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Comparative SEM Evaluation of Penetration of Adhesive Systems in Human Dentin with a Non-rinse Conditioner and a Self-etching Primer

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The purpose of this study was to compare the effect of a self-etching primer and a non-rinse conditioner with the effect of a conventional adhesive system on the penetration depth in dentin of human teeth, using scanning electronic microscopy (SEM). Fifteen human third molar teeth were sectioned into 2 pieces. All pieces were flattened with grade 600 to 1200 silicon carbide paper and divided into 6 groups: group 1 - Prime & Bond NT (NT), negative control; group 2 - 37% phosphoric acid + Prime & Bond NT (PANT), positive control; group 3 - Non-rinse Conditioner (NRC) + Prime & Bond NT (NRCNT); group 4 - NRC + Prime & Bond 2.1 (NRCPB); group 5 - NRC + Scotchbond MP (NRCSB); group 6 - Prompt L-Pop (PLP). All teeth were covered with Dyract AP. The tooth fragments were decalcified, and its resin replicas were evaluated by SEM by three previously standardized examiners. The penetration was scored from 0 (no penetration) to 3 (maximum penetration). The Kruskal-Wallis and Mann-Whitney U tests ($p<0.05$) showed three statistically homogeneous groups: {NT, NRCPB, NRCSB and PLP}, {NRCNT} and {PANT}. The authors concluded that the self-etching primer and the non-rinse conditioner provide a lower penetration depth in human tooth dentin than the conventional adhesive system.

Key Words: adhesive systems, dentin, resin-dentin interface, self-etching primers, scanning electron microscopy.

INTRODUCTION

The acid etching technique, presented by Buonocore (1) in 1955, generated a revolution in Dentistry, stimulating the development of several new products. Recently, acid primers, single-bottle agents and self-etching bonding systems have become known in a very short period of time. However, these products must be evaluated and tested before they are used on a large-scale basis. As several factors must be analyzed, scanning electron microscopy (SEM) has been used to evaluate the micromechanical bonding mechanism offered by the current adhesives. This research is done through observation of dentinal tissue samples or replicas in resin (2), which clearly reveals that bonding has a biomechanical nature, based on hybridization of the demineralized dentinal tissues, with resin tag and lateral branch formation (3-5).

Some of the adhesive systems have an etching and primer combination that is applied in only one step (acid primers), in order to render their clinical use more practical and fast. Others include a primer and adhesive in only one bottle (single-bottle agents); these are used after enamel and dentin etching procedures with phosphoric acid have been performed (6,7). Although possibilities of error are minimized during their use, the total application procedure is not simple because several layers must be applied, therefore time saving procedures regarding conventional systems are not that significant (8,9).

Thus, efforts aiming to develop new materials are being carried out, in order to simplify the total acid etching system and to prevent the phenomenon known as “nanoleakage”, by avoiding a greater dentin demineralization where the total penetration of the adhesive many times did not occur (10,11). Many products are

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available on the market, such Non-rinse Conditioner (Dentsply), which is an acid that needs no rinsing, and Prompt L-Pop (ESPE), which is a new self-etching primer which, in fact, combines all the etching and bonding process in only one stage. However, the action of these new materials on dentinal tissues is not yet well known and must be analyzed under different aspects, in order to be properly used by professionals so that they can make the best of their properties during clinical application.

The purpose of this study was to evaluate the effect of a non-rinse conditioner associated to adhesives and also to evaluate a self-etching primer, comparing them with the effects of 37% phosphoric acid, regarding regularity and infiltration depth of adhesives in human teeth dentin using SEM.

MATERIAL AND METHODS

Fifteen recently extracted erupted third human molars were selected for this study. These teeth had no caries, nor previous restorations. They were stored in distilled water at room temperature for 3 months.

In order to evaluate adhesive infiltration in the dentin, the teeth were sectioned longitudinally following the buccal-lingual groove direction, obtaining 2 halves. The occlusal surfaces were removed 1 mm below the dentinal-enamel junction, which corresponded to the central groove. With a transversal section, their roots were also eliminated. They underwent cutting procedures under the occlusal dentin surface, so as to obtain 3-mm thick discs for a total of 30 specimens. These were cut with a double-faced diamond disc and water was used for cooling. The dentin samples were flattened and polished using number 600 to 1200 wet silicon carbide paper.

Teeth fragments were divided into 6 groups of 5 dentin samples each (Table 1). The materials used and the corresponding manufacturer and composition are presented in Table 2.

The etching agents and adhesive systems were applied following manufacturer recommendations. The dentin discs were coated with Dyract AP (Dentsply), making a cylinder of approximately 6 mm high with a diameter of 4 mm. In order to do this, 3 increments of 2 mm each were superimposed, and each one was photopolymerized for 40 s with a QHL 75 Curing Lite

Table 1. Etching and adhesive agents of the 6 experimental groups.

Group	Etching agent	Adhesive agent	Symbol
1	———	Prime & Bond NT	NT
2	37% Phosphoric Acid	Prime & Bond NT	PANT
3	Non-Rinse Conditioner	Prime & Bond NT	NRCNT
4	Non Rinse Conditioner	Prime & Bond 2.1	NRCPB
5	Non Rinse Conditioner	Scotch Bond MP	NRCSB
6	Prompt L-Pop		PLP

Table 2. Materials used and their corresponding composition and manufacturers.

Material	Composition	Manufacturer
Non-Rinse Conditioner (NRC)	Itaconic acid, Maleic ácid, Water, Solvent	Dentsply/De Trey Konstanz, Germany
Prime & Bond NT	Di and trimethacrylate resins, Functionalized amorphous silica, PENTA, Photoinitiators, Stabilizers, Cetylamine hydrofluoride, Acetone	Dentsply/De Trey Konstanz, Germany
Prime & Bond 2.1	Elastomeric dimethacrylate resins, PENTA, Photoinitiators, Stabilizers, Cetylamine hydrofluoride, Acetone	Dentsply/De Trey Konstanz, Germany
Scotchbond Multi-Purpose	Primer: HEMA, photo-activated polymer, water Adhesive: BIS-GMA, HEMA, Photoinitiators	3M Dental Products St. Paul, MN, USA
Prompt L-Pop	Liquid 1(red blister): Methacrylated phosphoric esters, Initiators, Stabilizers Liquid 2 (yellow blister): Water, Fluoride complex, Stabilizers	ESPE Dental AG Seefeld, Germany

equipment (Dentsply; intensity>500 mW/cm[2]). All samples were stored for one week in distilled water at room temperature.

In order to observe the adhesive penetration micromorphology, specimens were submitted to cycles of demineralization, which led to complete decalcification and dissolution of the dentinal structures. Approximately 5 consecutive cycles were performed consisting of a 10% hydrochloric acid bath for 5 h, and a 5% sodium hypochlorite bath for 1 h; baths and cycles were intercalated with a 5-min distilled water rinse.

After all dentinal tissues had been completely eliminated, resin replicas were then coated with a gold layer (Sputer equipment) and examined with a scanning electron microscope (DSM 960 - Zeiss, Germany).

Photomicrographs were made at 1000X and 3000X, for each sample obtained in the dentin, near the pulp area. These photomicrographs were evaluated by three specialized professionals who attributed comparative scores, according to the following penetration

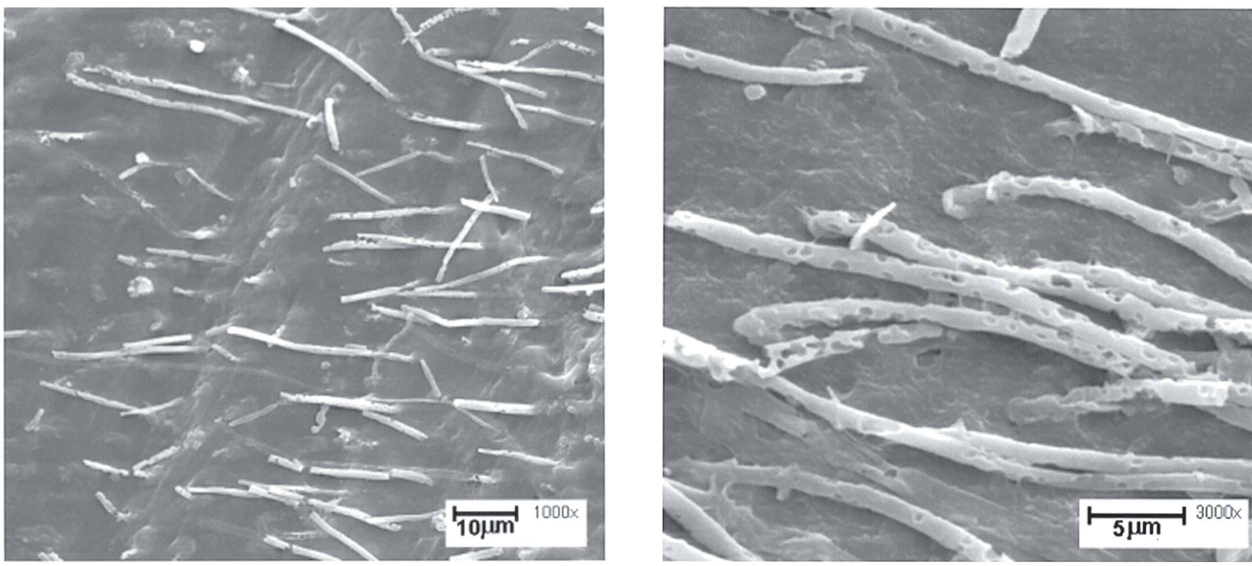

Figure 1. Group 1. *Left*: Resin replica of the dentinal surface. Only some areas presented resin tags (1000X). *Right*: Higher magnification revealing an irregular shape of the resin tags (3000X).

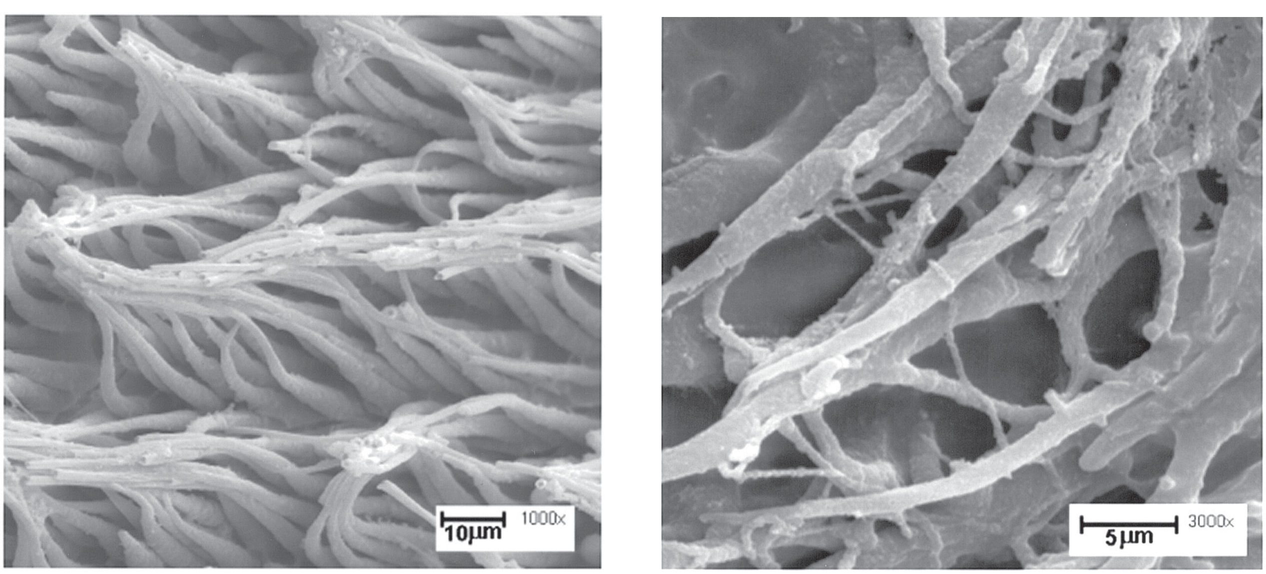

Figure 2. Group 2. *Left*: Resin replica of the dentin where a regular tag distribution can be observed, with deep penetration (1000X). *Right*: Higher magnification revealing long tags, with an inverted cone shape and a large quantity of lateral bond branches (3000X).

scale: 0 = without penetration; 1 = minimum penetration; 2 = intermediate penetration and 3 = maximum penetration. Characteristic photomicrographs of each score were used for calibration among evaluators.

Data obtained was statistically analyzed by Kruskal-Wallis and Mann-Whitney U non-parametric tests ($p<0.05$).

RESULTS

Group 1: On the dentin samples where only Prime & Bond NT was applied, no acceptable adhesive infiltration was revealed. In many areas there was no evidence of tag formation and when tags were present, they were of irregular shape (Figure 1).

Group 2: Good penetration was verified all over the sample. The resin tags were long and presented an inverted cone shape, showing an adequate tubular sealing. The presence of several lateral adhesive branches was also observed. (Figure 2).

Group 3: Variable penetration was observed and tags were generally short, presenting an irregular shape

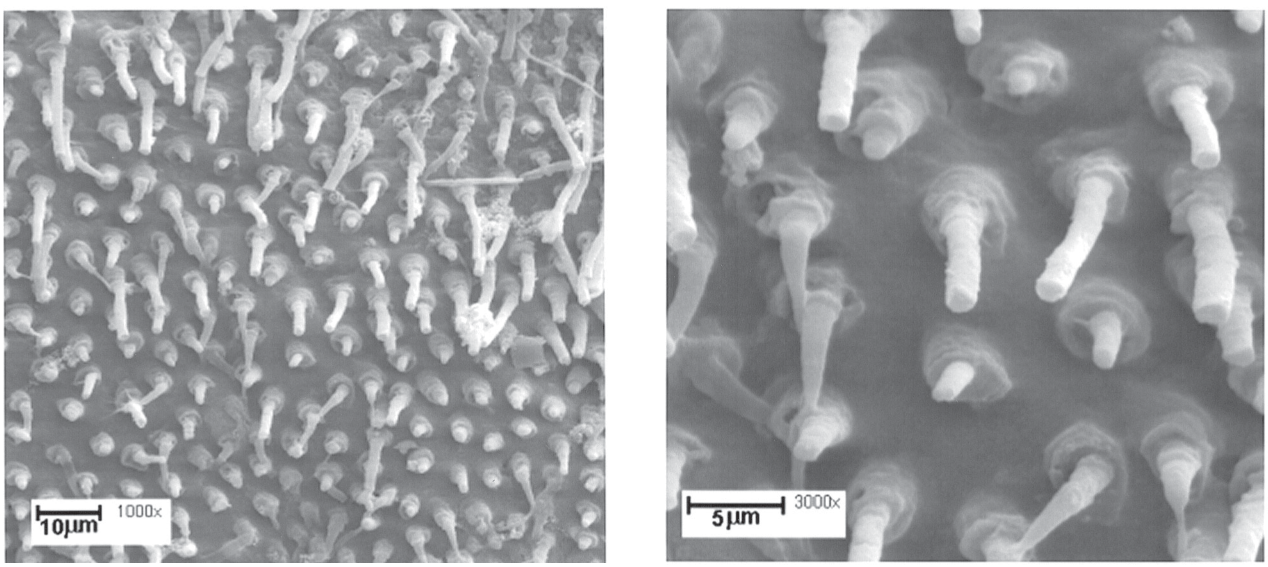

Figure 3. Group 3. *Left*: Resin replica of the dentinal surface where an irregular penetration was observed (1000X). *Right*: Higher magnification revealing short tags, irregularly shaped, in the entrance of tubular orifices (3000X).

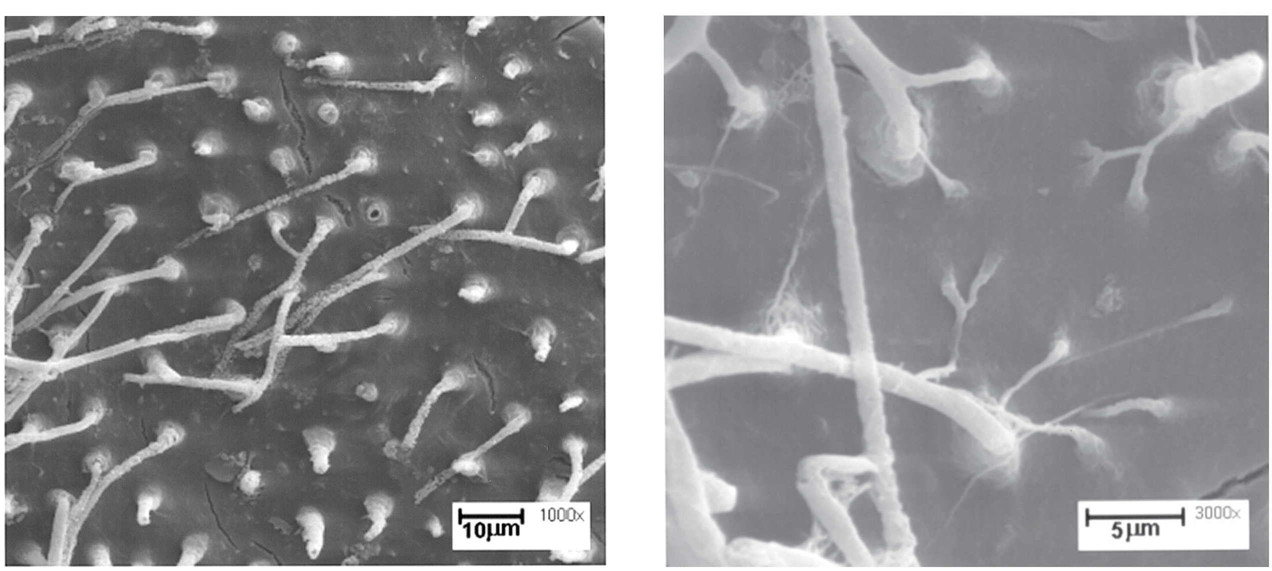

Figure 4. Group 4. *Left*: An irregular etch pattern was observed in the dentin, with small island formation with good penetration (1000X). *Right*: Higher magnification revealing irregular shaped resin tags (3000X).

at the entrance of the tubular orifice. This might indicate imperfect sealing. (Figure 3).

Group 4: When NRC was applied associated to Prime & Bond 2.1 on the dentinal surface, an irregular etch pattern was observed with some areas with good penetration, and others without resin tag formation (Figure 4).

Group 5: Dentin samples treated with NRC associated with Scotchbond MP presented a poor etch pattern, with few resin branches, irregular in shape (Figure 5).

Table 3. Mean ranks regarding penetration in dentin.

Material	Mean Rank
NT	14.67^{a}
PANT	50.00^{c}
NRCNT	34.67^{b}
NRCPB	23.33^{a}
NRCSB	21.17^{a}
PLP	21.17^{a}

Different letters indicate statistical differences ($p<0.05$)

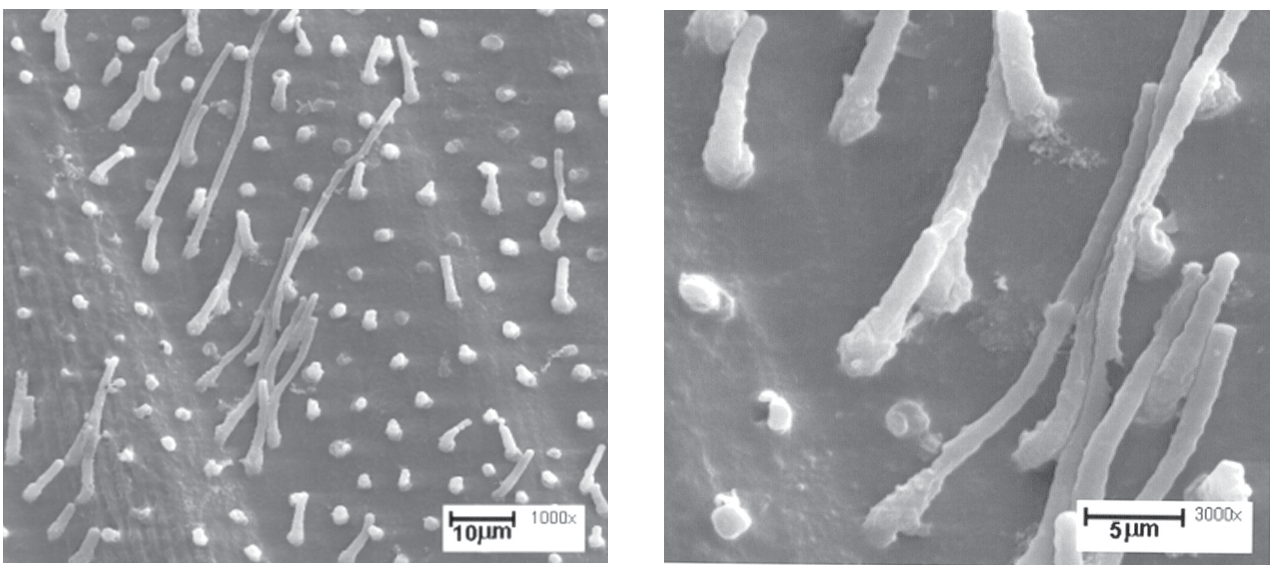

Figure 5. Group 5. *Left*: A poor etch pattern was observed on the dentinal surface (1000X). *Right*: With a higher magnification, few resin branches with irregular shapes were observed (3000X).

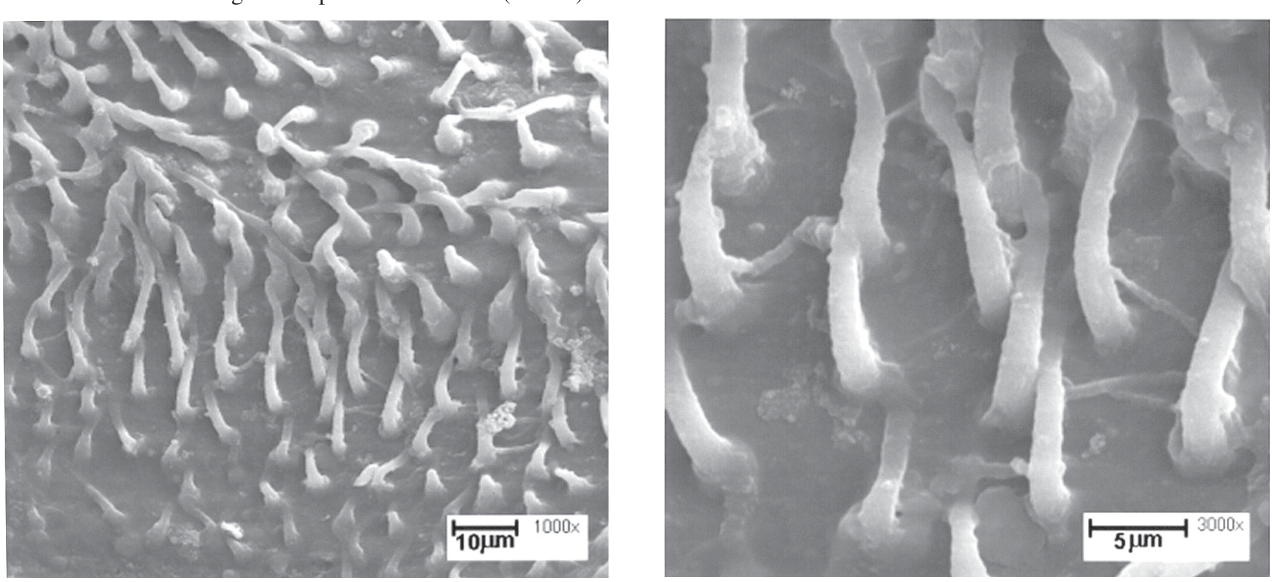

Figure 6. Group 6. *Left*: Resin sample of the dentinal surface. A poor and irregular etch pattern was observed (1000X). *Right*: Higher magnification (3000X).

Group 6: The Prompt L-Pop self-etching system presented a poor etch pattern that was quite irregular with areas with no tags and other areas with tag formation, although these tags were short (Figure 6).

Statistical analysis revealed homogeneous groups for dentin penetration. The decreasing penetration order was as follows: {PANT}, {NRCNT} and {NT, NRCPB, NRCSB and PLP} (Table 3).

DISCUSSION

The method used in this study, SEM of total dental tissues decalcification, is extremely simple and useful for evaluation of characteristics of resin tags and also for evaluation of their lateral branches and the dentinal surface etch pattern (12). When the dentinal tubule orifices are totally sealed, the initial parts of the tags present a reverse cone shape, generally having adhesive lateral branches, indicating a very good etch pattern. Some studies (3,5) have suggested that these lateral branches or anastomoses between the dentinal tubules may contribute to the micromechanical retention mechanism, resulting in a positive influence on the junction between the dentinal tissue and the composite resin.

According to Ferrari et al. (2), lower SEM magnifications allow dentinal enamel and dentin etch pattern verification, where tag density and depth can also be observed, whereas higher magnifications reveal morphological characteristics of resin tags penetrating both substrates.

In studies regarding "nanoleakage", Sano et al. (11) suggested that adhesive systems should be developed using the least possible demineralized dentin thickness, as well as monomers having a great affinity with collagen and with hydroxyapatite, because this would enable a more even penetration and full polymerization. Thus, more careful investigations on acid solution concentrations were carried out in order to avoid dentinal decalcification in such magnitude that the adhesive would not be able to penetrate as deeply, leaving a collagen basal portion unprotected. This could in the future involve running risks regarding bond strength (7,10). Self-etching systems may be a solution for this problem, because they provide less dentinal demineralization, simultaneous to resin monomer penetration (13).

When Prime & Bond NT was associated with 37% phosphoric acid (PANT) on the dentinal surface, an even etch pattern with long tags was observed, as well as a large number of adhesive lateral branches. This process clearly revealed the micromechanical bonding mechanism. Currently, it is believed that tag length is not as important for bond strength as was once believed; however, the importance of its shape, as well as the presence of lateral branches, must be noted (10,14,15).

When NRC was associated with Prime & Bond NT, a lesser tag density was observed; in fact, these tags were much shorter. When NRC was associated with Prime & Bond 2.1 or Scotchbond MP, in addition to shorter, less dense tags, their distribution was quite irregular, indicating the worst results.

A shorter tag formation was observed with Prompt L-Pop, as expected because this is a characteristic of the self-etching systems (4,11,16-18). A quite irregular etch pattern was observed, some areas were properly etched and others had no etching at all; thus, this is cause for alarm. However, other studies report good results in bond strength tests (19).

The use of the Prompt L-Pop system is extremely attractive due to its practical application, and perhaps even promising. It represents a time-saving clinical procedure, facilitating its use, reducing failures in the hybridization process (13,17). However, it is too early to recommend this procedure without restrictions. This product still requires improvement and longer clinical studies before it can be recommended on a large scale for clinical use.

Although the manufacturer recommends the sole use of Prime & Bond NT without previous etching in some situations, it does not seem adequate. During observations with SEM, there was no evidence of the micromechanical bonding mechanism on the dentin, and a low density tag formation with quite irregular distribution in both dental tissues was observed.

According to the results of this study, the need for an etching agent was observed. Phosphoric acid presented clear evidence of the resin bonding mechanism to the dentin. In spite of this, the use of adhesive systems with low concentration acid solutions seems to be the path to be followed, considering the advantages and positive results obtained in some research (7,10,11,20). This tendency is supported by the principle that the quality of the hybrid layer is more important than its thickness (16). We conclude that the self-etching system and the non-rinse conditioner provide a lower penetration depth in human teeth dentin than the conventional adhesive system.

RESUMO

O objetivo desse estudo foi comparar o efeito de um adesivo autocondicionante e de um ácido não lavável quanto a profundidade de penetração na dentina de dentes humanos, através da observação em Microscopia Eletrônica de Varredura (MEV). Quinze terceiros molares tiveram a dentina exposta, cortando-se a face oclusal, planificados com lixa d'água 600, cortados transversalmente e distribuídos em 6 grupos de 5 cada: grupo 1 – Prime Bond NT (NT), controle negativo; grupo 2 – ácido fosfórico 37% + Prime Bond NT (PANT), controle positivo; grupo 3 – Non-Rinse Conditioner (NRC) + Prime Bond NT (NRCNT); grupo 4 – NRC + Prime Bond 2.1 (NRCPB); grupo 5 – NRC + Scotch Bond MP (NRCSB) e grupo 6 – Prompt L-Pop (PLP). Todos foram recobertos com Dyract AP. Os fragmentos dentários foram descalcificados com HCl a 10% e NaOH a 5% e as réplicas em resina examinadas em MEV. Três examinadores calibrados avaliaram as fotomicrografias e atribuíram escores de 0 = sem penetração a 3 = penetração máxima. Os testes de Kruskal-Wallis e Mann-Whitney (P<0,05) evidenciaram três grupos homogêneos {NT, NRCPB, NRCSB e PLP}; {NRCNT} e {PANT}. Os autores concluíram que o sistema adesivo autocondicionante e o ácido não lavável apresentaram menor penetração em dentina do que o sistema adesivo convencional.

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