Original Article

Effectiveness of Caries Infiltration and CPP-ACP Containing Paste on Color Change and Surface Hardness of Artificial White Spot Enamel Lesions

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Abstract

Initial enamel caries is characterized as a whitish colored area with subsurface porosities that may have progressed to a more advanced lesion. Several approaches have been proposed to manage the non-cavitated enamel caries at different levels. This study aimed to determine the color improvement and surface hardness recovery of artificial white spot lesions after two conservative treatments - caries infiltration (Icon) and CPP-ACP containing paste (Tooth Mousse). Enamel carious lesions on extracted human premolars were created and underwent the two respective treatments following each manufacturer's instructions. Groups with or without lesion formation, but no further treatments, were included as negative and positive controls. All specimens were subjected to the demineralization-remineralization pH cycles at 37 °C for 8 weeks. At designated time points, the surface microhardness of the lesions was assessed using Vickers diamond indenter and the difference in color of the lesions to the time before treatment was evaluated using a spectrophotometer. The lesions were also microscopically observed from both top and cross-sectional views using SEM. Parametric and non-parametric statistics were conducted to analyze all data at 95% confidence level. Surface hardness of the lesions decreased significantly after artificial enamel caries formation. Following the caries infiltration treatment, hardness value recovered immediately and maintained throughout the period of pH cycles, even though not be comparable to that of the positive control. Difference in color of the lesions could be significantly detected after single treatment of the resin material. On the other hand, daily application of CPP-ACP containing paste did not regain the surface hardness of the lesions and the color change could not be significantly observed within 8 weeks of pH cycle. In conclusion, the caries infiltration is superior to the CPP-ACP treatment in color and surface hardness improvements of artificial white spot enamel lesions.

Key words: Caries infiltration; Casein phosphopeptide-amorphous calcium phosphate (CPP-ACP); Color difference; Enamel caries; Surface hardness

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Introduction

Dental caries is one of the major infectious diseases affecting oral health, especially in children and adolescent populations.[1,2] The process of dental caries is initiated by an accumulation of a complex microbial community, termed as 'dental biofilm', on the tooth surface, subsequently creating a unique microenvironment. Frequent consumption of high-sugar diet, for example, can increase the acidity of such an environment, which in turn disrupts the homeostasis of closely packed bacterial plaque.[2] As supported by the ecological plaque hypothesis, a shift in the plaque microflora to the high proportion of acid-producing (acidogenic) and acid-resistant (aciduric) bacteria causes the decrease of pH at the tooth surface and therefore, initiates the caries-conducive conditions.[3] If the pH of interstitial plaque fluid falls below the critical pH of 5.2 - 5.5, the dissolution of enamel minerals, mainly apatite crystals, can occur. Conversely, once the pH rises above the critical pH, dissolution will cease. Repeated demineralization process would then result in a formation of enamel caries, where increasing intercrystalline spaces and porosities of enamel surface can be detected.[2,3]

Characteristics of enamel carious lesions comprise the subsurface body of the lesion, which is porous and shows high degree of demineralization and the outermost surface layer, which contains high mineral content.[4] Thus, initial enamel caries is usually non-cavitated and detected as a subsurface demineralized zone underneath the intact remineralized surface. The clinical signs of enamel caries are the so-called 'white spot' lesions due to the difference of the refractive indices of adjacent sound enamel and air or electrolytes contained in the porosities of the lesion.[2] A greater proportion of the incoming light is scattered, resulting to the whitish look of enamel caries compared with the surrounding normal enamel. The white spot lesions are an esthetic point of concern for the patients and several treatment methods have been proposed to manage this unpleasing appearance.[5-7]

Currently, the concept of minimal intervention in restorative dentistry has been widely accepted. Initial enamel caries should be detected as early as possible to promote the remineralization before a cavitation occurs as well as to stop the progression of the lesions by conservative treatments. A number of noninvasive or microinvasive procedures have been introduced to both arrest caries progression (or further remineralize the lesion) and also improve the esthetic appearance in a suitable period of time.[2] One modality is the use of casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) containing agents. CPP-ACP nanocomplexes are milk-derived compounds, in which amorphous calcium phosphate is stabilized. CPP-ACP has been shown to reduce demineralization and promote remineralization of initial carious lesions by elevating the level of free calcium and phosphate ions at the tooth surfaces.[8] Also, clinical studies have confirmed the efficacy of CPP-ACP containing paste in reducing the area of enamel white spot lesion after daily application for 4-12 weeks.[6,7]

Another conservative approach for the

treatment of initial lesions is the caries infiltration. This technique aims to arrest the progression of the initial enamel caries by filling up the subsurface porosities of the lesions with a low-viscosity, light-curing material, so-called resin infiltrants.[9] The conjugated features would block the diffusion pathways for acids to further dissolve the mineral contents of enamel, hence preventing the progression of caries.[10] Moreover, caries infiltration of the initial enamel lesions is also useful for esthetic reasons. As the infiltrant has a visual appearance similar to normal enamel with the relatively slight difference between both refractive indices, light scattering at the surface can be modified and the white spot lesions can, therefore, be masked and become less noticeable.[5,9,11]

Regarding the previous information, the use of either CPP-ACP or caries infiltration treatment is likely to potentially protect the enamel caries from further acid challenge and esthetically improve the appearance of the lesions. As there is still no literature comparing the effectiveness of both strategies, it is noteworthy to investigate such benefits of these two conservative approaches. The aims of this *in vitro* study, therefore, were to assess the masking ability on artificial white spot lesions by means of CPP-ACP remineralizing paste and the caries infiltration technique and also to evaluate the protective capability of both treatments to prevent the lesions from acid attack under pH cycle regimen. The null hypotheses were that there would be no difference in the color change and in the surface hardness of enamel white spot lesions, for either the use of CPP-ACP containing paste or caries infiltration, at different evaluating time points up to 8 weeks of pH cycle.

Materials and methods

Figure 1 shows the overall flowchart illustrating the specimen preparation in the current observation. Intact, non-carious, non-restored human upper premolars extracted for orthodontic purposes were collected as the substrate with the protocol ethically approved by the Institutional Review Board (MU-DT/PY-IRB 2014/DT 086). The teeth were stored in 0.1 % thymol solution at 4 °C and used within 6 months following extraction. Soft tissues and/or calculus were removed and the enamel surfaces were polished with pumice-water slurry using a rubber cup mounted in a slow-speed handpiece. Verification of enamel defects under magnification loupes (×3.5) was performed and the teeth with defects, previous demineralization or cracks were excluded from the study. Roots were cut at the cementum-enamel junction and the crowns were buccolingually sectioned into halves using a diamond blade under water coolant (IsoMet; Buehler, Lake Bluff, IL, USA). Each half was embedded in acrylic block with the proximal surface facing outside and then polished as little enamel as possible with wet 4000-grit silicon carbide (SiC) abrasive paper, exposing approximately 1×1 mm^2 flat area of the enamel surface. Two layers of acid-resistant nail varnish were coated, leaving a window of polished enamel surface.

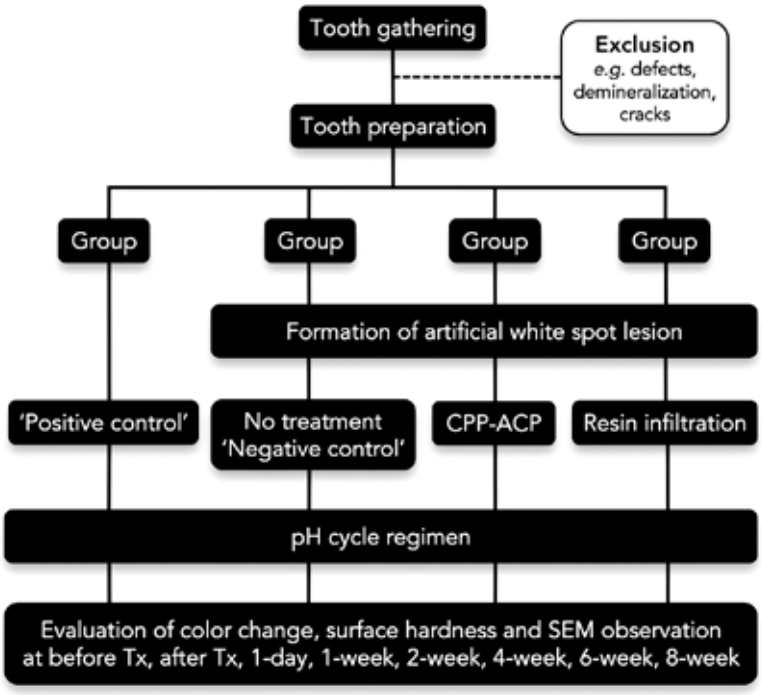


Figure 1 *Flowchart of specimen preparation*

1. Artificial white spot lesion formation

One group of the specimens was not processed to the formation of artificial white spot lesion, served as a positive control. For the remaining specimens, white spot lesion at the unprotected area was created by immersion of an individual in a 2 mL demineralizing solution, modified from Mukai and others[12], containing 50 mM acetic acid, 1.5 mM $CaCl_2$ and 0.9 mM KH_2PO_4 adjusted to pH 5.0 with 1M KOH, at 37 °C for 14 days. After the period, the specimens were removed from the solution, rinsed with distilled deionized water (DDW) for 1 min and blot dried with absorbent paper. To produce a naturally mimicking surface layer of white spot lesions, the demineralized specimens were individually placed in a 2 mL remineralizing solution[12] containing 1.5 mM $CaCl_2$, 0.9 mM KH_2PO_4, 130 mM KCl and 20 mM HEPES adjusted to pH 7.0 with 1M KOH, at 37 °C for further 7 days, then again rinsed thoroughly with DDW after immersion. The pH was periodically monitored using a pH meter (Orion 3-Star; Expotech USA, Houston, TX, USA), and the respective solutions were renewed daily after each immersion.

2. Treatments of the artificial white spot lesion

One group of the specimens with artificial white spot lesions was left untreated, served as a negative control. The remaining specimens after lesion formation were randomly treated by either resin infiltration material (Icon; DMG, Hamburg, Germany) or CPP-ACP containing paste (Tooth Mousse; GC Corporation, Tokyo, Japan) following each manufacturer's instructions. Caries infiltration was done to the lesions only one time prior to the pH cycle as described below, while the CPP-ACP containing paste was

applied before the remineralization step of the pH cycle process at each day. The materials used, compositions and treatment procedures are listed in Table 1.

Table 1 *List of materials used in the study*

Material	Compositions	Treatment procedures
Resin infiltration material - Icon	*Icon-Etch:* 15-20% hydrochloric acid *Icon-Dry:* 99% ethanol	Apply Icon-Etch and leave undisturbed for 2 min; remove using high-power suction and rinse thoroughly with air-water spray for 30 s.
(DMG, Hamburg, Germany)	*Icon-Infiltrant:* TEGDMA-based resin, initiators, stabilizers	Apply Icon-Dry for 30 s and dry for 5 s. (repeat the etching process if the white spot lesions are still visible after the application of Icon-Dry) Apply Icon-Infiltrant and let set for 3 min; remove excess material with microbrush and light-cure for 40 s; reapply and leave for 1 min; light-cure for 40 s; gently finish the surface using Astropol polishing system.
CPP-ACP containing paste - Tooth Mousse (GC Corporation, Tokyo, Japan)	Recaldent® CPP-ACP, glycerol, D-sorbitol, water, sodium carboxymethyl cellulose, propylene glycol, xylitol, sodium saccharine, phosphoric acid, guar gum, silicon dioxide, titanium dioxide, zinc oxide, ethyl paraben, butyl paraben, propyl paraben	Apply 0.01 g of CPP-ACP containing paste and leave in place for 5 min; wipe away with cotton pellet.

CPP-ACP = Casein phosphopeptide-amorphous calcium phosphate; TEGDMA = Triethylene glycol dimethacrylate

3. Regimen of pH cycle

Each group of the specimens - intact enamel (positive control), white spot lesion (negative control) and white spot lesions treated with either resin infiltration material or CPP-ACP paste - was subjected to a pH cycle to simulate the demineralizing-remineralizing cycle in the oral cavity. For each one-day cycle, the specimens were separately immersed in the demineralizing solution for 6 h and in the remineralizing solution for 18 h at 37 °C. During each change, the specimens were rinsed with DDW for 1 min, blot dried with absorbent paper and then immersed in the new respective solutions. The pH cycle process was repeatedly performed for a total of 8 weeks.

4. Evaluation of the color change of artificial white spot lesion

Twenty specimens of each group were employed in this part of the study. The color of each specimen was measured using a spectrophotometer (Spectro Shade Micro; MHT Italy S.p.A, Verona, Italy) at each designated time

point. Each specimen was rinsed, blot dried and the lesion color was measured immediately to prevent any influences from surface dehydration. The images were captured at the same position and analyzed using the software (SpectroShade software version 2.40; MHT Italy S.p.A). The values of *L** (difference in lightness), *a** (green-red coordinate) and *b** (blue-yellow coordinate) were recorded and the color change (Δ*E**) was calculated according to the following equation:

$$\Delta E^*_{(T_a - T_0)} = \sqrt{(L^*_{T_a} - L^*_{T_0})^2 + (a^*_{T_a} - a^*_{T_0})^2 + (b^*_{T_a} - b^*_{T_0})^2}$$

where Δ*E** is the color difference of artificial white spot lesion at each of the designated evaluating time points (T_a), i.e., immediate after treatment (T_1), 1 day (T_2), 1 week (T_3), 2 weeks (T_4), 4 weeks (T_5), 6 weeks (T_6), and 8 weeks (T_7) after treatment, to the time before treatment (T_0).

5. Measurement of surface hardness

One hundred and fifty specimens were used in this part - 35 each for white spot lesion groups with treatments and 40 each for positive and negative control groups. Prior to the test, baseline surface microhardness was assessed to verify that all specimens had Vickers hardness number (VHN) in the range of sound enamel (370-420 VHN).[2] The specimens were then assigned to each subgroup in balanced manner (4 groups and 8 time points, in which the same specimens were used at both T_0 and T_1 for the positive and negative controls). The formation of artificial white spot lesion, the application procedures of each treatment and the pH cycle regimen were performed as described previously. At each time point, the surface microhardness was determined using a fully automatic tester with Vickers diamond indenter under a microscope at ×500 magnification (Model ARS 9000; Future-Tech Corporation, Kanagawa, Japan). A load of 100 g was applied onto the surface for 15 s. Three indentations, 100 μm apart, were made and the average VHN was calculated to represent hardness value of each specimen.

6. Scanning electron microscopic (SEM) assessment

The representative specimens from each tested subgroup were sectioned vertically into halves through the middle of the area of interest. One half was used to observe the surface morphology from top view. Cut surface of another half was lightly polished with a series of increasingly finer grit-size of SiC abrasive papers, followed by a diamond suspension down to 1 μm and used to microscopically inspect the cross-sectional view of the surface. All specimens were rinsed with DDW, dried, mounted on aluminium stubs and sputter-coated with gold, then examined under a scanning electron microscope (JSM 6610 LV; JEOL, Tokyo, Japan).

7. Statistical analysis

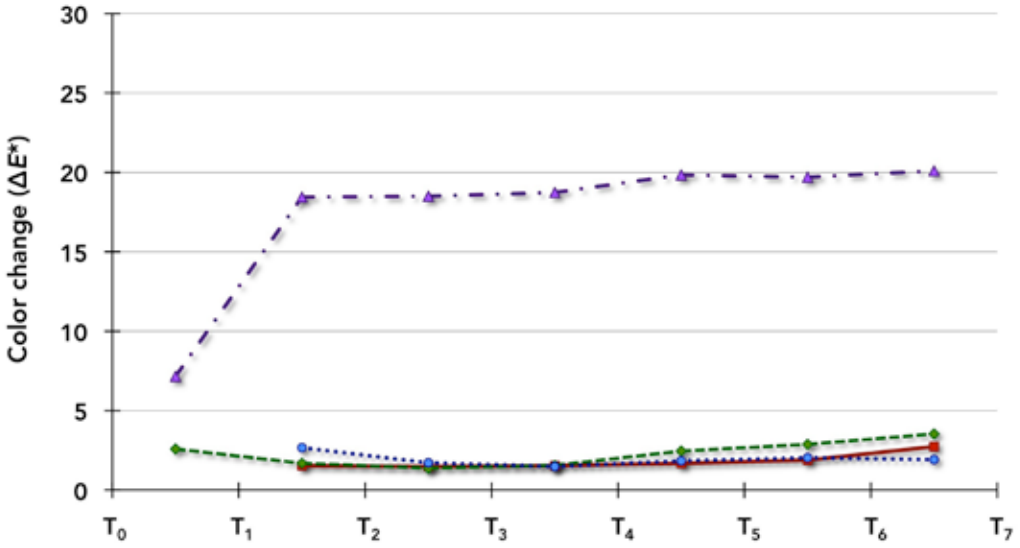
Collected data were analyzed using a statistical software system (SPSS 17.0; SPSS, Chicago, IL, USA). The central tendencies of Δ*E** and VHN values were calculated. Non-parametric Kruskal-Wallis, Wilcoxon signed-rank and Mann-Whitney *U* tests were carried out for analysis of the color difference within and between subgroups as the Shapiro-Wilk test did

not assume the normal distribution of all ΔE^* data. For hardness values, parametric One-way ANOVA with Dunnett's T3 multiple comparison test was used since Levene's method indicated heterogeneity among the variances. The significance level was set at $p < 0.05$.

Results

1. Color change of artificial white spot lesion

Line graph presenting medians, including the first and third quartiles, of ΔE^* values between each evaluating time to the time before treatment (T_0) is shown in Figure 2. Immediately after the caries infiltration treatment, color of the lesion changed significantly and was statistically different from other groups during the period of pH cycle ($p < 0.001$). For CPP-ACP application, similarity in ΔE^* values to those of the negative control was observed ($p \geq 0.057$), indicating no significant color change of white spot lesion after treatment.

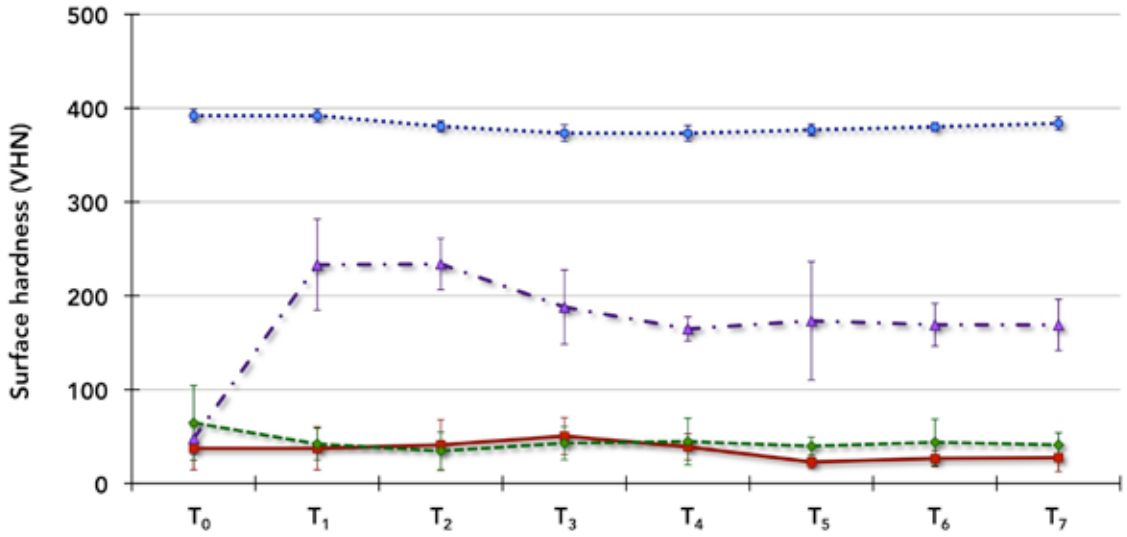


Group	$T_1 - T_0$	$T_2 - T_0$	$T_3 - T_0$	$T_4 - T_0$	$T_5 - T_0$	$T_6 - T_0$	$T_7 - T_0$
Caries infiltration	7.1 (5.2 / 13.0)	18.4^{a} (14.7 / 22.3)	18.5^{a} (15.0 / 22.1)	18.7 (16.2 / 22.8)	19.8^{b} (16.5 / 23.1)	19.7^{b} (16.7 / 23.2)	20.1 (16.5 / 23.8)
CPP-ACP	2.6^{c} (1.3 / 3.3)	1.7^{A} (1.2 / 3.6)	1.4^{B} (1.2 / 1.7)	1.5^{C} (1.1 / 2.5)	$2.4^{c,D}$ (1.3 / 3.7)	2.9 (2.1 / 4.6)	3.5^{F} (2.9 / 4.7)
Positive control	n/a	2.7 (2.4 / 3.3)	$1.7^{d,B}$ (1.2 / 2.8)	1.5^{C} (1.1 / 1.8)	$1.8^{d,D}$ (1.3 / 2.3)	$2.0^{e,E}$ (1.4 / 3.1)	$1.9^{e,G}$ (1.3 / 3.4)
Negative control	n/a	$1.5^{f,g,A}$ (1.2 / 2.3)	$1.5^{h,B}$ (0.9 / 2.6)	$1.5^{f,h,C}$ (1.0 / 2.3)	$1.7^{g,D}$ (1.2 / 2.6)	1.9^{E} (1.5 / 3.3)	$2.7^{F,G}$ (1.9 / 4.0)

Figure 2 *Color differences [median ΔE^* (25^{th}/75^{th} percentiles)] of artificial white spot lesion at each designated evaluating time point to the time before treatment (T_0). Same lowercase superscript letters indicate no statistically significant difference between subgroups in the same row ($p > 0.05$). Same uppercase superscript letters indicate no statistically significant difference between subgroups in the same column ($p > 0.05$)*

2. Surface hardness

Figure 3 depicts the line graph displaying means and standard deviations of VHN for each subgroup at different evaluating time points. Baseline enamel surface hardness was noted at 393.8 ± 10.2 VHN. After artificial white spot lesion formation, VHN decreased significantly to approximately 37-64 VHN ($p \leq 0.002$). Throughout the pH cycle process, daily application of CPP-ACP containing paste did not improve the surface hardness ($p \geq 0.999$), which also was statistically similar to that of the white spot lesion with no treatment at every time points ($p \geq 0.596$). On the other hand, a single application of resin infiltration material immediately increased VHN significantly ($p = 0.026$) and remained stable up to 8 weeks of pH cycle ($p \geq 0.137$); however, such values were not comparable to those of intact enamel ($p < 0.001$).



Group	Before Tx	After Tx	1 day	1 week	2 weeks	4 weeks	6 weeks	8 weeks
Positive control	392.01[a]		380.56[a]	373.38[a]	373.18[a]	376.83[a]	380.05[a]	383.80[a]
Caries infiltration	47.70[c]	233.17[b]	233.84[b]	187.98[b]	164.74[b]	173.27[b]	169.09[b]	168.92[b]
CPP-ACP	64.50[c]	42.04[c]	34.59[c]	42.90[c]	44.65[c]	39.78[c]	43.89[c]	41.08[c]
Negative control	37.36[c]		41.02[c]	50.44[c]	39.01[c]	22.85[c]	26.59[c]	27.45[c]

Figure 3 *Means and standard deviations of enamel surface hardness (VHN) of each subgroup at different evaluating time points. Same superscript letters show no statistically significant difference between subgroups ($p > 0.05$)*

3. SEM assessment

Figure 4 represents the surface characteristics and cross-sectional microstructures of the enamel surfaces at immediately after each treatment (T_1). Smooth and homogenous surfaces were discerned for the positive control group (Fig. 4A and 4B). Characteristics of white spot lesion, i.e., surface erosion and microcavities (Fig. 4C), including intact surface layer and subsurface demineralization (Fig. 4D) could be

clearly observed for the negative control group with lesion depth of 30-40 µm. After immediate application of resin infiltration material, typical keyhole appearance of demineralized enamel surface was not apparent and seemed to be covered with resin material (Fig. 4E). The cross-sectional view of white spot lesion after caries infiltration also showed disappearance of subsurface lesion (Fig. 4F), which was similar to what has been detected in the group without lesion formation. For the CPP-ACP-treated group, similar features as compared to the untreated enamel white spot lesion were shown (Fig. 4G and 4H).

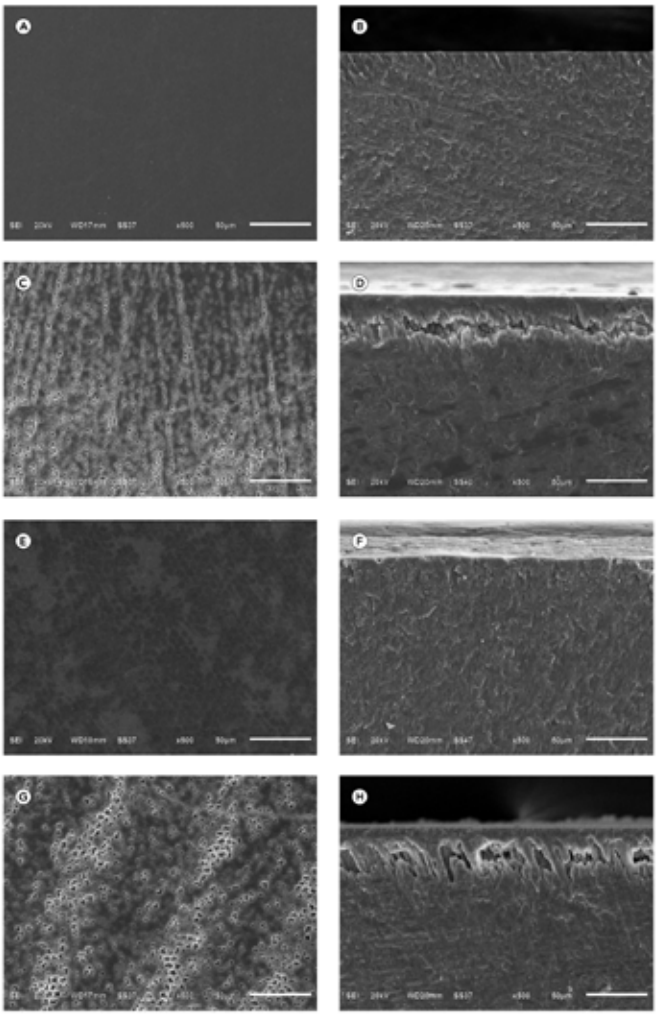


Figure 4 *Representative SEM micrographs (×500 magnification) of the surface characteristics (left) and cross-sectional microstructures (right) of enamel surfaces at immediately after each treatment (T_1). (A and B) Positive control group; (C and D) Artificial white spot lesion without treatment (Negative control group); (E and F) Artificial white spot lesion treated with resin infiltration material; (G and H) Artificial white spot lesion treated with CPP-ACP containing paste*

Microscopic characteristics of enamel, both top and cross-sectional views, after pH cycle for 8 weeks (T_7) are shown in Figure 5. In general, all subgroups showed similar enamel morphologies to those observed immediately after treatments. Enamel surfaces of both positive control and negative control groups seemed to not significantly alter after 8-week period of pH cycle (Fig. 5A-5D). Coverage of resin material to the artificial white spot lesions was still intact (Fig. 5E and 5F). For CPP-ACP application, similar features of both views of the lesions compared to those prior to the treatment were observed (Fig. 5G and 5H), in spite of the daily application of Tooth Mousse throughout the pH cycle period.

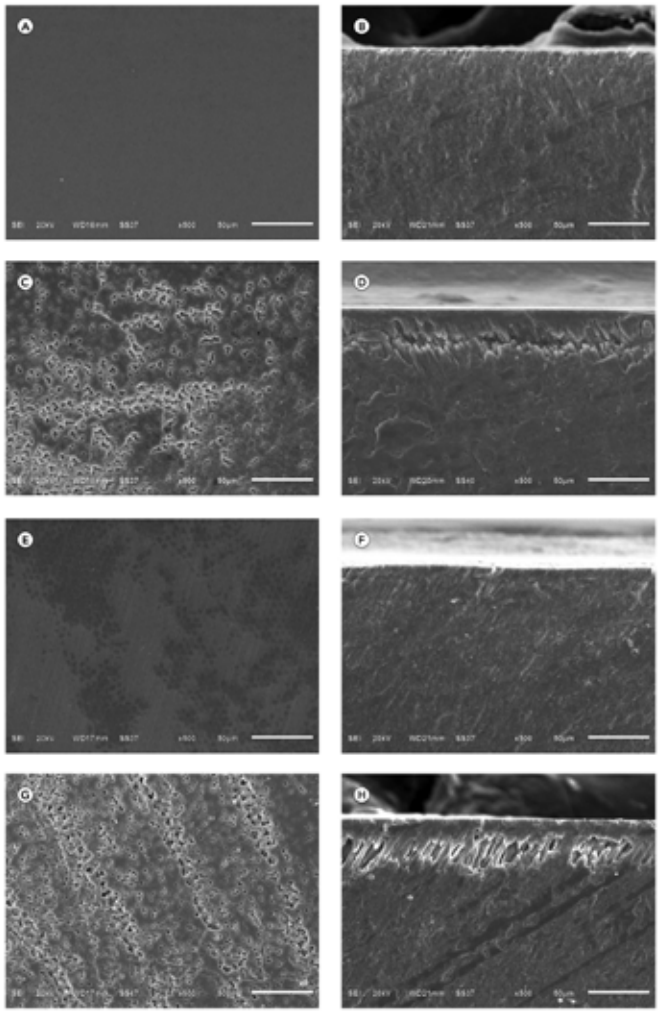


Figure 5 *Representative SEM micrographs (×500 magnification) of the surface characteristics (left) and cross-sectional microstructures (right) of enamel surfaces after pH cycle for 8 weeks (T_7). (A and B) Positive control group; (C and D) Artificial white spot lesion without treatment (Negative control group); (E and F) Artificial white spot lesion treated with resin infiltration material; (G and H) Artificial white spot lesion treated with CPP-ACP containing paste*

Discussion

Typical characteristics of initial non-cavitated enamel caries are a whitish opaque appearance with loss of luster and micro-porosities at the surface, which extend deeper into the enamel as subsurface lesion.[2] Such features not only affect the patient's look, especially at the esthetically relevant teeth, but also facilitate the caries progression, even though the less acid-soluble surface zone is presented.[2,4] Currently, there are several approaches aimed to treat and prevent the caries process. The noninvasive and microinvasive measures are of interest in everyday dental practice as to promote maximum conservation of tooth structure and defer the operative intervention as long as possible. Casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) remineralizing agent and the caries infiltration technique are two strategies, which claim to prevent enamel demineralization (or further promote remineralization) and improve the teeth to their natural color.[5-11] The results of the current investigation, however, clearly demonstrated that both approaches provided different results with positive outcomes derived from the caries infiltration. Only single application of resin material to the artificial enamel caries immediately enhanced the surface hardness and improved color of the lesion compared with the daily use of CPP-ACP containing paste. Throughout the period of pH cycle for 8 weeks, both parameters were also maintained, wherein those after daily application of CPP-ACP seemed not to be ameliorated. The null hypotheses that there would be a similarity in the color change and in the surface hardness of enamel white spot lesions between the two treatments, therefore, were rejected.

The general principle behind caries infiltration is to penetrate the lesions with the low-viscosity, light-polymerizable resin material.[9] After the surface layer of enamel caries has been etched with hydrochloric acid gel (Icon-Etch) and is fully dried with ethanol (Icon-Dry), the TEGDMA-based resin (Icon-Infiltrant) is able to penetrate the lesions up to a few hundred micrometers.[9,13] Following photopolymerization, the resin seals the lesions externally and internally, as clearly observed from both views of SEM micrographs. Low-viscosity resin material then formed the diffusion barrier on and within the caries, and re-hardened the lesions by increasing the enamel surface hardness, confirming the results of previous investigations.[14,15] However, the structure of TEGDMA molecule has low molecular weight and is highly flexible.[13,16] Mechanical property of resin-infiltrated lesion would, therefore, not be comparable to that of the intact enamel. Nevertheless, infiltration of the lesions could prevent further acid demineralization. Stability of enamel surface hardness was detected, at least during the 8-week period of pH cycle. It would hence affirm the efficacy of caries infiltration to arrest the progression of caries, as supported by a number of laboratory and clinical studies.[10,14,15] Slight decrease of hardness value, but not statistically different, after a week of pH cycle might be due to the water softening of TEGDMA polymer networks.[16]

An alternative avenue to improve the enamel lesion hardness is an enhancement of calcium and phosphate delivery, with the application of CPP-ACP containing paste. High concentrations of calcium and phosphate ions at the tooth surface after CPP-ACP treatment are freely bioavailable to diffuse down the concentration gradients into enamel subsurface lesions, thereby promoting remineralization by recrystallization of existing crystal remnants.[8] However, throughout the period of pH cycle in this study, the surface of enamel carious lesion could not be strengthened despite the daily treatment of CPP-ACP. Typical characteristics of white spot lesions were still detected micro-scopically, which resemble those observed in the negative control group. These findings are not in agreement with the results of previous literature.[17] The typical intact or pseudo-intact surface layer of initial enamel caries, which was created to mimic the natural carious lesion, might have blocked the diffusion of free calcium and phosphate ions into the subsurface body of the lesion[4,18], especially if the CPP-ACP has been applied solely in the form of cream, rather than in slurry preparation as used in the former observations.[8,17,19] Furthermore, this experimental study provided the absence of dental plaque, which is essential for the action of CPP-ACP molecules, no reservoir for a high concentration of both ions was available. It has been previously claimed that sole use of CPP-ACP containing paste in the *in vitro* investigation might not be sufficient to demonstrate the remineralizing potential of CPP-ACP.[17]

In addition to protecting the enamel lesions from acid challenge, esthetic improvement has also been a concern for the treatments of white spot appearance.[5-7] It is clearly shown from the current outcomes that, immediately after the caries infiltration, the color of the lesion altered significantly. Lightness reduction was observed (data not shown) and the color difference could be noticed visually as median ΔE^* value exceeded that considering clinically perceptible at higher than 3.3. Esthetic appearance hence can be significantly enhanced within a relatively short period of time. This improvement would confirm the masking ability of caries infiltration, based on the modification of light refraction within the enamel lesion.[5,9,11] Greatly significant difference in color change after 1 day of pH cycle, however, were beyond expectation. It might be assumed that the resin-infiltrated enamel lesions would show improved appearance and better color mask after 1 day following treatment.[11] Or else, it could possibly owing to some interactions between the resin material with demineralizing and remineralizing solutions during pH cycle regimen, even though the surface finishing was performed carefully to remove the outermost oxygen inhibition layer. Due to the hydrophilic nature of TEGDMA, impact on water sorption and discoloration during storage in aqueous solutions might be anticipated.[16,20] Clinically, final polishing after polymerization of the infiltrant material is therefore, important to avoid discoloration of superficial unpolymerized resin components.

On the other hand, CPP-ACP containing paste showed no significant influence on the color improvement of enamel white spot lesions.

Only slight difference in color change could be detected for the lesions in spite of the daily application of Tooth Mousse. Such difference could, however, not be noticeable ($\Delta E^* \leq 3.3$), meaning that the white spot appearance was not significantly improved. The result is in contrast to what have been found in the previous clinical studies.[6,7] As mentioned above, the sole use of CPP-ACP containing paste showed insufficient effect on enamel remineralization in the laboratory study.[17] Color of the white spot lesions, therefore, may also not be recovered for similar reasons. The observation period of this study, however, was limited to 8 weeks with pH cycle protocols. Besides, the current regimen of acid challenge tends to favor the remineralizing model, which might influence the formation of intact surface layer to prevent the diffusion of calcium and phosphate ions.[21] It is important to take these factors into account because the thickness of the outermost zone of enamel caries possibly affects the subsequent demineralization and remineralization.[4,18]

Within the limitations of this experimental study, caries infiltration could stabilize the artificial enamel lesions by infiltrating the subsurface porosities of the defects and increasing the surface hardness immediately after treatment. Enhanced mechanical property of the resin-infiltrated lesions was maintained throughout the 8-week period of acid challenge. Color improvement of the white spot appearance was also detected following the single application of resin infiltration material. Daily treatment with CPP-ACP containing paste, in contrast, was unable to regain the surface microhardness as well as failed to improve whitish discoloration of the initial enamel caries during pH cycle process for 8 weeks. Future investigations are needed to further elucidate these two minimal invasive treatments to enamel carious lesions in various conditions of laboratory models and most importantly in the clinical situations.

Conclusion

Caries infiltration with resin material is shown to immediately improve the surface hardness and esthetic appearance of artificial white spot lesions *in vitro*. On the other hand, CPP-ACP remineralizing paste is unsuccessful in the enhancement of physical and mechanical characteristics of initial enamel caries during 8 weeks of demineralizing-remineralizing regimen.

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