano proprietà di idrofilia in seguito alla polimerizzazione. Infine un ulteriore sviluppo consiste nello stabilizzare l’hybrid layer che è soggetto a stress ciclici in ambiente.

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Pubblications


