In vitro permeability of etch-and-rinse and self-etch adhesives used for immediate dentin sealing

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To investigate the permeability of deep dentin following immediate sealing with different etch-and-rinse and self-etch adhesives (Single Bond 2, Adper Prompt L-Pop, Clearfil Protect Bond, Clearfil S3 Bond, G-Bond) and a dentin desensitizer (Gluma). Fluid-transport model was used to measure fluid conductance during and after application of adhesives. Polyvinylsiloxane impressions of bonded dentin were taken to monitor fluid transudation from the surface of the adhesives. The area and number of dentinal fluid droplets and/or blisters were calculated using image analysis. None of the adhesives were able to block fluid conductance completely. The fluid conductance values of the adhesives displayed the following statistical ranking (p<0.05): G-Bond≤Clearfil Protect Bond<Smear-layer-covered dentin<S3 Bond<Single Bond 2<Adper Prompt L-Pop<Gluma Desensitizer<Acid-etched dentin. Highly significant correlation was observed between the permeability of the tested adhesives and the area fraction of fluid droplets/blisters on the adhesive surfaces (r=0.98, p<0.01).

Keywords: Permeability, Water droplets, Hybrid layer, Dentin bonding systems, Image analysis

INTRODUCTION

Exposure of the dentin tubules is inevitable during cavity or crown preparations. Once the tubules are opened, they act as channels that transmit mechanical, chemical, and bacterial stimuli to the pulp. Provisional sealing materials do not cohesively bind to dentin and may permit leakage of bacteria and their products before the luting of final restoration. During the provisional stage, the dentin may also encounter external stimuli that include impression taking, rinsing, drying, and removal of temporary sealing, which may all encourage tooth sensitivity and potential pulp damage. Thus, coating should be performed immediately after cutting the dentin to provide a “biological seal” that acts as a dentin-pulp protector. To serve this aim, it has been suggested that freshly cut dentin surfaces of indirect restorations (i.e., inlay or onlay, veneer, and crown preparations) be sealed with resin-based adhesives prior to the taking of impressions. In addition to its favorable effects on the reduction of post-preparation and post-cementation sensitivity, this so-called “resin coating” or “immediate dentin sealing” technique can result in significantly increased retention, reduced marginal leakage, and improved bond strengths, when used for traditional crown preparations of vital teeth. Reportedly, the bond of definitive restoration to resin-coated dentin can be obtained following extended provisional restoration phases up to 2 weeks. Ideally, polymerization of the resin-based adhesive system should reduce the permeability of the exposed dentin, by formation of hybridized resin tags inside the tubule lining and lateral branches forming anastomoses, thus causing lateral hybridization of the peritubular dentin. Owing to technique sensitivity of etch-and-rinse (total-etch) adhesives, optimal hybridization and sealing of the dentinal tubules with the so-called wet-bonding technique may differ with each bonding system; and a common clinical manifestation of such inconsistent bonding is the patient’s complaint of postoperative sensitivity. Milder versions of self-etching primers may help reduce hydraulic conductance through the dentinal tubules, as these adhesive systems preserve smear plugs if the primers are not agitated during etching. 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 the dentin permeability during the processes of simultaneous etching and priming. Nevertheless, in vital teeth that exhibit positive pulpal pressure, transudation of dentinal fluid across polymerized adhesive layers may avoid near-perfect dentin sealing through the use of simplified adhesive systems. In light of these observations, the two-fold purpose of this study was: (1) to evaluate the permeability of deep dentin bonded with etch-and-rinse and self-etching adhesives in the presence of simulated pulpal pressure, and (2) to investigate the extent of fluid transudation across resin-bonded dentin by SEM-replica technique, in order to identify the relationship between adhesive permeability and its manifestation as fluid droplets and/or blisters on the adhesive surfaces. Accordingly, the null hypotheses tested were that (1) there is no difference in the ability of the tested adhesive systems to reduce hydraulic conductance of dentin, and (2) there is no correlation between the permeability of the tested adhesive systems and the area and number of fluid droplets and/or blisters.
droplets and/or blisters on the adhesive surfaces.

**MATERIALS AND METHODS**

**Specimen preparation**

Seventy non-caries, human mandibular third molars, extracted from patients 18–25 years old were used in the present study. The use of extracted teeth and the experimental protocol of the study were approved by the human subjects ethical committee. Soft tissue remnants were removed from the root surfaces, after which the teeth were stored in an aqueous solution of 0.5% chloramine-T at 4°C before use (a maximum of 1 month). The teeth were transferred to room temperature 24 h before experimental procedures and were carefully evaluated under a stereomicroscope to discard the presence of cracks and/or defects. Each selected tooth was prepared by first removing the occlusal enamel using a slow-speed diamond-impregnated saw (Isomet 4000, Buehler Ltd., Lake Bluff, IL) under copious water-cooling. The roots were removed from each tooth at 2 mm below the cemento-enamel junction by means of the Isomet saw. The pulp tissue was then gently removed without altering the predentin surface. To create a standardized bonding substrate in deep dentin, the dentin surface was abraded with 180-grit silicon carbide paper under running water13,14). A remaining dentin thickness of 0.7–0.8 mm was achieved from at least one region of the ground surface to the highest pulp horn, as measured with a pincer-type caliper14). The flat dentin surfaces were then rinsed with water for 2 min.

**Bonding procedures and measurement of permeability**

Each crown segment was attached to a plexiglas platform (5 cm×5 cm×1 cm) that was perforated by an 18-gauge stainless steel tubing that ended flush with the top, using cyanoacrylate adhesive (Super glue, Bison, Goes, The Netherlands). This tubing permitted the pulp chamber to be filled with deionized water and to be connected to a hydraulic pressure device, which delivered 20-cm water pressure16) for measurement of the fluid movement across the dentin surface. Ten randomly selected crown segments were used for each test material.

Six materials were examined. They included 1 two-step self-etch adhesive system (Clearfil Protect Bond, Kuraray Medical Inc., Okayama, Japan); 1 two-component, all-in-one self-etch adhesive (Adper Prompt L-Pop, 3M ESPE, Seefeld, Germany); 2 one-component self-etch adhesives (Clearfil S3 Bond, Kuraray and G-Bond, GC Corp., Tokyo, Japan); 1 etch-and-rinse (total-etch) adhesive (Single Bond 2, 3M ESPE); and 1 dentin desensitizer (Gluma Desensitizer, Heraeus Kulzer, Hanau, Germany). All materials were used according to the manufacturers’ instructions. Light activation of the bonding agents was performed using a halogen light-curing unit (XL-2500, 3M ESPE, St. Paul, MN, USA) with an output power intensity of 600 mW/cm², at a standardized distance of 5 mm from the bonding surface16). As per the manufacturer’s instructions, Gluma Desensitizer was applied with a pellet and left undisturbed for 60 s, after which the surface was dried carefully by applying a stream of compressed air until the fluid film had disappeared and the surface was no longer shiny. Then, the dentin was thoroughly rinsed with water. All test materials were applied while connected to the permeability device in the presence of pulpal pressure application. The chemical compositions of the test materials are presented in Table 1.

The method employed for measurement of hydraulic conductance was derived from Pashley and Depew's

<table>
<thead>
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<th>Table 1 Chemical composition of the test materials</th>
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<tr>
<td><strong>Product (Manufacturer)</strong></td>
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<tr>
<td>Single Bond 2</td>
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<tr>
<td>G-Bond (GC Corp., Tokyo, Japan)</td>
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<tr>
<td>Clearfil S3 Bond (Kuraray, Okayama, Japan)</td>
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<tr>
<td>Clearfil Protect Bond (Kuraray; Okayama, Japan)</td>
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<tr>
<td>Adper Prompt L-Pop (3MESPE; Seefeld, Germany)</td>
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<tr>
<td>Gluma Desensitizer (Heraeus, Kulzer, Hanau, Germany)</td>
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HEMA: 2-hydroxyethyl methacrylate, Bis-GMA: bisphenol-A-dimethacrylate, UDMA: urethane dimethacrylate, TEGDMA: triethyleneglycol dimethacrylate, MDP: 10-methacryloyloxydecyl dihydrogen phosphate, 4-MET: 4-methacryloyloxyethyl trimellitic acid
protocol\textsuperscript{17}, which allows measurement of fluid flow by following the movement of an air bubble trapped within a glass capillary tube that is positioned between the pressure reservoir and the crown segment (Fig. 1). First, the absence of fluid conductance before exposure of occlusal dentin was confirmed by attaching 5 intact crown segments separately to the testing apparatus as described and observing the (absence of) fluid movement for 2 h. Thereafter, a separate group of teeth ($n=10$), in which the smear-layer-covered dentin was not bonded with any adhesive, was used to measure the fluid filtration in the presence and absence of the smear layer. The dentin permeability of smear-layer-covered specimens was measured at a hydraulic pressure of 20 cm water for 1 h. The permeability was reassessed in this group after removing the smear layer by treating the dentin surface with 37% phosphoric acid for 15 s. Then, using the remaining teeth that were assigned to different test groups ($n=10$ group), the permeability of each specimen was measured independently, after application of the test material on the dentin surface, according to the manufacturer’s instructions. The fluid flow was measured by following the movement of an air bubble in a glass capillary tube of 0.1 mL. The linear displacement of the bubble was converted to volume by using Darcy’s law\textsuperscript{18}:

$$L_p = J_v / A \cdot \Delta P t$$

Where, $L_p$ is hydraulic conductance in $\mu$L$\cdot$cm$^{-2}$$\cdot$min$^{-1}$$\cdot$cm$^{-1}$$\cdot$H$_2$O, $J_v$ is fluid flow in $\mu$L, $A$ is dentin surface area in cm$^2$, $\Delta P$ is hydrostatic pressure in cm H$_2$O, and $t$ is time in min. The dentin surface area of each specimen was calculated on digital photographs taken perpendicular to the occlusal surface at 16$\times$ magnification under a stereomicroscope (Olympus, Tokyo, Japan). The digital images were transferred to a Macintosh workstation, and the areas of specimens were calculated using open-source image analysis software (ImageJ for MacOSX; V.1.34, National Institutes of Health, Bethesda, MD). The results of fluid permeability were statistically analyzed by Kruskal-Wallis multiple comparisons test with significance set at $\alpha=0.05$.

**SEM evaluation and image analysis**

Impressions of water transudation from smear-layer-covered and smear-layer-removed dentin, and from dentin-hybrid layers were obtained using low viscosity polyvinylsiloxane impression material, after which positive replicas were produced from these impressions (negative replicas), with a thermoplastic epoxy-resin material (Araldite, Huntsman advanced materials, Basel, Switzerland). Just prior to taking impressions, unpolymerized oxygen-inhibition layer was removed from each bonded resin surface with a cotton pellet\textsuperscript{14}. The araldite replicas were sputter-coated with 20 A° gold/palladium and examined with an SEM (JEOL 6400, JEOL Corp., Tokyo, Japan) operating at 20 kV. Three digital photographs were obtained from each occlusal surface (1 from the middle of the mesiodistal axis and 2 respective pictures, each 1 mm from the mesial and distal aspects of the first picture) at 500$\times$ standard magnification and were saved as high-resolution (2400$\times$1800 pixels) tagged image file format (TIFF) files, which correspond to a surface area of 32,800 µm$^2$, as calculated with ImageJ. The digital images were transferred to a Macintosh workstation, and the number and area fraction of water droplets and/or blisters in each specimen were calculated using the ImageJ software. To count the droplets, the particle analysis algorithm was used. Accordingly, the droplets were first digitally “removed” from the background (dentin surface) by a thresholding procedure, after which the number of droplets was quantified using the particle-counting
function. Likewise, the area of droplets was measured using the area-measurement function of ImageJ. For both the area fraction and number of droplets and/or blisters, the mean values of 3 pictures were recorded as the value for that sample. The results were analyzed statistically by one-way ANOVA and Tamhane’s T2 statistics with significance set at α=0.05. The correlations between the mean conductance values and the area fraction of droplets and the number of droplets were investigated using Pearson’s correlation with statistical significance set at α=0.05.

RESULTS

Fluid conductance measurements

The fluid conductance values (µL/min at 20 cm H₂O) of the test groups are presented in Table 2 as mean±standard deviation. As expected, acid-etched dentin showed the highest fluid conductance (48.64±7.05×10⁻⁴, p<0.05). The lowest conductance values were obtained with G-Bond followed by Clearfil Protect Bond (p<0.05, Table 2), both of which yielded significantly lower fluid conductance values than that of smear-layer-covered deep dentin (11.81±2.35×10⁻⁴, p<0.05). Pairwise comparisons between the mean fluid conductance of S3 Bond and Single Bond 2 and of Single Bond 2 and Adper Prompt L-Pop showed no significant differences (both p>0.05), followed by Gluma Desensitizer, which had the highest permeability among the materials tested (p<0.05; Table 2).

SEM examination and image analysis

Representative SEM micrographs of the positive-impression replicas of dentin surfaces bonded under pulpal pressure are presented in Fig. 2. Regardless of the adhesive system applied, the existence of water droplets and/or blisters, varying in shape and size, was observed consistently in all specimens. Deep dentin bonded with Adper Prompt L-Pop displayed several areas of fluid transudation that were morphologically regarded as water trees (Fig. 3). The water tree pattern was observed in all specimens but displayed nonuniform distribution and size. Being limited to the Adper Prompt L-Pop group only, the water trees were not considered as water droplets and/or blisters and were not included in the area and number measurements.

Table 2 shows the mean total area and number of water droplets per 32,800 µm². Under pulpal pressure, G-Bond exhibited the smallest area fraction of water droplets (12.19%±0.49%), followed by Clearfil Protect Bond (15.02%±0.14%), Clearfil S3 Bond (22.64%±0.11%), Single Bond 2 (23.48%±0.24%), and Adper Prompt L-Pop (25.59%±0.24%) (all p<0.05). The greatest area fraction of water droplets was observed in deep dentin treated with Gluma Desensitizer (28.60%±0.42%, p<0.05). A highly significant correlation was identified between the fluid conductance of the tested materials and the total area of fluid droplets and/or blisters on the adhesive surfaces (r=0.99, p<0.01; Fig. 4). Contrarily, the fluid conductance values were not highly correlated with the number of fluid droplets and/or blisters (r=0.54, p<0.05; Fig. 4).

DISCUSSION

Tooth preparation leads to an open dentin wound that should be sealed immediately. Among the several treatment modalities available, the use of adhesive resins seem to provide a good-to-satisfactory level of seal, as verified under laboratory and clinical conditions. Apparently, variations in the permeability of adhesively sealed dentin depends largely on the adhesive system used, with some proprietary adhesives being even less effective at sealing dentin than the original smear layer. These observations justify the aim of testing the effect of different adhesive resin systems on the hydraulic conductance of deep human dentin. In an attempt to simulate the clinical condition, the tested adhesives were bonded in the presence of initial pressure application. Thus, some of the results obtained herein may differ from those of other laboratory studies, in which the pressure was applied after bonding of adhesive resins to the dentin surfaces. It should also be noted that the clinical reality of teeth being prepared for fillings and crowns may vary, especially when teeth are anesthetized with adrenalin-containing solutions.
Fig. 2  Representative SEM micrographs of the replicas of bonded dentin surfaces under 20 cm H$_2$O pressure. A: G Bond; B: Clearfil Protect Bond; C: Clearfil S3 Bond; D: Single Bond II; E: Adper Prompt L-Pop; F: Gluma Desensitizer.

Fig. 3  SEM micrographs of deep dentin bonded with Adper Prompt L-Pop, showing regions of water-treeing.
Among the tested adhesives, G-Bond and Clearfil Protect Bond displayed the lowest permeability values, respectively, both of which were significantly lower than that of smear-layer-covered deep dentin. Thus, the first null hypothesis that there is no difference in the ability of the tested adhesive systems to reduce hydraulic conductance of dentin was rejected. Our results show some similarities with those of Sauro et al. who reported that Clearfil Protect Bond had the lowest fluid permeability, followed by G-Bond, which displayed lower but statistically similar adhesive permeability with that of Clearfil S3 Bond. The authors explained the low fluid flow rate of G-Bond by its 4-MET component, which results in less water uptake when stored in water. They also suggested that apart from differences in chemical composition and the presence of less hydrophilic co-monomers, the low fluid flow rate of G-Bond may be due to the manufacturer’s recommendation of applying a strong, continuous air blast to remove the solvent prior to polymerization. Stronger or longer air blast improves the quality of the adhesive film and reduces its permeability, thereby lowering the influence of water contamination. Together with the favorable effect of the 4-MET component, the significance of air blasting on the reduction of G-Bond’s adhesive permeability may be even more pronounced when the adhesive is applied on initially perfused “water-contaminated” deep dentin, as performed here. Accordingly, G-Bond demonstrated lower permeability than Clearfil Protect Bond whose adhesive permeability can be explained by its hydrophilic components (e.g., HEMA), which are capable of imbibing large amounts of water that eventually remain entrapped at the resin/dentin interface. This explanation may also account for the permeability values achieved with the HEMA-based self-etching adhesive Clearfil S3 Bond, although this simplified, one-component adhesive was not able to seal deep dentin as good as its two-component predecessor. One-component self-etch adhesives contain higher concentrations of organic solvent and water than other adhesive systems. Thus, incomplete water removal and evaporation are likely.

For the 2 more permeable adhesives —Single Bond 2 and Adper Prompt L-Pop—in vitro fluid conductance was greater than that of smear-layer-covered dentin. Single Bond 2 is a HEMA-based, etch-and-rinse adhesive, whose preliminary etching step inevitably increases the permeability of dentin owing to the removal of smear layer and smear plugs. In addition to this inherent disadvantage, the adhesive contains 13% polyalkenoic acid polymer in an ethanol-water solvent. Polyalkenoic acid copolymers have multiple pendant carboxylic acids along a linear backbone, which tend not only to bind water in the adhesive but also to preclude its penetration into interfibrillar spaces due to its high molecular weight. Moreover, the interfibrillar spaces in the walls of etched tubules are filled with water, which probably dilutes and interferes with resin infiltration. Such incomplete hybridization of resin tags to tubule walls are known to permit fluid movement from dentinal tubules to adhesive interfaces in etch-and-rinse adhesives. For the two-component adhesive Adper Prompt L-Pop, observation of the areas of water transudation regarded morphologically as water treeing is interesting but should be validated with further studies. Provided that our assumption is valid, demonstration of water treeing on the SEM micrographs of the adhesive surfaces are highly suggestive of the extent of convective fluid flow across the bonded interface, while the nonuniform distribution of the water-treeing patterns observed herein confirm the variation in permeability from different regions of tooth preparation. Using transmission electron microscopy, Tay et al. observed the water-treeing phenomenon in this type of adhesive and the patterns of nanoleakage within both adhesive and hybrid layers. The vertically oriented water trees were explained by the outward movement of water from...
the dentin tubules during etching. SEM studies have revealed that Prompt L-Pop is as acidic and aggressive as 32–37% phosphoric acid, which might be responsible for dissolving the smear plugs. Under simulated pulpal pressure, the loss of integrity of these hydraulic barriers may account for the permeability of Adper Prompt L-Pop, which is similar to that of the etch-and-rinse adhesive Single Bond 2.

Unlike other test materials, Gluma Desensitizer is not a dentin bonding system, and the present results showed that it does not seal dentin well. This material has been shown to reduce the dentinal fluid flow by coagulating plasma proteins and forming partitions within the dentinal tubules. Apparently, the extent of such reduction was significantly lower than those provided by the tested adhesives and even the smear-layer-covered dentin under pulpal pressure application. It should be noted that Gluma Desensitizer is not photocurable, and as such, the osmotic action of HEMA can increase fluid conductance.

Following the connection of previously sealed deep dentin specimens to a pressure apparatus at 20 cm of water pressure, Sauro et al. demonstrated a highly significant correlation between the relative permeability of 4 proprietary self-etch adhesives and the number of fluid droplets and/or blisters on the adhesive surfaces. In contrast to their findings, the present fluid conductance values were not highly correlated with the number of such globular elevations. Two reasons could be responsible for this finding: (1) In the latter study, all the tested adhesives were bonded to (intrinsically wet) dentin while connected to the permeability device but in the absence of initial pulpal pressure application. This might have resulted in a relatively less “water-contaminated” bonding substrate, which could affect the number of blisters and/or droplets observed after administration of pulpal pressure. (2) Perfusion of fluid from the pulp chamber tends to displace or lift the resin coating from the dentin, which in turn may force the formation of new water droplets that vary in shape and size, and to the coalescence of several droplets. Clinically, such coalescence may occur both before and during the application of bonding resin, and with time, blisters may even enlarge to a point where the adjacent blisters coalesce and disfigure the true number of droplets and/or blisters. Owing to this diagnostic challenge, a statistical analysis that involved the area fraction of water droplets and/or blisters was also made. Indeed, the results showed that fluid conductance was highly correlated with the area fraction of droplets and/or blisters on the adhesive surfaces. Thus, the second null hypothesis was partially accepted. It should be cautioned that these findings are based on the quantified morphological analysis of two-dimensional images, which definitely merit confirmation by the use of techniques that enable three-dimensional (volumetric) quantification of water movement at the resin/dentin interface.

CONCLUSIONS

Within the experimental conditions of this study, the following conclusions were drawn:

1. None of the tested adhesive systems were able to block the permeability of initially perfused deep human dentin. Among the tested adhesives, only the one-step self-etch system G-Bond and the two-step self-etch adhesive Clearfil Protect Bond were more effective at sealing the dentin than the original smear layer.

2. For the tested adhesives, greater permeability values were highly correlated with increased area fraction of water droplets and/or blisters.

REFERENCES


