Effect of acid-etching on remineralization of enamel white spot lesions

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This in vitro study aimed at investigating whether full remineralization would occur in white spot lesions when the surface porosity was increased by acid-etching. The effect of fluoride was also investigated. Enamel blocks with in vitro produced white spot lesions were used. Group A was exposed to a remineralizing solution only. In group B, the lesions were etched with 35% phosphoric acid for 30 s, then treated as in group A. Group C was treated as group A + daily treatment with a fluoride toothpaste slurry (1000 ppm) for 5 min. Group D was treated as group B + the daily fluoride treatment of group C. The remineralization was measured weekly with Quantitative Light-induced Fluorescence during the experimental period. After 10 weeks of remineralization, mineral profiles were assessed with transverse microradiography. The enamel fluorescence was partly regained. There were significant differences in the lesion depth, mineral content at the surface layer, and integrated mineral loss between the groups. Addition of fluoride accelerated the remineralization only in the beginning; in later stages the process leveled out and even reached a plateau in all the groups. It was concluded that full remineralization was not achieved by etching, by the addition of fluoride, nor by the combination of both treatments in this in vitro study. □ *Fluorescence; fluoride; microradiography*

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Since the introduction of fluoride, arrested white spot lesions are more common (1). It has been documented that one of the fluoride effects on white spot lesions is that preferential deposition of minerals is caused in the surface layer of the enamel, resulting in arrestment of these lesions (2, 3). From a prophylactic point of view, it is of benefit to reach the stage of arrested lesion with a highly mineralized surface zone, since this will prevent lesion progression. These arrested lesions will persist lifelong, exhibiting a white color, as in white spot lesions; or they might become yellowish or dark brown in color because of exogenous uptake of stains (4). When considering esthetics, the presence of these discolored lesions might not be acceptable. In orthodontic patients in particular, discoloration of lesions in the labial surfaces of incisors is a problem, as most of these patients seek orthodontic treatment for esthetic reasons.

Etching of sound enamel causes the removal of the fluoride-rich outer layer, exposing crystals that are more reactive towards de- and remineralization processes (5–7). Furthermore, it increases the surface area of the enamel and produces a certain degree of subsurface porosity. Etching may also affect the porosity and permeability of caries lesions (8, 9). The effect of acid etching is essentially confined to a thin outermost layer of enamel, irrespective of the porosity of the underlying tissue and does not cause mineral loss from the lesion body (10). On the other hand, structural alterations associated with acid application have been observed by confocal laser scanning microscopy to occur up to 100 μm below the surface in sound enamel (11). Therefore, etching of enamel caries lesions has been suggested to enhance remineralization of the incipient lesions (12, 13).

Given the phenomenon of surface layer deposition, leading to incomplete remineralization, the hypothesis of the current study was that etching would result in a more effective remineralization. In addition, the effect of fluoride was studied to see if fluoride-induced enhancement of remineralization, also in the case of an etched surface layer, would lead to incomplete remineralization.

The aim of this in vitro study was therefore to investigate the longitudinal effect of acid etching on the remineralization rate of white spot lesions, and to ascertain whether complete remineralization would occur. The role of fluoride in changing the characteristics of the surface layer, in particular whether it inhibits complete remineralization, was also investigated. The remineralization was measured at various time points by a quantitative light-induced fluorescence method (QLF). Additionally, the mineral profile of the remineralized tissue was analyzed by transversal microradiography (TMR).

Materials and methods

Lesion formation

From sound premolar teeth, extracted for orthodontic reasons from young teenagers, 74 enamel blocks were cut from the buccal side, one from each tooth and fixed to

plastic holders. The natural surface of the enamel was kept unpolished. The tooth-blocks were covered with acid-resistant nail varnish, leaving a window of $3 \times 3\ mm^2$. Subsurface lesions were produced in methylcellulose gel covered with a solution of lactic acid buffer (0.1 M, pH 4.6, 37°C). After 14 days, the enamel blocks were removed from the gel, and the layer of nail varnish was removed from the tooth surface, which was then cleaned with acetone on a piece of gauze to remove any remnants. The teeth were also washed with water jet from a three-in-one syringe to remove any gel remnants from the lesion surface.

Baseline lesion determination and effect of acid etching

Lesion depth, integrated mineral loss, the mineral volume percentage of the surface layer of the produced lesions, and the amounts of mineral lost due to the acid etch procedure were determined after the lesion formation. For this purpose, 14 of the enamel blocks were used. On each of these blocks, part of the lesion and the surrounding sound enamel were protected with nail varnish. The exposed parts of the lesions were treated with 35% phosphoric acid for 30 s, and then washed with a water jet for another 30 s as done in clinical practice. The enamel blocks were then sectioned and examined with transverse microradiography (TMR). The sections were obtained from the middle of the tooth blocks, including the etched and non-etched parts of the lesion in each section.

Group assignment

The remaining enamel blocks ($n = 60$) were used for the remineralization experiments. The blocks were divided into four groups: A, B, C, and D. Two of the groups (B and D) were etched according to the procedure described above; groups A and C were left without etching. The four groups were then exposed to remineralizing solution containing 1.5 mM $CaCl_2$, 0.9 mM KH_2PO_4, 130 mM KCl, and 20 mM HEPES at pH 7.0 and 37°C. The enamel blocks in groups C and D were exposed to a daily fluoride treatment (30% wt of 1000 ppm fluoride from a NaF dentifrice slurry) for 5 min (Table 1). The tooth blocks were randomly assigned to the different experimental groups and the remineralization process was carried on for 10 weeks, 24 h per day.

Table 1. Experimental group assignment

Group	Treatment
A	Remin. solution
B	Etching + remin. solution
C	Remin. solution + daily F treatment
D	Etching + remin. solution + daily F treatment

Quantitative light-induced fluorescence measurements

To quantify mineral changes, enamel fluorescence was measured by the QLF method (14) for the sound enamel, after lesion formation, and once a week during the remineralization period. Each enamel block was removed from the solution, blotted with tissue paper, and left to dry for 2 min before the fluorescence measurements were conducted. With this method, the enamel surface is illuminated with white light emerging from an arch lamp based on Xenon technology. A 370-nm filter (full width half measure of 80 nm) was used to produce blue light. Images of the enamel samples were captured with a color CCD micro-video camera (Panasonic WV-KS 152) connected to a computer. To enable detection of enamel autofluorescence, a yellow high pass filter (Hoya Y-50) was used to exclude light with wavelengths < 500 nm. The combination of the lamp and the filters was optimized in such a way as to maximize the fluorescence and minimize reflection (14).

The fluorescence loss (ΔL) in the lesion was calculated according to the method described by de Josselin de Jong et al. (15), where the mean enamel fluorescence at the lesion site was calculated as a percentage of the surrounding sound values (ΔL_{mean} in %).

Transverse microradiographic measurements

At the end of the 10th week, 8 enamel blocks from each group were sectioned for TMR analysis. Two 100-μm sections were obtained from each block and placed on a perspex holder containing an aluminum stepwedge of 12 steps (16). In each of these steps, the thickness increased by 25 μm. The enamel sections and the stepwedge were radiographed on a high-resolution photographic plate (type 1A, Kodak) with Ni-filtered Cu-Kα radiation at 20 mA and 20 kV for 10 min. The photographic plate was placed at 60 cm distance from the X-ray source to ensure parallel X-rays and to minimize blurring of the image.

Table 2. QLF data of the four groups as a percentage of the baseline fluorescence loss

Group	After 1 week of remin. (mean ± SD)	After 4 weeks of remin. (mean ± SD)	After 10 weeks of remin. (mean ± SD)
A *(no etch, no F)*	2.6 ± 7.4	9.5 ± 9.7	14.7 ± 8.1
B *(etch, no F)*	10.2 ± 7.2	22.6 ± 9.8	26.9 ± 13.0
C *(no etch, F)*	13.3 ± 10.2	22.6 ± 15.9	26.7 ± 19.1
D *(etch, F)*	20.9 ± 13.5	27.9 ± 14.6	22.3 ± 13.4

Table 3. TMR data for the etched and non-etched baseline groups

Baseline	IML (vol% · μm) (mean ± SD)	LD (μm) (mean ± SD)	SL (vol%) (mean ± SD)
Non-etched *baseline for groups A & C*	5625 ± 1310	152 ± 17	67 ± 8
Etched *baseline for groups B & D*	6336 ± 1573	159 ± 20	58 ± 7

IML = integrated mineral loss. LD = lesion depth. SL = mineral content of the surface layer of the enamel.

The microradiograms were scanned with a CCD video camera connected to a microscope with a computer-operated XY table. Mineral content depth profiles were measured with software for TMR (TMR 1.24; Inspektor Research Systems, Amsterdam, The Netherlands). The mineral content was calculated in accordance with the formula of Angmar et al. (17). Three parameters were measured; integrated mineral loss (IML), lesion depth (LD), and the mineral volume percentage of the surface layer (SL).

Statistical analysis

For the QLF results, the Kruskal-Wallis test was performed. Two factors were analyzed: time of remineralization (in weeks) and treatment. For TMR results, ANOVA was used to compare between the changes in TMR parameters in the different groups. The differences between each group and its corresponding, etched or non-etched, baseline value were also compared. Duncan's multiple range test was employed for the multiple comparisons. A simple factorial comparison was made with the etching and fluoride as the two factors.

Results

QLF

The regain of fluorescence after 1, 4, and 10 weeks of remineralization is presented in Table 2 as a percentage of the baseline fluorescence loss of the lesions. There was no statistical difference in the fluorescence gain among the different groups at the end of the experiment. However, after the first week there was a significant difference ($P < 0.01$) between the etched and non-etched groups when the comparisons were made within the non-fluoride treated groups (A and B) and within the fluoride-treated groups (C and D). After 4 weeks, the highest rate was in group D ($P < 0.01$), followed by groups B and C ($P < 0.01$), and then group A. After this period, the remineralization process slowed down considerably, until it reached a plateau.

TMR

Etching. The values of integrated mineral loss (IML), lesion depth (LD), and mineral content of the surface layer (SL) are presented in Table 3 as mean ± SD for the 14 enamel blocks that were sectioned at the beginning of the study to determine the initial lesion parameters for the etched and non-etched lesions. There were no statistically significant differences between the etched and non-etched initial lesions for any of the parameters.

Remineralization. The mean and standard deviation of IML, LD, and SL for the four experimental groups after the 10 weeks of remineralization are presented in Table 4. The differences between each group and its corresponding, etched or non-etched, baseline value are summarized in Table 5. Both tables also present the results of the statistical analysis. Fig. 1 shows the mean mineral profiles of the experimental groups.

Statistical analysis by ANOVA showed a significantly higher value of IML (Table 4) in group C compared with groups A, B, and D ($P = 0.029$). For the mineral regain from the corresponding baseline for each group (Table 5),

Table 4. TMR data for the experimental groups after 10 weeks of remineralization

Group	IML (vol% · μm) (mean ± SD)	LD (μm) (mean ± SD)	SL (vol%) (mean ± SD)
A *no etch, no F*	2785 ± 612	111 ± 13	76 ± 6
B *etch, no F*	2934 ± 429	101 ± 4	69 ± 9
C *no etch, F*	3767 ± 645	126 ± 11	80 ± 4
D *etch, F*	2839 ± 545	110 ± 15	75 ± 4
Statistical analysis:			
P value (by ANOVA)	0.029	0.017	0.073
Duncan's multiple range test (groups not significantly different are connected by vertical lines)	A | D | B | C	B | D | A | C	

IML = integrated mineral loss. LD = lesion depth. SL = mineral content of the surface layer of the enamel.

Table 5. TMR incremental data for the experimental groups after 10 weeks of remineralization. *Change from baseline values*

Group	IML (vol% · μm) (mean ± SD)	LD (μm) (mean ± SD)	SL (vol%) (mean ± SD)
A *no etch, no F*	2978 ± 612	42 ± 13	8.9 ± 5.7
B *etch, no F*	3367 ± 429	57 ± 4	13.7 ± 8.9
C *no etch, F*	1997 ± 645	27 ± 11	12.5 ± 3.6
D *etch, F*	3462 ± 545	48 ± 15	18.9 ± 4.0
Statistical analysis:			
P value (by ANOVA)	0.001	0.003	0.038
Duncan's multiple range test (groups not significantly different are connected by vertical lines)	C A B D	C A D B	D B C A

IML = integrated mineral loss. LD = lesion depth. SL = mineral content of the surface layer of the enamel.

the largest remineralization was observed in the etched groups (B and D). Statistically, there was less remineralization in group C than in the other groups ($P = 0.001$).

The difference in the lesion depth (Table 4) was also significant between the different groups. Group C exhibited the deepest lesion of all the groups ($P = 0.017$), indicating least reduction of lesion depth. Group B, on the other hand, showed the shallowest lesion, though the difference with groups A and D just failed significance. The biggest reduction in lesion depth compared to the baseline values (Table 5) occurred in the two etched groups ($P = 0.003$) with reductions of 57 and 48 μm for B and D, respectively, values which were significant from group C.

When the mineral volume percentage of the surface layer for the 4 groups was compared (Table 4), the values were not significantly different. Significance was reached when the mineral regain in the surface was later calculated from the baseline values (Table 5). The highest recovery of the minerals of the surface layer occurred in group D, followed by group B ($P = 0.038$); the least recovery was in groups A and C. The etch step persisted in both the B and D groups at the end of the remineralization period. Fig. 2 presents the scans and the mineral profile of one section from each of the two groups.

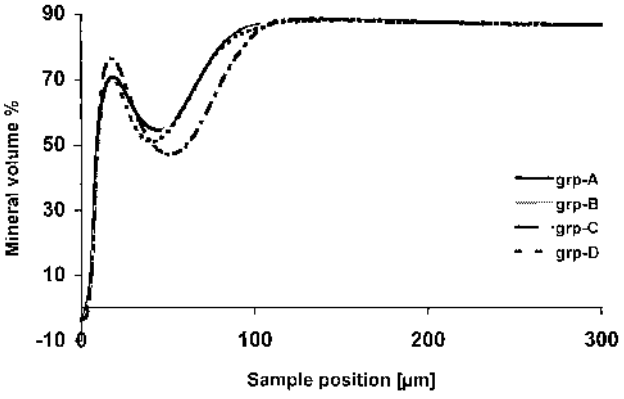

Fig. 1. Mean mineral content depth profiles of the 4 experimental groups.

Discussion

The surface layer of an incipient caries lesion is, by definition, high in mineral content (18). Removing part of this surface layer by acid etching of the caries lesion was thought to be an option to increase surface porosity (19, 20), and to make the underlying body of the lesion accessible for mineral ions (21, 22). This study was planned to investigate longitudinally the remineralization in etched and non-etched caries lesions in the presence or absence of fluoride, as well as the characteristics of the resulting surface layer.

The results from this study showed that the etched enamel lesions exhibited a more pronounced reduction of the lesion depth after remineralization than the non-etched lesions. Similar results were reported by Flaitz & Hicks (19). In the latter study, lesion depth had decreased significantly more in the etched group than in the non-etched group. The results were consistent for two different degrees of saturation of the remineralizing fluids that were used. In their study, Flaitz & Hicks also reported a thicker surface layer for the etched groups after the remineralization had taken place. In our study, the mineral volume of the surface layer was measured instead of its thickness. Although less mineral content of the surface layer was found in the etched groups than in the non-etched at the end of the remineralization period, the amounts of deposited mineral were higher in the etched groups. The deposition of mineral in groups B and D also compensated for the mineral loss due to the etching procedure.

It has been reported that fluoride uptake by the enamel is inversely proportional to its initial fluoride content (23, 24). Since the highest fluoride content is found in the outermost layer of the enamel, and exponentially decreasing as the depth increases (25, 26), removal of the surface by etching will not only increase the porosity of the enamel

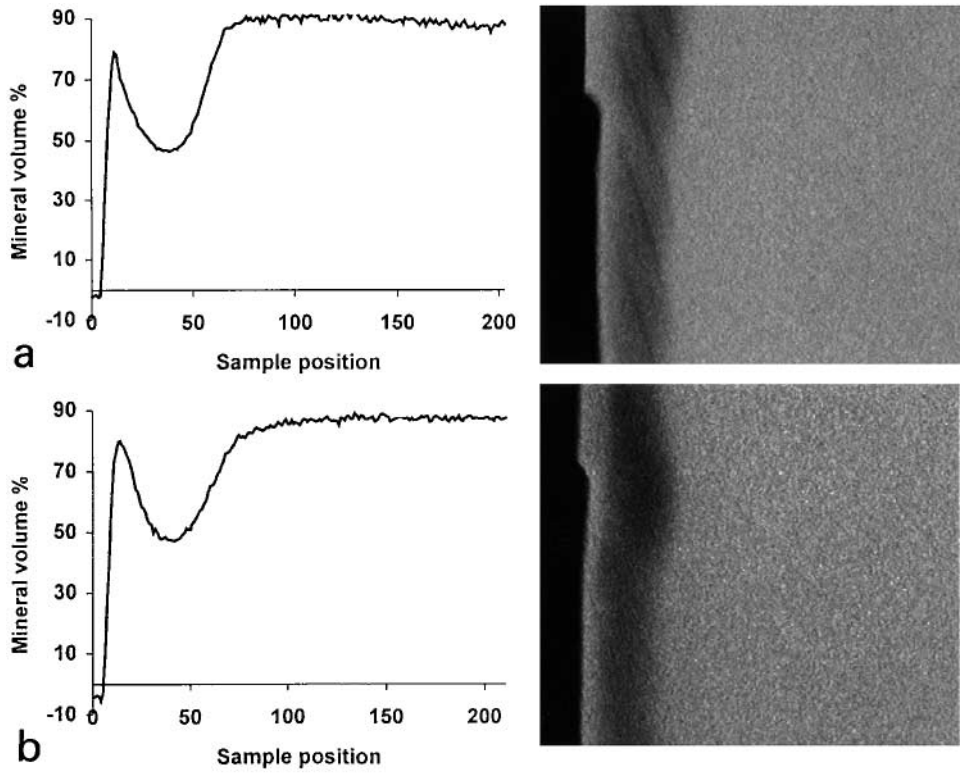

Fig. 2. Mineral content depth profile of 1 enamel block from group B (2a), and 1 from group D (2b) showing the persisting etch-step.

but also enhance the possibility for fluoride uptake. This effect was demonstrated in group D, which showed the highest repair of the mineral volume of the surface layer compared to the other groups.

All lesion parameters obtained by TMR analysis showed more repairs in the etched groups than in the non-etched groups in either presence or absence of fluoride. In the presence of fluoride, there was more repair when expressed as the integrated mineral loss and the mineral content of the surface layer, but the repair in the lesion depth was highest in the etched, non-fluoride treated group.

In the non-etched, fluoride-treated group, a highly remineralized surface layer was observed. This surface layer is probably the result of the fluoride-enhanced precipitation of minerals originating from the remineralizing fluid (3, 27, 28), an observation which was supported by SEM findings (data not shown). The other source for mineral supply in this layer probably came from the lesion body. Fluoride has been reported to draw the free mineral ions from the lesion body towards the surface (3, 29), a process that results in redistribution of the mineral in the lesion. This surface layer seemed to act as a barrier that hindered remineralization.

Interesting additional information can be obtained by comparing the QLF and TMR data. In the present study the regain of the fluorescence amounted to about 25%, while the change in mineral loss (expressed as IML) was, on average, around 50%. The reduction in lesion depth varied between groups but averaged around 25%. It has been postulated that a loss of fluorescence reflects an increase in scattering, which in turn depends on porosity (30, 31), and vice versa. The current data would indicate that an increase in fluorescence during remineralization is a more complex function of the various mineral loss parameters and not directly proportional to mineral uptake alone (IML). We assume that this is due to the inability to achieve a complete ('perfect') remineralization (32). This observation would apply particularly in situations with preferential mineral depositions at various depth, as occurs during lesion arrestment.

Irrespective of treatment, a substantial lesion persisted at the end of the experiment, and full remineralization was not reached. Although the degree of remineralization varied significantly between the different groups in the first weeks of the experiment, with the fluoride accelerating the process, the enhancing effect was only temporary and the curves of remineralization for all groups reached a plateau after a few weeks. We conclude from this study that acid etching enhanced the remineralization process of white spot enamel lesions. The addition of fluoride accelerated the remineralization process during the first few weeks,

especially in the etched group. Furthermore, fluoride treatment caused redistribution of the mineral content within the lesion. The etched groups retained a porous structure of their surface layer even after a relatively long period of in vitro remineralization.

Full remineralization, which is desirable for esthetic reasons, particularly in cases of "orthodontic" caries, is difficult to attain. Additional measures might be needed to trigger the remineralization process after a period of initial exposure to the remineralizing environment; a second etch might be an option in such cases. However, further investigations are needed to elucidate this dilemma, including clinical studies, since the differences between in vitro and clinical conditions should be considered.

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