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Original Research

Nanoleakage Patterns of Two Dentin Adhesives using Confocal Microscope

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Abstract:
Background: Gap-free margin at the dentin/restoration interface is the primary requisite for the success of a composite restoration. Although gap free margins at the dentin/restoration interface were achieved with some recent dentin bonding systems, Sano *et al.* (1994) have described certain leakage pattern by observing the penetration of silver nitrate along the gap free margins under scanning electron microscope or transmission electron microscope, which he termed as "nanoleakage." Nanoleakage may allow the penetration of bacterial products and dentinal/oral fluid along the interface, which may compromise the stability of the resin-dentin bond. To evaluate the nanoleakage patterns of two different adhesives using the confocal laser scanning microscope (CLSM).

Materials and Methods: Occlusal enamel of forty freshly extracted human maxillary premolars was removed by transverse sectioning, using a diamond disk to expose a flat dentin surface and polished them with 600 grit silicon carbide disk for 60 s. A few grains of Rhodamine B was mixed and dissolved in the bonding adhesives before their application. The teeth were divided into four groups of ten each: Group 1 - Adper self-etch plus (6th generation) and Group 2 - Fuji II LC. The bonded surfaces were then restored with a layer of 2 mm thick universal composite (Z250). After 24 h storage in normal saline, the roots of all the teeth were horizontally sectioned at the level of CEJ. The coronal specimens were then sectioned into two and polished with 600 grit silicon carbide for the confocal laser scanning microscopic evaluation, for the analysis of nanoleakage patterns in hybrid layer (HL) and the adhesive layer.

Results: Both the groups exhibited typical nanoleakage patterns, both within the HL and the adhesive layer. Structures labeled with rhodamine B appear red in the CLSM.

Conclusion: Different leakage patterns were observed with the both groups was due to their composition.

Key Words: Adhesive layer, bonding agent, composite, confocal laser scanning microscope, fluorescence, hybrid layer, interface, nanoleakage, rhodamine B, scanning electron microscope, transmission electron microscope

Introduction

Polymerization shrinkage and the resultant contraction gaps at the tooth-restoration interfaces continue to be a significant problem associated with composite resin restorations. To minimize contraction gap formation and the potential for bacterial leakage, dentin bonding agents, glass ionomer cements, and liners has been used Sano *et al.*[1-3] have described a pattern of leakage, by observing the penetration of silver nitrate along gap-free margins with several dentin bonding systems under scanning electron microscopy (SEM) or transmission electron microscopy (TEM), which they termed "nanoleakage." It represents permeation laterally through the hybrid layer (HL) and may be the result of the incomplete infiltration of the adhesive resin into the demineralized dentine.

Resin-based restorative materials (composite resins, compomers, and resin modified glass ionomer cements) are used in different dentistry applications mainly as esthetic restorative materials, substitutes of amalgam posterior restorations, dentin/restoration bonding systems, orthodontic corrections. Bonding of resin-based composites to dentin can be accomplished by means of etch-and-rinse or self-etching (SE) adhesive systems. The etch-and-rinse approach has been considered as technique-sensitive.[2-6] Incomplete resin infiltration and evidence of phase separation within resin-dentin interfaces and its detrimental effects have been demonstrated.[7] To overcome the shortcomings of total-etch adhesives, SE adhesives have been developed in an attempt to simplify the procedure steps. Thus, eliminating, conditioning, rinsing, and drying steps which are critical to conventional total etch technique, yet extremely difficult to standardize.[8]

The development and marketing of new bonding agents continues to be rapid, the quality of the dentin bond was reported to be material dependent in certain situations and associated with the chemistry of individual materials.[1,2] On the other hand, resin-modified glass ionomer liners have been used successfully under composite filling materials providing superior retention. Resin modified glass ionomers have developed and commercialized for improved handling and

physical properties; as light-cured glass ionomer cements.[4] They have dual setting reactions. One is a classic acid/base reaction of the conventional glass-ionomer by the mixing of their two components, and the other is a polymerization of resin monomers by light irradiation.[9,10] Meanwhile they can be considered as adhering to tooth tissue through a kind of SE approach. The basic difference with the resin-based SE approach is that glass-ionomer is SE through the use of a relatively high molecular weight (8000-20000) polycarboxylic-based polymer.[11-13]
The purpose of the study was to assess the pattern of nanoleakage that associate the use of one type of SE adhesive system and the use of a glass ionomer liners with a composite restoration.

Materials and Methods
Twenty non-carious human premolars extracted for orthodontic treatment purpose were selected, and they were ultrasonically cleaned. The buccal, lingual, and occlusal enamel was ground to obtain flat occlusal dentin surface using high-speed handpiece with flat disk diamond stone with constant water spray. The surfaces were polished with wet #600 grit silicon carbide paper for 60 s to create a standardized smear layer. The teeth were divided into two groups; ten samples for each material. A few grains of rhodamine B (BATCH NO. J09Z/4108/0411/71, SDFCL LAB) were mixed and dissolved in the bonding adhesives before their application.

The occlusal surfaces of the first group were treated with the antibacterial SE adhesive system (Adper SE plus) and the second group were treated with the glass ionomer liner according to the manufacturer's instructions.

A layer of 2 mm thickness of the composite was applied to the bonded surfaces to protect the bonded layer from erosion and desiccation and was light cured for 20 s. All the specimens were left for 24 h in physiologic saline.

Nano leakage assessment
The teeth were sectioned buccolingually across the bonding surface with a low-speed diamond saw (model 650, South Bay Technology Inc., CA, USA). The sectioned teeth surfaces were finished using 600 grit silicon carbide paper. The specimens were analyzed in confocal laser scanning microscope (CLSM) ultima confocal system (meridian instruments).

Materials
Composition and manufacturer: Composite resin Z250 R_{es}in--BIS-GMA&TEGDMA Fillers-- zirconia and silica, 3M-ESPE Germany.

Adper SE plus two-step SE adhesive
Primer (SE primer)
10-Methacryoyoxydecyl dihydrogen phosphate (MDP), 12-methacryoyloxydodecyl-pyridmium bromide, 2-hydroxyethyl methacrylate (HEMA), hydrophilic dimethacrylate, water.

Bonding agent
10-MDP, Bisphenol A diglycidyl methacrylate (Bis-GMA), HEMA, hydrophobic dimethacrylate, dl-Camphorquinone, N, N-Diethanol-p-toluidine, silanated colloidal silica, surface treated sodium fluoride (Kurary Medical Inc. Osaka, Japan).

Fuji 11 LC
Powder: Fluoro alumino silicate glass
Liquid: Modified polyacrylic acid with pendant methacrylate groups, HEMA, photoinitiator, water, 3M-ESPE Germany.

Results
The CLSM image of the surface composition of the dentin side surface of the specimen that received one layer of Adper SE plus at low magnifications (×10) are shown in Figure 1a and b. It was noticed that very thin HL is present between the adhesive and the dentin measuring about 2-3 um. CLSM analysis revealed the presence of FL dye deposits which were represented by the low peak seen in the area of FL dye detection. Figure 1a and b represent CLSM images of one of the samples which gave a different appearance. The resin tags extending in the dentine appeared as shiny white color rods. As previously mentioned, CLSM analysis revealed that the shiny red color rods were due to the presence of FL dye. Silver granules could not be seen except in the areas of the resin tags even in the images with higher magnification.

Glass ionomer
The results of the CLSM analysis are shown as backscatter electron images of surface of the specimen that received glass ionomer liner at low magnifications (×10) showed extensive amount of fluorescent dye red shiny deposits could be seen just beneath the glass ionomer layer and diffusing into the dentin. These deposits were seen as spots not connected to each other and spreading along the interface in a non-uniform pattern (Figure 2a and b). The CLSM analysis indicated that this characteristic red shiny appearance was deposits of FL dye granules.

Other images showed that the FL dye granules are concentrated more in the top layer of the interaction zones and seen to diffuse within the dentin.

Discussion
Fear of bacterial ingress at the restoration/tooth interface and the subsequent caries recurrence has pushed the interest of researchers to assess the adaptation and adhesion of restorative materials to the tooth. Several studies[14,15] had the concern to evaluate the source of adhesive-dentin bond through assessing the interface characterization. Evaluating the microleakage and recently the nanoleakage represents the importance of assessing the adaptation of materials.[16,17]

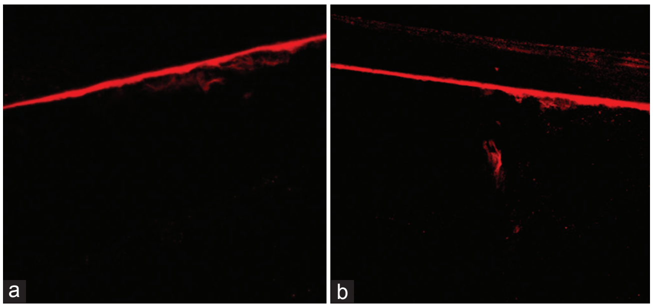

Figure 1: (a and b) Group 1: Adper self-etch plus showing nanoleakage in the form of spotted type patterns and reticular patterns near the adhesive layer.

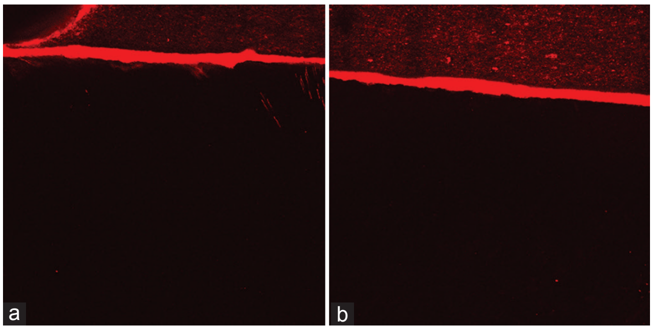

Figure 2: (a and b) Group 2: Fuji 11 LC showing the spotted type of nanoleakage pattern all over the hybrid layer.

The use of FL dye (rhodamine B) was effective in detecting the nanoleakage than the silver nitrate dye to eliminate the drawback of demineralizing the dentin and disintegrating the glass ionomer. In this study, it is evident that the Adper SE plus created a thin HL which results from the mild acid-etching effects of SE primers, which only modify the smear layer rather than remove.[5] It may reduce the outward fluid flow and result in superior dentin sealing due to retained hybridized smear plugs within the tubules, and this results in minimal FL dye deposition which was revealed by CLSM analysis. Surprisingly, with the use of Adper SE plus FL dye deposits could not be seen in SEM images of most of the samples but rather it was detected with the CLSM analysis. This was proved that Adper SE plus chemically interacts with hydroxyapatite, forming a very stable bond with low dissolution rate in water.[18,19]

It is worth to be noticed that in one of the samples CLSM images revealed the presence of FL dye granules in the bottom of the HL surrounding the resin tags as red deposits. This indicates incapability of the adhesive to infiltrate completely into the micro porosities which may be the result of retained water which creates pathways for the silver granules.[20,21]

The CLSM evaluation (Figure 2a and b) of the RMGI liner showed dense silver deposits within the interaction zone between RMGI and dentin.[22,23] The silver deposits exhibited a spotted pattern, suggesting a porous layer that is prone to silver stain uptake. This pattern is suggestive of the presence of water particles that allowed silver particle's movement.[24-26] This water is the characteristic feature of the RMGI's hydrogel structure which depends essentially on the presence of water. Moreover, the presence of hydrophilic monomers (as HEMA) after its polymerization creates three-dimensional copolymer network which may attract water and swell, like a hydrogel.

Resin-modified glass-ionomer probably holds the possibility to bond to enamel and dentin by the same chemically based bonding mechanism. For conventional glass ionomer, the underlying mechanism of adhesion is thought to be based on a dynamic ion-exchange process, in which the polyalkeonic acid softens and infiltrates the hydroxyapatite structure.[27,28] In RMGI, it is hypothesized to displace calcium and phosphate ions out of the substrate and to form an intermediate adsorption layer of calcium- and aluminum-phosphates and polyacrylates at the glass-ionomer-hydroxyapatite interface. To assure for completion of the chemical reaction of the RMGI liner, the specimens were left for 24 h before proceeding to the test.[29,30]

The results also revealed the presence of gaps at the interface between dentin and RMGI, which may be due to the curing shrinkage. Polymerization reaction occurs with the HEMA and urethane dimethacrylate-based monomers contained in the matrix, and this may serve to produce subsequent shrinkage that appears within 5 min after polymerization, and proceeds for the next 24 h. This shrinkage gives rise to contraction stress which can damage the adhesive interface and create marginal gaps.[25,26]

In conclusions, within the limits of this study it can be drawn that the SE adhesive system (Adper SE plus) can provide better sealing ability than the use of resin-modified glass ionomer liner with the composite resin restorations which is evaluated by the decreased probability of nanoleakage.

Conclusion

Within the limitations of study, following conclusions were drawn:

- Different types of leakage patterns were observed in all the dentin bonding agent groups tested. This is most likely due to the difference in the composition of each adhesive system tested.
- The various nanoleakage patterns observed in this study were: water tree-like patterns, rosette or cluster like pattern, reticular, and spotted type of patterns.
- The results of this study indicate the existence of nanoleakage in all materials tested, both in HL and the adhesive layer.
- Even though the current generation bonding agents were said to be gap-free, still the leakage persists in them.
- Although nanoleakage tests can provide much useful information on the sealing ability of restorations and the quality of HL, the current knowledge about this phenomenon is limited.

- Further studies are needed to evaluate the clinical significance of this nanoleakage phenomenon and, if necessary, to develop adhesive systems minimizing nanoleakage in order to optimize dentinal bonding.

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