Original Research

Nanoleakage of Class V Resin Restorations Using Two Nanofilled Adhesive Systems

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How to cite the article:

Al-Agha EI, Alagha MI. Nanoleakage of class V resin restorations using two nanofilled adhesive systems. J Int Oral Health 2015;7(7):6-11.

Abstract:

Background: This study was carried out to evaluate the nanoleakage of two types of nanofilled adhesive systems in Class V composite resin restorations.

Materials and Methods: Totally 60 human premolars were randomly assigned to two groups ($n = 30$). Standardized round Class V cavities (enamel and dentin margins) were prepared. A total-etch (N-Bond total etch) (Ivoclar Vivadent) and self-etching (N-Bond self-etch) (Ivoclar Vivadent) adhesive system were evaluated. The cavities were restored incrementally with nanohybird composite resin (Tetric N-Ceram). The teeth were sectioned into a series of 1 mm thick beams then they were immersed in the prepared ammoniacal silver nitrate tracer solution for 24 h in a black photo-film container to ensure total darkness. The beams were then rinsed with distilled water, and immersed in photo-developing solution for eight hours then they were subjected to the nanoleakage evaluation. The specimens were analyzed in the environmental scanning electron operated with backscattered electron mode at ×1000 magnification.

Results: Self-etch adhesive recorded higher nanoleakage % mean value than the total-etch adhesive. The difference in nanoleakage % mean values between total and self-etch adhesive was statistically significant.

Conclusion: The self-etch adhesive had statistically significant higher nanoleakage mean values than the total-etch adhesive.

Key Words: Class V restoration, composite resin, nanofilled adhesives, nanoleakage

Introduction

Resin composites are increasingly used for restorative purpose because of good esthetic and the capability of establishing a bond to enamel and dentin.[1] Improvements of mechanical properties of the composite have permitted its use in posterior teeth with greater reliability than was the case some years ago.[2]

It has been suggested that adding filler particles to adhesive systems can generally improve the mechanical properties of

adhesive layer and bonding to dentin.[3] This leads to penetration of bonding agents into the dentin tubules where they form resin tags and by forming an interdiffusion "resin-dentin" surface, which is known as the "hybrid layer."[4]

The formation of an effective hybrid layer is achieved by diffusion of monomers in the collagen fibers, and this is the main bonding mechanism of the total-etch adhesive systems.[5] Due to the technique-complexity and sensitivity, the innovations in the adhesive systems were directed toward a simplified application process. The self-etching adhesive systems were developed to avoid the collapse of the collagen network and to simplify the clinical technique.[6] Thus, while total-etch systems have accumulated more years of data, self-etch adhesives offer a compelling, patient-friendly advantage.[7]

Although gap-free margins at the dentin/restoration interface could be achieved with some adhesive systems.[8] Sano *et al.*, have described another pattern of leakage, by observing the penetration of silver nitrate along gap-free margins by the aid of scanning (scanning electron microscope [SEM]). Since the leakage was found to occur within the nanometer-sized spaces around the collagen fibrils within the hybrid layer, this leakage was termed "nanoleakage."[9] Nanoleakage may result in hydrolytic breakdown of either the adhesive resin or collagen within the hybrid layer, thereby compromising the stability of the resin-dentin bond.[10] Therefore, it was found worthy to study the effect of different thickness of smear layer on nanoleakage. The purpose of this study was to evaluate the nanoleakage of two types of nanofilled adhesive systems in Class V composite resin restorations.

A total number of 60 upper premolar teeth which were extracted for orthodontic treatment were collected. The teeth were randomly divided into two main groups of 30 teeth each, according to the adhesive system used ($n = 30$); either total-etch bonding system (A), or self-etch bonding system (B). Standardized rounded Class V cavities were prepared (2 mm diameter and 2 mm depth) in the gingival one third of the labial surface of teeth. For standardization of the cavity width, a round carbide bur size #023 with high-speed handpiece was used under water coolant. The depth of the cavity was adjusted to 2 mm by inserting the entire head of the bur. No bevels were made at any of the enamel margins of the prepared cavities.[11] A new bur was used with each 10 cavities to avoid dullness. In the group (A), the dentin surfaces were etched with 37% phosphoric acid gel (N-etch) (Ivocalr Vivadent) for 15 s, according to the manufacturer's instructions. Then,

samples were rinsed with water using a plastic syringe for 10 s and blot-dried using minisponges, leaving a moist surface.

Adhesive system application

A. Total etch bonding agent: In the first group (A) the (total etch Tetric N Bond)(Ivocalr Vivadent) was applied by using a fully saturated microbrush with slight agitation to cover the entire surface and was gently air dried approximately 0.5 mm away from the prepared surface for 1-3 s, to allow the solvent to evaporate. The adhesive was then light cured using the visible light curing unit for 20 s according to the manufacturer's instructions.

B. Self-etch bonding agent: In the second group (B) the (self-etch Tetric N Bond) (Ivocalr Vivadent) was applied similarly, by using fully saturated microbrush with slight agitation to cover the entire surface and was gently air dried approximately 0.5 mm away from the prepared surface for 1-3 s, to allow the solvent to evaporate. The adhesive was then cured using light curing unit for 20 s according to the manufacturer's instructions.

All the prepared cavities were restored incrementally using the nanohybrid resin composite (Tetric N-Ceram) (Ivocalr Vivadent). The material was placed in two increments using a plastic condenser to avoid sticking of composite to the instrument and cured for 20 s according to the manufacturer's instructions. Each tooth was mounted on the cutting machine (Bronwill). Two-thirds of the tooth was ground until exposing the occlusal surface of the composite restoration of Class V cavities. The teeth were placed vertically and sectioned into a series of 1 mm thick slabs under water cooling then they were mounted horizontally and again sectioned longitudinally into a series of 1 mm thickness. The sectioning was performed using a diamond disc of 4 diameter × 0.3 mm thickness with diamond cutting blades and water-resistant titanium coating with low speed saw to obtain beams of 1.0 mm^2 cross-section area. The specimens were then subjected to the nanoleakage evaluation.[12] The specimens were stored in distilled water until subjected to the nanoleakage evaluation after 24 h.[13] The solution was prepared by dissolution of 25 g of silver nitrate crystals in 25 ml of distilled water. Concentrated (28%) ammonium hydroxide was used to titrate the black solution until it becomes clear, as ammonium ions complexed the silver into diamine silver ions. The solution was diluted to 50 ml with distilled water yielding a 50% solution pH = 9.5.[14] The beams were immersed in the prepared ammoniacal silver nitrate tracer solution for 24 h in a black photo-film container to ensure total darkness. The beams were then rinsed with distilled water, and immersed in photo-developing solution for 8 h under the effect of florescent light, to reduce silver ions into metallic silver grains along the bonded interface.[15] Using the energy dispersive analytical X-ray (EDAX), the amount of silver nitrate within the dentin adhesive interface, adhesive layer, hybrid layer and resin tags, in each specimen was measured in a point at ×1000 magnification

directly on Environmental SEM (ESEM) microscope monitor within the different beams. The specimens were analyzed in the environmental scanning electron operated with backscattered electron mode at ×1000 magnification. The mean of all regions was calculated. The silver nitrate uptake was expressed as a weight percentage of the total area evaluated. Initially, descriptive statistics for each group results. Two-factorial analysis of variance ANOVA tests of significance comparing variables affecting mean values. Student's *t*-test was performed to detect significance between the main groups. Statistical analysis was performed using Aasistat 7.6 statistics software for Windows (Campina Grande, Paraiba state, Brazil). $P \leq 0.05$ are considered to be statistically significant in all tests.

Results

Self-etch adhesive recorded higher nanoleakage % mean value than the total etch adhesive. The difference in nanoleakage % mean values between total and self-etch adhesive was statistically significant ($P < 0.05$) (Table 1 and Graph 1).
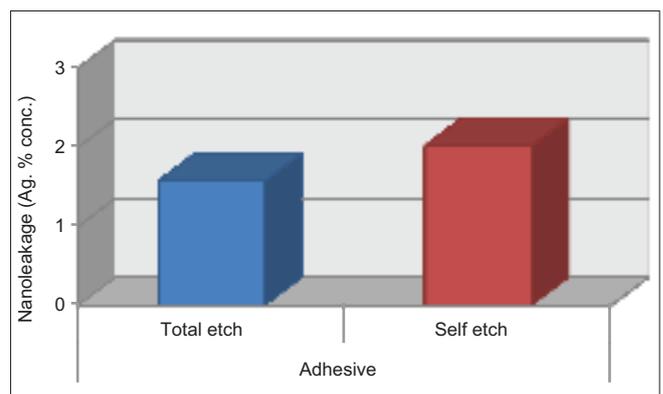
Figure 1 shows ESEM photomicrograph of nanoleakage pattern of nanofilled resin composite restoration with nanofilled total-etch adhesive system. The nanoleakage pattern showed a thin, uniform thickness of continuous silver deposition along the resin-dentin interface (green arrow) with micro-gaps (pink arrow).

Figure 2 shows ESEM photomicrograph of nanoleakage pattern of nanofilled resin composite restoration with nanofilled self-etch adhesive system. The nanoleakage pattern showed a thick continuous line of silver deposition along the resin-dentin interface (green arrow) with presence of micro-gaps (pink arrow).

Table 1: Comparison of total nanoleakage % results (mean values±SDs) between total and self-etch adhesives.		
Variable	**Mean±SD**	**Statistics**
Adhesive		$P=0.0033*$
Total etch	1.569±0.2828	
Self-etch	2.013±0.1169	

*Significant (*P*<0.05), ns: Non-significant (*P*>0.05), SD: Standard deviation

Graph 1: A column chart of total nanoleakage % mean values for total and self-etch adhesives.
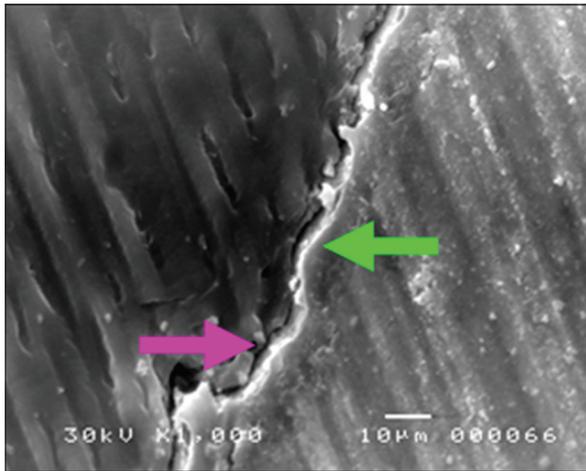
Figure 1: Environmental scanning electron microscope photomicrograph of nanoleakage of nanofilled resin composite restoration with nanofilled total-etch adhesive system.
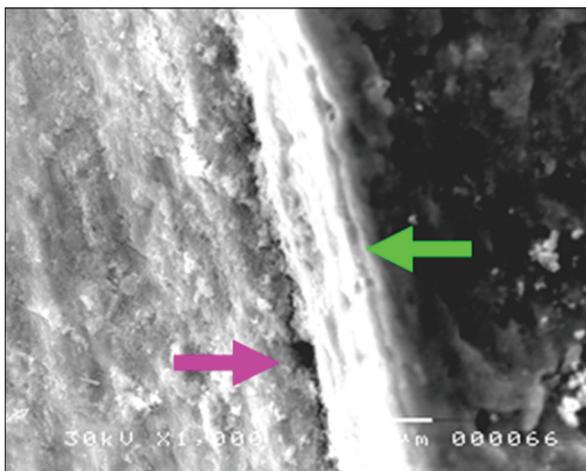

Figure 2: Environmental scanning electron microscope photomicrograph of nanoleakage of nanofilled resin composite restoration with nanofilled self-etch adhesive system.

Discussion

Nanoleakage phenomenon was first described by Sano *et al.*, 1994 who studied the diffusion of ions or molecules throughout the nanometric spaces located inside the hybrid layer. It was reported that even in the absence of gaps, silver ions could penetrate the hybrid layer and that this finding could be linked to inefficient monomer diffusion through a demineralized collagen layer. This kind of leakage may allow the penetration of bacterial products or oral fluids along the interface, which may result in hydrolytic breakdown of either the adhesive resin or collagen within the hybrid layer, thereby compromising the stability of the resin-dentin bond.[10] Bonding to dentin constitutes one of the topics that warrant considerable attention. The bonding agents are known to improve the adhesion of composite resins because it creates micro-mechanical interlock between the dentin collagen and resin by forming the hybrid layer.[16] This could be obtained by different strategies: Total-etch and self-etch.[17] Total-etch is technique sensitive,[18] whereas self-etch adhesives represent an alternative approach in enamel and dentin bonding.[19]

In this study, Class V cavities were chosen as they are regarded ideal for testing leakage. They present no macro-mechanical undercuts, they require for at least 50% bonding to dentin, they are usually found in anterior teeth or premolars and when restored, they result in an enamel and dentin margins.[20] The burs used were replaced every 10 preparations because the efficiency of the instruments used for cavity preparation has a great effect on quantity and quality of the smear layer.[21] Two nanofilled adhesive systems were used (Tetric N-Bond Self-Etch and Tetric N-Bond Total Etch) as the filler content reinforce the hybrid layer and help to create some gradient of elasticity and the collagen fibrils network mostly filter out the nanofillers, holding them at the hybrid layer surface thus acting as intermediate shock absorber. Filler particles may also reduce the shrinkage of the adhesive, thus helping to prevent microleakage.[22] Visible light cured nanofilled resin composite (Tetric N-Ceram) was used in this study due to the fact that filler technology might influence the performance of the dental composite significantly.[23] In the present study, ammoniacal silver nitrate solution $[Ag(NH_3)_2]^+$ 50% (wt/vol) (pH = 9.5) was used because it is the most commonly used material for nanoleakage evaluation as it easily migrates within the interface zone due to its extremely small diameter molecule (0.059 nm).[24] This small size and high reactivity to stain after binding tightly to any exposed collagen fibrils that are not enveloped by the adhesive resin makes sliver nitrate the most appropriate agent to detect the nanoporosities within the hybrid layer.[25] Moreover, silver nitrate induces an electron microscopic measurable contrast providing a sharp picture of the degree of penetration into the interface. Following its penetration, it has the potential to immobilize, which prevents further penetration during specimen preparation.[26] There is a possibility, however, that the acidic conventional silver nitrate (pH = 3.4) may over-demineralize dentin and create artificial paths along the dentin-adhesive interface, thus masking the nanoleakage results. Consequently, some studies proposed the use of less acidic silver nitrate solution to evaluate the nanoleakage phenomenon.[27] Therefore, in this study ammoniacal silver nitrate solution was used with a concentration of 50% (wt/vol) and pH = 9.5. Nanoleakage evaluation was performed using SEM in conjunction with EDAX which enables distinct images to be captured together with sensitive and accurate analysis. The concurrent EDAX analysis was carried out in order to identify the existence of metallic silver particles.[28] When SEM was used alone for nanoleakage examination, with secondary and backscattered electron imaging, erroneous interpretations were common. EDAX, in contrast, provides accurate quantitative analysis and distribution for the various existing elements.[29]

The results of this study showed that the self-etch adhesive recorded significantly higher nanoleakage mean values than the total-etch adhesive. The ESEM finding showed that the total etched dentin surfaces were covered continuously by the adhesive and sent very fine processes into the anastomosing

tubules. This result was in agreement with Kukletová *et al.*, 2007,[30] and Gateva and Dikov 2012[31] who studied nanoleakage of adhesive systems by transmission electron microscopy (TEM), and reported that, although a certain amount of nanoleakage was observed in all the tested groups, it was more pronounced for the self-etch adhesives when compared to the total-etch adhesives. Similarly, Kubo *et al.*, 2002[32] found that conventional total-etch adhesive systems tended to show less nanoleakage than the self-etch adhesives when they used the field emission-SEM (FE-SEM). This may be due to the specific characteristics of different adhesive systems that determine the degree of smear layer removal, demineralization of the underlying dentin, as well as the ability of the adhesive to wet and penetrate the dentin The difference in nanoleakage seemed to be dependent on the dentin adhesive systems used due to variable infiltration ability or difference in resin monomer composition or solvent. The self-etching adhesives are more hydrophilic than the total-etch adhesive systems and more permeable to water originated from dentin and, therefore more susceptible to degradation of resin-dentin bonds.[33] This holds true specifically that other studies performed by Hashimoto *et al.*, 2010[34] and Malekipour *et al.*, 2010[35] considered the type of adhesive system as influential in the leakage of composite restorations as different nanoleakage patterns were observed with different adhesive systems. Furthermore, different parameters of the application technique (e.g. etching time, dentin moisture) could also play a role in this respect.[26] However, Owens *et al.*, 2006[36] found no statistically significant difference in nanoleakage between dentin bonding agents and this may be due to the difference in methodology as they applied thermocycling protocol. The results of EDAX in this study revealed higher silver nitrate uptake that was expressed in weight percentage (wt%) of total area evaluated for self-etching bonding agent than the total-etching. It has been shown that hybrid layer is somewhat porous and is accessible to silver dye, even in the absence of marginal gap and this leakage is a result of penetration paths through the network of interfibrillar spaces with a size in the range of a few nanometers.[13] It was worthy to mention that the self-etch adhesive is not totally removing the smear layer or opens all the tubules since the self-etching materials have higher pH values than that used with total-etch adhesive systems, and self-etching materials are not rinsed away, thus, the smear layer or its components are incorporated into the bonded layer.[37] Furthermore, as the self-etch adhesive systems only modify the smear layer, the presence of residual water may lead to incomplete polymerization of the adhesive, limiting resin-dentin bond quality.[38] Another explanation could be related to the fact that self-etching adhesives contain increased concentrations of water, acidic monomers and hydroxyethylmethacrylate which makes these polymers very hydrophilic and likely to absorb water into the dentin interface and act even after polymerization as semi-permeable membranes, this means increased silver uptake into the hybrid and adhesive layers resulting in higher amount of nanoleakage.[39] Furthermore, Sano *et al.*, 1995[9] found that the resin-dentin interfaces bonded by self-etching primers demonstrated the presence of a fine network of silver deposits within thin hybrid layers formed by these systems. However, results are not consistent with Duarte *et al.*, 2006[13] who concluded that self-etch adhesive systems resulted in the least penetration of silver nitrate within the hybrid layer using FE-SEM and TEM as they have the potential to form a hybrid layer and seal dentin and the collagen fibrils are not completely deprived from hydroxyapetite in contrast to total-etch adhesives. This could be due to different techniques in adhesives application. Also, the results are not harmonizing with Vaysman *et al.*, 2003[40] who observed that the highest reduction in bonded dentin permeability and increase in sealing ability occurred when the self-etching adhesive systems was applied to the roughest dentin surface. The difference in results may be because they used different bur cutting surface roughness (conventional straight edge bur, cross-cut serrated bur and extensively serrated bur) which are different than the cutting burs used in this study. However, the high surface roughness they produced in their samples might have increased dentin surface area, allowing a better contact between adhesive and dental substrate. Also, Abdel-Wahab *et al.*, 2008[41] found that the thin hybrid layer which results from mild acid etching effects of self-etching primers, only modify the smear layer rather than remove it and result in superior dentin sealing due to retained hybridized smear plugs within the tubules and this showed minimal silver deposition, which was revealed by EDAX analysis. This difference could be material related.

Conclusion

A comparison of dental adhesive systems variables proved that the self-etch adhesive had statistically significant higher nanoleakage mean values than the total-etch adhesive at the margins of Class V restorations.

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