Evaluation of microshear bond strength and nanoleakage of etch-and-rinse and self-etch adhesives to dentin pretreated with silver diamine fluoride/potassium iodide: An in vitro study

Karthik Selvaraj, Vidhya Sampath, Sujatha V, Mahalaxmi S

ABSTRACT

Aims: The aim of this in vitro study was to comparatively evaluate the microshear bond strength (MSBS) of etch-and-rinse and self-etch (ER and SE) bonding systems to dentin pretreated with silver diamine fluoride/potassium iodide (SDF/KI) and nanoleakage at the resin-dentin interface using transmission electron microscope (TEM).

Subjects and Methods: Seventy-two dentin slabs of 3 mm thickness were prepared from extracted human permanent third molars and divided into four groups (n = 18) based on the dentin surface treatment as follows: (1) ER adhesive bonding without dentin pretreatment; (2) SDF/KI pretreatment of dentin followed by ER adhesive bonding; (3) SE adhesive bonding without dentin pretreatment; and (4) SDF/KI pretreatment of dentin followed by SE adhesive bonding. Resin composite was built on the dentin slabs to a height of 4 mm incrementally, and dentin-composite beams of approximately 1 mm² cross-sectional area were prepared. The beams were subjected to MSBS analysis, and the fractured surface was observed under scanning electron microscope to determine the mode of failure. The resin-dentin interface was examined under TEM for evaluation of nanoleakage.

Statistical Analysis Used: One-way ANOVA followed by Tukey’s post hoc multiple comparison tests.

Results: Pretreatment of dentin with SDF/KI increased the MSBS of ER and SE adhesives, though not statistically significant, except between Groups 2 and 3. In all the groups, the predominant mode of failure was adhesive followed by cohesive in resin, mixed and cohesive in dentin. TEM examination of resin-dentin interface showed that pretreatment with 38% SDF/KI reduced nanoleakage regardless of the type of bonding system used.

Conclusions: Pretreatment of dentin with SDF/KI minimized nanoleakage at the resin-dentin interface without adversely affecting the bond strength of resin composite to dentin.

Key words: Etch-and-rinse adhesives, microshear bond strength, nanoleakage, potassium iodide, self-etch adhesives, silver diamine fluoride, transmission electron microscope

The ultimate objective in adhesive dentistry is to establish an effective adhesion to dental tissues. Dentin bonding agents adhere to dentin substrate by micromechanical hybridization. Formation of a perfect resin-infiltrated hybrid layer is essential to provide a durable bond to dentin. The extent of resin infiltration into the demineralized and exposed collagen determines the quality of resin-dentin bond. Loss of integrity of either the resin component or the dentin matrix within this resin-dentin interface has been directly related to the loss of stability of the hybrid layer. Incomplete resin infiltration into the hybrid layer not only results in nanoleakage but also degradation of dentin matrix due to matrix metalloproteinases (MMP) activation.

Access this article online

Quick Response Code: 10.4103/0970-9290.191893

Website: www.ijdr.in

For reprint contact: reprints@medknow.com

How to cite this article: Selvaraj K, Sampath V, Sujatha V, Mahalaxmi S. Evaluation of microshear bond strength and nanoleakage of etch-and-rinse and self-etch adhesives to dentin pretreated with silver diamine fluoride/potassium iodide: An in vitro study. Indian J Dent Res 2016;27:421-5.
Sano et al. have shown morphologic evidence of the presence of nanometer-sized porosities ranging in size from 20 to 100 nm in the hybrid layer using silver nitrate as a tracer. The penetration of any substance into these nanometer-sized porosities is termed as nanoleakage. These spaces serve as pathways for movement of water and enzymes like MMPs that can cause degradation of collagen fibrils, exposed by suboptimally infiltrated adhesives, resulting in a reduction of bond strength over time.

Various strategies have been tried to eliminate or minimize nanoleakage at the resin-dentin interface such as the application of multiple consecutive coatings of the adhesive resin to dentin and pretreatment of dentin using tubular occluding agents such as oxalates or poly (glutamic) acid-modified, diluted ceramicrete (PADC). Silver diamine fluoride (SDF), primarily a cariostatic agent has been shown to inhibit MMP-2, MMP-8, MMP-9, and cysteine cathepsins. It has also been shown to possess tubule occluding property. In addition, it possesses a broad spectrum of antibacterial action against Streptococcus mutans, Streptococcus sobrinus, Lactobacillus acidophilus, Lactobacillus rhamnosus, and Actinomyces naeslundii. Quock et al. showed that pretreatment of dentin with 38% SDF did not adversely affect the bond strength of composite resin to dentin. However, it resulted in discoloration of dentin. Hence, the use of potassium iodide (KI) to mask this staining effect had been advocated. However, till date, there are no studies to evaluate the effect of the combination of SDF and KI on the bond strength of etch-and-rinse and self-etch (ER and SE) adhesives to dentin. The null hypothesis tested were that (1) SDF/KI has no effect on the bond strength of composite resin to dentin and (2) the type of adhesive, ER or SE, does not affect the bond strength of composite resin to SDF/KI pretreated dentin surface. Hence, the aim of this study was to comparatively evaluate the microshear bond strength (MSBS) of ER and SE adhesives to dentin. In this study consists of 38% (w/v) of diamine silver fluoride and a saturated solution of KI. The groupings and specimen preparation protocol are given in Table 1.

In each group (n = 18), 15 dentin-composite slabs were used to evaluate MSBS, and the remaining three slabs were used to evaluate nanoleakage at the resin-dentin interface under TEM. These slabs were sectioned into 1 mm × 1 mm beams by means of a low-speed diamond disk under water coolant. Five such dentin-composite beams were prepared from each slab.

**Microshear bond strength testing**

The beams were embedded in acrylic resin block and fixed to a universal testing machine (Instron 3382, Canton, MA, USA). Forces were applied to the dentin-composite bonded interface at a crosshead speed of 1 mm per min until fracture occurred. An average value of five beams from each sample was considered as the mean MSBS for that sample, and the average MSBS value of fifteen such samples in the group was considered as the mean for that group. The fractured samples were analyzed using a SEM (JSM-5600, JEOL, Tokyo, Japan) and the mode of failure was determined.

**Nanoleakage evaluation**

Fifteen dentin-composite beams (1 mm × 1 mm) from each group were immersed in 50 wt% ammoniacal silver nitrate (Chenchems, Chennai, Tamil Nadu, India) tracer solution for 24 h. The silver impregnated samples were rinsed with distilled water and placed in the photo-developing solution for 8 h under a fluorescent light. The beams were then dehydrated in an ascending ethanol series (30% to 100%) and embedded in epoxy resin. Nondemineralized 90–100 nm thick sections were prepared with an ultramicrotome (Leica EM UC6, Leica Microsystems GmbH, Vienna, Austria) and the resin-dentin interface was examined for nanoleakage without additional staining using TEM (Philips CM20, Philips, Eindhoven, The Netherlands).

**Statistical analysis**

The results were statistically analyzed using one-way ANOVA followed by Tukey’s post hoc multiple comparison tests (P < 0.05).

**RESULTS**

The mean MSBS values (in MPa) and the distribution of failure modes (% of the groups are given in Table 2. Group 2 showed the highest MSBS value (29.52 ± 1.13) compared to the other groups. This was significantly higher than the mean MSBS of group 3 (28.47 ± 0.53). No significant
Thepredominantmodeoffailurewaseadhesivefollowedbycohesiveinresinandmixedcohesiveindentin.

The TEM photomicrographs of Group 1 (ER) [Figure 1a] and Group 3 (SE) [Figure 1c] showed large reticular pattern of nanoleakage within the hybrid layer whereas the TEM photomicrographs of Group 2 (SDF/KI-ER) [Figure 1b] and Group 4 (SDF/KI-SE) [Figure 1d] showed spotted pattern of nanoleakage.

**DISCUSSION**

From the results of this study, it was observed that pretreatment with SDF/KI did not adversely affect the bond strength of ER and SE bonding systems to dentin. This is the first study to report the effect of the combination of SDF/KI, both on the bond strength to dentin and on nanoleakage. Quock et al. found similar results of bond strength with SDF alone, but they observed dark staining of the dentin samples.[17] The disadvantage of using SDF alone is that they cause black staining of the tooth structure. Knight et al. showed that this untoward side effect of staining can be eliminated by application of KI following SDF application, and washing off the white precipitate formed.[19] Hence, in this study, the combination of SDF/KI was used. KI reacts with free silver ions and forms a creamy white precipitate of silver iodide, which eliminates the black staining caused by SDF application. Knight et al.[20] inferred that leaving the silver fluoride/KI precipitate on the surface significantly reduced the bond strength of auto cure glass ionomer cement to dentin. They also showed that the use of SDF/
KI combination, followed by washing away the white precipitate formed with water and air drying the dentin did not adversely affect the bond strength of auto cure glass ionomer cement to dentin.\[20\] Hence, in the present study, the reaction products were washed away before dentin bonding. Furthermore, SDF/KI application was done after acid etching the dentin surface with 37% phosphoric acid in the ER groups in contrast to Quoc et al.’s study,\[17\] where etching of dentin with 35% phosphoric acid was done after dentin surface treatment with 38% SDF. This method was followed based on a study by Knight et al.\[21\] who showed that increased levels of silver and fluoride were present on demineralized dentin samples compared to nondemineralized samples following application of SDF/KI using an electron probe microanalysis.

In this study, the MSBS values between the control groups, and between the control and experimental groups of each bonding system were not statistically significant \[Table 2\]. This implies that SDF pretreatment of dentin did not adversely affect the bond strength, irrespective of the bonding system used. Only experimental ER group showed significantly higher bond strength (29.52 ± 1.13) compared to control SE (28.47 ± 0.53) group.

The use of ER adhesives on dentin results in the formation of a continuous, uniform, and thicker hybrid layer; whereas, in SE adhesives the hybrid layer is thin and often associated with droplets between adhesive and resin composite. Moreover, the resin tags obtained with ER adhesives has conical swelling at their bases which are a sign of proper adaptation and sealing. Formed resin tags also contain numerous small lateral extensions of microtags branching off at right angles that offer supplementary retention and sealing; whereas SE adhesives show short and funnel-shaped tags with no lateral branches.\[1,22\] This difference in the physical appearance of the hybridized complex, however, will not jeopardize the optimal bond strength achieved with SE bonding systems, as long as a uniform demineralization front is created at the underlying dentin and it is fully impregnated by resin.\[22\] This could be the reason for the absence of a significant difference between the ER and SE groups in the present study.

Resin–dentin bonds created by contemporary adhesives are susceptible to fluid permeation, more so with 2-hydroxyethyl methacrylate (HEMA)-based adhesives, resulting in significant reduction in their bond strengths.\[23,24\] Adequate infiltration of monomers into the etched dentin and a molecular interaction of adhesive with apatite-depleted collagen remains a challenge with the ER systems.\[25\] Often, a discrepancy exists between the depth of demineralization and the degree of resin infiltration.\[25,26\] Nanoleakage may occur due to the continuous transudation of dentinal fluid through open dentinal tubules, around resin tags, thus adversely affecting bond strength.\[27\] Furthermore, during wet bonding, residual water in interfibrillar spaces might decrease the degree of polymerization of the adhesive and/or lead to hydrogel formation of HEMA within the hybrid layer leading to debonding.\[4,23\]

Craig et al.\[15\] suggested that application of SDF/KI to dentin could precipitate proteins in the dentinal tubules,\[28\] block dentinal tubules by forming deposits of calcium fluoride\[29\] and reduce dentin tubule patency by the formation of silver iodide. Sadek et al.\[11\] showed that the use of potassium tetraoxalate, a tubule occluding agent increased the bond strength of total-etch adhesives to dentin. Similar kind of tubule occlusion could have been produced by SDF/KI and may be the reason for the marginal increase in bond strength in the experimental groups compared to the control groups. Tubule occlusion caused by SDF could have prevented the intrinsic fluid movement at the adhesive interface of ER adhesives. This could be responsible for the significant increase in the bond strength of the experimental ER group in the present study as compared to the control SE group.

Mode of failure tells not only about the reliability of stress distribution during the test but also about the weakest area in the complex dentin–adhesive interface.\[30\] In all the groups, the predominant mode of failure was adhesive followed by cohesive in resin, mixed and cohesive in dentin.

TEM examination of resin–dentin interface revealed large reticular patterns of nanoleakage, suggestive of extensive silver uptake in both the control (ER and SE) groups. In this pattern, the silver deposits were oriented perpendicular to the surface of the hybrid layer and are the morphological manifestation of water–treeing.\[31\] They represent water filled resin blisters that osmotically draw water from dentin and adhesive into hypertonic regions created by solubilized calcium and phosphate. These sites would debond leading to adhesive failure.\[3\] A less extensive and spotted pattern of nanoleakage was seen in both the experimental groups.
This reduction in silver uptake might be attributed to the tubule occluding property of SDF. Tay et al. in 2003 reported that when oxalate desensitizers are applied to acid-etched dentin; the oxalate reacts with the calcium ions within the dentinal tubules to form precipitates of calcium oxalates. These precipitates occlude the dentinal tubules and minimize outward fluid movement to the dentin surface. This may prevent fluid contamination from the underlying dentinal tubules during the application of adhesives, thereby reducing nanoleakage.\[11\] Sadek et al. in 2007 showed that PADC, a desensitizer was also able to reduce dentin permeability by occluding the dentinal tubules.

In this study, pretreatment with SDF/KI before adhesive bonding minimized nanoleakage by occluding the dentinal tubules without adversely affecting the bond strength. Therefore, both the null hypotheses are accepted. However, further studies are needed to find the long-term effects of the use of SDF/KI on the bond strength of resin composite to dentin and its effect on nanoleakage at the resin-dentin interface.

CONCLUSIONS

Within the limitations of this in vitro study, it can be concluded that application of SDF/KI to dentin before bonding did not adversely affect the MSBS of resin composite to dentin. Specimens bonded with ER adhesive system following SDF/KI application showed significantly higher MSBS than specimens bonded with SE adhesive system alone. Pretreatment with SDF/KI reduced nanoleakage at the resin-dentin interface regardless of the type of bonding system used.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. De Munck J, Van Landuyt K, Peumans M, Poitevin A, Lambrechts P, Braem M, et al. A critical review of the durability of adhesion to tooth tissue: Methods and results. J Dent Res 2005;84:118-32.
2. Nakabayashi N, Nakamura M, Yasuda N. Hybrid layer as a dentin-bonding mechanism. J Esthet Dent 1991;3:133-8.
3. Hashimoto M, De Munck J, Ito S, Sano H, Kaga M, Oguchi H, et al. In vitro effect of nanoleakage expression on resin-dentin bond strengths analyzed by microtensile bond test, SEM/EDX and TEM. Biomaterials 2004;25:5565-74.
4. Tay FR, Pashley DH, Suh BI, Carvalho RM, Itthagarun A. Single-step adhesives are permeable membranes. J Dent 2002;30:371-82.
5. Breschi L, Mazzoni A, Ruggeri A, Cadenaro M, Di Lenarda R, De Stefano Dorigo E. Dental adhesion review: Aging and stability of the bonded interface. Dent Mater 2008;24:90-101.
6. Breschi L, Martin P, Mazzoni A, Nato F, Carrilho M, Tjaderhane L, et al. Use of a specific MMP-inhibitor (galdarin) for preservation of hybrid layer. Dent Mater 2010;26:571-8.
7. Sano H, Shono T, Takatsu T, Hosoda H. Microporous dentin zone beneath resin-impregnated layer. Oper Dent 1994;19:59-64.
8. Sano H, Takatsu T, Ciucchi B, Horner JA, Matthews WC, Pashley DH. Nanoleakage: leakage within the hybrid layer. Oper Dent 1995;20:18-25.
9. Mazzoni A, Scafla P, Carrilho M, Tjaderhane L, Di Lenarda R, Polimeni A, et al. Effects of etch-and-rinse and self-etch adhesives on dentin MMP-2 and MMP-9. J Dent Res 2013;92:82-6.
10. Hashimoto M, Sano H, Yoshida E, Hori M, Kaga M, Oguchi H, et al. Effects of multiple adhesive coatings on dentin bonding. Oper Dent 2004;29:416-23.
11. Sadek FT, Pashley DH, Ferrari M, Tay FR. Tubular occlusion optimizes bonding of hydrophobic resins to dentin. J Dent Res 2007;86:524-8.
12. Rosenblatt A, Stamford TC, Niederman R. Silver diamine fluoride: A caries “silver-sulfide bullet”. J Dent Res 2009;88:116-25.
13. Mei ML, Li QL, Chu CH, Yi CK, Lo EC. The inhibitory effects of silver diamine fluoride at different concentrations on matrix metalloproteinases. Dent Mater 2012;28:903-8.
14. Mei ML, Ito L, Cao Y, Li QL, Chu CH, Lo EC. The inhibitory effects of silver diamine fluorides on cysteine cathepsins. J Dent 2014;42:329-35.
15. Craig GG, Knight GM, McIntyre JM. Clinical evaluation of diamine silver fluoride/potassium iodide as a dentine desensitizing agent. A pilot study. Aust Dent J 2012;57:308-11.
16. Mei ML, Li QL, Chu CH, Lo EC. Samaranaayake LP. Antibacterial effects of silver diamine fluoride on multi-species cariogenic biofilm on caries. Ann Clin Microbiol Antimicrob 2013;12:4.
17. Quocck RL, Barros JA, Yang SW, Patel SA. Effect of silver diamine fluoride on microtensile bond strength to dentin. Oper Dent 2012;37:610-6.
18. Obwegeser H, Von Wachter R. The treatment of hyperesthetic dentin with nascent silver iodide. Zahnarztl Welt 1954;9:429-30.
19. Knight GM, McIntyre JM, Craig GG, Mulmani, Zilm PS, Gully NJ. An in vitro model to measure the effect of a silver fluoride and potassium iodide treatment on the permeability of demineralized dentine to Streptococcus mutans. Aust Dent J 2005;50:242-5.
20. Knight GM, McIntyre JM, Mulmani. The effect of silver fluoride and potassium iodide on the bond strength of auto cure glass ionomer cement to dentine. Aust Dent J 2006;51:42-5.
21. Knight GM, McIntyre JM, Craig GG, Mulmani, Zilm PS, Gully NJ. Differences between normal and demineralized dentine pretreated with silver fluoride and potassium iodide after an in vitro challenge by Streptococcus mutans. Aust Dent J 2007;52:16-21.
22. Albaladejo A, Osorio R, Toledano M, Ferrari M. Hybrid layers of etch-and-rinse versus self-etching adhesive systems. Med Oral Patol Oral Cir Bucal 2010;15:e112-8.
23. Tay FR, King NM, Chan KM, Pashley DH. How can nanoleakage occur in self-etching adhesive systems that demineralize and infiltrate simultaneously? J Adhes Dent 2002;4:255-69.
24. Sauro S, Pashley DH, Montanari M, Chersonsi S, Carvalho RM, Toledano M, et al. Effect of simulated pulpal pressure on dentin permeability and adhesion of self-etch adhesives. Dent Mater 2007;23:705-13.
25. Nuroohman H, Nikaido T, Takagaki T, Sadi A, Ichinose S, Tagami J. Apatite crystal protection against acid-attack beneath resin-dentin interface with four adhesives: TEM and crystallography evidence. Dent Mater 2012;28:e89-98.
26. Spencer P, Swafford JR. Unprotected protein at the dentin-adhesive interface. Quintessence Int 1999;30:501-7.
27. Pashley DH, Pashley EL, Carvalho RM, Tay FR. The effects of dentin permeability on restorative dentistry. Dent Clin North Am 2002;46:211-45, v-vi.
28. Greenhill JD, Pashley DH. The effects of desensitizing agents on the hydraulic conductance of human dentin in vitro. J Dent Res 1991;60:686-98.
29. Thrash WJ, Jones DL, Dodds WJ. Effect of a fluoride solution on dentinal hypersensitivity. Am J Dent 1992;5:299-302.
30. Thrash WJ, Jones DL, Dodds WJ. Effect of a fluoride solution on dentinal hypersensitivity. Am J Dent 1992;5:299-302.
31. Tay FR, King NM, Chan KM, Pashley DH. How can nanoleakage occur in self-etching adhesive systems that demineralize and infiltrate simultaneously? J Adhes Dent 2002;4:255-69.
32. Sauro S, Pashley DH, Montanari M, Chersonsi S, Carvalho RM, Toledano M, et al. Effect of simulated pulpal pressure on dentin permeability and adhesion of self-etch adhesives. Dent Mater 2007;23:705-13.
33. Nuroohman H, Nikaido T, Takagaki T, Sadi A, Ichinose S, Tagami J. Apatite crystal protection against acid-attack beneath resin-dentin interface with four adhesives: TEM and crystallography evidence. Dent Mater 2012;28:e89-98.
34. Spencer P, Swafford JR. Unprotected protein at the dentin-adhesive interface. Quintessence Int 1999;30:501-7.
35. Pashley DH, Pashley EL, Carvalho RM, Tay FR. The effects of dentin permeability on restorative dentistry. Dent Clin North Am 2002;46:211-45, v-vi.
36. Greenhill JD, Pashley DH. The effects of desensitizing agents on the hydraulic conductance of human dentin in vitro. J Dent Res 1991;60:686-98.
37. Thrash WJ, Jones DL, Dodds WJ. Effect of a fluoride solution on dentinal hypersensitivity. Am J Dent 1992;5:299-302.
38. Shimaoka AM, de Andrade AP, Cardoso MV, de Carvalho RC. The importance of adhesive area delimitation in a microshear bond strength experimental design. J Adhes Dent 2011;13:307-14.
39. Tay FR, Pashley DH, Yoshiyama M. Two modes of nanoleakage expression in single-step adhesives. J Dent Res 2002;81:472-6.
40. Tay FR, Pashley DH, Mak YF, Carvalho RM, Lai SC, Sub BI. Integrating oxalate desensitizers with total-etch two-step adhesive. J Dent Res 2003;82:703-7.