The Nanoleakage Patterns of Different Dentin Adhesive Systems

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국문초록

상아질 접착제의 nanoleakage 양상에 관한 연구

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본 연구는 total etching (Scotchbond Multi-Purpose; MP) 및 self-etching (Clearfil SE Bond; SE 과 Prompt L-pop; LP) 상아질 접착제의 nanoleakage 양상을 관찰하고 열 순환 후의 nanoleakage 양상의 변화를 분석하고자 하였다. 30개의 발거된 치아의 교합면 및 협, 설측 법랑질을 제거 하였다. 열 순환 시행 여부에 따라 두 군으로 나누어 실험 하였으며 각각의 상아질 접착제 도포 후 Z-250으로 교합면을 수복하였다. Silver nitrate 용액 및 현상액에 침적 후 치아의 협설 방향으로 평행하게 절단하여 SEM으로 관찰하였다. 서로 다른 양상의 nanoleakage가 관찰 되었다. MP의 경우는 resin tag 주위로 뚜렷한 은의 침착을 관찰 할 수 있었으며 혼합층 전체 두께에 띠 및 점상으로 흩어져 침착된 은을 관찰 할 수 있었다. SE의 경우는 혼합층의 하층을 따라 은으로 침착된 선을 관찰 할 수 있었으며 혼합층과 adhesive 경계를 따라 무정형의 은 침착물 들을 관찰할 수 있었다. LP의 경우는 혼합층의 하부 및 혼합층 내에 띠 모양으로 은의 침착을 관찰 할 수 있었으며 혼합층의 하부에서는 관찰되지 않고 혼합층의 내부에서만 관찰되는 경우도 있었다. 열 순환을 시행한 군에서는 전반적 nanoleakage 양상은 열 순환을 시행하지 않은 군과 유사하였으나 은 침착의 증가를 관찰 할 수 있었다.

주요어 : 상아질, 접착, 혼합층, Self-etching, Thermocycling, Nanoleakage

I. Introduction

Previous studies have focused on marginal gaps as the major pathway of microleakage. But Sano et al.[1] described the occurrence of microleakage in the absence of gaps and the subsurface porosity of demineralized dentin. Yu and Davis[2] described microleakage within or beneath smear layers and resin-bonded dentin using silver nitrate and SEM/EDS techniques. These results indicate that investigators should examine microleakage pathways at high magnification rather than simply relying on macroscopic dye penetration.

Dentin adhesive systems with their micromechanical adhesion to dentin has greatly reduced microleakage[3]. However, the ideal sealing that the adhesive resin completely penetrates the demineralized dentin is rarely achieved, leaving some porous regions within the exposed collagen network[4]. These porosities can be penetrated by solution such as silver nitrate, even in gap-free bonding between denin and restorative composite resin, and this has been termed nanoleakage[5]. The nanoleakage tests can provide much useful information on the sealing ability of dentin adhesive systems and the quality of the hybrid layer [6].

The pore size of nanoleakage is so small that penetration of bacteria into the compound zone

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can be excluded, but not of metabolic products like acids[4]. It should also be considered that marginal gap formation may be caused by the degradation of the adhesive bonding components whereas nanoleakage cannot be seen as an indicator for this loss of attachment.

Poor adhesion to tooth structure and the polymerization contraction of restorative materials are the main causes of microleakage, which can be greater if there is a mismatch in coefficients of thermal expansion of teeth and restorative material. Many leakage tests have used thermal stress to stimulate the clinical situation[7]. This phenomenon may cause the breakdown of the bond and the subsequent failure of restoration. The effects of thermal stress have often shown increased microleakage[8], but in the case of nanoleakage, there is not sufficient information.

Dentin adhesive systems are currently available as three-step, two-step, and single-step systems, depending on how the three cardinal steps of etching, priming, and bonding to tooth substrates are accomplished or simplified[9]. Two-step systems are sub-divided into single-bottle self-etching primers that require an additional bonding step. The recently introduced single-step, self-etching adhesives further combined these three bonding procedures into a single-step application.

Self-etching adhesive systems are attractive in that prior removal of the smear layer and smear plugs is not required. This reduces the potential for post-operative sensitivity and bonding problems associated with movement of dentinal fluid through patent dentinal tubules. The technique-sensitivity associated with bonding to optimally wet collagen matrix is also eliminated[10]. Since they do not require a separated acid-etching step, they are less likely to result in a discrepancy between the depth of demineralization and the depth of resin infiltration[11] because both processes occur simultaneously.

The purpose of this study was: (1) to compare nanoleakage patterns of total etching system and self-etching adhesives and (2) to investigate the change of the nanoleakage patterns after thermocycling.

Ⅱ. Materials and Methods

Thirty freshly extracted human molars stored in 0.5% chloramine-T solution were used. Occlusal, buccal and lingual enamel was removed using a model trimmer under running water. The flat occlusal dentin surfaces were examined under a microscope to insure that no enamel remnants remained. The dentin surfaces were wet ground with 500-grit silicon carbide paper (Buehler Ltd., Lake Bluf, IL, USA) for 60 sec.

Three dentin adhesive systems were used in this study (Table 1). Scotchbond Multi-Purpose (MP) was used as total etching adhesive system. Clearfil SE Bond (SE) and Prompt L-pop (LP) were used as self-etching adhesives. Each 10 dentin surfaces were treated with one of the dentin adhesive systems following each manufacturer's instruction (Table 2). 2mm thick of Z-250 hybrid resin composite (3M Dental Products Inc., St. Paul, USA) was light-cured for 40 sec using XL 3000 curing light (3M) on the treated surface. After storage in tap water at 37℃ for 24 hr, the margins were finished and polished with Sof-lex disks (3M). The specimens were checked using a microscope to ensure that no flash was left along the exposed dentin surfaces.

Five teeth of each dentin adhesive group were thermocycled at 5-55℃ water for 30,000 cycles. Dwell time was 1 min and transfer time was 15 sec for each cycle. Root apices of all teeth were sealed with Z-250 composite (3M), and the entire tooth surface except for the bonded interface and 1mm of the tooth surface adjacent to the interface, was coated with two layers of nail varnish. The teeth were placed in 50% (w/v) silver nitrate solution in total darkness for 24 hr, rinsed in running water for 5 min, and immersed in photo-developing solution under fluorescent light for 8 hr in order to reduce the silver ions to metallic silver. After removal from the developing solution, the teeth were placed in running water for 5 min. The teeth were embedded in translucent resin (Epofix; Struers, Denmark), and sectioned bucco-lingually across the bonded surface, making a total

Table 1. Materials, manufacturers and compositions

Adhesive system	Etchant	Primer	Adhesive resin	Manufacturer
Scotchbond Multi- Purpose	35% H_3PO_4	HEMA, light-cured polymer, water	Bis-GMA, HEMA, photoinitiator	3M Dental Products Inc., St. Paul, USA
Clearfil SE Bond	Self-etching primer MDP, HEMA, hydrophilic dimethacrylate, dl-camphoroquinone, N,N-diethanol-p-toluidine, water		MDP, Bis-GMA, HEMA, hydrophobicdimethacrylate, dl-camphorquinone, N,N-diethanol-p-toluidine, silanated colloidal silica	Kuraray Co, Ltd., Osaka, Japan
Prompt L-Pop	One-step Water, stabilizer, parabenes; methacrylated phosphoric acid eaters; fluoride complex; photoinitiator (BAPO)			3M-ESPE, Seefeld, Germany

BAPO, bis-acyl phosphine oxide; HEMA, hydroxyethylmethacrylate;
Bis-GMA, bisphenol glycidyl dimethacrylate;
MDP, 10-methacryloyloxydecyl dihydrogen phosphatee

Table 2. Bonding procedures according to the manufacturer's instructions.

Scotchbond Multi-Purpose	Etching : apply 15 sec, rinse 10 sec, dry gently Priming : apply primer for 20 sec, air dry mildly Bonding : apply adhesive, air thin gently, light-cure for 20 sec
Clearfil SE Bond	Self-etching priming : apply with a brush, leave undisturbed for 20 sec, air dry mildly Bonding : apply with a brush, air thin gently, light-cure 10 sec
Prompt L-Pop	Apply with continuous scrubbing motion for 15 sec, gently air dry, light cure for 10sec

of 20 specimens for each dentin adhesive system.

All the sectioned surfaces were polished with increasingly fine diamond pastes (6, 3, 1㎛; Buehler Ltd., Lake Bluf, IL, USA). The specimens were cleaned ultra-sonically, air dried, mounted on aluminum stubs and placed in a desiccator for 24 hr, and coated with thin gold. 10 specimens for each group were examined under scanning electron microscope (SEM; JSM840A Jeol, Japan) in backscattering electron mode. The silver particles were confirmed with energy dispersive X-ray system (EDS; Oxford, England).

Ⅲ. Results

All specimens in the six groups showed nanoleakage at the adhesive/ dentin interface. Seven specimens (2 in MP, 2 in SE, 3 in LP thermocycled groups) were excluded from the analysis because of definite marginal microleakage as visible gaps between dentin and composite (Fig. 2(a)). The silver particles were confirmed with EDS (Fig. 2(b)). The conical shape of global silver penetration pattern was shown in Fig. 1(a). And the gap free margins are illustrated in Fig. 1(b). Typical leakage patterns at the adhesive/ dentin interfaces for each dentin adhesive system are illustrated in Fig. 2(a)-(d) under the SEM observation.

Scotchbond Multi-Purpose produced a very distinct hybrid layer which was approximately 15-20㎛ thick. Distinct resin tags were also observed in Fig. 3(a). Accumulation of silver particles were noted at the base of the hybrid layer. And in some parts, loosely distributed patches of silver

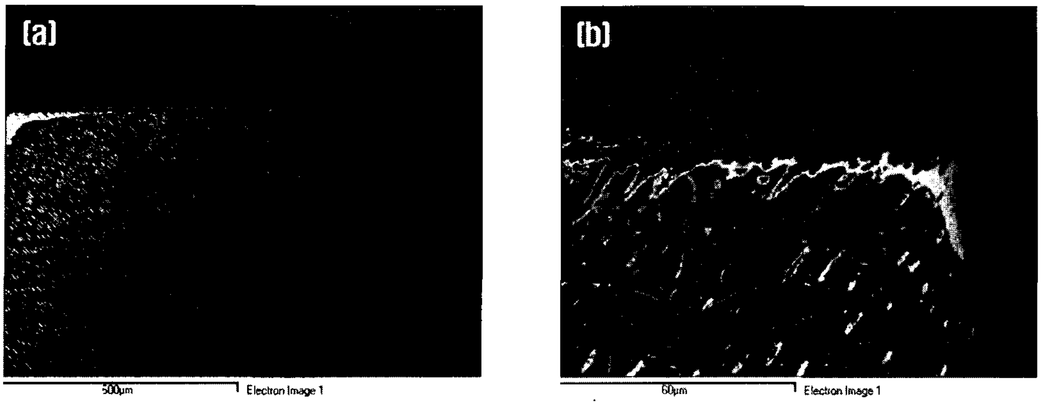

Fig. 1. [a] Backscattering SEM images of Scotchbond Multi-Purpose specimens demonstrate the conical shape of global silver penetration pattern at low magnification (100X). [b] Note the gap free margin at high magnification (1000X). C:composite resin, D:demineralized dentin

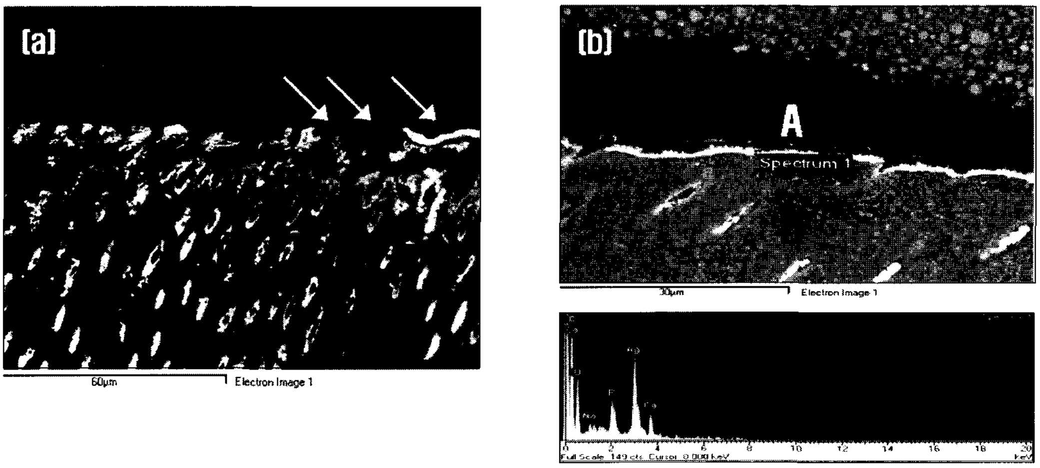

Fig. 2. [a] Backscattering SEM image (2000X) of Scotchbond Multi-Purpose specimen shows the visible gaps (white arrow). [b] Backscattering SEM image of Clearfil SE Bond : EDS proves the white line is silver. A:adhesive, C:composite resin, D:demineralized dentin

deposite appeared in the hybrid layer. Silver was also deposited around the resin tags. Peri and lateral tubules were filled with silver.

The leakage patterns for Clearfil SE Bond was illustrated in Fig. 3[b]. The hybrid layer was approximately 2-3㎛ thick. The resin tags were not clear. Silver particles were deposited along the lower half of the hybrid layer by thin line. At top of the hybrid layer, amorphous silver particles were also identified beneath the layer of composite resin

Prompt L-Pop produced a thin hybrid layer of approximately 2-3㎛ (Fig. 3[c] and [d]). This system showed few resin tags. Silver deposited along the base of the hybrid layer, with isolated deposits located throughout its whole thickness in many specimens. In another specimens, distinct bands of silver deposits could be detected within the hybrid layer, but silver deposition was not distinct in the base of the hybrid layer.

There were no changes in leakage patterns in thermocycled groups. But the silver distribution in the base of and within the hybrid layer was somewhat more intense (Fig. 4[a]-[d]).

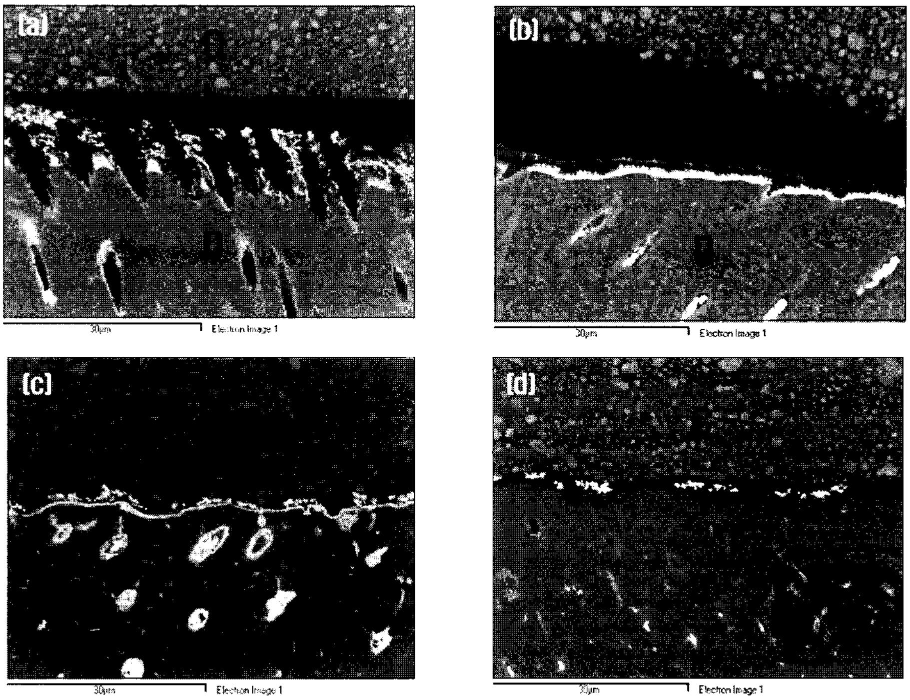

Fig. 3. The nanoleakage patterns : (a) Scotchbond Multi-Purpose, (b) Clearfil SE Bond, (c) and (d) Prompt L-Pop. (a) shows silver accumulation at the base of hybrid layer. In (b), thin line of silver was noted in the base of hybrid layer. And distinct bands of silver deposits were observed within the hybrid layer in (c) and (d). C:composite resin, D:demineralized dentin

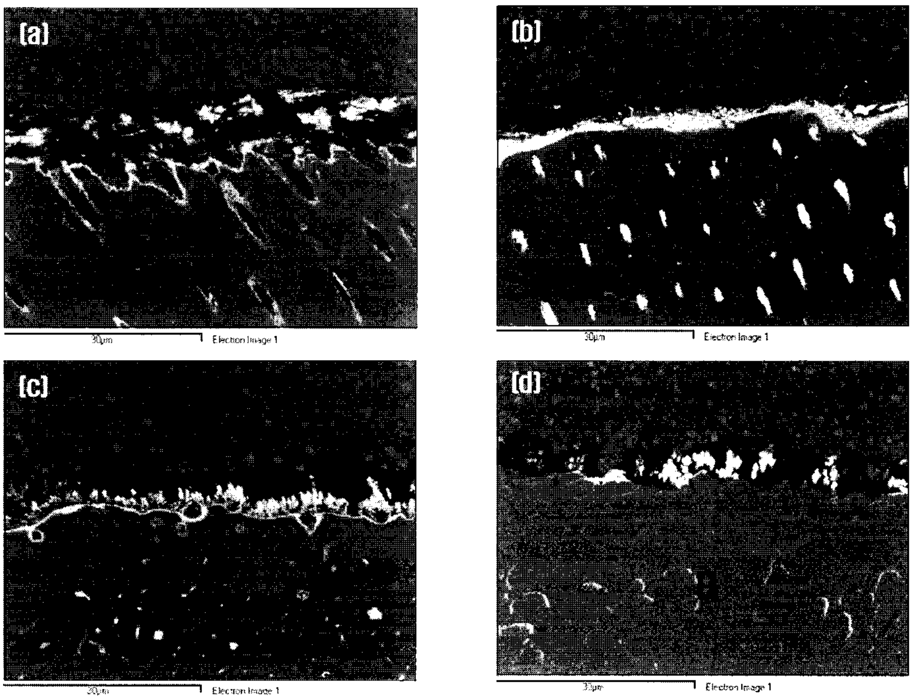

Fig. 4. The nanoleakage patterns of thermocycled groups : (a) Scotchbond Multi-Purpose, (b) Clearfil SE Bond, (c) and (d) Prompt L-Pop. Note that there were no differences in nanoleakage patterns between Fig.3(a)-(d) and Fig.4(a)-(d) except more intense silver penetration in Fig.4(a)-(d). C:composite resin, D:demineralized dentin

Ⅳ. Discussion

In general, all dentin adhesive systems used in this study showed nanoleakage within the hybrid layer and adhesive resin to some extent. This may be a consequence of incomplete penetration of the adhesive resin into the demineralized dentin, leaving the collagen not enveloped by resin, or this may result from polymerization shrinkage[12]. The nanoleakage pathway may be located within the adhesive resin, within the hybrid layer and within partially- or fully-demineralized dentin[13]. Porosity in the hybrid layer not infiltrated by adhesive resin is the most important pathway for nanoleakage and influences the durability of the bond, since solutions can penetrate into these nanometer-sized spaces and hydrolyze the exposed collagen and adhesive resin[12]. The spaces are too small to allow bacterial immigration. However, bacterial products, e.g. acids, might use nanoleakage as penetration pathways through the hybrid layer and dentinal tubules. And the unprotected protein at the dentin adhesive interface may be accessible to bacterial proteolytic enzyme[33].

The silver crystals have a minimum diameter of 3 to 5 nm; after longer treatment these small crystals fuse to form large aggregates of 15 to 50 nm diameter[14]. Collagen can be stained strongly with silver[15]. The resin of current dentin adhesive systems has been reported not to permeate uniformly within the spaces around the collagen fibrils, leaving them exposed. It may be speculated that when the specimens were immersed in the silver nitrate solutions, the silver penetrated the spaces existing within the hybrid layer by seeping around and precipitating onto the exposed collagen fibrils[16].

In the dye penetration studies reported in the literatures, the thermocycling regimens varied in temperature number of cycles and dwell time. Temperature extremes have varied among researchers with a mean low temperature of 6.6℃ and mean high temperature of 55.5℃[17]. The time of immersion of specimens in hot or cold fluids has varied from a few seconds to several minutes. Harper et al.[18] pointed out that a patient would not tolerate direct contact of vital tooth with extremely hot or cold substances for extended periods of time, so dwell time longer than 15 sec would seem to be inappropriate. According to the study of Miyazaky et al.[19], the bond strength of dentin adhesive systems decreased after 30,000 cycles, especially in the self etching systems. Thus, temperature of 5-55℃, dwell time of 15 sec and 30,000 cycling numbers were selected in this study.

During the thermal cycling test, the hot water may accelerate hydrolysis of the resin composite as well as the bonding agent and extract poorly polymerized resin oligomers[20]. The results of this study showed that thermocycling did not affect nanoleakage greatly for dentin adhesive systems used. The results are in agreement with those of previous microleakage studies[21,22]. Harper et al.[18] showed that the rate of temperature change beneath resin restorations was relatively low after taking cold or hot fluids, indicating that the linear coefficient of thermal expansion would not greatly influence the dimensional change of the material and therefore not affect microleakage. Stryom et al.[23] suggested that the bonding of the adhesive to tooth structure is micro-mechanical in nature and would not be adversely affected by temperature cycling. High bond strength and the formation of a hybrid layer at the adhesive/dentin interface resulted in gap-free restorations[16]. Although porosities are still present within the hybrid layer and may permit molecular leakage, it is not believed that thermal stresses imposed on the restorations would be great enough to enlarge the porosities or create gaps at the interface and subsequently increase the dye penetration[24]. The nanoleakage phenomenon is related to the process of bonding and is not caused by a later fatigue of the hybrid layer[25].

The bonding agents used in this study achieved almost totally gap-free margins at the dentin-restoration interfaces. This may be attributed to several factors. The stress generated by polymerization contraction of resin composites has been reported to be about 13-17 MPa and shear bond strength of approximately 21 MPa is believed to be necessary to prevent marginal contraction gaps[26]. All three adhesive systems in this study are capable of achieving higher bond strengths, which theoretically resist the polymerization contraction stresses. Feilzer et al.[27] found that the resulting stress inside a restoration was proportional to the ratio of its bonded to non-bonded surfaces. The flat occlusal composite restorations in this study have almost as much free surface area as bonded surface area, and hence have a C-factor of less than 1. Feilzer et al.[27] stated that restorations with C<1 are the only restorations likely to survive polymerization contraction stresses. All these factors may explain the gap-free margins observed in this study, even though bulk placement technique was employed. The composite thickness was less than 2mm, which is the recommended initial thickness for the incremental technique. Using silver nitrate staining, the artificial gaps created upon high vacuum dehydration during specimen preparation can be easily differentiated from true gaps by the absence of silver staining along the gap border with the backscattered electron mode[28]. The conical shape of the penetration-induced structure can be explained by the diffusion behavior (Fick's first law) of the silver nitrate solution during the exposure time of 24 hr[25].

Scotchbond Multi-Purpose demonstrated more intense accumulation of silver in the base of hybrid layer than Clearfil SE bond and Prompt L-Pop. This may mean that Scotchbond Multi-Purpose has much more porosity in the base of hybrid layer than other systems. Silver ions are easily reduced to metallic silver by organic material, especially in the presence of collagen fibers. Intense silver accumulation along the base of hybrid layer may have resulted from that silver ions had filled collagen-rich and resin-poor spaces at the bottom half of deminerailzed dentin[29]. Silver deposition in the hybrid layer may represent incomplete removal of water. Using air stream to dry applied primer may not effectively remove residual water from a HEMA/water primer[30]. Remaining water interrupts the formation of adequate polymer network, leaving silver penetrable porosity.

Clearfil SE Bond simultaneously etches and primes the dentin, allowing the adhesive resin to fully penetrate the demineralized dentin. So theoretically silver deposition may not occur at the base of hybrid layer. However, in this study, this system could not ensure complete penetration of adhesive resin into the inter-fibrilar collagen spaces, otherwise, silver would not appear within the hybrid layer.

Prompt L-Pop demonstrated minimal deposition of silver in the base of hybrid layer and the distributed silver particles in the whole hybrid layer. This nanoleakage patterns cannot be attributed to incomplete resin infiltration. These micro-voids more likely represent areas in which water was incompletely removed from the resin-dentin interfaces. The presence of residual water within the adhesive may lead to domains of incomplete polymerization of the adhesive or sequestrations of more hydrophilic oligomers in these particular regions[9]. All self-etch systems contain water to ionize the acidic monomers for effective demineralization of dentinal hard tissues[9]. Whereas water is comparatively easy to remove with the use of acetone-based adhesives, because its azeotrope contains more water and it has a higher vapor pressure, it is difficult to remove the last traces of water from ethanol-based adhesives, due to the increased capacity of ethanol to form hydrogen bonds with water[9].

The deeper the demineralized dentin and thicker the hybrid layer, the greater is the potential for nanoleakage of silver ions within the partially- or fully-demineralized dentin and the hybrid layer, or within partially polymerized adhesive resin[31]. The critical index of the quality of a hybrid layer may be how much silver it takes up per unit volume or per unit depth. Self-etching systems that do not etch as deeply as total etch systems may have as much or more silver uptake per unit volume of hybrid layer than more aggressive etching systems[31].

Although the mechanisms leading to the nanoleakage phenomenon are not completely explored, it seems to be evident from our study that nanolealage is mainly due to a discrepancy between the depth of etching and the depth of resin penetration in the cases of total etching systems like Scotchbond Multi-Purpose. Also, it should be considered that non-polymerized primer monomers and residual water additionally might affect porosities in the hybrid layer region which form nanoleakage as well[32] in the cases of self-etching systems like Clearfil SE Bond and Prompt L-Pop. It is too early to judge the clinical relevance of nanoleakage. But the influences in marginal discoloration, recurrent caries, post-operative symptoms and the longevity of the composite restoration cannot be excluded. Further studies are needed to evaluate the clinical significance of this phenomenon and, if necessary, to develop adhesive systems minimizing nanoleakage in order to optimize dentin bonding.

Ⅴ. Conclusions

1. Different nanoleakage patterns were observed with the different adhesive systems.
   - In the group of MP, accumulation of silver particles were noted at the base of the hybrid layer. And in some parts, loosely distributed patches of silver particles appeared in the hybrid layer. Silver was also deposited around the resin tags, the peri and lateral tubules.
   - In the group of SE, silver particles were deposited along the lower half of the hybrid layer by thin line. At top of the hybrid layer, amorphous silver particles were also identified beneath the layer of composite resin.
   - In the group of LP, silver deposited along the base of the hybrid layer, with isolated deposits located throughout its whole thickness in many specimens. In another specimens, distinct bands of silver deposits could be detected within the hybrid layer, but silver deposition was not distinct in the base of hybrid layer.
2. The dentin adhesive systems used in this study did not achieve perfect sealing at the composite/dentin interface.
3. There were no changes in nanoleakage patterns in thermocycled groups. But the silver distribution in the base of and within the hybrid layer was somewhat more intense.

References

1. Sano H, Shono T and Takatsu T : Microporous dentin zone beneath resin-impregnated layer. *Oper Dent* 19:59-64, 1994.
2. Yu XY and Davis EL : Orientation and progression of microleakage in restoration with smear layer-mediated dental bonding agent. *Quintessence Int* 23:551-555, 1992.
3. Retief DH : Do adhesive prevent micro-leakage? *Int Dent J* 44:19-26, 1994.
4. Eick JD, Gwinnett AJ, Pashly DH and Robinson SJ : Current concepts to adhesion to dentin. *Crit Rev Oral boil Med* 8:306-335, 1997.
5. Sano H, Takatsu T, Ciucchi B, Horner JA, Matthews WG and Pashley DH : Nanoleakage:leakage within the hybrid layer. *Oper Dent* 20(1):18-25, 1995.
6. Li H, Burrow MF and Tyas MJ : Nanoleakage patterns of four dentin bonding systems. *Dent Mater* 16(1):48-56, 2000.
7. Trowbrigde HO : Model systems for determining biologic effects of micro-leakage. *Oper Dent* 12:164-172, 1987.
8. Alani AH and Toh CG : Detection of micro-leakage around dental restorations: a reviw. *Oper Dent* 22:173-85, 1997.
9. Tay FR, Pashley DH and Yoshiyama : Tow modes of nanoleakage expression in single-step adhesives. *J Dent Res* 81(7):472-476, 2002.
10. Perdiao J, Van Meerbeek B, Lopes MM and Ambrose WW : The effect of a re-wetting agent on dentin bonding. *Dent Mater* 15:282-295, 1999.
11. Spencer P, Wang Y and Walker MP : Interfacial chemintry of the dentin/adhesive bond. *J Dent Res* 79:1458-1463, 2000.
12. Sano H, Yoshiyama M, Ebisu S, Burrow MF, Takatsu T, Ciucchi B, Carvalho R and Pashley DH : Comparative SEM and TEM observations of nanoleakage within the hybrid layer. *Oper Dent* 20(4):160-7, 1995.
13. Sano H, Yoshikawa T, Pereira PNR, Kanemura N, Morigami M, Tagami J and Pashley DH : Long term durability of dentin bonds made with a self-etching primer, in vivo. *J Dent Res* 78:906-911, 1999.
14. Hayet MA : Silver in Positive Staining for electron microscopy. *New York: Van Nostrand Reinhold*:124-146, 1975.
15. Eick JD, Miller RG, Robinson SJ and Bowles CQ : Quantitative analysis of the dentin adhesive interface by Auger spectroscopy. *J Dent Res* 75:1027-1033, 1995.
16. Li H, Burrow MF and Tyas MJ : Nanoleakage of cervical restorations of four dentin bonding systems. *J Adhes Dent* 2(1):57-65, 2000.
17. Gale MS and Darvell BW : Controlling dentin penetration in computer micro-leakage tracer mapping. *J Dent*

25:129-36, 1997.
18. Harper RH, Schnell RJ and Swarts ML : Phillips RW. In vivo measurements of thermal diffusion through restorations of various materials. *J Prosth Dent* 43:180-5, 1980.
19. Miyazaki M, Sato M and Onose H : Durability of enamel bond strength of simplified bonding systems. *Oper Dent* 25:75-80, 2000.
20. Bastioli C, Romano G and Migliaresi C : Water sorption and mechanical properties of dental composites. *Biomaterials* 11:219-223, 1990.
21. Rossomando KJ and Wendt Jr SL : Thermocycling and dwell times in micro-leakage evaluation for bonded restorations. *Dent Mater* 11:47-51, 1995.
22. Prati C, Tao L, Simpson M and Pashley DH : Permeability and microleakage of resin composite restorations. *J Dent* 22:49-56, 1994.
23. Strydom C, Retief DH, Russell CM and Denys FR : Laboratory evaluation of the Gluma 3-step bonding system. *Am J Dent* 8:93-8, 1995.
24. Li H, Burrow MF and Tyas MJ : The effect of thermocycling regimens on the nanoleakage of dentin bonding systems. *Dent Mater* 18(3):189-96, 2002.
25. Dorfer CE, Staehle HJ, Wurst MW, Duschner H and Pioch T : The nanoleakage phenomenon:influence of different dentin bonding agents, thermocycling and etching time. *Eur J Oral Sci* 108(4):346-51, 2000.
26. Retief DH, Mandras RS and Russell CM : Shear bond strength required to prevent microleakage of the dentin/restoration interface. *Am J Dent* 7:44-46, 1994.
27. Feilzer AJ, de-Gee AJ and Davidson CL : Setting stress in resin composite in relation to configuration of the restoration. *J Dent Res* 66:1636-1639, 1987.
28. Tay FR, Gwinnett AJ, Pang KM and Wei SHY : Variability in microleakage observed in a total-etch wet bonding technique under different handling conditions. *J Dent Res* 74:1168-1178, 1995.
29. Pashley DH, Ciucchi B, Sano H and Horner JA : Permeability of dentin to adhesive agent. *Quintessence International* 24(9):618-631, 1993.
30. Paul SJ, Welter DA, Ghazi M and Pashley D : Nanoleakage at the dentin adhesive interface vs microtensile bond strength. *Oper Dent* 24(3):181-8, 1999.
31. Pereira PN, Okuda M, Nakajima M, Sano H, Tagami J and Pashley DH : Relationship between bond strengths and nanoleakage:evaluation of a new assessment method. *Am J Dent* 14(2):100-4, 2001.
32. Griffiths BM and Watson TF : Variable polymerization shirinkage and the interfacial micropermeability of a dentin bonding system. *J Adhesive Dent* 1:119-131, 1999.
33. Pioch T, Staehle HJ, Duschner H and Garcia-Godoy F : Nanoleakage at the composite-dentin interface:a review. *Am J Dent* 14(4):252-8, 2001.

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