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*Original Research Article*

# Evaluation of adhesive systems in primary dentin by nanoleakage: effects of aging

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## Abstract

**Introduction:** Nanoleakage evaluation by silver uptake in permanent teeth provides good spatial resolution of submicron defects at the hybrid layer, which have not been tested in primary teeth. **Objective:** This study evaluated the nanoleakage at the dentin-adhesive interface in primary teeth by two methods, for three adhesive systems, immediately (IM) and six months (6M) after adhesive procedures. **Material and methods:** Crowns of primary molars were occlusal flat grounded and divided into three groups according to the adhesive system tested (n = 6-7). Scotchbond Multi-purpose/SMP, Single Bond/SB and Clearfil SE Bond/CSB adhesive systems were applied with a composite resin (Filtek Z-250). Crowns were sectioned so that 0.8 mm² sticks were obtained and subdivided depending on the time of evaluation: IM or 6M. They were immersed into silver nitrate solution and evaluated by SEM-EDS. Data (%) were analyzed by ANOVA ($p < 0.05$) and the scores by Kruskal-Wallis and U Mann-Whitney tests ($p < 0.05$). **Results:** There was no difference between groups regarding to the evaluation time (aging) percentage. In terms of scores, there was a significant difference for the adhesive variable: SMP and SB showed similar results with less leakage, while CSB demonstrated higher leakage. **Conclusion:** Nanoleakage was not influenced by aging, but noticeable difference was observed between total-etch and self-etching adhesives. Total-etch showed better performance.

## Introduction

Adhesion to dentin is primarily based on the hybridization mechanism, which is a micromechanical bond between adhesive polymers and collagen fibrils from the demineralized dentin [15]. Thus, modern adhesive systems may remove total or partially the smear layer as well as the subjacent dentin's mineral, which are replaced by resin monomers [3, 27].

Some adhesive systems showed a reduction in the time and number of application steps in order to reduce defects of handling [27], which is attractive for pediatric dentistry [22, 23], due to the child's management. However, it is known that this simplification does not necessarily improve bonding effectiveness over time [2-4, 32]. Adhesion between dentin and resin deteriorates over time, limiting adhesive restorations longevity [28]. Studies demonstrated that resin-dentin bond strength decreases over time and that the hydrolysis of collagen fibrils is responsible for such reduction, even in the absence of interfacial gaps. The degradation of the bond could be the result of water movement within the hybrid and adhesive layers. This hydrolysis may extract unconverted monomers from the hybrid layer rendering a weak interface [1, 16, 19-21, 26]. Also, not encapsulated collagen fibrils can be hydrolyzed by metalloproteinase enzymes [21, 25, 29].

Interfacial gaps inside of the hybrid and adhesive layers [21] are explained by nanoleakage, described for the first time by Sano et al. (1995) [19, 20]. The evaluation of nanoleakage by silver uptake provides good spatial resolution of submicron defects in resin infiltration or inadequate polymerization [25, 27], which have been evaluated in many studies involving permanent teeth [5, 6, 10, 13, 14, 16, 18, 22, 24-26, 30-32]. However, few studies so far, have attempted to show this phenomenon in primary teeth [7, 8, 11, 17].

Therefore, the objective of this study was to evaluate the nanoleakage in primary dentin for three different adhesive systems by means of silver nitrate uptake, immediately and six months after bonding aged specimens.

## Material and methods

### Teeth selection, storage and preparation

This study was approved by Institutional Review Board (under protocol no. #205/07). Forty extracted caries-free human primary molars were stored into 0.1% thymol solution, 0.9% saline solution, pH = 7 at room temperature. A flat superficial dentin surface of each tooth was exposed after wet grinding the occlusal enamel with a #200 grit silicon carbide paper (SiC). The surface was further wet polished with a #400 and #600 grit SiC paper in four different directions, during 10 seconds each, to standardize the smear layer.

### Bonding procedures

All the bonding procedures were carried out by the same operator, at room temperature. After cleaning with distilled water, specimens were divided into three groups (n= 6-7 teeth) for each adhesive systems: *Scotchbond Multi-Purpose (3M ESPE, St. Paul, MN, USA), Single Bond (3M ESPE, St. Paul, MN, USA*, and *Clearfil SE Bond (Kuraray Medical, Tokyo, Japan)* (Table I). The adhesives were applied according to the manufacturer's instructions and light cured with a LED light unit with a power output of 400 mW/cm$^2$ (Radii, SDI, Bayswater, Australia). Resin composite build ups (Filtek Z250 - *3M ESPE, St. Paul, MN, USA*) were then constructed on the bonded occlusal surfaces in three increments of 1.5 mm, which were light cured for 20 seconds each with the same light intensity.

Sections of 0.9 mm thickness each were made in a longitudinal direction (perpendicular to the adhesive interface) with a 0.3 mm diamond disc (Buehler, Lake Bluff, IL,USA) in an Isomet 1000 machine (Isomet 1000, Buehler, Lake Bluff, IL, USA), under water refrigeration at 250 rpm. Initially those sections were cut in a mesial-distal direction in order to obtain specimen's slices. A sticky wax was applied in order to keep the slices together. After that, another buccal-lingual sectioning was performed to provide sticks with 0.8 mm$^2$ area. The bonded sticks from each tooth were then randomly subdivided into two groups: one assigned to be tested immediately and the other six months after storage in distilled water containing 0.4% sodium azide, at 37°C.

**Table I** – Adhesive systems. Composition, application mode, batch number

| Adhesive System | Composition | pH (primer) | Steps | Batch number |
|---|---|---|---|---|
| **Scotchbond Multi-Purpose** | Primer: Aqueous solution of HEMA, polyalkenoic acid copolymer. Bond: Bis-GMA, 2-HEMA, photo initiator component. | 3.3 * | a; b; d; e; f; i | Primer: 7BJ<br>Bond: 7PX |
| **Single Bond** | Water, ethanol, HEMA, Bis-GMA, dimethacrylates, photo initiator systems, methacrylate functional copolymer (polyacrylic, polyitaconic and polyalkenoic acid. | 4.7 * | a; c; d; g; h; i | 7LY |
| **Clearfil SE Bond** | Primer: MDP, HEMA, hydrophilic dimethacrylate, di-camphorquinone, N,N-p-toluidine diethanol, water. Bond: MDP, HEMA, Bis-GMA, hydrophobic dimethacrylate, di-camphorquinone, N,N-p-toluidine diethanol, colloidal silanated silica. | 1.9 ** | j; f; h; i | Primer: 00760[a]<br>Bond: 01094[a] |

Abbreviations - HEMA: 2-hydroxyethyl methacrylate, Bis-GMA: bisphenyl-glycidyl methacrylate, 10- MDP: 10-methacryloyloxydecyl-dihydrogen-phosphate.
* Manufacture information
**Van Meerbeek et al. (2003)
Application Mode - a) acid etch – phosphoric acid 35% (15s); b) rinsing (15s); c) rinsing (10s); d) drying with absorbent papers – with no dentin over dry; e) primer application and drying (5s); f) adhesive application; g) 2 coats of adhesive; h) drying the adhesive (2-5s); i) light cure (10s); j) primer (20s) and air dry.

## Nanoleakage evaluation

Bonded sticks were coated with two layers of nail varnish applied up to within 1 mm of the bonded interface. They were then rehydrated in distilled water for 10 minutes and immersed in ammoniacal silver nitrate tracer solution for 24h (Cennabras Indústria e Comércio Ltda., Guarulhos, São Paulo, Brazil). The solution was prepared according to the protocol previously described by Tay et al. (2002) [26]. The specimens were then rinsed thoroughly in distilled water and immersed in photo developing solution (Caithec Materiais Odontológicos, Rio do Sul, Santa Catarina, Brazil) for 8h, under a fluorescent light to reduce silver ions. All sticks were then placed inside an acrylic ring, which was attached to a double-sided adhesive tape, and embedded in epoxy resin. The specimens were wet polished with #600 SiC paper to remove the nail varnish, and further polished with a #1200 grit SiC paper with a 1; 0.3; and 0.05 $\mu$m diamond paste (Buehler, Lake Bluff, IL, USA). They were then ultrasonic cleaned, air dried and gold coated (SCD 005, Bal-tec, Balzers, Liechtenstein) in order to analyse the resin-dentin interfaces by SEM (Philips XL-30, Philips Eletric Corporation, Eindhoven, Netherlands). Two analysis were performed to verify the silver uptake: a) A percentage of silver uptake evaluated [18]; b) the silver nitrate uptake expressed by scores [32].

For the first method (a), the analysis was performed in the backscattered electron mode (BSE) and by the use of energy dispersive X-ray spectrometry (EDS) (EDAX, Ametec Inc., USA). Analysis of each stick was performed at three regions (center, right and left) of the bonded stick (adhesive layer, hybrid layer and resin tags) (1000X) (Figure 1). The silver nitrate uptake was expressed as a percentage, according to the mean values observed by EDS for each tooth.

In the second method (b), photomicrographs of the whole stick were taken in BSE mode (90X) and one single operator analysed each one of the photomicrographs in a 14" laptop (Aspire 4520, Acer Inc., China). The evaluation was performed in total area by the use of scores, following an adaptation of the method suggested by Yuan et al. (2007) [32]:

0 - no leakage;
1 - Mild leakage - less than 25% of the evaluated area;
2 - Clear leakage - between 25 and 50% of the evaluated area;
3 - Large leakage - more than 50% of the evaluated area.

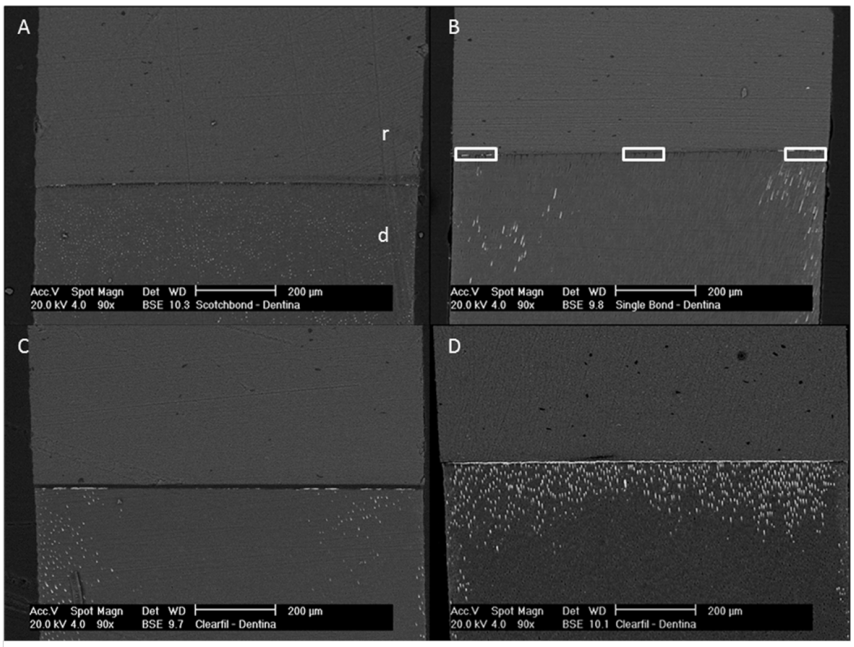


**Figure 1** – Photomicrographs of the dentin-resin interface (90X). Different degrees of silver nitrate penetration (white) can be observed: discrete **(A)**; clear and localized **(B)**; clear and disperse **(C)**; intense deposition of silver uptake **(D)**. Images show lower silver uptake penetration for Scotchbond Multi-purpose and Single Bond **(A and B)**, and higher penetration for Clearfil SE Bond **(C and D)**. Photomicrograph **B** shows areas evaluated by EDS. (r- resin; d-dentin; A and C: 6M groups; B and D: baseline)

### Data treatment

The percentages calculated from the mean values of silver uptake for each tooth were analyzed by ANOVA. Analysis of each stick's score was performed by Kruskal-Wallis and U Mann-Whitnney Test ($p < 0.05$).

## Results

### Silver nitrate penetration expressed by percentage

The mean values of silver penetration for each tooth, as well as the results of the statistical analysis are expressed in table II.

**Table II** – Mean values (%) silver nitrate leakage

| Adhesive | Time | | | |
|---|---|---|---|---|
| | Baseline | | 6 months | |
| | n | Mean (SD) | n | Mean (SD) |
| Scotchbond MP | 7 | 27.15 (8.26) | 6 | 30.54 (11.74) |
| Single Bond | 7 | 32.16 (11.54) | 7 | 33.18 (9.94) |
| Clearfil SE Bond | 6 | 30.31 (18.73) | 7 | 31.39 (13.3) |

n= number of teeth; SD = Standard deviation

## Silver nitrate penetration expressed by scores

The score distribution according to the silver nitrate penetration for each adhesive system is presented in table III. Kruskal-Wallis test revealed that there was no significant difference for the variable aging ($p = 0.79$), but there was a statistical difference among the adhesives ($p = 0.00$). The U of Mann-Whitney test ($p < 0.05$) showed that Scotchbond Multi-purpose and Single Bond adhesives had similar performance and allowed less silver nitrate uptake than Clearfil SE Bond adhesive.

**Table III** – Score distribution from the silver nitrate leakage to each adhesive system. Immediate (baseline) and six months evaluation

| Time | Adhesive | 0 | 1 | 2 | 3 | Total |
|---|---|---|---|---|---|---|
| **Baseline** | Scotchbond MP | 0 | 11 | 5 | 2 | 18 |
| | Single Bond | 0 | 9 | 7 | 1 | 17 |
| | Clearfil SE Bond | 0 | 7 | 5 | 6 | 18 |
| **6 months** | Scotchbond MP | 0 | 13 | 5 | 2 | 20 |
| | Single Bond | 0 | 9 | 9 | 1 | 19 |
| | Clearfil SE Bond | 1 | 4 | 4 | 8 | 17 |
| | | | | | **Total** | 109 |

Kruskal-Wallis test (Adhesive, p = 0.00 / time, p = 0.79)
U Mann-Whitnney test: (Scotchbond MP = Single Bond) < (Clearfil SE Bond)

## Photomicrographs

Figures 1-4 show representative photomicrography observed in SEM.

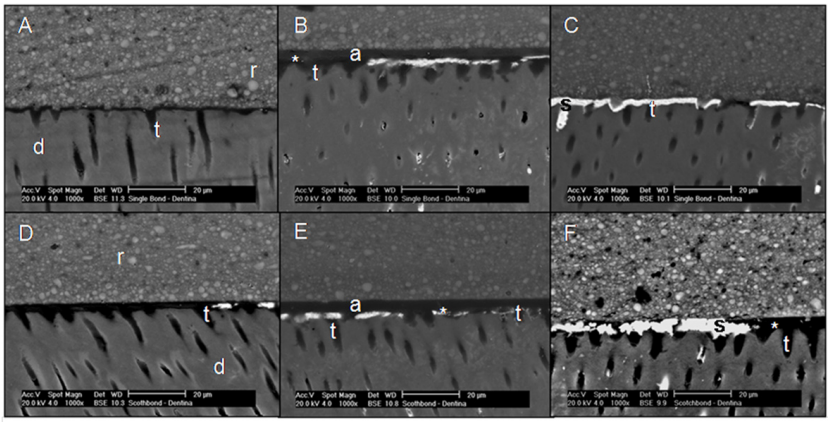


**Figure 2** – Photomicrographs of dentin-resin interface (1000X) of total-etch adhesives baseline. For Single Bond **(A-C)** and Scotchbond Multi-purpose **(D-F)** can be observed: absence **(A)** and discrete silver nitrate penetration (spot) **(D)**; infiltration at the base of hybrid layer (reticular) **(B and E)**; large penetration at the hybrid layer and at the adhesive layer (reticular) **(C, F)**. (r-resin, d-dentin, a-adhesive, s-silver uptake, *-hybrid layer, t- resin tags)

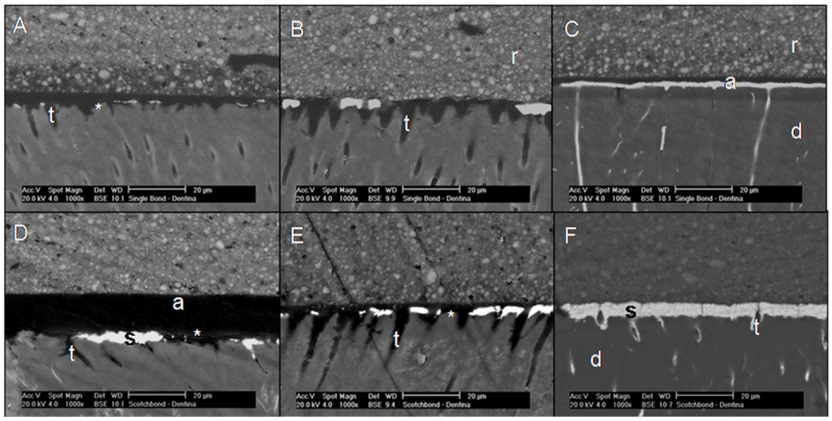


**Figure 3** – Photomicrographs dentin-resin interface of total-etch adhesives (1000X) after six months. Single Bond **(A-C)** and Scotchbond Multi-purpose **(D-F)** show discrete **(A)** and clear **(D)** silver nitrate penetration at the base of the hybrid layer (spot); clear penetration of silver nitrate at the adhesive layer and hybrid layer (spot) **(B)**; intense infiltration at the hybrid layer and at the adhesive layer **(F)** (reticular); and intense penetration of silver nitrate at the hybrid layer **(C and E)** (reticular). (r-resin, d-dentin, a-adhesive, s-silver uptake, *-hybrid layer, t- resin tags)

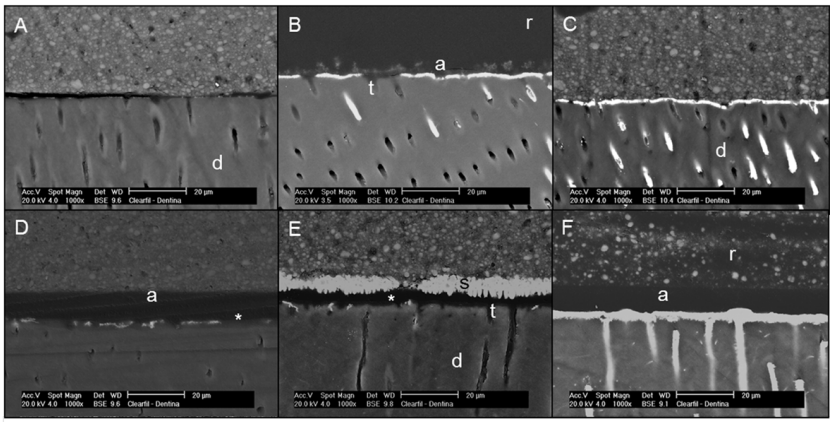


**Figure 4** – Photomicrographs of dentin-resin interface (1000X) after self-etch adhesive application at baseline and after six months. Images of Clearfil SE Bond show: absence **(A)** and discrete silver nitrate penetration (spot) **(D)**; infiltration at adhesive layer (water tree) **(B)**; higher penetration at hybrid layer and adhesive layer (reticular) **(C)**; intense deposition at the superior layer of the adhesive (reticular) **(E)** and at the base of hybrid layer (reticular) **(F)**. (r-resin, d-dentin, a-adhesive, s-silver uptake, *-hybrid layer, t- resin tags)

## Discussion

Nanoleakage was first described in 1995 by Sano et al. (1995) [19, 20] when a little diffusion of small ions was observed inside of the hybrid layer even with the absence of interfacial gaps. The most commonly technique to analyze these defects uses silver nitrate immersion technique in conjunction with Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) analyses [4, 6-8, 11, 16, 17, 26, 31, 32].

The results of this study (in percentage) showed that there was no significant difference among the groups studied. Regarding score's evaluation, there was no significant difference for aging, but there was a difference among adhesives.

Scotchbond Multi-purpose and Single Bond showed similarity between them with less silver nitrate penetration than Clearfil SE Bond. One can note that the adhesive that demonstrated higher leakage (Clearfil SE Bond) is self-etching adhesive, contrasting with the ones that require total etching (Scotchbond Multi-purpose and Single Bond) which have lower leakage (Figure 1). These findings can be related to higher water content in the self-etching adhesives, once they need it to activate the acidic

monomers and produce an efficacious hard tissue demineralization. This necessary water decreases the hydrolytic stability of the adhesive system due to water sorption [7, 26].

Some studies have related the influence of aging in nanoleakage [16, 18, 31], by means of hydrolysis degradation, as well as polymer's plastification, which was not observed in this study. One of the reasons could be the absence of the storage liquid's renewal, since there is an acceleration of adhesion aging when the solution is changed periodically [9].

The short time of evaluation as well as the other factor's interaction over adhesion's aging should be considered, as observed by Ernhardt et al. (2008) [4], who reported a stable adhesion in sound dentin after 6 months, suggesting that other factors may contribute for the physico-chemical degradation of the interface, as pH changes, occlusal load and enzyme's variations [4].

The nanoleakage patterns observed in this study were similar to those reported in the literature (reticular, spot and "water tree type") as well as the location of the silver nitrate at the adhesive interface (hybrid layer and adhesive layer) (Figure 2-4). All patterns and location of the nanoleakage were found in all adhesives, except for the "water tree type" pattern, observed only for self-etching adhesive, probably due to the higher water content of those systems (Figure 4) [6-7, 26]. The analyses of resin-dentin interface produced by total-etch adhesives revealed a hybrid layer with high amount and long length of the resin tags, meanwhile the self-etching adhesive showed a hybrid layer with a lower quantity of tags (Figure 2-4) confirming previous studies [12, 23]. Further studies should be conducted in order to evaluate bonding mechanism in primary dentin.

According to results, the two methods of evaluation showed that nanoleakage at the adhesive interface in primary dentin was not influenced by aging of the three adhesive systems tested. However, the analysis of the scores showed that there was more silver uptake for specimens of the self-etch group than those of the total-etch group.

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