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# SCIENTIFIC ARTICLES

# Effects of Current and Potential Dental Etchants on Nerve Compound Action Potentials

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**In this study, a 35% phosphoric acid gel (3M Scotchbond etchant), a nonrinse etchant (NRC), and two EDTA-containing conditioners (RC-Prep and File-Eze) were tested in vitro for blocking nerve conductance evoked in the rat sciatic nerve after local application. The phosphoric acid gel and NRC completely and irreversibly inhibited conductance. On the other hand, RC-Prep reduced the compound action potentials (cAPs) by 50% in 120 min. With File-Eze, the reduction in cAPs was less than 50% after an application time of 160 min (61.8 ± 1.8%). At 160 min the cAPs in the RC-Prep group had been inhibited by 62.4%. These results indicated strong neurotoxic effects of phosphoric acid and NRC when applied directly on exposed pulp in the total etch procedure.**

Mineralized dentin, covered with a smear layer, is not sufficiently porous to permit much infiltration of adhesive monomers in a clinically relevant period of time, which has led to the use of acidic conditioners to remove the smear layer and to solubilize the mineral crystallites from around the collagen fibrils of the underlying dentin matrix to provide space for resin monomer permeation (1). Today, the simultaneous application of an acid to enamel and dentin, known as the total-etch technique, is an almost universal strategy of dentin bonding (2).

Similar to cervical dentin sensitivity, postoperative sensitivity and pain subsequent to the placement of bonded restorations is a common clinical problem whose mechanism is poorly understood. Such teeth are particularly sensitive to heat and cold, and evoked pain is generally of short duration and moderate intensity (3). This clinical finding has been attributed to the occurrence of the polymerization stress of resin-based composites, which leads to internal stresses that can exceed the strength of the bond with the surrounding tooth structure and cause the interface to fail (4). Eventually, the resulting marginal gap can lead to microleakage, and thus, to postoperative sensitivity (4). However, postoperative sensitivity is a rapidly developing clinical occurrence, and it is highly unlikely that within 24 to 48 h posttreatment enough microleakage would occur to produce pain (5), suggesting that other factors could be involved in this phenomenon.

The details of the mechanism of pain perception in dentin have not been fully clarified. There are several hypotheses regarding how pain is conducted through dentin to the pulp nerve system. Of these, the hydrodynamic theory of dentin sensitivity (6) is believed to be the best explanation and implicates fluid flow through patent tubules and activation of nerves (7). Accordingly, effective prevention for postoperative sensitivity should involve the use of agents that either occlude dentinal tubules or modify nerve sensitivity (7), or both. Because one major reason for the use of etchants in the total-etch procedure is to open up dentin tubules that have been previously occluded by the smear layer, the ability of the etchant to modify or block the nerve transmission could be considered a valuable compensation for the hydrodynamic forces that it will lead to occur.

A commercially available EDTA gel has been recently shown to produce similar etching patterns with 37% phosphoric acid on cut dentin and enamel, suggesting that EDTA could be considered a potential conditioner for use in the total etch procedure (8). Pretreatment of dentin with EDTA not only removes the smear layer, but depletes calcium and phosphate from the surface to open microporosities much smaller than the dentinal tubules, thus allowing penetration of hydrophilic resins (8). Similarly, the use of nonrinse etchants, which do not require rinsing, is another recent approach to the simplification of conditioning enamel and dentin simultaneously. Structural changes in enamel and dentin have been analyzed by scanning electron microscopy after the application of a nonrinse conditioner and showed that the demineralization pattern of both enamel and dentin clearly resembles the effects created by phosphoric acid etching (9).

This study investigated the effects of one conventional phosphoric acid gel (3M Scotchbond etchant (3M Dental Products Division, St. Paul, MN)), one nonrinse etchant (NRC), and two EDTA containing conditioners [RC-Prep (Medical Products Laboratories, Philadelphia, PA) and File-Eze (Ultradent Products Inc., South Jordan, UT)] on modification of nerve sensitivity, using a neurotoxicity study model on the rat sciatic nerve.

TABLE 1. Etching agents

| Material | Composition | Manufacturer |
|---|---|---|
| 3M Scotchbond etchant | 35% phosphoric acid | 3M Dental Products Division, St. Paul, MN |
| NRC | Maleic acid, itatonic acid, water | Dentsply DeTrey, Konstanz, Germany |
| RC-Prep | 10% urea peroxide and 15% EDTA in a water-soluble vehicle | Medical Products Laboratories, Philadelphia, PA |
| File-Eze | 19% EDTA in an aqueous, water soluble solution, with lubricating base | Ultradent Products Inc., South Jordan, UT |

## MATERIALS AND METHODS

The etchants/conditioners used and their compositions are listed in Table 1. Locally bred female albino rats (*Rattus rattus*) weighing 300 to 340 g were anesthetized with ether, decapitated, and both sciatic nerves were dissected free from adherent tissue under a microscope and placed in a Pyrex bath. The bath contained tyrode solution (150 mM $Na^+$, 2.7 mM $K^+$, 1.8 nM $Ca^{2+}$, 0.1 mM $Mg^{2+}$, 140 mM $Cl^-$, 12 mM $HCO_3^-$, 0.4 mM $H_2PO_4^-$, 11 mM dextrose) and was continuously gassed with $O_2$ and $CO_2$ (95% and 5%, respectively). The pH was 7.5 and the temperature was kept at 37°C. The nerve was placed between two suction electrodes, which allowed for stimulation and recording of compound action potentials (cAPs). The nerves were continuously perfused with tyrode solution with a rate of 2 ml/min at 37°C. Rectangular stimulus pulses (0.1 ms in duration and 0.8 Hz) were delivered by a 0811A Pulse Generator (Hewlett Packard, Quincy, MA) through a Schwarzer AK1W161B Stimulus isolation unit. The cAP was amplified by a Schwarzer Polygraph EW 561 Amplifier, displayed on a Telequipment DM 63 Dual Beam Storage Oscilloscope and photographed for permanent records of the experiments. The minimal threshold that was required for evoking a cAP for each nerve was determined. The experiment was performed at a voltage three times that value. The nerves were stimulated for 20 min before the actual experiments. Glass capillary rods were used to bring the test materials into contact with the nerves. The capillary rods also ensured an equal area of contact that was 2 mm in width and approximately 3 mm in length. The etchants were applied on these rods. Each material was tested on five separate nerves.

The test materials were kept in contact with the nerves until cAP amplitudes were completely inhibited. Material application was discontinued after inhibition of the cAPs, the capillary rod was changed, and the tissues were perfused for at least 120 min. cAPs were recorded every 5 minutes to determine any recovery in amplitude, and the readings were terminated when three subsequent measurements of the cAP amplitude were the same.

Statistical comparisons between the test materials were carried out using the Friedman test with significance levels set at $p < 0.01$ and $p < 0.05$, followed by a post hoc Mann-Whitney *U* test (at $p < 0.01$ and $p < 0.05$) for intramaterial comparisons of the readings versus time.

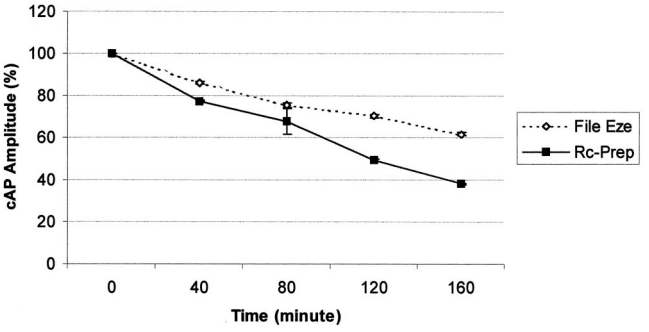


FIG. 1. Effects of RC-Prep and File-Eze on cAPs. Both materials caused reversible inhibition of the cAPs. However, the reduction was less than 50% for File-Eze after an application time of 160 min.

## RESULTS

NRC and the 3M etchant caused a rapid inhibition of the nerve activity and depressed the amplitude of the cAP to zero in all nerves, irreversibly. This was confirmed with subsequent washing of the nerves with fresh tyrode solution, after which none of the samples exhibited recovery of the cAPs. Both the NRC and the 3M etchant were, therefore, excluded from statistical analyses.

Reductions in the amplitude of cAPs by File-Eze and RC-Prep versus time are presented in Fig. 1 as means ± SD. With File-Eze, the cAPs were inhibited less than 50% despite a test period for as long as 160 min (61.8 ± 1.8% at 160 min). Testing procedures were terminated after this period and recovery of the cAPs was maintained. For RC-Prep, cAPs were reduced by 50% (49.36 ± 0.8) in all nerves in 120 min. Readings were conducted for 160 min, as with File-Eze, at which time the average amplitude of the cAP had been reduced to 37.6 ± 0.8%. Recovery of the cAPs was also observed upon subsequent washing with tyrode solution.

For both File-Eze and RC-Prep, differences in the reduction of cAPs versus time within each group were significant ($p < 0.01$, Friedman test). Similarly, intermaterial comparisons revealed significant differences between the materials in reduction versus time ($p < 0.01$, Mann-Whitney *U* test). In an attempt to rank the test materials according to their potency to produce total inhibition of the cAP, the NRC and the 3M etchant were the fastest, followed by RC-Prep and File-Eze.

## DISCUSSION

In the present study, both the 3M etchant and NRC caused rapid inhibition of the cAPs that could not be reversed by subsequent irrigation with tyrode solution, implying that their effects on the nerve samples after direct contact were most probably detrimental. Such an effect from the 3M etchant was anticipated (5). However, it was surprising to observe a similar potency from NRC, which is a mixture of relatively weak acids in water (Table 1).

Initial work on dentin desensitization using resin impregnation was carried out by Brännström et al. (10) and Nordenvall et al. (11). This technique relied on purely mechanical interactions between dentin and conventional unfilled resin (Concise Enamel Bond) after treatment of the dentin using EDTA and air drying for 20 s. Such treatment has been shown to reduce pulpal nerve activity

when stimulated (12) and perceived clinical sensitivity in humans (13). The findings obtained herein with RC-Prep and File-Eze, which both contain EDTA, may corroborate those findings. However, both conditioners were unable to produce rapid depression of the cAPs, although they had been brought to direct contact with the nerve. When applied to intact dentin, which is a relatively indirect access to the pulpal nerves, these conditioners would presumably not be able to alter the composition of the dentinal fluid in a single application. Thus, the neurotoxic effect could never occur. It was concluded that neither RC-Prep nor File-Eze were found suitable for the purpose of preventing postoperative dentinal pain.

Accordingly, it is also unlikely that neither phosphoric acid gels, such as the 3M etchant, nor the nonrinse conditioner could contribute to desensitization of cut dentin, because there seems to be no way that these conditioners could be titrated or applied in such a way as to balance these two opposite effects and reach the desired clinical result. Interpreting the changes in action potentials that were observed in vitro has been possible to some extent, although extrapolation into the clinical situation must be tempered with caution.

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