

The removal of phosphoric acid and calcium phosphate precipitates: an analysis of rinse time

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Abstract

The acid-etch technique is used extensively in dentistry. Variations in rinse time are found throughout the dental literature. Even though controversies concerning the appropriate rinse time exist, all investigators agree that copious water lavage is important.

This study compared different rinse times using both liquid and gel etchants labeled with ³²Phosphorus. Each etchant type was evaluated on the time necessary to remove the residual soluble and insoluble radiolabel from the human enamel surfaces. The radiolabeled etchants were removed in much shorter rinse times than is currently suggested by dental manufacturers and investigators.

The acid-etch technique is used extensively in dentistry to facilitate bonding to enamel. This procedure involves etching the outermost layer of enamel with phosphoric acid to form a porous retentive surface. Buonocore (1955) was the first to demonstrate the use of acid to etch enamel surfaces. Since then, controversies concerning the type and concentration of etchant, length of etch, and rinse time have developed. Until recently, the recommended etchant was 50% w/w (weight-to-weight) orthophosphoric acid buffered with 7% zinc oxide (Williams and von Fraunhofer 1977). Silverstone (1974) found that phosphoric acid concentrations of 30-40% with a 60-sec etch time produced the most retentive enamel surface topography. Most commonly used today is 37% phosphoric acid with an application time of 60 sec, followed by copious rinsing with water for 15-60 sec.

The effect of maintaining constant acid concentrations and varying both etch and rinse times is not well documented. Williams and von Fraunhofer (1977) studied bond strength in which the acid concentration was constant (62% w/w orthophosphoric acid) and the etch and rinse times varied from 10 to 60 sec. They demonstrated in short etch times that bond strength increased as rinse time increased. For long etch times, the bond

strength decreased as the rinse time increased. This research demonstrated that a 60-sec etch time with a 10-sec rinse time produced the best bond strength. Other reports based on clinical observations suggest rinse times ranging from 5 to 60 sec.¹

Even though controversies exist on the appropriate length of rinse time, all investigators agree that copious water lavage is necessary. Etching enamel with phosphoric acid results in deposition of calcium phosphates which must be removed to give optimal bonding (Williams and von Fraunhofer 1977; Soetopo and Hardwick 1978). Therefore, rinse time must be adequate for removal of both the etching medium and precipitated calcium phosphates. In pediatric dental patients, prolonged rinsing time may be impractical due to the increased likelihood of salivary contamination. The purpose of this study was to evaluate the efficacy of rinse time on removing residual etchant and calcium phosphate salts from the etched enamel surface.

Methods and Materials

Experimental Etch and Rinse Procedure

Sixty-four extracted permanent posterior teeth were used in this study. The teeth were polished with pumice, the roots removed with a separating disk, and the teeth paired based on morphology. Using an adhesive dot on a nonfissured, smooth surface, an area of 0.32 cm² was covered. The teeth were coated in melted baseplate wax and the dot was removed exposing a standardized area of enamel. To ensure that no adhesive material remained on the enamel surface, a cotton-tipped applicator dipped in acetone was used to dissolve any remaining adhesive. Each matched pair was assigned randomly to one of eight rinse times which ranged from 0 to 60 sec (Marks 1982). Each tooth pair then was assigned to receive an etching

¹Horn 1981; Jordan et al. 1981; Simonsen 1981.

treatment with either 37% gel^a or liquid^b etchant.

A modified vacuum filter apparatus^c was used to contain radioactivity associated with the etching procedure. The filter paper was removed from this apparatus and a hole placed in the center of the lid (Fig 1). A small piece of wax was placed on the exposed grating to stabilize the teeth during the etch and rinse procedures. A 1:500 dilution of ³²P-labeled orthophosphoric acid^d (specific activity 9120 Ci/mmol, concentration = 2.0 mCi/ml) was prepared in both liquid and gel etchants. Twenty-five µl of either gel or liquid etchant was placed on the prepared area of each tooth for 60 sec. Each tooth then was washed within the containment apparatus for the assigned rinse time using a standard dental air/water syringe. Both air and water controls were fully depressed during this rinse procedure.

Scintillation Counting

After rinsing, the teeth were placed in 5-ml scintillation vials containing 3 ml of scintillation fluid^e and vortexed once each hour for 4 hr. The control groups included triplicate scintillation vials containing: (1) scintillation fluid only; (2) 25 µl of 1:500 dilution of liquid etchant in scintillation fluid; and (3) 25 µl of 1:500 dilution of gel etchant in scintillation fluid. All samples were evaluated on a liquid scintillation counter^f using the ³²P channel to determine counts per minute (CPM).

Autoradiography

In order to determine the level of residual radioactivity on the tooth surface, a modified autoradiographic technique was employed. The etched surface of each tooth was placed in contact with the emulsified surface of standard dental radiographic film.^g Three unetched teeth were similarly placed to serve as negative controls. The teeth and film were wrapped individually in aluminum foil and incubated at -20° C for four days.

In addition, 1 µl of ³²P-labeled orthophosphoric acid was added to 2.5 ml of liquid etchant, making a 1:2500 dilution. From this initial dilution, the following were made: 1:5000, 1:10,000, 1:20,000, 1:25,000, and 1:30,000. Subsequently, 25 µl of each was placed on a 1 x 1.5-inch strip of glass microfiber filter paper.^h The filter paper was dried for approximately 3 hr, then placed in contact with the emulsified surface of the radiographic film. Film and

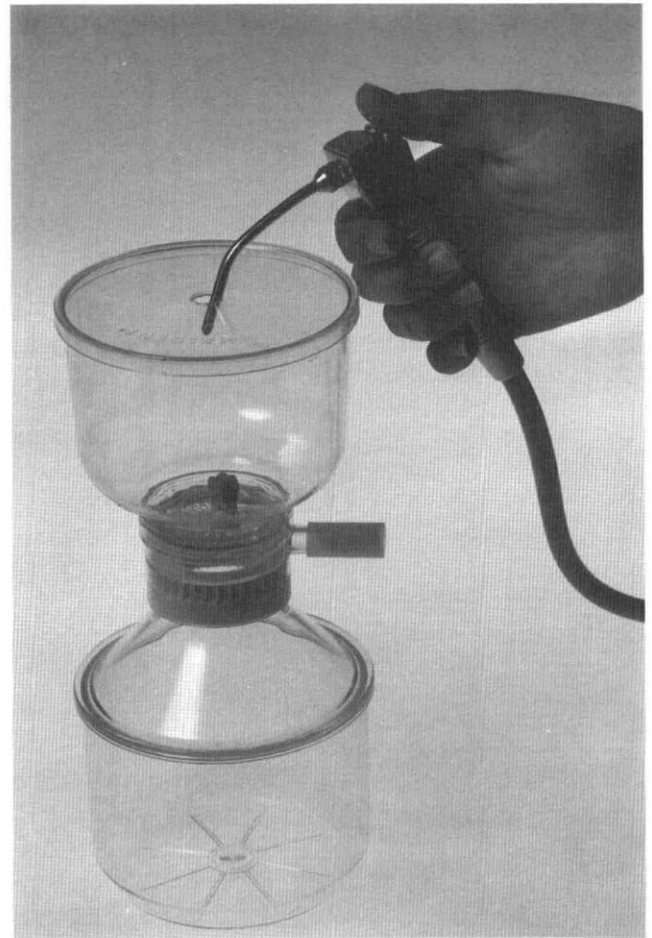


FIG. 1. The filter paper was removed from the apparatus and a hole placed in the center of the lid.

filter paper were wrapped in aluminum foil and stored at -20° C for four days. After the indicated storage time, the film was developed in a dental film developer.ⁱ Additionally, 25 µl from each of these dilutions was placed in a scintillation vial with 5 ml of scintillation fluid. The CPM associated with these samples was determined as before.

Data Evaluation

Statistically significant differences in residual radioactivity between the soluble and insoluble etchants were determined by using a paired Student's *t*-test. The Kruskal-Wallis test, a nonparametric statistic, was used to determine statistically significant differences in residual radioactivity between different rinse times for each etchant.

Results

The soluble ³²Phosphorus remaining after various times of air-water spray rinsing is shown in Table 1 (next page). Residual soluble radioactivity was reduced to

ⁱ Auveloper — SS White Dental Products Int'l; Philadelphia, PA.

^a Scotchguard Etching Gel-Concise Brand — Dental Products/3M; St. Paul, MN.

^b Enamel Bond System Etching Liquid — Dental Products/3M; St. Paul, MN.

^c Sterilization Filter Unit-500 ml — Nalge Co; Rochester, NY.

^d Orthophosphoric acid (³²P) in water — New England Nuclear; Boston, MA.

^e Aqueous Counting Scintillant — Amersham Corp; Arlington Heights, IL.

^f Beckman LS-230 — Beckman Instruments Inc; Palo Alto, CA.

^g Ultra-Speed DF75 Safety 2 Film — Eastman Kodak; Atlanta, GA.

^h Glass Microfiber Filters 934-AH — Whatman Inc; Clifton, NJ.

background levels after 5 sec of rinsing in both the liquid and gel etchant groups. Significant residual radioactivity in both liquid and gel groups was present only after 2.5 sec of air-water spray rinsing as determined by a Student's *t*-test ($P < 0.05$).

Table 2 shows the sensitivity of standard Kodak® dental radiographic film when exposed to known levels of ^{32}P Phosphorus. Approximately 500 CPM of radioactivity could be detected by this modified autoradiographic technique. This level was judged to be sufficiently sensitive to evaluate residual ^{32}P Phosphorus after the etch-rinse procedure. The levels of residual insoluble precipitate with both liquid and gel rinses are summarized in Table 3. All detectable precipitable radioactivity was removed from the liquid etchant group after 7.5 sec of air-water spray rinsing (Fig 2). Alternatively, all detectable precipitated radioactivity was removed from the gel etchant group after 30 sec of air-water spray rinsing (Fig 3). The distributions of residual radioactive precipitate were judged to be significantly different between the liquid and the gel etchant groups as determined by the Kruskal-Wallis nonparametric analysis ($P < 0.05$).

Discussion

The findings demonstrated that soluble radioactivity associated with acid-etching agents was removed rapidly by air-water spray rinsing. These findings were verified for both liquid and gel etchant groups (Table 1). Statistically significant levels of radioactivity were not present in rinse times greater than 2.5 sec. This indicated that removal of soluble, unbound etchant materials from smooth enamel surfaces was accomplished easily with minimal air-water spray rinsing.

Insoluble or physically bound radiolabeled ^{32}P required longer rinse periods for complete removal in both the liquid and the gel etchant groups (Table 3). Previous studies (Williams and von Fraunhofer 1977; Soetopo and Hardwick 1978) have shown that calcium phosphate precipitates are formed during the acid-etching procedure. The authors interpret the residual radioactivity that was present after air-water rinsing and absorption in scintillation fluid to be a calcium phosphate precipitate formed during the etching procedure. This precipitate should be radioactive since the radiolabel would form a phosphate precipitate with calcium liberated from the etched enamel. The presence of these radioactive precipitates was confirmed in the autoradiographic evaluation of the etched enamel. It is important to remove this precipitate in order to gain optimal resin penetration and bond strength (Williams and von Fraunhofer 1977; Soetopo and Hardwick 1978).

Statistically significant differences between the air-water spray removal of the insoluble precipitates were seen when gel and liquid etchants were compared (Figs 2, 3). These data indicated that an optimal rinse time for

TABLE 1. Residual Soluble ^{32}P Phosphorus After Rinse Procedure

Rinse Time (seconds)	Liquid			Gel		
	Mean (CPM)	SD	(N)	Mean (CPM)	SD	(N)
0.0	75,084*	2378	3	56,223*	1867	3
2.5	57*	20	4	74*	36	4
5.0	26	6	4	28	14	4
7.5	17	3	4	22	3	4
10	25	4	4	20	2	3
15	26	4	4	22	3	4
30	24	7	4	26	8	4
60	19	4	4	20	4	4

Background = 22 cpm \pm 4

* Significantly different from background levels as determined by Student's *t* test ($P < 0.01$).

37% v/v (volume-to-volume) liquid etchants was ~ 15 sec, and optimal rinse time for gel etchants of the same concentration was ~ 30 sec. This difference may be associated with reduced diffusion of calcium phosphate salts from the enamel-etchant interface in the gel etchant. This phenomenon may be secondary to the reduced diffusion characteristics of the gel matrix.

The use of this novel radiolabel technique yielded specific data that permitted quantitation of residual etchant and demonstrated the presence of surface calcium phosphate precipitates. The differentiation of soluble and insoluble radiolabel in these experiments was difficult. The procedure utilized probably reflected freely soluble and poorly soluble radiolabel. The method used for quantitation of calcium phosphate precipitate was sufficient to detect approximately 500 CPM of ^{32}P . Since low levels of precipitates potentially could interfere with resin penetration, a more sensitive technique may need to be developed. Future studies should compare the removal of radiolabel to changes in resin-to-enamel bond

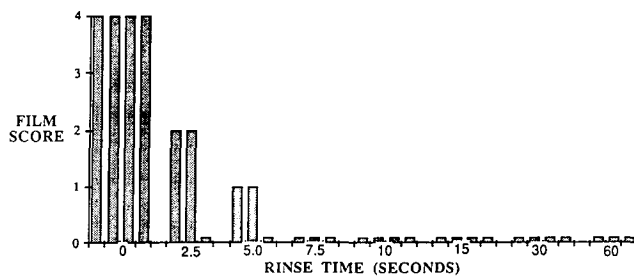


FIG. 2. Insoluble precipitate following liquid etching procedure.

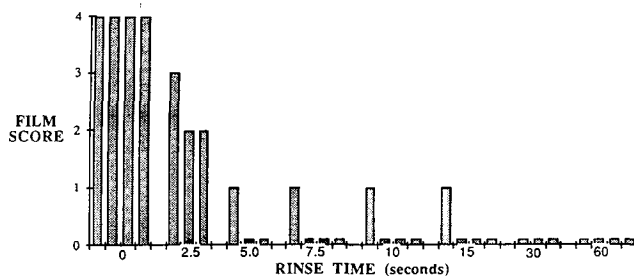


FIG. 3. Insoluble precipitate following gel etching procedure.

TABLE 2. Standardization of ³²Phosphoric Acid Autoradiographic Technique

Dilution of ³² P (10 ⁻⁴)	Counts/min	Film Score (4 day exposure)
4.0	4575	4 (2-3 mm dark halo)
2.0	2043	3 (1-2 mm light halo)
1.0	976	2 (dark central development)
0.5	537	1 (light central development)
0.33	306	0 (no activity)
Control	53	0 (no activity)

strength. In addition, studies should be undertaken to evaluate removal of radiolabel from pit and fissure surfaces.

A clinically successful acid-etch procedure is dependent on a number of factors such as a desirable etch pattern and adequate resin penetration. Of equal importance in the child patient is prevention of contamination of etched enamel surfaces before placement of resin. This is especially true in respect to placement of pit and fissure sealants. By reducing rinse times to the shortest period that effectively removes calcium phosphate precipitates, one may be able to achieve greater clinical success by reducing the incidence of salivary contamination.

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TABLE 3. Residual Radioactive Precipitate After Rinse Procedure*

Rinse Time (seconds)	Film Score					
	Liquid			Gel		
	Mean	SD	(N)	Mean	SD	(N)
0.0	4.0	0.0	4	4.0	0.0	4
2.5	1.33	1.16	3	2.33	0.58	3
5.0	0.5	0.58	3	0.33	0.58	3
7.5	0.0	0.0	3	0.25	0.5	4
10	0.0	0.0	4	0.25	0.5	4
15	0.0	0.0	4	0.33	0.58	4
30	0.0	0.0	4	0.0	0.0	3
60	0.0	0.0	3	0.0	0.0	4

* The level of residual radioactivity was significantly greater in the Gel group as determined by Kruskal-Wallis analysis ($P < 0.05$).

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Xylitol benefits

Xylitol consumption can significantly reduce dental caries formation in children, according to two long-term studies conducted in Finland.

Children in the studies chewed gum containing Xylitol two to three times per day while maintaining their existing caries prevention programs, based on fluoridated water and fluoride toothpaste. One study showed a 30-60% reduction in new dental caries among children consuming seven to 10 g of Xylitol per day in gum. In the other study involving 11- to 12-year-old children at high risk for caries development, the Xylitol gum chewers developed 50-80% fewer cavities than the comparable control group of children using no gum.

Xylitol, a naturally occurring sugar alcohol, is found in many fruits, vegetables, and plants. It is as sweet as sucrose (table sugar) and has no artificial aftertaste. Because it dissolves very quickly in the mouth, rapidly absorbing heat, Xylitol produces a unique "cooling" sensation. The product is currently used in many countries in foods, pharmaceuticals, and oral health products.