Effect of different acid etchants on the remineralization process of white-spot lesions: An in vitro study.

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Abstract: Purpose: To investigate the effect of acid etchants with different low concentrations on remineralization of white spot lesion (WSL). **Methods:** WSL were prepared on buccal surfaces of 100 intact premolars using the methyl cellulose gel/lactic acid method. The samples were then placed in a remineralizing solution in addition to fluoride application twice daily for 5 minutes. The changes were quantified weekly using the Quantitative Light-induced Fluorescence (QLF) system. When changes in fluorescence radiance approached zero, each sample was etched with one of the following acids; 5% phosphoric acid, 10% phosphoric acid, 5% polyacrylic acid or 10% polyacrylic acid for 15 seconds, washed, dried, and placed again in the remineralizing solution. Two samples were randomly selected from each group for transverse microradiography (TMR) and scanning electron microscopy (SEM) analysis. **Results:** The 10% polyacrylic acid group showed the most significant improvement in fluorescence gain over the second phase of remineralization. It also showed partial loss of surface minerals without affecting enamel thickness as the phosphoric acid did. Additionally, 10% polyacrylic acid created the largest number of pores and smallest in size when compared to phosphoric acid, thus enhancing remineralization more efficiently than phosphoric acid without compromising the enamel outermost layer. (*Am J Dent* 2020;33:43-47).

Clinical significance: The findings of this study may improve the remineralization of WSL from the bottom of the lesion instead of precipitation on the outermost layer of the lesion leaving a better quality of enamel. 10% polyacrylic acid enhanced remineralization more efficiently than phosphoric acid without compromising the enamel outermost layer.

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Introduction

Decalcification of enamel surface and development of white spot lesions (WSL) in adolescents is a prevalent problem especially in those using orthodontic appliances and showing poor compliance with oral hygiene instructions.[1,2] If left untreated, the decalcification may progress to produce carious cavitation, and present an esthetic problem.[3] Thus, prevention, diagnosis and treatment are of paramount importance to reach satisfactory esthetic results at the end of orthodontic treatment.[4]

Several methods have been suggested to prevent and reduce the incidence of white spot lesions (WSL) in general, and in orthodontic patients in particular, such as fluoride application in its different forms.[1,4,5] The application of casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) was reported as effective in the prevention of WSLs.[6-10] Additionally, consumption of probiotics can cause a significant decrease in the *S mutans* levels in plaque, thus helping to prevent WSL formation.[11] Chewing sorbitol or sorbitol/xylitol sweetened gum has also been reported to have a similar effect.[12] The effect of these products, however, is controversial as reported in some randomized clinical trials.[13,14]

Despite all of the attempts to remineralize WSLs, only partial remineralization had occurred in most of the studies.[3] One of the reasons advocated for incomplete remineralization of the body of the lesion is the deposition of minerals on the surface layer, which decreases porosity and prevents further minerals from reaching deeper lesion areas.[15-17]

One of the proposed methods to enhance remineralization is acid etching of the enamel surface layer; acid etching would remove the fluoride rich layer and expose more reactive crystals.[18,19] A more pronounced reduction in lesion depth after remineralization in acid etched WSL was reported in several studies.[16,17,20] The effect of acid etching with high acid concentration (phosphoric acid ~38%), applied at a delayed stage of remineralization to trigger the process after it reached a plateau, was investigated.[21] In that study, the use of high concentration of phosphoric acid caused excessive loss of minerals from the enamel surface.

Accordingly, the rationale for conducting this in vitro study was to investigate different acid etchant types and concentrations on the remineralization process after WSLs were exposed to a remineralizing regimen and reached a plateau, without adversely affecting the enamel structure. Changes in mineral content were assessed using quantitative light induced fluorescence (QLF). The null hypothesis was that there is no difference between the effect of different acid etchants on the remineralization process of WSL.

Materials and Methods

This was an in vitro study that consisted of a demineralization phase and two remineralization phases; before and after the acid etching procedure. QLF was used for the quantification of remineralization rate during the study.

Sample selection - A total of 100 premolar teeth with sound buccal enamel surfaces were selected from a pool of 180 teeth extracted for orthodontic reasons. After polishing with a non-fluoridated prophylaxis paste using a bristle brush, the teeth were examined using a magnifying lens; all teeth with defects, hypomineralization, hypoplasia or cracks were excluded. The teeth were stored in deionized water with azide to prevent overgrowth of microorganisms. Sample size was determined

to be at least 22 samples (per group) to yield a power of 85% in detecting 3% difference change in lesion fluorescence (ΔF). Significance level was set at $P \leq 0.05$.

The selected teeth were cut perpendicularly from mesial to distal surface using a fissure diamond bur on a high speed handpiece with water cooling. A diamond disc was used to flatten the fitting surfaces of the teeth. The teeth were used to prepare 100 slabs of healthy buccal enamel, which were then fixed onto 13 mm diameter black plastic sample holders using cyanoacrylate adhesive (Superglue[a]), numbered from 1 to 100 (engraved using a small round bur on high speed handpiece on the back of each holder).

Artificial WSL preparation - The enamel surface was coated with acid-proof nail varnish (Revlon[b] nail enamel) leaving a window of ~ 3 × 3 mm^2. Subsurface artificial lesions were produced using the lactic acid-methyl cellulose gel method[16] (protocol # CEP002, Department of Cariology, Endodontology and Paedodontology, ACTA, Amsterdam, The Netherlands) with a pH of 4.6 maintained using a WTW 3401 pH meter,[c] with exposure time of 10 days at 37°C.

Remineralizing regimens - During Phase I of remineralization, samples were kept in a glass tray filled with remineralizing solution. The remineralizing solution contained 1.5 mM $CaCl_2$, 0.9 mM KH_2PO_4, 130 mM KCl and 20 mM HEPES at PH 7.0. The glass tray was placed in a water bath at a temperature of 37°C which was monitored using a thermostat inside the lab incubator. Fluoride slurry (30% wt of 1,000 ppm fluoride from NaF dentifrice, Colgate Maximum Fresh 1,000 ppm), was applied for 5 minutes twice daily. The remineralizing solution was refreshed on weekly basis.

The fluorescence radiance was measured once a week on all the samples. When the fluorescence change in the remineralizing WSL approached zero change (after 6 weeks of remineralization), the samples were randomly divided into four groups by a research assistant to ensure blindness of the investigator, marking the beginning of Phase II. The samples were then acid etched with one of the following acids for 15 seconds, then washed for 10 seconds and dried for 10 seconds:

Group 1: surfaces etched with 10% phosphoric acid.
Group 2: surfaces etched with 5% phosphoric acid.
Group 3: surfaces etched with 10% polyacrylic acid.
Group 4: surfaces etched with 5% polyacrylic acid.

QLF images were captured before and after etching of WSL (W6-A and W6-B) and once a week for all specimens throughout the second phase of the study.

The remineralization protocol during this phase was similar to the protocol followed during Phase I.

Quantitative light-induced fluorescence measurements - To quantify mineral loss after demineralization, enamel fluorescence was measured by QLF. Each enamel block was removed from the solution, blotted with tissue paper and left to dry for 2 minutes before the QLF images were captured using the QLF system which was comprised of a digital single lens reflex camera (model EOS 550D[e]) fitted with a 60 mm macro lens (model EF-S, f/2.8 USM macro lens) and a biluminator tube (112 mm length and 70 mm diameter). This tube covered the sample completely excluding other light, and ensured a constant distance between the sample and the lens. Enamel was illuminated with white light emerging from an arc lamp based on xenon technology and violet-blue illumination for fluorescence excitation was from 12 high performance light emitting diodes (LED) with a wavelength peak: 405 nm, full width at half maximum (FWHM): 15 nm. To enable detection of enamel auto-fluorescence, a yellow high-pass filter was used to exclude light with wavelengths < 500 nm. The combination of the lamp and filters was optimized in such a way as to maximize the fluorescence and minimize reflection. Images were then captured with the camera and stored into a computer to be analyzed.

The QLF images of the samples were analyzed using special software (QA2[f] version 1.18) provided with the QLF system. For this purpose, a line tracing was drawn surrounding the lesion site with its borders on sound enamel. Inside this tracing, the fluorescence levels of sound tissues were reconstructed using the fluorescence radiance of surrounding sound enamel. Subsequently, the difference between the reconstructed and original fluorescence level was calculated as a percentage.

Transverse microradiography (TMR) - Four samples, randomly selected (one sample from each group) after the end of Phase I (after etching), and one non-etched sample were sent for TMR. Two sections (approximately 500 μm thick each) were cut from each tooth sample. They were lapped to a final thickness of approximately 100 μm. The sections were radiographed (with Cu K-alpha radiation, using a Ni-filter, at 20 KV and 20 mA) and analyzed by TMR2006.[f]

Scanning electron microscopy (SEM) - Four randomly selected samples (one sample from each group) after the end of Phase I (after etching) were sent for scanning electron microscopy (SEM) study. The specimens were dried under high pressure air flow to ensure complete dryness of the samples for imaging. They were then mounted on stubs using a vacuum resistant adhesive. The specimens were mounted in such a way that the area to be studied faced upwards. Each stub contained one specimen. The stubs were then placed on a tray which was later placed in the vacuum chamber of the SEM. The surface was scanned and observed on the screen at 50×, 500×, 1,000×, 2,500×, 5,000× and 20,000× magnifications. The number of lesions produced by each of the acid etchants per 100 μm^2 was calculated on each specimen at 1,000× magnification. The size of the lesion was measured at 5000× magnification, and the depth was descriptively analyzed at 20,000× magnification.

Error of the method - Twenty samples were randomly selected from all groups and re-analyzed after a 20-day interval from the initial analysis using QLF to determine the measurement error which was calculated for the study. The error of method was calculated using Dahlberg's double determination formula.[22] Houston coefficient of reliability[23] was also calculated and was above 90%. The value of the Dahlberg error was 0.89% for ΔF.

Statistical analysis - Means and standard deviations of ΔF at each time point were calculated using the SPSS[g] version 20. Repeated measures ANOVA was used to compare between the weekly changes in each group and between the groups. Bonferroni test was used to identify the significant differences

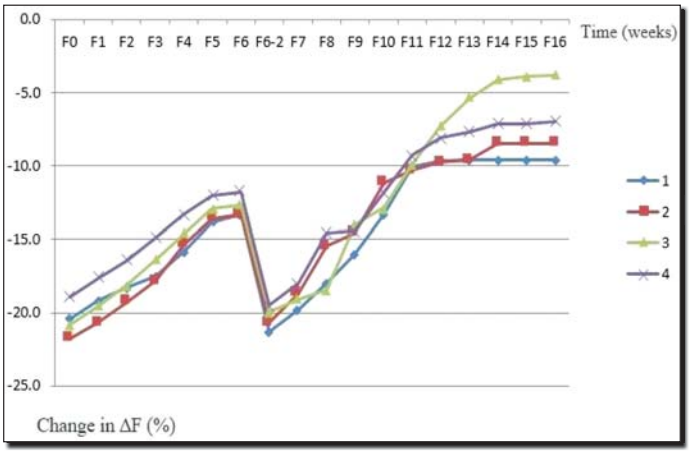

Fig. 1. Change in fluorescence radiance during Phases I and II in the different groups.

Table. Mean ± SD of fluorescence radiance loss (ΔF) at base line, before and after acid etching, end of study and the differences with their significance.

	Mean ΔF + SD				Mean difference	
Group	Week 0 Baseline	Week 6A Pre-etch	Week 6B Post-etch	Week 16 End of study	Week 16- Week 0	Week 16- Week 6B
1	-20.4± 4.1	-13.3± 3.0	-21.3± 5.5	-9.6± 2.2	10.8*	11.7*
2	-21.8± 4.7	-13.3± 4.0	-20.7± 4.7	-8.5± 4.9	13.3*	12.2*
3	-20.8± 4.9	-12.7± 3.3	-19.9± 4.7	-3.8± 0.8	17*	16.1*
4	-18.9± 4.6	-11.8± 3.0	-19.5± 8.1	-7.0± 3.1	11.9*	12.5*

* P value < 0.001.

between the follow up measurements. Normality test for the data sets was performed. All data was under Gaussian normal distribution curve. The results were considered to be significant at P≤ 0.05.

Results

After Phase I, there was a significant regain in fluorescence radiance at the end of Phase I of remineralization (week 6) compared to the baseline (P< 0.001).

Almost a steady rate of fluorescence gain was observed over time. When minimal changes in the fluorescence radiance were reached, between weeks 5 and 6, Phase II of remineralization (after etching of enamel surface) was started as seen in Fig. 1.

Following the application of the acid etchants, enamel samples exhibited fluorescence radiance loss in all the experimental groups when compared to the pre-etching images (P< 0.001) as seen in the Table. All the acid etchants showed a similar effect on fluorescence radiance loss in the different groups.

During Phase II, all the groups showed steady increase in fluorescence radiance (Fig. 1) with a significant difference (P< 0.001) between week 6-B (post-etching) and week 16 (end of the study). Groups 2 (5% phosphoric acid) and 3 (10% polyacrylic acid) showed more fluorescence gain than the other groups (P< 0.05).

TMR results - Figure 2a shows a typical subsurface lesion with intact smooth non-etched WSL with a band of radiolucency beneath the enamel.

Figure 2b shows one of the samples after the application of 10% phosphoric acid. There was loss of the most superficial part of the highly mineralized enamel surface leaving a thin band of enamel profile.

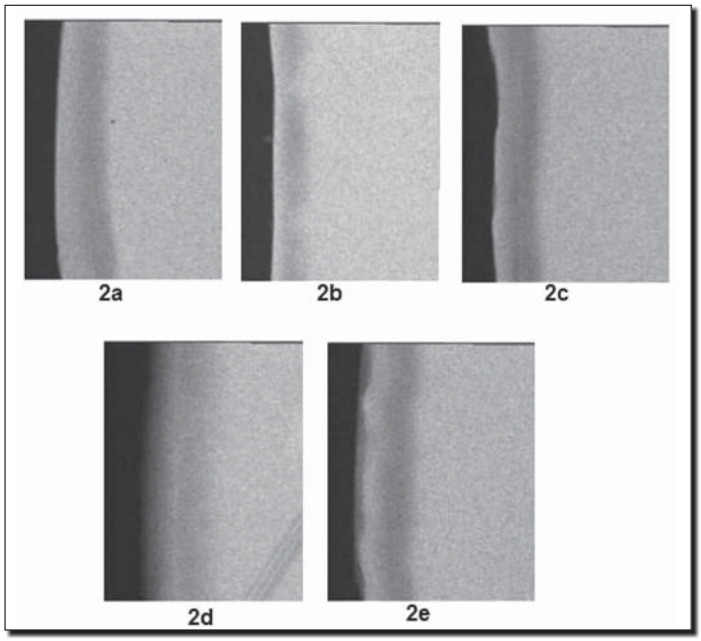

Fig. 2. TMR scans of samples from the different groups.

Figure 2c shows a sample after the application of 5% phosphoric acid. A similar pattern of enamel loss had occurred as in 10% phosphoric acid; the thickness of lost enamel, however, was less than that in the 10% phosphoric acid group.

Figure 2d shows a sample after the application of 10% polyacrylic acid. The scans show that the convexity and thickness of the enamel outermost was maintained with less mineral content in this layer and more radiolucency of the enamel at the application site.

Figure 2e shows a sample after the application of 5% polyacrylic acid. The scans show a similar effect of etching as in the sample treated by 10% polyacrylic acid, but the radiolucent change in enamel surface did not involve the full thickness of the enamel. More than half of the enamel thickness was left intact.

Scanning electron microscope results - As for the SEM results, Fig. 3 shows enamel samples from all the study groups at different magnifications (1,000×, 5,000x, 20,000×). The sample which was treated by 10% phosphoric acid showed porous enamel with lesions created by the acid covering most of the surface of the WSL. The surface showed little mineral precipitation since the acid etching removed most of the precipitants and part of the enamel. The number of lesions created by the acid was ~18/100 μm^2, lesion area was 244 μm^2. The acid left deep penetrating pores in the enamel layer.

The samples treated by 5% phosphoric acid showed less porous enamel than the 10% phosphoric acid ones with a reduced amount of mineral precipitations. The number of lesions created by the acid was ~16/100 μm^2, lesion area was 50 μm^2. The lesions were similar to those in the 10% phosphoric acid, with less depth of penetration.

The 10% polyacrylic acid 10% application created a larger

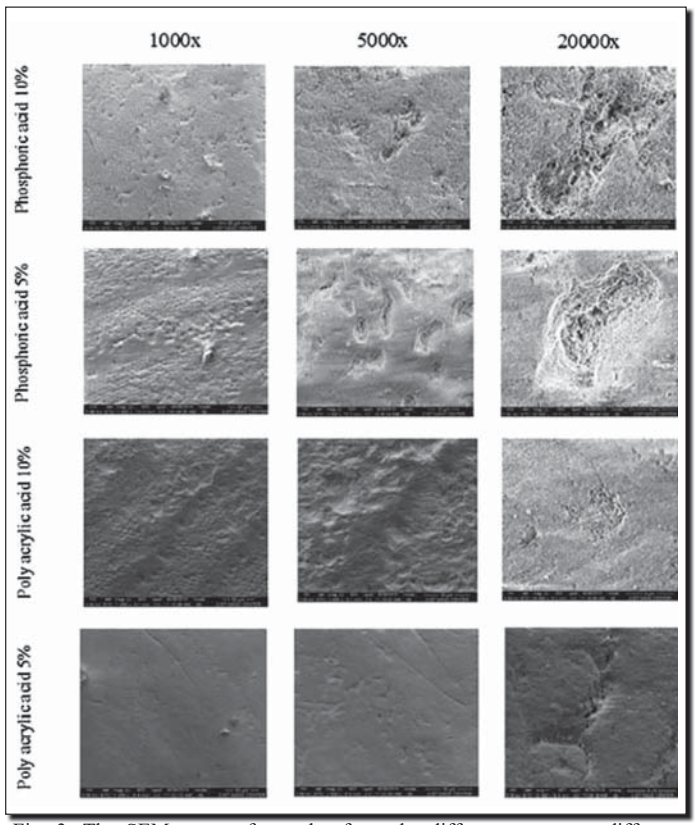

Fig. 3. The SEM scans of samples from the different groups at different magnifications

number of lesions on the enamel surface when compared to the phosphoric acid; the number of lesions was about 32/100 μm^2. The lesion area was ~54 μm^2. In comparison to phosphoric acid etchant, 10% polyacrylic acid showed less depth of penetration in the enamel surface.

The 5% polyacrylic acid group showed less effect on the enamel surface where the appearance of the etched porous surface was not as evident when compared to the other acid etched surfaces. The number of lesions created by this acid was ~11/100 μm^2 and the size of the lesion was ~19 μm^2. It showed minimal depth of penetration into the enamel surface.

Discussion

Natural remineralization through saliva involving mineral gain in the surface layer of WSL has little improvement on esthetics and structural properties of deeper lesions.[24,25] Therefore, remineralizing agents are needed to repair the deeper parts of WSL for better esthetic results.[25] The current study investigated the ability of different acid etchants with different concentrations to re-open the pores in the outermost layer of enamel after an initial stage of remineralization where the enamel surface had been blocked and no further remineralization could be reached.

During Phase I of remineralization, remineralizing solution similar in composition to that of saliva was used. The samples were kept at a temperature similar to that of a healthy individual. In addition to the saliva substitute, fluoride dentifrice was applied twice daily to all the samples to mimic the recommended hygiene measure practiced by the average individual. The combination of the remineralizing solution and fluoride dentifrice should cause remineralization of WSLs. The remineralization process, however, might be adversely affected when high calcium and phosphate concentration (5 Mm) is used;[26] high levels of calcium and phosphate may lead to rapid precipitation of minerals on the enamel surface.[27,28] These mineral precipitations, in addition to the presence of fluoride, may occlude the pores that would allow access to the demineralized subsurface, limiting the remineralization process.[27,29,30]

Although the fluorescence loss was similar in all the groups due to the different acid etchants indicating a similar loss of mineral amount from the enamel surface, the pattern of mineral loss was different in the different groups. The phosphoric acid caused a complete loss of the whole outermost layer of the enamel. The effect of the 10% phosphoric acid concentration, however, was more pronounced; it removed the whole outermost layer, while the 5% phosphoric acid removed some enamel as patches on the enamel surface rather than the whole surface. The remaining outermost layer of the enamel seemed to be highly mineralized as seen in the mineral profiles of TMR. Polyacrylic acid, on the other hand, caused loss of minerals from within the outermost layer of enamel. This layer kept its thickness but with less mineral content.

The 10% polyacrylic acid penetrated the whole thickness of this layer reaching to the body of the lesion, while the 5% penetrated it partially without reaching to the end of the thickness of this layer. To our knowledge, no previous studies investigated the pattern of mineral loss of enamel with different acid etchants from the surface of enamel to compare our results with.

Additionally, 10% phosphoric acid caused the formation of larger lesions on the remaining part of the outermost surface layer of the WSL when compared to the 5% phosphoric acid and the polyacrylic acid groups. The size of the lesions was almost five times larger than those produced by the other acid groups. The 10% polyacrylic acid group showed similar sized lesions to those produced by the 5% phosphoric acid. The number of these lesions, however, was almost double that of the 5% phosphoric acid. Five percent polyacrylic acid showed the least number and size of lesions on the enamel surface with the least depth of penetration in all the groups.

The pattern of mineral loss from the outermost surface layer of the WSL due to the different acids affected the amount of remineralization in Phase II of the study. The 10% polyacrylic acid group showed the most fluorescence gain indicating that the best remineralization occurred in that group. It was significantly better than 10% phosphoric acid and 5% polyacrylic acid. The difference from Group 2, however, did not reach a significant level.

Al-Khateeb et al[21] investigated the effect of 40% phosphoric acid on the remineralization process. In that study, the etched samples exhibited better remineralization than the non-etched ones, however, the difference did not reach a significant level. The proposed explanation for the lack of difference was that minerals gained in the etched groups were consumed to replace those lost by the etching procedure. The high concentration of phosphoric acid caused excessive loss of surface minerals.[30]

Hidaka et al[31] investigated lower concentrations of 2% and 10% phosphoric acid, respectively and 10% polyacrylic acid.

They reported better effect with 10% polyacrylic acid and more mineral loss with 10% phosphoric acid. The 2% concentration had little effect on the remineralization process.

Incomplete remineralization occurred despite the etching regimen used. Further investigations are needed to reach to full recovery of the WSLs in regard to their mineral content and esthetics.

In conclusion, the null hypothesis was rejected; treatment of WSLs was improved by the use of 10% polyacrylic acid etchant after it reached a plateau stage, the use of the other types of acid etchants seemed to enhance the remineralization, but to a lesser degree. Phosphoric acid etchant showed an unnecessarily aggressive etching behavior when applied on the WSL. A recommendation for further studies is to investigate if the addition of other remineralizing agents with the 10% polyacrylic acid etchant would enhance the remineralization process.

a. UHU, GmbH & Co KG, Germany.
b. Revlon, New York, NY, USA.
c. Wissenschaftlich-Technische Werkstätten GmbH & Co. KG, Weilheim, Germany.
d. Colgate-Palmolive, Bangkok, Thailand.
e. Canon Inc., Tokyo, Japan.
f. Inspektor Research B.V., Amsterdam, The Netherlands.
g. SPSS, Chicago, III, USA.

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