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Assessment of the nanoleakage between different adhesive systems and deproteinized dentin with Nd:YAG laser and 10% NaOCl

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ASSESSMENT OF THE NANOLEAKAGE BETWEEN DIFFERENT ADHESIVE SYSTEMS AND DEPROTEINIZED DENTIN WITH ND:YAG LASER AND 10%NAOCL

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Abstract

The objective of this study was to evaluate the global leakage scores on the interface between two adhesive systems and the acid-etched dentin and to estimate the nanoleakage after removing the collagen network by Nd:YAG laser or 10% NaOCl. Thirty extracted human upper premolars were selected to receive standardized buccal and lingual class V cavities. The teeth were randomly divided into two groups of fifteen teeth each: in the first group, the Excite® bonding system was used, whereas in the second group, the Solobond Plus adhesive system was applied.

In each group, teeth were equally allocated to one of the following treatments prior to bonding application: a) acid etching / Nd:YAG laser; b) acid etching /10% NaOCl and c) acid etching. Global leakage score of each specimen was calculated as the percent of the total cut dentin surface penetrated by silver nitrate.

The results showed that treating the acid etched-dentin with Nd:YAG laser led to a significant decrease in the global leakage scores; these latter were significantly less than those of acid-etched / 10% NaOCl treated dentin. The Excite® bonding system showed higher global leakage scores than the Solobond Plus bonding system.

Keywords: Nanoleakage - Nd: YAG laser – adhesive – acid etching.

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EVALUATION DE LA NANOPERCOLATION ENTRE DIFFÉRENTS SYSTÈMES ADHÉSIFS ET LA DENTINE DÉPROTÉINISÉE AU LASER ND: YAG ET AU NAOCL 10%

Résumé

Le but de cette étude était d'évaluer l'étanchéité du collage à l'interface entre deux types différents d'adhésifs dentaires et la dentine mordancée à l'acide; la déprotéinisation de la dentine a été réalisée au laser Nd:YAG ou par application de NaOCl à 10%.

Sur trente prémolaires supérieures, des cavités classe V standardisées ont été préparées au niveau des faces vestibulaires et linguales. Les dents ont été réparties au hasard en deux groupes de quinze dents chacun: le système « Excite® » a été utilisé pour le premier groupe et le système adhésif «Solobond Plus » pour le second. Dans chaque groupe, les dents ont été alloués à l'un des traitements suivants avant l'application de l'adhésif: a) mordançage à l'acide / laser Nd:YAG; b) mordançage à l'acide / NaOCl à 10% et c) mordançage à l'acide. Le score global d'infiltration a été calculé pour chaque dent. Les résultats ont montré que la déprotéinisation de la dentine mordancée à l'acide au laser Nd: YAG a entrainé une diminution significative des scores d'infiltration; ces derniers étaient significativement inférieurs à ceux de la dentine mordancée et déprotéinisée par le NaOCl à 10%. Le système adhésif Excite® a montré des scores d'infiltration plus élevés que le système de collage Solobond Plus.

Mots – clés : mordançage – adhésif - laser Nd:YAG.

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Introduction

Dentin structure has been characterized as a biological composite of collagen matrix filled with submicron to nanometer-sized, calcium deficient, carbonate-rich apatite crystallites dispersed between parallel micron-sized hypermineralized, collagen poor, hollow cylinders (dentinal tubule containing peritubular dentin) [1] .
Hybridization of dentin is a process that creates a molecular level mixture of adhesive polymers and dental hard tissues [2]. Hybridized dentin is prepared in the subsurface of acid-etched tissues by the polymerization of resin monomers that have impregnated the tissues. It has a gradient structure because it is prepared by diffusion of monomers placed on the conditioned surface and their polymerization in situ.

Despite the significant improvements that have been made to adhesive systems, the bonded interface continues to remain the weakest point. Ideally, the adhesive would allow restorative material bio-integration and provide strong and durable bonds that would seal the tooth-restoration interface, preventing microleakage and the subsequent ingress of microorganisms [3, 4]. There are several explanations suggested for the bond failure namely- incomplete resin infiltration, the collapsed relatively impermeable region or a poor polymerization because of lack of sufficient irradiation [5].
The term "nanoleakage" has been introduced to explain a penetration pathway within hybrid layers of the dentin-composite junction in the absence of gap formation. This phenomenon is argued in the literature to be a risk factor for the quality of the dentin bonding [6].

Prior to the description of the phenomenon of nanoleakage in 1994, numerous studies on the quality of restoration margins were carried out [3]. The penetration depths were evaluated using conventional light microscopy. However, the limited lateral resolution as well as the inadequate depth of focus did not allow detailed structure analysis within the hybrid layers. Such experiments should be conducted using higher lateral resolutions techniques than conventional light microscopy to differentiate between the various paths of penetrations [7].

A significant factor in achieving satisfactory adhesion of restorative resins to dentine substrate is the method by which the dentine surface is treated before an adhesive is applied. Removal of the collagen fibers with a deproteinizing agent would facilitate the access of the adhesive resins to a substrate that is more permeable and less sensitive to water content. Using deproteinization processes to remove the superficial destabilized collagen layer and subsurface remnants from etched dentine surfaces has been proposed since the 1990's [8].

Sodium hypochlorite (NaOCl) is a non-specific proteolytic agent that effectively removes organic components at room temperature. Literature shows that sodium hypochlorite treatment removes the dentine's organic components and changes its chemical composition, so that it becomes similar to etched enamel. This substrate is also rich in exposed hydroxyapatite crystals and may result in a stable interface over time, because it is made of mineral. An increase in "wettability" is expected on deproteinized dentine surfaces, since they are hydrophilic [9] and are more permeable [10]. Thus, chemical interactions between resin and the deproteinized dentine surface are most likely to occur, since the surface has been described as having wider tubule openings with finer irregularities on the intertubular dentine, after treatment with a deproteinizing agent [8].

The neodymium: yttrium-aluminumgarnet (Nd:YAG) laser is an excellent surgical instrument for tissue coagulation, vaporization and incision. The Nd:YAG laser is attracted mostly to the pigmented tissue. The collagen network has the ability to be stained by special dye. So, if the dentin was acid-etched and the partially demineralized dentin was stained with special dye selective for collagen network, the Nd:YAG laser would ablate the collagen network selectively and deproteinize the dentin without affecting the mineralized tissue [11].

The objective of this study was to evaluate the global leakage scores on the interface between two adhesive systems and the acid-etched dentin and to estimate the nanoleakage after removing the collagen network by Nd:YAG laser or 10% NaOCl.

Materials and Methods

Thirty sound human upper premolars were used in this study. Following extraction, they were cleaned and stored in 50% ethanol at 8°C for a maximum of 1 month in order to hinder bacterial growth. Prior to the experiments, the teeth were immersed in water for 24 hours at 20 °C [12].

Cavity preparation

Standardized class V cavities were prepared on the buccal and lingual surfaces (3mm high, 3mm wide and 2mm depth) using a medium grain diamond bur # 848 on a high speed handpiece under water coolant. The outline of the cavity was drawn on the tooth surface with a 0.5 mechanical pencil using a matrix band with a pre-cut hole of 3mm x 3 mm; the matrix was fixed on the tooth with a retainer so that the gingival floor of the cavity was within the cemento-enamel junction.
The cavity form was completed with round bur #2, on a low speed handpiece under water coolant; the enamel margins were not beveled.

The teeth were randomly divided into two groups of fifteen teeth each; the Excite® (Ivoclar, Vivadent) and the Solobond Plus (VOCO GmbH) adhesive systems were applied in the first and the second groups, respectively.

Within each group, three modalities were adopted for teeth etching / demineralization prior to bonding application:
a- Acid etching / Nd:YAG laser.
b- Acid etching /10% NaOCl.
c- Acid etching.

Conditioning of enamel and dentin

The teeth were etched using the total etch technique (37% phosphoric acid); the etching was applied onto enamel and dentin beginning with the enamel margins for 15 seconds.
The cavities were thoroughly rinsed from phosphoric acid with water. The dentin surface was dried with an air syringe for two seconds to achieve a slightly moist surface with no visible excess water.

Treatment with Nd:YAG laser

Van Gieson stain was prepared by mixing 1% of aqueous fuchsin solution (9 cm^3) with saturated aqueous picric acid solution (50 cm^3) and distilled water (50 cm^3). A thin layer of Van Gieson stain was placed on the internal surfaces of the cavity using a Vivadent brush applicator; in a fine motion the dye was brushed gently onto the dentin. The teeth were stabilized in their positions on the laboratory manikin; this latter was fixed on the tray of the Nd:YAG device. The teeth were treated with Nd: YAG laser in a non-contact mode at its fundamental wavelength of 1064 nm, 10 mj, for three pulses with water cooling system [13].

NaOCl application

The application of 10% NaOCl was done after acid etching using a disposable brush for a dwell time of one minute and the NaOCl was removed with 5 ml distilled water.

Application of the adhesive system and teeth restoration

The Excite® or the Solobond Plus bond was applied onto the conditioned tooth structure. In a light motion the material was brushed gently for 10 seconds.
The bonding was left for 10 seconds, and the excess was removed with air stream free of water and oil. The bonding was light cured for 20 seconds.
The resin-based composite was applied in three incremental horizontal layers. Each layer was separately light cured for 20 seconds from all surfaces to ensure complete polymerization. Before curing the final increment, a transparent matrix was placed to contour the restoration. The margins were finished and polished with sandpaper disc.

Ability to resist Nanoleakage

The teeth were checked using a dissecting microscope to ensure that no flash was left along margins. Root apices were sealed with Tetric® Ceram (Ivoclar, Vivadent) composite and the entire teeth - except for the bonded interface and 1 mm of the teeth surface adjacent to the interface - were coated with two layers of nail varnish. After that, the teeth were placed in a 50% (weight/volume) silver nitrate solution in total darkness for 24 hours, rinsed in running water for 5 minutes, immersed in photo-developing solution and exposed to a fluorescent light for 8 hours in order to reduce the silver ions to metallic silver [6]. After their removal from the developing solution, the teeth were placed under running water for 5 minutes and sectioned longitudinally across the bonded surface in order to obtain two sections of each sample. The sections were mounted on microscope glass slides using resin adhesive. All the cut surfaces were polished with increasing fine diamond pastes (3, 1 µm) obtaining a 4µ thickness sections and covered with slide covers. Examination was carried out using a polarized light microscope.
Global leakage score of each specimen was calculated as the percent of the total cut dentin surface penetrated by silver nitrate:
Global leakage score =P/L x 100
where: P=length of silver nitrate penetration along the resin/dentin interface; L= total length of dentinal cavity wall on the cut surface [14].
Cavities per tooth were not interrelated. The data was analyzed as if they were independent replica cavities for each of the treatments.
The collected data was subjected to one-way ANOVA followed by Tukey post hoc comparisons test to explore significant difference in mean between the groups. The paired t-tests were used to compare the global leakage scores between each pair of groups of Excite bonding system and Solobond Plus bonding system. The alpha error was set at 0.05.

Results

All the samples in this study showed nanoleakage at the adhesive resin interface, but to a varying degree.
Figures 1 and 2 represent the means of the global leakage scores for the different groups and subgroups.

When the Nd:YAG laser was used after acid conditioning of the dentin, the polarized light microscopy showed the least amount of silver penetration; a very thin line or some few areas of silver deposition beneath the layer of Excite® bonding system was detected. The mean of the global leakage scores were 5.8 ± 0.28.,.
When the 10% NaOCl was used after acid etching, the polarized light microscopy showed a relatively thick silver line with some tubules filled with silver deposition. The mean of the global leakage scores were 13.33 ±0.583.
When the dentin was not treated with any deproteinizing agent after acid conditioning, the polarized light microscopy showed thick areas of silver penetration involving the lower interface of the interdiffusion zone. The mean of the global leakage scores were 31.17 ±0.76,.

The analysis of variance revealed a highly significant difference (p<0.001) in the global leakage scores among the three subgroups when using the Excite® adhesive system (Table 2).

		Scores											
		1	2	3	4	5	6	7	8	9	10	Mean	SD
Groups	Acid etching + Nd:YAG laser	6.2	6.1	6.2	5.5	5.7	5.8	5.4	5.7	5.8	5.6	5.8	±0.28
	Acid etching + 10% NaOCl	12.4	13.5	12.9	12.8	12.9	13.7	13.5	13.3	14.1	14.2	13.33	±0.583
	Acid etching	30.3	31.2	32.1	30.8	30.2	30.3	31.4	32.2	32	31.2	31.17	±0.76

Table 1: Mean and standard deviation values of the global leakage scores of each treatment modality for the Excite® bonding system.

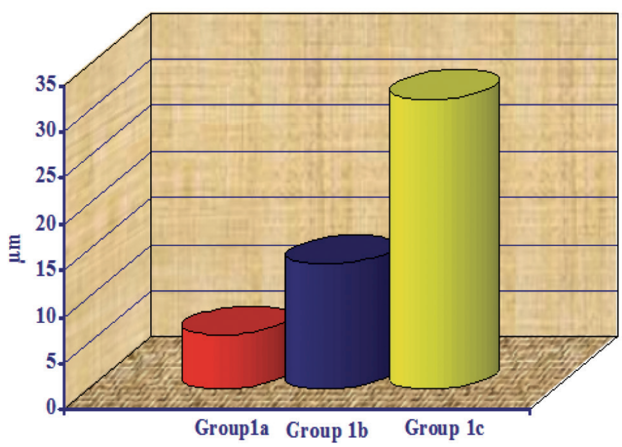

Fig. 1: The means of the global leakage scores of each treatment for the Exite® bonding system (1a: acid etching / Nd:YAG laser / Exite®; 1b: acid etching /10% NaOCl / Exite® and 1c: acid etching / Exite®).

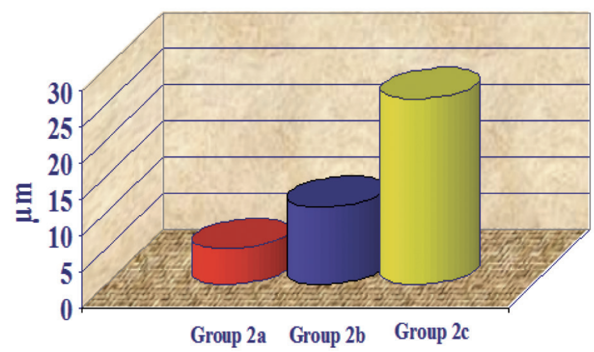

Fig. 2: The means of the global leakage scores of each treatment for the Solobond Plus bonding system (2a: acid etching / Nd:YAG laser / Solobond Plus; 2b: acid etching /10% NaOCl / Solobond Plus and 2c: acid etching / Solobond Plus).

The Tukey's multiple range test showed highly significant differences among all subgroups (Table 3).

Table 4: Mean and standard deviation values of the global leakage scores of each treatment for the Solobond Plus bonding system.

When the Nd:YAG laser was used after acid conditioning of the dentin, the polarized light microscopy showed the least amount of silver penetration; a very thin line or some few areas of silver deposition beneath the layer of Solobond plus bonding system was observed. The mean of the global leakage scores were 4.91% ± 0.35.

When 10% NaOCl was used after acid conditioning of the dentin, the polarized light microscopy showed a relatively thick silver line with some tubules filled with silver deposition. The mean of the global leakage scores were 10.7 ±0.46.

When the dentin was not treated with any deproteinizing agent after acid conditioning, the polarized light microscopy showed thick areas of silver penetration. The mean of the global leakage scores were 25.59 ±0.52 .

Table 5 shows the statistical analysis of the data using one-way ANOVA; a highly significant difference was found ($p<0.001$) in the global leakage scores among the three subgroups. The Tukey's multiple range test showed highly significant differences among all the subgroups when using the Solobond Plus adhesive system (Table 6).

Table 7 represents the paired t-test results of the global leakage scores when comparing each treatment modality using the Excite® and the Solobond Plus bonding systems. A highly significant difference was found between the corresponding treatments with higher scores obtained with the Excite® system ($p<0.05$).

Discussion

The longevity of composite fillings depends, among other factors, on the

Source of variance	Sum of Squares	df	Mean Squar	F	Sig
Between groups	3394.345	2	1697.672	5069.36050	H.S
Within groups	9.042	27	0.335		
Total	3404.387	29			

Table 2: Analysis of variance of global leakage scores of all treatments modalities for the Excite® bonding system.

Groups	Difference between means	HSD (5%)	HSD (1%)	Sig.
Group 1a v/s 1b	7.53	0.642	0.823	H.S
Group 1a v/s 1c	25.37			H.S
Group 1b v/s 1c	17.84			H.S

Table 3: The results of Tukey's HSD test applied on the mean changes in values of the global leakage scores of each treatment modality using the Excite® bonding system.

creation of a hybrid layer and the interactions between the tooth substances and the filling materials.
Marginal discoloration, recurrent caries, postoperative sensitivity and the loss of restorations are the most frequent consequences of an insufficient or incorrect hybrid layer formation.

Many studies have been carried out to detect the amount of microleakage based on gap formation. Godoy and Finger [7] showed that with the restoration in place, the exact location of leakages cannot be detected in 75% of the cases.
To distinguish this type of leakage within the hybrid layer from the typical microleakage associated with gap formation, Sano et al. [14] introduced the term "nanoleakage". Penetration pathways in nanoleakage are porosities of less than 50 nm located between the untreated dentin and the superficial collagen-rich fibrous network.
This nanoleakage may be the result of residual water around collagen fibrils, collapse of the collagen network, or incomplete resin infiltration into exposed collagen network and polymerization [15]. Although the amount of nanoleakage may be very small (nanometer size) in the bonded assembly, over time, it has the potential to serve as a pathway for water movement within the adhesive-resin interface. Such water movement may extract unconverted monomers from the resin adhesive or hybrid layer. Therefore, the effect of nanoleakage on the integrity of resin-dentin bonding has become an issue of interest, not only for short-term, but for long-term adhesion in particular [16]. The formation of nanoleakages within the hybrid layer and the adhesive-resin interface is an important indicator for judging the material's sealing ability and for the quality of the hybrid layer, which, in turn, affects the longevity of the restoration [14].

In order to quantify the amount of nanoleakage, silver nitrate was used to visualize the depth of the dye penetration pathway within the hybrid layer [14]. The silver nitrate staining is one of the most commonly used methods for microleakage evaluation at tooth-restoration margins levels as it easily migrates within the interface zone due to its extremely small diameter molecule (0.059 nm) [17]. 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 [18, 19]. Other organic dyes (including methylene blue and basic fuchsin) have been used for leakage evaluation, but these substances have larger molecules and exhibit a propensity for bonding to tooth structure that can potentially demonstrate a wider and deeper gap than actually exists.

Although the mechanisms leading to the nanoleakage phenomenon are not completely explored, our study showed nanoleakage at the adhesive-resin interface, to varying degrees; this

		Scores											
		1	2	3	4	5	6	7	8	9	10	Mean	SD
Groups	Acid etching + Nd:YAG laser	5.1	5.4	4.8	5.2	4.3	4.8	4.5	5.2	5.1	4.7	4.91	±0.35
	Acid etching + 10% NaOCl	10.2	10.6	11.1	10.8	10	10.3	11.2	11.4	10.9	10.5	10.7	±0.46
	Acid etching	25	25.4	24.8	24.7	25.1	25.2	25.5	26.2	10.9	26.1	25.59	±0.52

Table 4 : The mean values of the global leakage scores and the SD of the Solobond Plus bonding system after acid etching and deproteinization with Nd:YAG laser or 10% NaOCl application or without dentin deproteinization.

Source of variance	Sum of Squares	df	Mean Squar	F	Sig
Between groups	2331.129	2	1165.564	230.312	H.S
Within groups	136.642	27	5.061		
Total	2194.487	29			

Table 5: Analysis of variance of global leakage scores of the three treatment modalities using the Solobond Plus bonding system.

Groups	Difference between means	HSD (5%)	HSD (1%)	Sig.
Group 2a v/s 2b	5.79	2.497	3.201	H.S
Group 2a v/s 2c	20.68			H.S
Group 2b v/s 2c	14.89			H.S

Table 6: The results of Tukey's HSD test applied on the mean changes in values of the global leakage scores of each treatment modality for the Solobond Plus bonding system.

Groups		Means	t-values	df	p- value
Acid etching + Nd:YAG Laser	Excite®	5.8	6.279	10	H.S
	Solobond Plus	4.91			
Acid etching + 10% NaOCl	Excite®	13.33	11.199	10	H.S
	Solobond Plus	10.7			
Acid etching	Excite®	31.17	19.162	10	H.S
	Solobond Plus	25.59			

Table 7: Paired t-test of the global leakage scores to compare between each pair of groups for each treatment.

might be due, in part, to a discrepancy between the depth of etching and of resin penetration. Our results comply with those of Li et al. [20] who found that all the tested specimens showed nanoleakage at the adhesive-dentin interfaces, and with those of Sano et al. [14] who found that even in the absence of marginal gaps, there was a varying amount of penetration of the silver ions through the “hybrid layer” and into the underlying tubules.
Our results were also in agreement with those of Pioch et al. [21] who reported that dye penetration when using 1% rhodamin-B solution was observed in all specimens.

The application of Nd:YAG laser on conditioned dentin significantly decreases the values of the global leakage score regardless of the type of the dentin adhesive system [13]. This is due to the ability of Nd:YAG laser to remove the collagen network from the acid-etched dentin, thus increasing the diffusion potential of the monomer into the intact dentin and minimizing the nanoleakage.
The depletion of collagen from the surface of acid etched dentin results in:
- an increase of the permeability of dentin substrate due to the dentinal tubules enlargement near the outer dentin surface; this will facilitate the spreading and diffusion of adhesive monomers through dentin (Barbosa et al. [22] and Inaba et al. [23]).
- an augmentation of the dentin surface energy and thus a potentialisation of the adhesive monomers diffusion through dentin (Bedran de Castro et al. [24]).

The proteolytic action of NaOCl is believed to involve extensive fragmentation of long peptide chains and formation of N-chloramines with terminal amine groups that further decompose to other by-products, including inter- and intra-molecular crosslinks via Schiff base formation [25, 26]. NaOCl-treated dentin is rich in exposed hydroxyapatite crystals [26] and could result in a more stable interface over time because it is essentially made of mineral [27]. In our study, the use of 10% NaOCl for one minute on the conditioned dentin had a decreasing effect on the global leakage scores. This result was in agreement with those of Pioch et al. [6]. Contrarily, Ferrari et al. [28] found that the application of 10% NaOCl did not reduce the global leakage scores nor improve the restoration seal.

The lowest values of global leakage scores were recorded when using Solobond Plus, an acetone-based bonding system; Inai et al. [29] reported similar results due to the relatively high volatility of solvents such as acetone. In fact, alcohol may displace the surface moistures into the micro- or nanoporosities of the etched dentin surface.

Conclusion

Within the limitations of this study, we found that treatment of the acid-etched dentin with Nd:YAG laser led to a significant decrease in the leakage scores of the adhesive bonding system. Nd:YAG laser can be therefore used for removing the collagen network from acid-etched dentin before applying the bonding agent to improve the quality of the dental restorations.
Also, better results were obtained with the application of 10% NaOCl after acid conditioning compared to acid conditioning alone.
The global leakage scores of Solobond Plus adhesive system were significantly less than those of the Excite® adhesive system.

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