Increased susceptibility for white spot lesions by surplus orthodontic etching exceeding bracket base area

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Introduction: There is a paucity of information with regard to the susceptibility of iatrogenic white spot lesion formation after inattentive, surplus orthodontic etching with 30% phosphoric acid and the subsequent provision or absence of adequate oral hygiene. Methods: Ninety sound enamel specimens were randomly allocated to 6 trial groups (n = 15 each) for etching with 30% phosphoric acid for either 15 seconds and standardized daily enamel brushing or no brushing, etching for 30 seconds with daily brushing or no brushing, or nonetched controls with brushing or no brushing. Nutritive acidic assaults were simulated by demineralization cycles 3 times per day for 1 hour with interim storage in artificial saliva. Lesion depths in terms of percentage of fluorescence loss (delta F, delta Q) and lesion extension compared with the baseline were assessed by using quantitative light-induced fluorescence after 2, 7, 14, 21, and 42 days. Etching duration, trial time elapse, and oral hygiene, as well as the significance of factor interactions, were analyzed with 3-way analysis of variance (α = 5%). Results: The impact of the factors of enamel brushing, trial time elapse, and etching each had a comparably significant effect on lesion progression. The effect of surplus etching on white spot lesion formation was significantly enhanced by the simultaneous absence of enamel brushing and also the progression of trial time. The combination of 30 seconds of surplus etching with inadequate oral hygiene was especially detrimental. Conclusions: Excessive surplus orthodontic etching of the complete labial enamel surface, instead of the bracket bases only, must be avoided to prevent iatrogenic white spot lesions. Etching times not exceeding 15 seconds are favorable. (Am J Orthod Dentofacial Orthop 2012;141:574-82)

The use of fixed orthodontic appliances and the conditioning of enamel surfaces by etching with phosphoric acid to achieve microretention for bonding brackets with composite adhesive systems are common components of fixed orthodontic therapy. Although the benefits of contemporary orthodontics are inconceivable without multi-bracket appliances, there is also a downside: poor oral hygiene is known to cause white spot lesions within weeks.1-6 Preventive strategies reported in the literature have focused on the application of fluoride-releasing varnishes,7,8 fluoride-releasing bonding materials,9,10 chlorhexidine for lowering the level of Mutans streptococci,11 and high concentrations of fluoride gels,12 or daily rinsing13-16 to prevent enamel demineralization.

White spot lesions are not only disturbing esthetically, especially in the anterior teeth,17 but also are an initial stage of enamel caries, producing outer enamel layers with significantly reduced mineralization18 that will progress to a stage at which restoration will be needed, if inadequate oral hygiene prevails.19

Previous experiments on bovine enamel, including extensive etching intervals, suggested that white spot lesions might be triggered iatrogenically under some circumstances: eg, by surplus etching of enamel areas that are subsequently not covered by bracket bases or bonding material, because etching removes parts of the outer enamel layer and results in a rough, retentive surface (Fig 1).20-22 This can be especially severe in teeth in which almost the complete facial enamel surface is etched but subsequently incompletely covered by bonding material or sealers. There is a lack of information in the literature regarding the question of whether these areas show increased susceptibility to
white spot formation and whether demineralization of these possible predilection sites is enhanced by oral hygiene measures, etching duration, and observation time.

This study was designed to evaluate the effect of enamel conditioning with 30% phosphoric acid for either 15 or 30 seconds with and without careful enamel brushing (oral hygiene) on the formation of cariogenic white spot lesions in a period of 6 weeks after etching and bonding by using quantitative light-induced fluorescence.

The null hypotheses were no significant differences in terms of demineralization depth and lesion extension compared with the baseline between a nonetched control group and (1) phosphoric acid enamel conditioning with either 15 or 30 seconds, and (2) subsequent adequate or inadequate oral hygiene at 2, 7, 14, 21, and 42 days after treatment.

**MATERIAL AND METHODS**

Enamel specimens were obtained from the labial surfaces of 90 permanent anterior teeth selected by using the exclusion criteria of decay, demineralization, or restorations (Fig 2, A). The teeth were stored in isotonic sodium chloride solution with 0.1% thymol before the trial to prevent desiccation and demineralization. For the trial, the specimens were mounted on acrylic base plates

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**Fig 1.** Schematic outline of the study: white spot lesions can be triggered iatrogenically by accidental surplus etching of enamel areas that are not covered by bracket bases or bonding material, thereby creating a rough, retentive surface structure. Scanning electron microscope photo courtesy of Prof. Thomas Attin, Zürich, Switzerland.

**Fig 2.** Standardized brushing and cleaning of enamel specimen (A) in groups A1-C1 was implemented by commercially available tooth brushes and a brushing machine (B) after Wiegand et al.27 See text for details.
and slightly polished to a graining grade of 1200 for standardization of enamel surfaces in terms of evenness and plane-parallel orientation during analysis. The enamel area to be measured had a diameter of 5 mm and was determined and automatically retrieved at the follow-up assessments with system-immanent quantitative light-induced fluorescence software.

Specimens fulfilling the selection criteria were randomly allocated to 6 trial groups (n = 15 each) for etching with 30% phosphoric acid for either 15 seconds and once a day brushing (A1) or no brushing (A2), or etching for 30 seconds with daily brushing (B1) or no daily brushing (B2), and nontreated controls with brushing (C1) or no brushing (C2) (Fig 3).

An objective method used for detecting white spot lesion formation and scoring the progress of enamel demineralization is to measure lesion depth in terms of percentages of fluorescence loss over time compared with the baseline by using quantitative light-induced fluorescence because enamel auto-fluorescence is directly related to the mineral content of the enamel, allowing for repeated, nondestructive, noninvasive measurements. A lowered fluorescence (by negative delta F values) indicates progress in demineralization.

In this study, demineralization was measured with 3 parameters: lesion depth expressed as a percentage of...
fluorescence loss compared with the baseline (delta F), lesion extension expressed as the area of demineralization (mm²), and delta Q values (defined as fluorescence radiance loss integrated over the lesion area [mm² · %]) compared with the baseline assessed by quantitative light-induced fluorescence (Inspektor Research Systems, Amsterdam, The Netherlands) and corresponding quantitative light-induced fluorescence software (version 2.0.0.43, Inspektor Pro Software, Inspektor Research Systems) at baseline and after 2, 7, 14, 21, and 42 days. The calculation of delta Q values compensated for minor fluctuations in measurement, since they were being leveled by putting fluorescence values in relation to the assessment of the area of demineralization.

The accuracy and reproducibility of the quantitative light-induced fluorescence results were ensured by excluding day and ambient light, and by an automatic calibration process implemented before each measurement cycle.

The experimental setup is illustrated in Figure 3. All quantitative light-induced fluorescence assessments were performed by 1 operator (M.B.).

Nutritive acidic assaults were simulated by demineralization cycles 3 times per day, each lasting 60 minutes, by using a demineralization solution that had previously been demonstrated to be suitable for causing white spot lesions (Table I). Between the demineralization cycles, the enamel specimens were stored in an artificial saliva and remineralization solution for 120 minutes. Therefore, a complete 1-day demineralization–remineralization program consisted of 3 hours of demineralization and 6 hours of remineralization, with subsequent overnight storage in the remineralization solution. Brushing in groups A1 through C1 was carried out each day after completion of all demineralization and remineralization cycles by using a brushing machine with commercially available toothbrushes (Dr. Best Flex Plus Medium; GlaxoSmithKline, Bühl, Germany) at a constant pressure of 2.5 N with a 1:3 slurry of artificial saliva and fluoridated dentifrice (Elmex; GABA, Lorrach, Germany) with subsequent water rinsing (Fig 2, B). Figure 3 provides the flow chart for the trial and the ingredients, and the mode of application of the demineralization and remineralization solutions is shown in Table I.

### Statistical analysis

The influence of the individual experimental factors—oral hygiene with enamel brushing, duration of etching, and trial time—as well as the interactions between those factors, on delta F, delta Q, and demineralization area extension values were analyzed both descriptively with mean values and standard deviations, and by using 3-way analysis of variance (ANOVA) for longitudinal data. In this mixed model, time was modeled as a repeated-measures factor. In the case of significant interactions between the experimental factors, the data were split and further analyzed by using 2-way ANOVA. The significance level was set to 5%. All analyses were performed with the software R (version 2.12; www.r-project.org).

### RESULTS

For all 3 assessment parameters—fluorescence loss, extension of demineralized enamel area, and delta Q—there were no changes in the daily brushed, non-etched control group C1. However, omitting daily brushing did have a demineralizing effect on the nonetched controls (Tables II and III; Figs 4-6). The results of the ANOVA for fluorescence (delta F) values (Table II) show that the impact of the factors of enamel brushing, trial time elapse, and etching all have comparably significant influences.

Interference between the parameters of etching duration, trial time elapse, and oral hygiene were analyzed by assessing the significance of parameter interactions. With the 2 exceptions of the interactions of brushing vs etching duration and etching duration vs trial time elapse, the interactions were not significant, so that it could be concluded that, for example, most factors (etching duration, trial time elapse, or absence of brushing) are not enhanced by interference with other factors (Table III). Although brushing or not brushing alone had no significant influence on the extension of the

### Table I. Ingredients, pH values, and concentration range (%) of the solutions used

<table>
<thead>
<tr>
<th>Product</th>
<th>Application</th>
<th>Contents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Demineralization solution at 37°C</td>
<td>30 mL/specimen</td>
<td>2.0 mmol/L Ca(NO3)2 × 4H2O, 2.0 mmol/L NaH2PO4 × 2H2O, 0.075 mmol/L acetate buffer, 0.02 ppm NaF, pH = 4.7</td>
</tr>
<tr>
<td>Remineralization solution at 37°C (Roth, Karlsruhe, Germany)</td>
<td>Storage solution/between demineralization cycles</td>
<td>1.5 mmol/L Ca(NO3)2 × 4H2O, 0.9 mmol/L NaH2PO4 × 2H2O, 150 mmol/L KCl, 0.1 mol/L Trisbuffer, 0.03 ppm NaF, pH = 7.0</td>
</tr>
<tr>
<td>Artificial saliva (remineralization) solution according to Klimek et al (Merck, Darmstadt, Germany)</td>
<td>Brushing slurry: artificial saliva vs dentifrice 1:3</td>
<td>Ascorbic acid 0.011 mmol/L, glucose 0.2 mmol/L, NaCl 9.9 mmol/L, CaCl2 × 2H2O 1.5 mmol/L, NH4Cl 3.0 mmol/L, KCl 17.0 mmol/L, NaSCN 2.0 mmol/L, KH2PO4 2.4 mmol/L, carbamide 3.3 mmol/L, Na2HPO4 2.4 mmol/L, mucine 2.7 g/L, Aqua Destillata 1000 mL</td>
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</table>
thereby triggering or enhancing the susceptibility to demineralized area during the 42 days of the trial, there was an influence when combined with the etching factor (Tables IV and V): further splitting of the ANOVA results by either brushing status or etching duration showed that trial time elapse had a significant effect only on the extension of the area of demineralization for 30 seconds of etching and that etching duration had a significant effect only on the delta Q values for nonbrushed teeth.

**DISCUSSION**

Composite adhesive systems used as orthodontic bracket adhesives act by means of enamel microretention. Whereas adequate shear bond strength is necessary for effective fixed orthodontic treatment, a known drawback is causing damage to the enamel during debonding. Our results suggest another downside: enamel sites prepared for enhancement of microretention but not covered by bonding materials or sealers are also likely to increase the retention of dental plaque, thereby triggering or enhancing the susceptibility to white spot lesion formation. Apart from the potential mechanical aspect of plaque adhesion, the iatrogenic damage that appears with surplus etching should be viewed as initial demineralization amplified by exposure to an acidic environment, as is typical for interbracket sites during stages of insufficient oral hygiene.

Previous research on the topic of enamel loss or demineralization by etching hints at demineralization...
damage from prolonged etching; however, those results are only within limits comparable with accidental etching during orthodontic bonding procedures, since they were either obtained from etching intervals that are considered much too long for orthodontic purposes (1–5 minutes)20,21 or performed with phosphoric acid concentrations that were much too high for orthodontic bonding purposes (up to 50%).20 Other authors used enamel of bovine instead of human origin but failed to detect demineralization by transverse microradiography after etching with 37% phosphoric acid for 30 seconds.22 In contrast to those studies, we focused on a typical orthodontic malpractice by accidental surplus etching during bracket bonding.

Although the application of fluorides in mouth rinses during fixed orthodontic treatment is a common practice today and has been shown to potentially inhibit lesion development by about 60%, white spot lesions are still a problem for many patients.1 In a recent study, 73% of orthodontic patients were reported to have developed at least 1 new white spot lesion during treatment.4 We assume, on the basis of our results, that the problem of white spot lesion development during orthodontic treatment can be reduced by more diligent application of etching gel and shorter intervals of orthodontic etching. As an additional strategy to support the inhibition of enamel demineralization, the application of fluoride-releasing sealants after bonding of brackets is common.
practice today and has been shown to be effective in reducing the depth of initial carious or white spot lesions by about 38%. However, the efficacy of sealants also depends on the diligence of their application. In relation to the duration of retention of those with different levels of oral hygiene and more or less abrasion by toothbrushing and interbracket brushing, no valid recommendations can be made currently based on the available evidence, particularly because it is not known how the durability of the sealing agents interferes with previous surplus etching. Even when etched areas are covered by bonding material, the question remains whether there is increased susceptibility to white spot lesion after fading or washing out of the bonding material after several months. It may well be that these areas are reduced in extension and smoothed by toothbrushing abrasion.

The effect of inadequate interbracket and enamel hygiene on the velocity of white spot lesion formation, especially during the first weeks of fixed appliance treatment, has been debated at length in the literature. The clear decrease in mineralization found in our study after 14 to 21 days agrees with previous investigations of the speed of development of white spot lesions. The null hypotheses of no significant differences in demineralization in terms of prior phosphoric acid enamel etching and subsequent oral hygiene were both rejected, since the impact of the factors of enamel brushing, trial time elapse, and etching were found to have comparable significant influences.

The major finding of this study was that there is also a white spot lesion formation enhancing effect from the duration of etching before orthodontic bracket placement. There was a clearly significant effect on lesion formation and progression (Table IV; Figs 4-6) with etching durations exceeding 15 seconds, especially without adequate enamel brushing. Previous research has indicated that etching durations of 15 seconds are sufficient for adequate shear bond strength for orthodontic purposes. The results of this study provide 1 more rationale for conservative etching durations.

Table IV. Because of the significant interaction between brushing and etching on the extension of the area of demineralization (Table III), ANOVA was split by brushing status, which showed that etching has a significant effect only for nonbrushed teeth.

<table>
<thead>
<tr>
<th>Subset</th>
<th>Effect</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brushed</td>
<td>Etching</td>
<td>0.13</td>
</tr>
<tr>
<td></td>
<td>Time</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>Etching × time</td>
<td>0.01</td>
</tr>
<tr>
<td>Nonbrushed</td>
<td>Etching</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td></td>
<td>Time</td>
<td>0.40</td>
</tr>
<tr>
<td></td>
<td>Etching × time</td>
<td>0.94</td>
</tr>
</tbody>
</table>

Table V. Because of the significant interaction effect between trial time elapse and etching duration on delta Q values, ANOVA was split by etching status, which showed that time elapse has a significant effect only for 30 seconds of etching.

<table>
<thead>
<tr>
<th>Subset</th>
<th>Effect</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Etching 0 s</td>
<td>Trial time elapse</td>
<td>0.13</td>
</tr>
<tr>
<td>Etching 15 s</td>
<td>Trial time elapse</td>
<td>0.68</td>
</tr>
<tr>
<td>Etching 30 s</td>
<td>Trial time elapse</td>
<td>0.02</td>
</tr>
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</table>

Fig 6. Temporal course of delta Q values for A, nonbrushed and B, brushed teeth. Mean values (± standard deviations) are shown. Duration of etching: black, 0 seconds; red, 15 seconds; blue, 30 seconds.
The rationale for the decision favoring an in-vitro setup was that society considers it unethical to involve human subjects in a trial when it is unknown whether damage might result to intact structures in vivo. Because of the paucity of information in the literature regarding iatrogenic damage from excessive etching with subsequent orthodontic therapy, it was unclear, at the start of the trial, whether the formation of white spot lesions is triggered or accelerated by the application of surplus etching gel. Therefore, we decided to use a standardized in-vitro setup.

Although the characteristics of artificial white spot lesions in vitro have proved to be similar to natural lesion formation, white spot lesion formation in vivo is quantitatively deeper. Therefore, the results of this pilot study provide a strong warning against underestimating the effects of surplus orthodontic etching, especially in the case of inadequate oral hygiene, because the enhancing effect of surplus orthodontic etching on white spot lesion formation is likely to be much more pronounced in an in-vivo setting.

There were some differences between the groups of nonetching and 15-second etching, hinting at a slightly lower fluorescence in the 15-second group. However, these differences were not significant (Table V) and were ascribed to minor fluctuations in measurements. It was leveled by the assessment of the area of demineralization, which was performed by obtaining delta Q values (Figs 5, A, and 6, A).

Excessive surplus orthodontic etching must be avoided to prevent iatrogenically triggered white spot lesions. Etching of almost the complete facial enamel surface instead of the bracket bases alone, without subsequent application of sealers, is considered to be outdated from the perspective of enamel protection. Since it is considered normal that there will often be some small areas of enamel that are not covered by bracket bases, careful application of bonding and sealers is necessary. From the viewpoint of white spot lesion prevention, etching durations of not more than 15 seconds are favorable. Diligent screening of oral hygiene in patients with fixed orthodontic appliances is a necessity, especially during the first weeks of treatment.

Further research is required about exfoliation or abrasion of fluoride-releasing sealants and how this interferes with overly etched enamel sites to establish sealer application intervals that should not be reduced to ensure continued protection.

Since the results of this pilot study showed an influence of surplus etching and insufficient oral hygiene on the potential in-vitro formation of white spot lesions, further investigations are planned, including premolars and third molars that are to be extracted because of a lack of space, in the context of orthodontic therapy, implementing recently developed in-vivo quantitative light-induced fluorescence devices for the analysis.

**CONCLUSIONS**

The following conclusions can be drawn from this study.

1. The factors of surplus orthodontic etching of more than 15 seconds, trial time elapse, and inadequate brushing each had a comparably significant influence on enamel demineralization.
2. The surplus etching effect on demineralization area extension is enhanced by the simultaneous absence of enamel brushing and an increase in trial time.
3. Excessive surplus orthodontic etching must be avoided to prevent iatrogenically triggered white spot lesions.
4. From the perspective of preventing white spot lesions, etching durations of not more than 15 seconds are favorable.

**REFERENCES**

11. Øgaard B, Larsson E, Glans R, Henriksson T, Birkhed D. Antimicrobial effect of a chlorhexidine-thymol varnish (Cervitec) in


