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SEM Evaluation of a Non-rinse Conditioner and a Self-etching Adhesive Regarding Enamel Penetration

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Clinical Relevance

Although the use of self-etching systems has resulted in regular etch patterns, they have provided less demineralization, preserving enamel surfaces.

SUMMARY

This study evaluated the effect of a non-rinse conditioner (NRC) associated with two adhesive systems, Prime & Bond NT and Prime & Bond 2.1. The study also evaluated a self-etching adhesive, comparing it with the 37% phosphoric acid effects related to the regularity and infiltration depth of adhesives in human tooth enamel via observation using Scanning Electronic Microscopy (SEM). Fifteen third molars were longitudinally sectioned into four parts by means of a mesio-distal cut and facio-lingual cut. All pieces were flattened with silicon carbide paper, randomly separated and divided into five groups where the enamel surfaces were treated with different materials according to the manufacturers' instructions as follows: Group 1–Prime & Bond NT (Dentsply); Group 2–37% phosphoric acid + Prime & Bond NT; Group 3–Non-Rinse Conditioner (Dentsply) + Prime & Bond NT; Group 4–NRC + Prime & Bond 2.1 (Dentsply); Group 5—Prompt L-Pop (3M ESPE). All teeth were covered with Dyract AP (Dentsply). Specimens were decalcified, metalized and the inner portions of Dyract were observed in SEM and evaluated by calibrated examiners to evaluate resin penetration on enamel. Resin penetration was ranked from 0 = no penetration, to 3 = maximum penetration. The Kruskal-Wallis and Mann-Whitney U tests ($p<0.05$) showed only three statistically homogeneous groups: {1}, {2,3} and {4,5}. The authors concluded that Prime & Bond NT showed the lowest penetration, NRC showed similar effects compared to phosphoric acid when associated with Prime & Bond NT, and concluded that Prompt L-Pop showed similar effects to NRC, which was associated with Prime & Bond 2.1.

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DOI: 10.2341/04-190

INTRODUCTION

The enamel acid-etch technique, presented by Buonocore (1955), generated a revolution in dentistry, stimulating the development of new products over the decades, including dental adhesives. The evolution of these dental adhesives allowed for more conservative dentistry, but a perfect dental adhesive system has not been developed, which fosters ongoing research. As part of this ongoing research, single-bottle agents and self-etch bonding systems have been developed, creating new products for evaluation. Among the evaluations that need to be done when a new dental adhesive is developed is the use of Scanning Electron Microscopy (SEM) to evaluate the micromechanical bonding mechanism created by this new adhesive using dental tissue samples or resin replicas (Ferrari & others, 1999). Usually, this technique confirms that almost all modern dental adhesive systems' bonding mechanisms are based on a biomechanical nature, based on hybridization of the demineralized dentinal tissues, with resin tag and lateral branch formation (Chappell & others, 1994; Titley & others, 1995; Ferrari & Davidson, 1996).

Some adhesive systems combine prime and etching, which is applied in one step, to render more practical and time-efficient clinical use; whereas, other systems include prime and adhesive in one bottle (single-bottle agents) and are used after enamel and dentin etching procedures with 37% phosphoric acid have been performed (Kanca, 1992) to render clinical use as more practical and time-efficient. Although possible errors are minimized during use, the total application procedure of these two adhesive systems (one-step and single-bottle) is not as simple as it appears and, as several layers are applied sequentially, time-saving is not a significant advantage when the total application procedure is compared to conventional systems (Finger & Fritz, 1996; Van Meerbeek, 1998).

Considering this, many efforts that aim for the development of new materials are being carried out in order to simplify the three-step systems (conventional systems) and prevent the phenomenon known as "nanoleakage," which usually occurs when the adhesive does not penetrate into whole, demineralized dentin (Prati, 1995; Sano & others, 1995). Based on this objective, many products have been launched, such as Non-Rinse Conditioner (NRC—Dentsply), which is an acid that requires no rinsing, and Prompt L–Pop (3M ESPE), which is an all-in-one adhesive system that combines the etching and bonding process into one stage. However, the effect of these new materials on dental tissues is not well known and must be studied and analyzed under different aspects so they can be properly used and their best properties achieved in clinical application.

Although some authors (Gordan & others, 1998; Prati & others, 1998; Ferrari & others, 1999) have already shown good results concerning the use of acidic primers in dentin, their use in enamel is not a consensual procedure, and their effect in cut or uncut enamel is still unknown.

This study evaluated the effect of a non-rinse conditioner associated with two adhesive systems, Prime & Bond NT and Prime & Bond 2.1. It also evaluated a self-etching adhesive, comparing all with the 37% phosphoric acid effects related to the regularity and infiltration depth of adhesives in human enamel by means of SEM observation.

METHODS AND MATERIALS

Fifteen recently extracted mandibular third molars, kept in distilled water at 37°C, were selected for this study. All the teeth were free from caries and previous restorations. The samples were cleaned with a periodontal curette, then cleaned with fine flour of pumice, using a rubber cup in a low-speed handpiece for 30 seconds. The samples were then stored in distilled water at 37°C; the distilled water was changed every seven days. These teeth were longitudinally sectioned into four parts by means of a mesio-distal and facio-lingual cut using a slow-speed diamond saw (Isomet, Buehler, Lake Bluff, IL, USA) under a coolant water flow. After the initial sections were cut, the occlusal surface was removed by means of a horizontal cut, using a low speed diamond saw at the DEJ. The enamel surfaces were then flattened and polished with 600 and 1200 silicon carbide paper.

Once 60 fragments were obtained, they were randomly separated and divided into five groups, where the enamel surfaces were treated with different materials (Table 1). The materials used and the corresponding manufacturers and compositions are presented in Table 2.

The etching agents and adhesive systems applied in each group were used according to the manufacturer's instructions. Once the agents had been used, three increments (2 mm high with a diameter of 4 mm) of Dyract AP (Dentsply) were applied in the enamel-flattened surfaces, making a cylinder approximately 6 mm high with a diameter of 4 mm. Each compomer increment was light cured for 40 seconds with a QHL 75 Curing Lite, Dentsply (intensity > 500mW/cm^2). The

Table 1: *Groups Tested*

Group	Etching Agent	Adhesive Agent
1	None	Prime & Bond NT
2	Phosphoric Acid 37%	Prime & Bond NT
3	Non Rinse Conditioner	Prime & Bond NT
4	Non Rinse Conditioner	Prime & Bond 2.1
5	None	Prompt L-Pop

Table 2: *Materials Used, Their Corresponding Composition and Manufacturers*

Material	Composition	Manufacturer
NRC	Itaconic acid, Maleic acid, Water, Solvent	Dentsply International, York, PA, USA
Prime & Bond NT	Di and trimethacrylate Resins, Functionalized Amorphous Silica, PENTA, Photoinitiators, Stabilizers, Cetilamine hydrofluoride, Acetone	Dentsply
Prime & Bond 2.1	Elastomeric dimethacrylate resins, PENTA, Photoinitiators, Stabilizers, Cetilamine hydrofluoride, Acetone	Dentsply
Prompt L-Pop	Liquid 1 (red blister): Methacrylated phosphoric esthers, initiators, stabilizers Liquid 2 (yellow blister): water, fluoride complex, stabilizers	3M ESPE Dental Products, St Paul, MN, USA

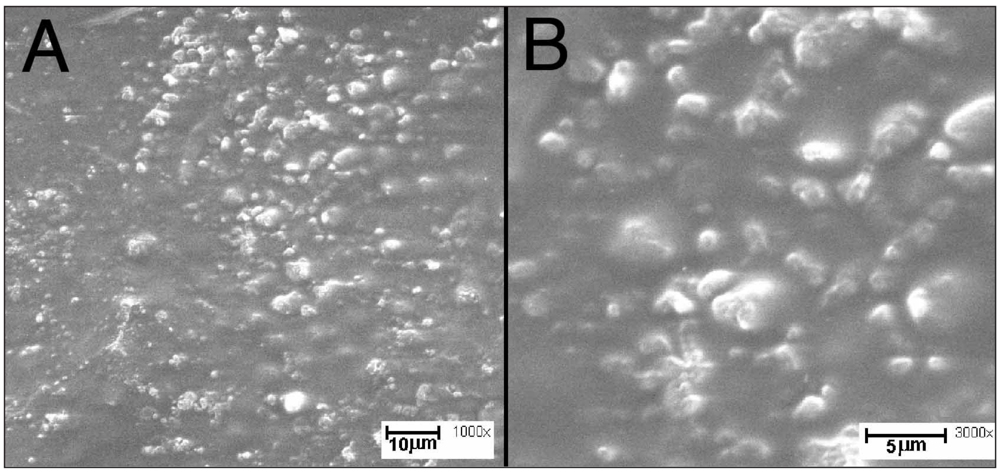

Figure 1. *Resin replica of the non-etched enamel, surface resulting after Prime & Bond NT was applied (Group 1). Figure 1A. SEM 1000x; Figure 1B. SEM 3000x.*

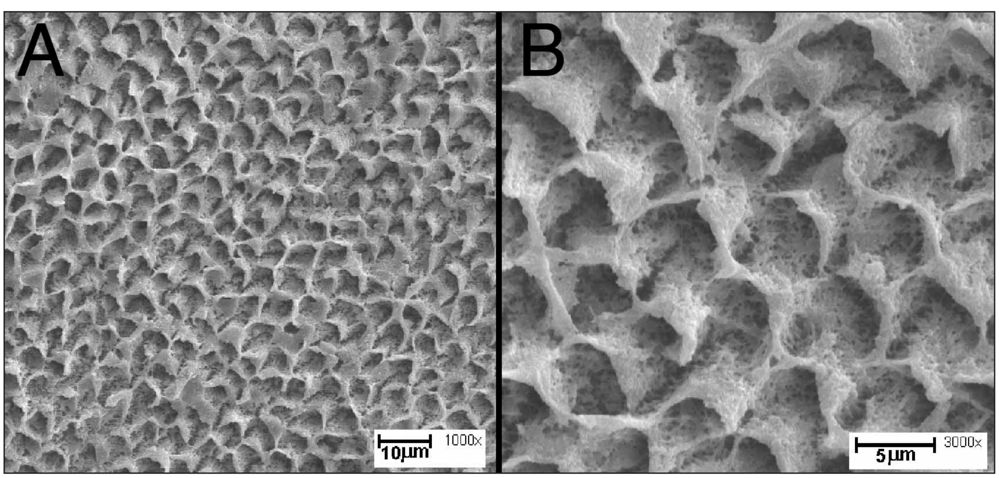

Figure 2. *Enamel etch pattern, obtained after the phosphoric acid procedure at 37% and Prime & Bond NT (Group 2) Figure 2A. SEM 1000x; Figure 2B. SEM 3000x.*

samples were then kept in distilled water for seven days.

In order to detect adhesive penetration micromorphology, all specimens were submitted to five consecutive demineralization cycles that led to complete decalcification and dissolution of the dental structures. These cycles consisted of a 10% hydrochloric acid bath over five hours, followed by a 5% sodium hypochlorite bath for one hour. The baths and cycles were intercalated with a five-minute distilled water rinse.

After all dental tissues had been completely eliminated, the inner portion of the Dyract cylinder, which was in touch with the dental tissue, was then placed on aluminum stubs and sputter-coated with gold (Edwards Coater S150B). The samples were then evaluated under JEOL-JSM T330A (Zeiss, Germany) Scanning Electron Microscopy.

Microphotographs of the center portion of the samples were taken at standard magnifications (1000x and 3000x). The microphotographs were evaluated by three calibrated professionals who attributed comparative scores to the following penetration 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 were statistically analyzed by Kruskal-Wallis and Mann-Whitney U (p<0,05) non-parametric tests.

RESULTS

In Group 1, where only Prime & Bond NT was applied, no acceptable adhesive infiltration pattern was revealed (Figures 1A and 1B).

In Group 2, where Prime & Bond NT was applied after the etching procedure had been carried out with phosphoric acid, the specimens showed an even etch pattern, presenting well-formed resin tags with good penetration (Figures 2A and 2B).

In Group 3, where all enamel samples were treated with NRC associated with Prime & Bond NT, a regular etch pattern was found along with tags that had good penetration, similar to the ones observed in Group 2 (Figures 3A and 3B).

In Group 4, where NRC was applied associated with Prime & Bond 2.1, an irregular distribution pattern of the resin tags was observed (Figures 4A and 4B).

In Group 5, the Prompt L–Pop self-etching adhesive system over the enamel specimens presented an even etch pattern, but this provided less demineralization, which resulted in a smaller magnitude tag formation (Figures 5A and 5B).

Statistical analysis (Table 3) revealed homogeneous groups for enamel penetration. The order of decreasing penetration was as follows: {Group 2 and Group 3} > {Group 4 and Group 5} > {Group 1}.

DISCUSSION

The method used in this study in SEM, using total dental tissue decalcification, is extremely simple and useful for resin tag characteristics and surface etch pattern evaluation (Cagidiaco & others, 1997). According to Ferrari and others (1999), during observation in SEM, lower magnifications allow dental 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.

Sano and others (1995) asserted that adhesive systems should be developed using the least possible demineralized dentin thickness and

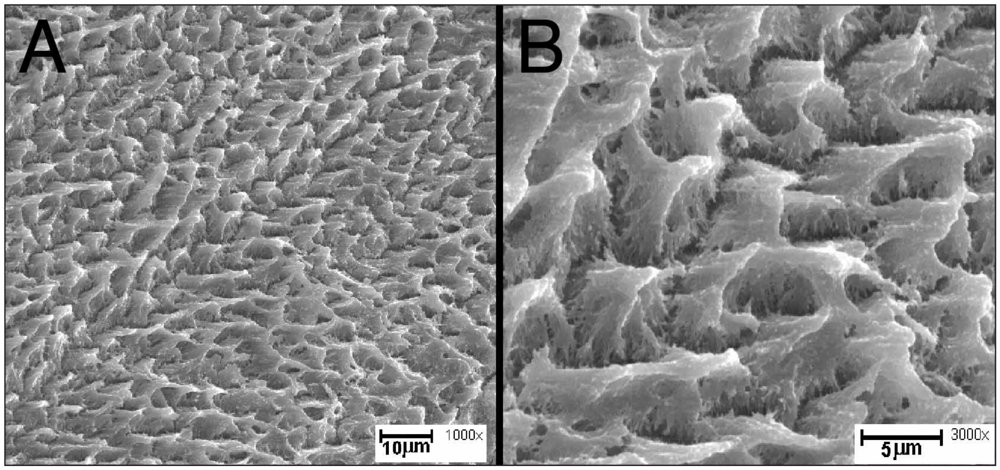

Figure 3. *Resin replica of enamel treated with NRC and associated with Prime & Bond NT (Group 3). Figure 3A. SEM 1000x; Figure 3B. SEM 3000x.*

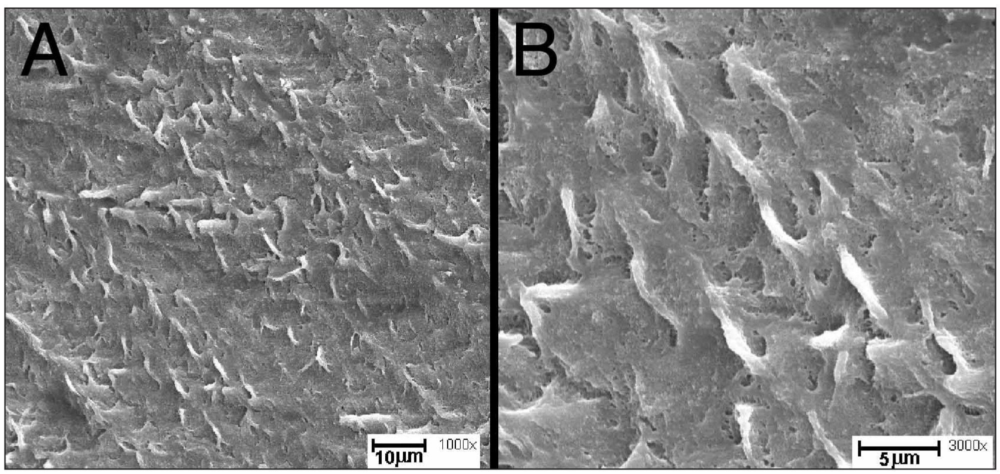

Figure 4. *Resin replica of the enamel surface, where Prime & Bond 2.1 was applied after the NRC procedure. An irregular tag distribution pattern was observed (Group 4). Figure 4A. SEM 1000x; Figure 4B. SEM 3000x.*

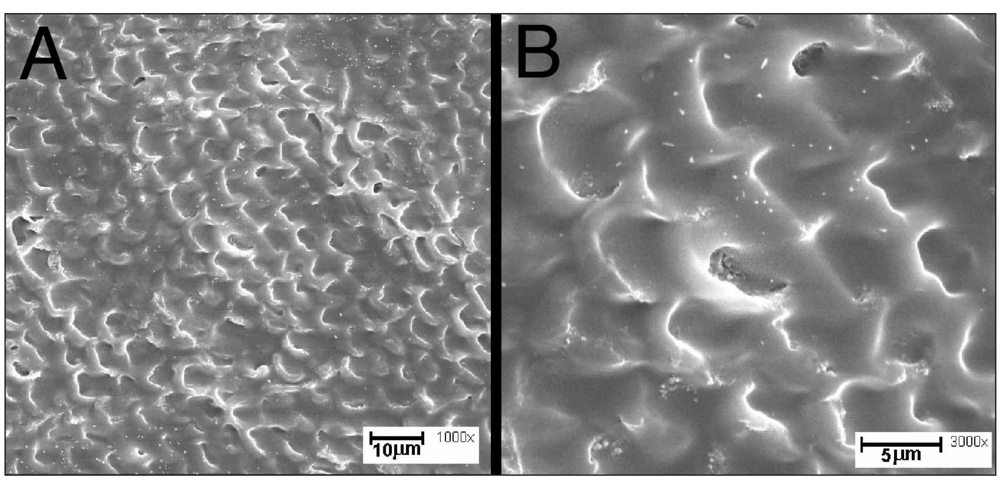

Figure 5. *The use of Prompt L-Pop in enamel resulted in a regular etch pattern, although it promoted a lesser degree of decalcification (Group 6). Figure 5A. SEM 1000x; Figure 5B. SEM 3000x.*

Table 3: *Mean Ranks Regarding Penetration in Enamel*

Material	Mean Rank*
Prime & Bond NT	6.18[a]
Phosphoric acid + Prime & Bond NT	44.50[c]
NRC+ Prime & Bond NT	44.50[c]
NRC+ Prime & Bond 2.1	22.41[b]
Prompt L-Pop	22.41[b]

**different letters indicate statistical difference (p<0.05)*

monomers that have a high affinity with collagen and hydroxyapatite, as this would enable better penetration and full polymerization. Thus, more careful investigations of acid solution concentrations were carried out to avoid dentinal decalcification in such magnitude that the adhesive would not penetrate as deeply, leaving a collagen basal portion unprotected, which could involve running a risk regarding bond strength in the future (Prati, 1995). Self-etching systems may be a solution for this problem once they provide less dentinal demineralization, simultaneous to resin monomer penetration (Watanabe, 1994).

In this study, when samples were evaluated, it was noted that, when NRC had been used in association with Prime & Bond NT, the results were similar to those obtained with phosphoric acid with Prime & Bond NT, where an even etch pattern and similar magnitude penetration were noted. When NRC was associated with Prime & Bond 2.1, the tag distribution pattern was somewhat irregular. On the other hand, Prompt L–Pop presented a regular etch pattern, although it produced smaller tags, which was expected, as its composition included a poor acid solution (Burrow & others, 1994; Perdigão & Swift Jr, 1994; Prati, 1995).

Due to its practical application, use of the Prompt L–Pop system is extremely attractive and, perhaps, even promising. It represents a time saving clinical procedure and is easy to use (Perdigão & Swift Jr, 1994; Watanabe, 1994). However, it seems too early to endorse this procedure without any restrictions and use by clinicians on a larger scale. This product still needs improvements and longer clinical studies. *In vitro* studies that evaluate bond strength or microleakage, for example, are also necessary to evaluate the influence of small tags on adhesion.

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

According to the results, the need for an etching agent was verified; thus, phosphoric acid presented clear evidence of the resin bonding mechanism to enamel. In spite of this, the use of adhesive systems with low concentration acid solutions seems to be the recommended procedure, considering the advantages and positive results obtained in different research studies (Prati, 1995; Sano & others, 1995; Gordan & others, 1998; Prati & others, 1998). This tendency is supported by the principle that the quality of the hybrid layer is more important than its thickness (Burrow & others, 1994).

CONCLUSIONS

The authors concluded that:

1. The use of Prime & Bond NT without previous acid self-etching did not produce the micromechanical retention mechanism.
2. The Non-Rinse Conditioner (NRC) presented similar results to phosphoric acid at 37% when associated with Prime & Bond NT.
3. The self-etching system Prompt L-Pop did not present similar results to phosphoric acid at 37%.

(Received 31 October 2004)

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