RESEARCH ARTICLE

Evaluation of Nanoleakage Depth and Pattern of Cervical Restorations Bonded with Different Adhesive Systems

Niveditha Bhupanapadu[1], Mohammed A Sattar[2], Arpita Deb[3]

Abstract

Aim: To evaluate nanoleakage depth and pattern of cervical restorations bonded with different adhesive systems.

Materials and methods: Thirty-six extracted human premolar teeth were used for the study and grouped according to different bonding agents.

Group I: fifth generation dentin bonding agent—ONE COAT SL.

Group II: sixth generation dentin bonding agent—PARABOND.

Group III: seventh generation dentin bonding agent—ONE COAT 7.0.

For nanoleakage depth evaluation, 36 teeth were divided into three groups of 12 teeth each, according to adhesive systems used. For each adhesive system, teeth were subdivided into three subgroups of four teeth each, according to storage period, 24 hours, 1 month, and 3 months before the examination. In each tooth, two cavities were prepared (buccal and lingual), each cavity was lined with different adhesive systems and restored using a nanohybrid composite. The restored teeth were then immersed in water bath at temperature 37°C for intended period of time and then stored in 50% silver nitrate for 24 hours and photo developing solution for 8 hours. After this, the teeth were cut in buccolingual direction and subjected to scanning electron microscope (SEM) analysis for nanoleakage depth analysis.

Results: Group II showed the highest nanoleakage at all three periods. At 24 hours, group III showed more leakage than group I (mean = 0.2869 > 0.2506). At 1 month storage period, there was no significant difference in the leakage. At 3 months storage period, group III showed less leakage than group I (mean = 0.5544 < 0.7313).

Keywords: Dentinbonding, Hybrid layer, Nanoleakage.

International Journal of Clinical Pediatric Dentistry (2022): 10.5005/jp-journals-10005-2391

Introduction

For many decades now, optimal adhesion is considered as the "holy grail" of restorative materials. In order to achieve maximum adhesion of restorative materials to tooth surface, the following conditions must be considered:

- Conservation of sound tooth structure.
- Retention of the restoration must be achieved.
- Prevention of micro/nanoleakage.[1]

Nanoleakage is defined as leakage of ions and molecules internally through spaces that are nanometer sized, usually around the collagen fibrils in hybrid layer at resin–tooth interface. These small ions permeate within the hybrid layer even without any gap formation, through spaces that are approximately 0.02 μm in size. The leakage occurs from the base of hybrid layer, exactly at the junction of resin monomers interfacing with decalcified/demineralized dentin and permeates laterally all over this layer. This lateral permeation has been reported to occur not only because of nanoleakage but also due to incomplete penetration of bonding agents/adhesives into demineralized dentin.[2]

Along with small ions, nanoleakage may lead to diffusion of microorganisms, bacterial products, and oral fluids along the resin–dentin interface. This will lead to "hydrolytic" breakdown of resin in bonding agent, and the collagen found in hybrid layer. Thus, weakening the bond strength of resin–dentin interface, which further compromises the retention of restoration.[3–5] And a compromised resin–dentin bond directly impacts the success of treatment, failing which, can lead to continued micro/nanoleakage and further, over a period of time affect the pulpal health and tooth vitality.

Through its evolution from generation one to eight, dentin adhesives have been continuously improvised through 30 years of development. Dentin bond agents are bonded to tooth surface using two techniques, total etch technique and self-etch technique.

In total etch technique, both enamel and dentin are simultaneously etched, which allows for removal of smear layer. But, the collagen fibrils in the hybrid layer incompletely expand which may diminish the resin infiltration thus compromising the bond between restoration and tooth.[6] The self-etch technique involves etching, priming, and bonding simultaneously to the

[1,2]Department of Conservative Dentistry and Endodontics, HKE Society's S. Nijalingappa Institute of Dental Sciences and Research, Kalaburagi, Karnataka, India

[3]Department of Conservative Dentistry and Endodontics, Dayananda Sagar College of Dental Sciences, Bengaluru, Karnataka, India

Corresponding Author: Niveditha Bhupanapadu, Department of Conservative Dentistry and Endodontics, HKE Society's S. Nijalingappa Institute of Dental Sciences and Research, Kalaburagi, Karnataka, India, Phone: +91 9886158894, e-mail: nivedithabhupanpadu@yahoo.co.in

How to cite this article: Bhupanapadu N, Sattar MA, Deb A. Evaluation of Nanoleakage Depth and Pattern of Cervical Restorations Bonded with Different Adhesive Systems. Int J Clin Pediatr Dent 2022;15(3):299–303.

Source of support: Nil

Conflict of interest: None

dentin surface,[7] however in this technique, smear layer remains intact. In self-etching technique, depth of demineralization and depth of resin infiltration is almost similar.[8]

As the dentin adhesive systems evolved from three-step technique to one-step technique, new products have been introduced into the market which require prior studies. For this reason, *in vitro* studies have to be done before clinical application.[9]

A variety of different techniques for assessing nanoleakage have been developed and utilized. SEM analysis with silver nitrate penetration has an advantage over other methods because SEM provides us with higher magnification of the specimen and the use of silver nitrate is second to the organic dyes in usage.

Materials and Methods

Thirty-six, sound, noncarious premolars with no anomalies were selected for the study, and divided into experimental groups as follows (Fig. 1):

- Group I: fifth generation dentin bonding agent—ONE COAT SL (*n* = 12).
- Group II: sixth generation dentin bonding agent—PARABOND (*n* = 12).
- Group III: seventh generation dentin bonding agent—ONE COAT 7.0 (*n* = 12).

The sample teeth in each experimental group were subdivided into three subgroups according to the storage period, that is, 24 hours, 1 month, and 3 months, and each of the subgroup had four sample teeth each. Any debris or calculus that was present on each sample tooth was then removed using a hand scaler and polished with pumice in rubber cups.

Standardized 3 mm × 2 mm wide and 2 mm deep, buccal and lingual cavities were prepared in each tooth (Fig. 2), at level of cemento-enamel junction (CEJ) in midline of buccal and lingual surfaces, using a round diamond bur, yielding us a total of eight cavities for each experimental group tested at the designated storage period.

Each cavity was lined with different adhesive systems and restored using the nanohybrid composite 3M Espe Filtek Z250 and light cured for 40 seconds (Fig. 3). After the restorations were done, the samples were divided accordingly to their respective groups and stored in water bath at 23°C for 24 hours, 1 month, and 3 months according to their respective subgroups. At the end of each storage period, the samples were removed from the water bath and further submerged in 50% silver nitrate solution in total darkness for 24 hours again.

Fig. 1: Armamentarium

At the end of 24 hours, the samples were taken out and rinsed in running water for 60 seconds. Further, the samples were sectioned through the center of the restorations buccolingually, such that the tooth is longitudinally split into mesial and distal half (Fig. 4) and the split surfaces are polished with abrasives to achieve debris free surfaces. For evaluation of nanoleakage pattern, the stored samples were additionally immersed in 10% ethylene diamine tetra-acetic acid (EDTA) solution for 5 seconds to remove the smear layer.

The samples were subjected to SEM analysis in backscattering mode.

Results

Nanoleakage Depth Analysis

For nanoleakage depth calculation, percentage of the depth of silver particles penetration from the bonded surface to total length of bonded surface was calculated for statistical analysis (Table 1).

After 24 hours, group II shows maximum leakage, followed by group III and group I (Fig. 5). After 1 month, group II shows maximum leakage, not much difference in mean of group I and group III

Fig. 2: Prepared cavity on buccal aspect

Fig. 3: Restored buccal cavity

(Fig. 6). After 3 months, group II shows maximum leakage, not much difference in mean of group I and group III (Fig. 7).

Two-way analysis of variance analysis shows that, *p*-value for intergroup comparison is 0.000 which indicates that types of groups are associated with different leakage values. *p*-value for subgroup is 0.000 which indicates that different subgroups values are also associated with leakage. *p*-value of interaction is also significant which indicates that leakage depends on relationship between groups and subgroups.

Result of two-way ANOVA indicated a significant effect for three different groups, $F = 1899.946$, $p = 0.000$ and a significant effect for subgroups for different durations, $F = 1217.586$, $p = 0.000$.

It indicates that there is sufficient evidence to conclude significant effect of different groups on leakage tendency of different materials at different duration. Additionally, the result shows no interaction between groups and subgroups, $F = 111.609$, $p = 0.000$.

Nanoleakage Pattern Analysis

Silver nitrate particle deposits were appreciable at higher magnification of 1000× and above. There was no distinct difference in the type of nanoleakage patterns observed, according to different type of adhesive system. However, the size and number of deposits increased as the storage time increased.

Also the pattern of deposits seemed to change from small circular dots to reticular patches distributed in the dentin from 24 hours sample to 3 months, respectively (Figs 8 and 9).

Discussion

The bonding agents used in this study were fifth (ONE COAT SL), sixth (PARABOND), and seventh generation (ONE COAT 7.0).

In the present study, the depth of silver nitrate penetration was compared between the fifth, sixth, and seventh generation bonding agents, stored in water at controlled temperature for three different periods, that is, 24 hours, 1 month, and 3 months. Results of the study show that:

- At 24 hours the maximum leakage was found in PARABOND (sixth generation; group II) followed by ONE COAT 7.0 (seventh generation; group III) and ONE COAT SL (fifth generation; group I).
- At 1 month storage period, there was no significant difference in the leakage seen in ONE COAT SL and ONE COAT 7.0, highest leakage was found in PARABOND (sixth generation; group III).
- At 3 months storage period, ONE COAT 7.0 (seventh generation; group III) showed less leakage than ONE COAT SL (fifth generation; group I). Group II, PARABOND showed highest leakage at all three time periods.

ONE COAT 7.0 is an ethanol based dentin adhesive. Ethanol is an alcohol, which has high vapor pressure in comparison to water, because of which water readily dissociates from demineralized dentin and helps in better resin infiltration thus facilitating nanoleakage reduction through the hybrid layer. Therefore, hydroxyethyl methacrylate (HEMA)/ethanol combination is advantageous than HEMA/water combination in adhesive resins. Ethanol also has high hydrogen bonding capacity [δh: 19.4 $(J/cm^3)^{1/2}$]. This helps to expand the demineralized collagen fibrils which remain collapsed due to air drying of the dentin surface.

ONE COAT SL contains water as solvent. Since water is already present all along the dentinal surface into the depth of demineralization. Therefore, incorporation of water is logical in dental adhesives. Although, the solvent effect is not the primary role of water in dentin bonding agents, hence it is used as secondary "co-solvent." According to Hiraishi et al., addition of water is unavoidable, but it should not be added excessively as this will affect the concentration of the resin monomers in the adhesives. Whether one-step or two-step dentin bonding agents, water based bonding agents tend to degrade with time when stored at higher than room temperatures.[10]

When water is used as solvent in two-step adhesives, the collapsed collagen fibrils caused due to air drying, get reexpanded, but the vapor pressure of water (17 mm Hg) is less than that of ethanol (40 mm Hg), also because water is a polar molecule, it has high affinity toward hydrogen bonds, this makes it difficult to remove the excess amount of residual water in the demineralized dentin which will further affect the integrity of the bond.

Jacobsen and Söderholm suggested that water is a poor solvent in HEMA containing dental adhesives.[11] The water from the HEMA–water mixture evaporates quickly leaving behind the concentrated HEMA in the solution, which reduces the vapor pressure of the residual water in dentin thus is more difficult for elimination.[12]

There are numerous studies which prove that, when the hybrid layer or the adhesive layer at the resin–dentin interface has excess amount of residual water, this will eventually lead to hydrolytic degradation of the bond due to water sorption, thus affecting the bond integrity, further leading to nanoleakage.[13]

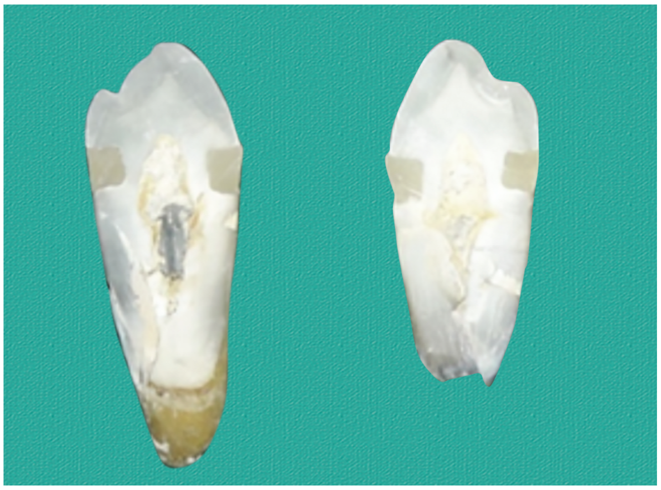

Fig. 4: Sectioning of tooth

Table 1: Mean and standard deviation of groups I, II, and III at 24 hours, 1 month, and 3 months

	Group I		*Group II*		*Group III*		
	Mean	*SD*	*Mean*	*SD*	*Mean*	*SD*	*p-value*
24 hours	0.2506	0. 02323	0.6106	0. 02863	0.2869	0. 01740	0.000
1 month	0.4069	0. 04771	0.9113	0. 03096	0.3838	0.0161	0.000
3 months	0.7313	0. 05427	0.9113	0. 03096	0.5544	0. 01788	0.000

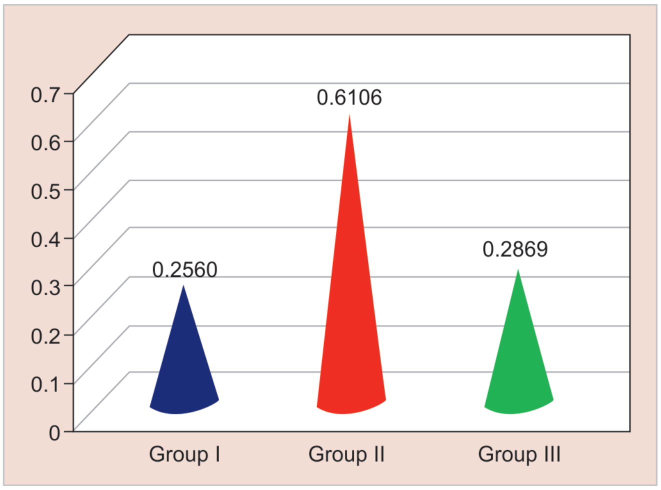

Fig. 5: After 24 hours group II shows maximum leakage, followed by group III and group I

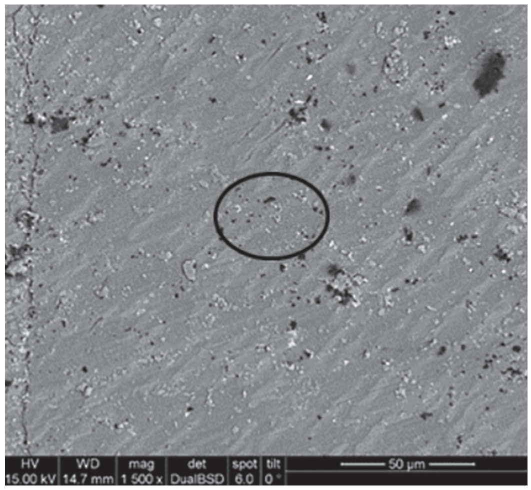

Fig. 8: SEM image of sample stored for 24 hours showing "circular dot" pattern

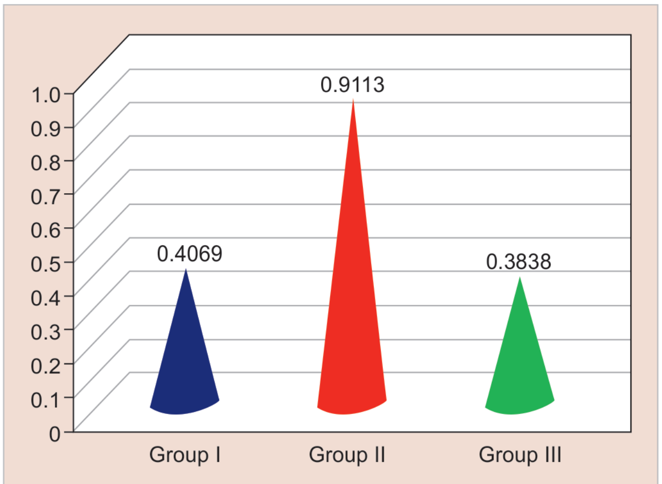

Fig. 6: After 1 month, group II shows maximum leakage, not much difference in mean of group I and group III

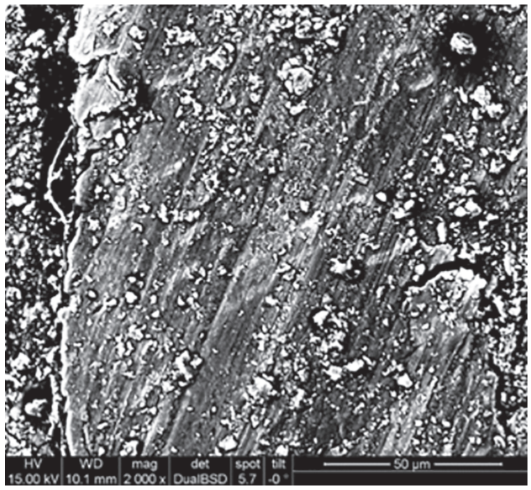

Fig. 9: SEM image of sample stored for 3 months showing reticular pattern

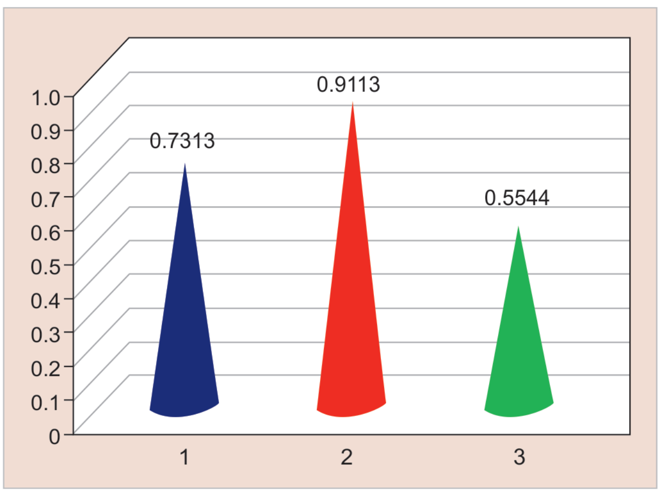

Fig. 7: After 3 months, group III showed least leakage. Group II showed highest, followed by group I

PARABOND, is a sixth generation chemically cured adhesive resin. It is well known that when chemically/self-cured resins are compared with light cured resins, the polymerization shrinkage of chemically cured resins is higher and in cervical class V restorations, which is bonded on five surfaces, has high c-factor which leads to bond failure at the adhesive and dentin interface.

Sixth generation dentin adhesives do not contain fillers, and absence of a separate primer affects the viscosity of the dentin adhesives wherein, the wettability of the dentinal surface reduces, thus reducing the adhesive property and sealing capacity leading to micro/nanoleakage.

The results of this study are comparable to Vinay and Shivanna's study "Comparative evaluation of microleakage of fifth, sixth, and seventh generation dentin bonding agents: an *in vitro* study." They concluded that seventh generation bonding agent showed least microleakage and sixth generation bonding agent showed the highest. The seventh generation adhesive they used was Clearfil S3 Bond, which is alcohol based like the one used in the present study.[14]

In another study conducted by Sánchez-Ayala et al., who compared marginal microleakage of six different one-step self-etch dentin bonding agents. Among the six different adhesive systems, ONE COAT 7.0 showed least amount of microleakage.[15]

Not many studies are available evaluating the nanoleakage of ONE COAT 7.0 which is HEMA based adhesive. The hydrophilic and hydrophobic components in the adhesive resin are retained in a mixed state because of the presence of HEMA, including the less-miscible dimethacrylate monomer molecule.

Nanoleakage Pattern

In the present study, nanoleakage pattern did not differ between the different type of adhesive resin used, as it was noted in all the experimental groups but it was dependent on the storage period.

At 24 hours storage period, the type of pattern observed was found to be unevenly distributed tiny circular dots of silver nitrate particles, which showed increase in number and size as the storage time increased to 1 month and 3 months. Three months samples showed more reticular type of pattern.

Conclusion

Under the limitations of this study, we can conclude that:

- Nanoleakage was present in all the groups at all three time periods. That is, nanoleakage was influenced not only by the type of dental adhesive, but also, by the duration of storage.
- Furthermore, nanoleakage was dependent on both technique of application and also in the chemistry of the adhesive resin. In this study, it was found that ethanol based dental adhesives showed least nanoleakage on long term analysis.
- The nanoleakage increased in depth, continuity, and its intensity because of storage in water.

Another conclusion to be drawn from this present study is that, further evaluation should be done by similar *in vitro* studies with larger sample sizes, longer storage period, and standardization of operator variability. Also long term clinical trials should be conducted to draw clinically relevant conclusions.

Clinical Significance

Dental hypersensitivity is one of the common postoperative complication we come across in clinical scenarios. Understanding the material aspect of bonding agents and their long term survivability helps us overcome such complications and adds to the benefit of the patients.

Orcid

Niveditha Bhupanapadu https://orcid.org/0000-0002-3638-008X

References

1. Anusavice KJ, Shen C, Rawls HR. Phillips' Science of Dental Materials. Elsevier Health Sciences; 2013. pp. 185–381.
2. Sano H, Takatsu T, Ciucchi B, et al. Nanoleakage: leakage within the hybrid layer. Oper Dent 1995;20(1):18–25.
3. Erick J, Gwinnett A, Pashley D, et al. Current concepts on adhesion to dentin. Crit Rev Oral Biol Med 1997;8(3):306–335. DOI: 10.1177/10454411970080030501
4. Carvalho RM, Tay F, Sano H, et al. Long term mechanical properties of EDTA-demineralized dentin matrix. J Adhes Dent 2000;2(3): 193–199.
5. Hashimoto M, Ohno H, Kaga M, et al. In-vivo degradation of resin-dentin bonds in humans over 1 to 3 years. J Dent Res 2000;79(6): 1385–1391. DOI: 10.1177/00220345000790060601
6. Van Meerbeek B, Yoshihara K, Yoshida Y, et al. State of the art of self-etch adhesives. Dent Mater 2011;27(1):17–28. DOI: 10.1016/j.dental.2010.10.023
7. Tay FR, Pashley DH. Aggressiveness of contemporary self-etching systems. I: depth of penetration beyond dentin smear layers. Dent Mater 2001;17(4):296–308. DOI: 10.1016/s0109-5641(00)00087-7
8. Carvalho RM, Chersoni S, Frankenberger R, et al. A challenge to the conventional wisdom that simultaneous etching and resin infiltration always occurs in self-etch adhesives. Biomaterials 2005;26(9): 1035–1042. DOI: 10.1016/j.biomaterials.2004.04.003
9. Lucena-Martin C, Gonzalez-Rodriguez MP, Ferrer-Luque CM, et al. Influence of time and thermocycling on marginal sealing of several dentin adhesive systems. Oper Dent 2001;26(6):550–555.
10. Hiraishi N, Nishiyama N, Ikemura K, et al. Water concentration in self-etching primers affects their aggressiveness and bonding efficacy to dentin. J Dent Res 2005;84(7):653–658. DOI: 10.1177/154405910508400714
11. Jacobsen T, Söderholm KJ. Some effects of water on dentin bonding. Dent Mater 1995;11(2):132–136. DOI: 10.1016/0109-5641(95)80048-4
12. Pashley EL, Zhang Y, Lockwood PE, et al. Effects of HEMA on water evaporation from water-HEMA mixtures. Dent Mater 1998;14(1):6–10. DOI: 10.1016/s0109-5641(98)00003-7
13. Yiu CKY, Pashley EL, Hiraishi N, et al. Solvent and water retention in dental adhesive blends after evaporation. Biomaterials 2005;26(34):6863–6872. DOI: 10.1016/j.biomaterials.2005.05.011
14. Vinay S, Shivanna V. Comparative evaluation of microleakage of fifth, sixth, and seventh generation dentin bonding agents: an *in vitro* study. J Conserv Dent 2010;13(3):136–140. DOI: 10.4103/0972-0707.71645
15. Sánchez-Ayala A, Farias-Neto A, Vilanova LSR, et al. Marginal microleakage of class V resin-based composite restorations bonded with six one-step self-etch systems. Braz Oral Res 2013;27(3):225–230. DOI: 10.1590/S1806-83242013000300003