Esthetic comparison of white-spot lesion treatment modalities using spectrometry and fluorescence

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ABSTRACT
Objective: To compare the esthetic improvements of white-spot lesions (WSLs) treated by fluoride, casein phosphopeptide amorphous calcium phosphate (CPP-ACP), or resin infiltration.

Materials and Methods: WSLs were created on human enamel and randomly assigned to four groups: NaF (500 ppm), CPP-ACP, resin infiltration (Icon), or distilled deionized water (DDW; control group). The color change (ΔE) of each specimen was measured with a Crystaleye spectrophotometer, and fluorescence loss (ΔQ) was measured by quantitative light-induced fluorescence (QLF), at different time points after treatment: baseline (0 weeks), 2 weeks, 4 weeks, and 6 weeks.

Results: The ΔE and ΔQ baseline values for the four groups before the treatments did not differ significantly. Icon treatment improved the WSL color significantly and gave the lowest ΔE (2.9 ± 1.2 on average) compared with other treatments (P < .01). The Icon treatment also resulted in a significant change in the ΔQ of WSLs compared with baseline (P < .01). In the NaF and CPP-ACP treatment groups, ΔQ showed significant recovery compared with the baseline values only after 4 weeks after treatment (P < .05).

Conclusions: Resin infiltration is more effective than NaF or CPP-ACP in providing esthetic improvement of WSLs. (Angle Orthod. 2014;84:343–349.)

KEY WORDS: White spot lesion; Enamel demineralization; Orthodontics; Resin infiltration; Fluoride; CPP-ACP

INTRODUCTION
White-spot lesions (WSLs) are one of the most prevalent side effects of fixed orthodontic treatments, affecting about 50% of the patients.1 The placement of fixed orthodontic appliances severely impedes oral hygiene and provides areas with low saliva flow that allow microbial adhesion and biofilm formation. If patients cannot maintain good oral hygiene during orthodontic treatment, the acid produced from biofilms will cause enamel demineralization with a clinical manifestation of WSLs.2 These WSLs can occur 1 month after the placement of fixed appliances and persist for 5 years after the removal of the appliances, severely affecting patients' esthetic appearance and quality of life.3,4

The measures adopted to prevent WSLs in orthodontics usually include routine oral hygiene instructions, application of fluoride, use of antimicrobial dentifrices and mouth rinses, and antimicrobial modifications of orthodontic biomaterials. Benson and colleagues5 recommended daily fluoride mouth rinsing (0.05% NaF) as an effective prophylactic measure to prevent WSLs. It has also been demonstrated that Listerine and Corsodyl mouth rinses inhibited biofilm
formation on orthodontic brackets. Shah et al. reported that stainless steel orthodontic brackets modified with a photocatalytic titanium oxide (TiO$_2$) coating resulted in reduced bacterial adherence and less biofilm accumulation. The prevention of biofilm formation has been studied extensively; however, according to recent studies, the prevalence of WSLs remains high.\(^8\)\(^\text{--}\)\(^11\)

Once WSLs occur, treatment usually includes noninvasive measures, such as remineralization or resin infiltration, and invasive measures, such as microabrasion or the placement of veneers or crowns. Invasive measures involve removal of enamel to various degrees. Clinicians therefore tend to prefer to start treatment with a relatively noninvasive approach.\(^12\) It was found that the depth of WSLs could be decreased after repeated remineralization using agents containing 5% fluoride.\(^13\) The severity, activity, and caries risk of WSLs could also be reduced by using casein phosphopeptide amorphous calcium phosphate (CPP-ACP).\(^14\)\(^,\)\(^15\) However, remineralization approaches are usually only applied in the early stage of WSL development and mainly affect the WSL surface layer only. In addition, the effects of these treatments are not satisfactory because the WSLs are often still visible clinically and radiographically, even after a time-consuming remineralization regimen, because of their limited remineralization potential.\(^16\)

Resin infiltration was developed to arrest caries lesion progression with low-viscous light-curing resins.\(^17\) This treatment creates a diffusion barrier on the surface and within the enamel, thus occluding pathways for acid entry into the enamel and leading to an esthetic improvement in caries lesions.\(^18\) Recent studies have found that resin infiltration could also restore the color of WSLs after orthodontic treatment.\(^19\)\(^--\)\(^21\) However, the effect of resin infiltration on recovering other aspects of WSLs, such as fluorescence loss is still not clear.

Currently, it is very difficult for clinicians to easily identify which approach works best for WSLs. The aim of this study was to compare the esthetic improvements of WSLs treated by three commonly used methods, fluoride, CPP-ACP, and resin infiltration using spectrophotometer measurements and quantitative light-induced fluorescence (QLF). Our hypothesis was that resin infiltration is more effective in the esthetic improvement of WSLs than fluoride or CPP-ACP.

**MATERIALS AND METHODS**

**Specimen Preparation**

One hundred fourteen human permanent premolars and molars were obtained from West China Hospital of Stomatology, Sichuan University, and selected by two investigators using the following exclusion criteria: presence of stain, demineralization, decay, fluorosis, enamel defects, or restorations. After extraction, the teeth were immediately cleaned with tap water and stored in 0.05% thymol solution at 4°C for less than 1 week before use in experiments.

Ethical approval was obtained from the medical ethics committee at West China School of Stomatology, Sichuan University.

WSLs were created by exposing the teeth to a demineralization gel (6% hydroxyethyl cellulose, 0.1 M lactic acid solution, pH 4.5) at 37°C for 1 week, as previously described.\(^22\) The acid-etched areas (WSLs) were restricted to 2 mm $\times$ 3 mm of the enamel surfaces by coating the surrounding enamel surfaces with two layers of acid-resistant nail varnish. To ensure equal demineralization of all teeth, a fresh demineralization gel was used for each tooth. After demineralization, specimens were washed thoroughly with distilled deionized water (DDW), and the nail varnish was removed using acetone before washing the teeth in DDW again. After this procedure each tooth displayed an artificial WSL of 2 mm $\times$ 3 mm (Figure 1).

**Specimen Screening and Baseline Evaluation**

After completion of WSL formation, baseline comparisons were performed by measuring the color ($\Delta E$) using a Crystaleye spectrophotometer (Basic Set CE100-DC/EU, Olympus, Tokyo, Japan) and fluorescence loss ($\Delta Q$, by QLF) of the WSLs compared to the adjacent sound enamel. Using these data, 52 specimens that had similar baseline $\Delta E$ and $\Delta Q$ were selected for inclusion in the study.

**Grouping and Treatment Interventions**

The 52 specimens selected for study were labeled with numbers and randomly divided into four groups of 13 (three test groups and one control group) by an investigator. All treatment interventions were performed by a trained dental investigator.

For the NaF group ($n = 13$), the specimens were immersed in 300 mL 500 ppm NaF solution (Kelong Chemical, Chengdu, China) and stirred at 100 rpm for 5 minutes to mimic the possible remineralization effected by daily brushing with fluoride toothpaste.

For the CPP-ACP group ($n = 13$), a CPP-ACP crème (GC tooth mousse, GC Corp, Tokyo, Japan) was applied according to the manufacturer’s instructions. In brief, the crème was applied to the specimen using a microbrush and left undisturbed for 5 minutes.

For the resin infiltration group ($n = 13$), the resin infiltration (Icon, DMG, Hamburg, Germany) was performed according to the manufacturer’s instructions.\(^23\) The WSLs were etched for 2 minutes with 15%...
hydrochloric acid (Icon Etch, DMG) and then rinsed with air-water-spray for 30 seconds. The WSLs were desiccated by air-blowing for 10 seconds, followed by application of ethanol (Icon Dry, DMG) for 30 seconds, and air-blowing again for 10 seconds. Then, the resin infiltrant (Icon Infiltrant, DMG) was applied to the WSLs with a sponge applicator provided by the Icon resin infiltration system and left for 3 minutes. Excess resin was removed by flossing. Subsequently, the resin was light-cured for 60 seconds from the buccal and occlusal aspects. The infiltration step was repeated once with a penetration time of 60 seconds to infiltrate remaining porosities. The prepared specimens were then polished with Astropol HP (Ivoclar Vivadent Inc, Amherst, NY) for 20 seconds according to the Icon Etch instructions.

DDW group (control, n = 13): the specimens were immersed in 300 ml DDW and stirred at 100 rpm for 5 minutes.

After the four treatment interventions, all the specimens were washed carefully with DDW and stored in 300 ml remineralizing solution (pH = 7) at 37°C and stirred at 100 rpm to mimic the natural remineralization from saliva in oral cavity (approximately 23 hours/day).24 The remineralization solution, which consisted of 1.5 mM CaCl₂, 0.9 mM KH₂PO₄, 130 mM KCl, 1 mM NaN₃, and 20 mM HEPES, was renewed once a week.25 The color and fluorescence measurements were repeated at 2-week intervals to see if, with time after the orthodontic treatment, there was improvement or worsening of the WSLs under conditions mimicking the natural oral environment.

**Crystaleye Spectrophotometer**

The enamel color of each specimen was measured using the Crystaleye spectrophotometer by a dental investigator who was blinded to the tooth treatment. The instrument was calibrated using a calibration plate (Olympus, Tokyo, Japan) before data acquisition. All measurements were performed under standardized ambient conditions to ensure accuracy and reproducibility.

Three images were randomly captured for each specimen and analyzed with the Crystaleye software (version 1.4, Olympus). The values of L* (differences in lightness), a* (green-red coordinate), and b* (blue-yellow coordinate) were recorded for the WSL and the adjacent sound enamel. Color change, \( \Delta E = (\Delta L^* + \Delta a^* + \Delta b^*)^{1/2} \), was calculated as the color difference between the treated and untreated enamel. The color measurements (\( \Delta E \)) were performed at four different treatment time points: baseline (0 weeks), 2 weeks, 4 weeks, and 6 weeks after treatment.

**Quantitative Light-induced Fluorescence**

The fluorescence loss of each specimen was measured using QLF by a dental investigator who was blinded to the tooth treatment. A fluorescence camera handpiece (Inspektor Research Systems BV, Amsterdam, Netherlands) connected to a personal computer with the image capturing software QLF Patient version 2.0.0.4 (Inspektor Research Systems BV) captured images of specimens.

To ensure that the images were always captured at the same camera position and angle, a video-repositioning technique was applied as described previously.26 The video-repositioning technique displayed the baseline and live image simultaneously, and images were automatically captured when the correlation based on a similar geometry of the fluorescence intensities reached 0.95. The images were stored for analysis by the system’s analysis...
software. A line was drawn surrounding the WSL site at its borders with sound enamel. The patch analysis and surface contour were copied and then matched for size, orientation, and location to this tooth surface in each consecutive image to ensure that the same area of tooth surface was analyzed at each time point.

The average fluorescence loss (ΔF) and lesion area (A) were recorded. The total fluorescence loss of the specimen, ΔQ = ΔF × A, was calculated. QLF analysis was also performed at four different time points: baseline (0 weeks), 2 weeks, 4 weeks, and 6 weeks after treatment.

**Statistical Analysis**

Statistical analysis was performed using SPSS 17.0 for Windows (SPSS Inc, Chicago, Ill). The mean ΔE and ΔQ values were calculated for all groups. The data were analyzed using multiple way analysis of variance and Tukey’s test, both with a significance level of P < .05.

**RESULTS**

The color change (ΔE) and fluorescence loss (ΔQ) of the four experimental groups did not differ significantly (P > .05) at the baseline measurement after completion of WSL formation at t = 0 (Figures 2 and 3). Before WSL treatment, the mean ΔE was 12.41 ± 3.47 for the control group and 12.91 ± 3.36 for the test groups. The mean ΔQ was −171.88 ± 53.26 for the control group and −170.06 ± 44.06 for the test groups.

The color of the WSLs in the Icon treatment group was improved significantly by the resin infiltration treatment and had the lowest mean ΔE (2.9 ± 1.2) compared with the other treatment groups (mean ΔE = 12.0 ± 3.6) regardless of time after treatment (P < .01) (Figure 2). There were no significant changes in ΔE with time during the 2–6 weeks after treatment in any group (P > .05) (Figures 1 and 2).

The fluorescence loss (ΔQ) in the Icon group was significantly ameliorated (P < .01) after the treatment (Figure 3). No significant change in ΔQ was found with time during the 2–6 weeks after infiltration (P > .05). In the DDW, NaF, and CPP-ACP groups, the ΔQ values showed significant recovery only at 4 weeks after treatment (remineralization) compared with untreated WSLs (P < .05). There were no significant differences in ΔQ values for the NaF, CPP-ACP, and DDW groups regardless of time after treatment (Figures 1 and 3).

**DISCUSSION**

This study compared the effects of three commonly used treatment modalities for WSLs against that of a control group (DDW) during a follow-up of 6 weeks after treatment. Significant decreases of ΔE and ΔQ values were found in the Icon group after the treatment (Figures 2 and 3), indicating that Icon had the best effectiveness of the three treatments in masking the WSLs and returning the enamel to its natural color. This is because the air or water in the microporosities of WSLs was replaced with resin, leading to less light scattering within the enamel.\(^{19}\)

Sound enamel has a refractive index (RI) of 1.62 while demineralized WSLs have many pores filled with water (RI = 1.33) or air (RI = 1.00). The difference in

![Figure 2. Color changes (ΔE) of WSLs exposed to the four different treatments with time after treatment (mean ± SD).](http://media.aaostd.com/angle-orthodontist/article-pdf/84/2/343/1397642/032113-232_1.pdf)
RIs between the enamel crystals and medium inside the porosities affects the light scattering and gives these lesions a whitish appearance, especially when desiccated.\textsuperscript{27} When the micropores of WSLs were infiltrated by resin (RI = 1.46), which has a similar RI as enamel and cannot evaporate, the difference in RIs between porosities and enamel was decreased to a negligible level, and the WSLs regained translucency, appearing similar to that of the surrounding sound enamel.\textsuperscript{21} Because a $\Delta E$ value of $<3.6$ is considered a clinically acceptable color difference,\textsuperscript{28} an average $\Delta E$ of 2.9 ± 1.2 after resin infiltration in our study indicated a better and acceptable color recovery compared with NaF or CPP-ACP treatment (Figure 2).

It was found that remineralization of WSLs by the artificial saliva for the NaF and CPP-ACP treatment groups was a slow process because of its dependence on the deposition of calcium ions.\textsuperscript{14,15} In contrast, the low-viscous resin could penetrate into deeper lesions and improve the esthetic appearance of the WSL immediately after treatment. That explains why the $\Delta Q$ immediately showed significant recovery for the Icon group, whereas there was improvement in $\Delta Q$ only after 4 weeks of remineralization in the NaF and CPP-ACP groups in this study.

Fluoride is used extensively to promote the remineralization of WSLs,\textsuperscript{13,29} and a daily NaF mouth rinse might reduce the occurrence and severity of WSLs during orthodontic treatment;\textsuperscript{30} however, the esthetic improvement of WSLs treated by fluoride is poorly understood. In this study, we found that the color of WSLs cannot be recovered using a single 500 ppm NaF treatment. Although the NaF group showed some improvement of $\Delta Q$ 4 weeks after treatment, there was no significant difference in $\Delta Q$ with the control group. Therefore, we cannot attribute the $\Delta Q$ recovery to the application of NaF; it is likely due to remineralization by the artificial saliva.

It has been reported that CPP-ACP promoted the formation of fluorapatite deep in WSLs in the presence of fluoride but that CPP-ACP alone may not have this effect.\textsuperscript{15} In our study, the CPP-ACP did not have a significant effect on the color recovery of WSLs. This might be because the treatment consisted of one short application. Based on clinical trials recently reported, there is insufficient evidence to make a conclusion regarding the long-term effectiveness of casein derivatives such as CPP-ACP in preventing caries in vivo.\textsuperscript{31} Further studies investigating the long-term effects of CPP-ACP on the recovery of WSLs are needed.

Resin infiltration was originally developed to obstruct the diffusion pathways for acids in order to protect internal enamel, with a masking effect being an additional advantage.\textsuperscript{23} This study shows that it could be used to arrest incipient WSLs instead of removing the lesions, which sacrifices healthy enamel. Microabrasion, which has been commonly used for treating WSLs, can remove up to 250 $\mu$m of enamel.\textsuperscript{32} In contrast, in the clinical practice of resin infiltration; the 15% HCl gel used to prepare the surface and open the pores of WSLs removes only about 40 $\mu$m of the surface layer. In our study, no cavitation occurred after etching with the HCl gel, and the subsequent resin infiltration helped strengthen the WSL structures.\textsuperscript{33} Because resin infiltration can immediately

\begin{figure}[h]
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\includegraphics[width=\textwidth]{figure3.png}
\caption{Fluorescence loss ($\Delta Q$) of WSLs exposed to the four different treatments with time after treatment (mean ± SD).}
\end{figure}
restore the color of WSLs, it saves time for patients and clinicians. In addition, resin infiltration maintained color stability for at least 6 weeks after application in our study. These features indicate that resin infiltration was the best of the techniques tested for the treatment of WSLs, which is consistent with a previous study that showed that resin infiltration was an effective treatment for masking WSLs and resisting a new acid challenge. However, the long-term effect of resin infiltration on WSLs in clinical practice should be studied further. The fluoride and CPP-ACP treatment and remineralization times should be extended to determine how long the remineralization modalities take to restore the WSLs to a normal enamel appearance. In addition, a randomized controlled clinical trial comparing these different treatment modalities would provide valuable information for dental practitioners.

CONCLUSION

- Resin infiltration can improve the esthetic characteristics (color and fluorescence) of orthodontic WSLs; however, the long-term effect of resin infiltration on WSLs in clinical practice should be studied further.

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