## Comparative Effect of Casein Phosphopeptide Amorphous Calcium Phosphate and Resin Infiltrant on Brown Spot Color Parameters after Using 20% Carbamide Peroxide

#### Farzaneh Shirani<sup>1</sup>, Hale Khalili<sup>2</sup>, Marjan Mehravaran<sup>3</sup><sup>™</sup>.

<sup>1</sup>Associate Professor, Dental Material Research Center, Department of Restorative Dentistry, School of Dentistry, Isfahan University of Medical Sciences, Isfahan, Iran

<sup>2</sup>Assistant Professor, Department of Color Science and Technology, School of Textile, Isfahan University of Technology, Isfahan, Iran
<sup>3</sup>Dental Students, Students Research Committee, School of Dentistry, Isfahan University of Medical Sciences, Isfahan, Iran

#### Abstract

**Background and Aim:** Tooth-colored restorations are the current strategy for treatment of aesthetically unpleasant brown spots (BSs). Considering the importance of conservative dentistry, this study aimed to assess the effect of resin infiltrant (RI) and casein phosphopeptide amorphous calcium phosphate (CPP-ACP) after the application of 20% carbamide peroxide (CP) on BS color parameters in the esthetic zone.

**Materials and Methods:** This experimental study was conducted on 70 extracted sound human teeth with BSs. The specimens were first subjected to the application of 20% CP and were then randomly divided into two groups of CPP-ACP and RI. Specimens were subjected to colorimetry before the intervention, after bleaching and after the application of RI or CPP-ACP using a spectrophotometer. Data were statistically analyzed using SPSS version 20, paired t-test, independent t-test and repeated measures ANOVA with p<0.05 level of significance.

**Results:** CP (20%) significantly increased the L\* and b\* and decreased the a\* parameter (p<0.001). CPP-ACP caused no change in a\* and b\* parameters of BS (p>0.05); however, the L\* significantly increased (p<0.001). RI significantly decreased the a\* parameter (p<0.001); but the reduction in L\* and increase in b\* were not significant (p>0.05). Comparison of the two groups revealed that only the color change ( $\Delta E$ ) during the bleaching procedure was significant (p<0.001).  $\Delta E$  of BS and the adjacent enamel did not change significantly in any step of the procedure in the two groups (p>0.05).

**Conclusion:** Based on the results, aesthetically unpleasant BSs on teeth may be treated using a bleaching agent along with RI or CPP-ACP. RI seems to have a superior efficacy in increasing the translucency compared to CPP-ACP.

**Key Words:** Spectrophotometry, Tooth discoloration, Esthetics, Dental, Carbamide peroxide, Casein phosphopeptide amorphous calcium phosphate

Received: 11 Aug 2014 Accepted: 24 Nov 2014

Corresponding author:

Committee, School of Dentistry, Isfahan University of Medical Sciences, Isfahan, Iran

Marjan mehr68@yahoo.com

Dental

Research

M. Mehravaran,

Students, Students

Journal of Islamic Dental Association of IRAN (JIDAI) Winter 2015 ;27, (1)

### Introduction

BS is a non-cavitated carious lesion, where subsurface demineralization has simultaneously occurred with intrinsic and extrinsic staining causing changes in the enamel appearance and its visual properties. These changes include reduction in enamel translucency and formation of a BS on the enamel surface [1]. Such discoloration occurs

Winter 2015; Vol. 27, No. 1

due to the entrapment of colored organic substances or metallic ions in the enamel structure. Such remineralized, discolored spots are more resistant to caries compared to the adjacent enamel; but are esthetically unpleasant. If located in the esthetic zone, an esthetic correction may be required for the BSs. Bleaching seems to be a more conservative treatment modality for color correction of BS lesions compared to restorations. Bleaching is defined as whitening/ lightening of tooth shade. The mechanism of action of bleaching with peroxide is via its penetration and diffusion into the enamel structure for the purpose of oxidation and lightening of the discoloration especially in dentin [2].

Evidence shows that use of remineralizing agents along with bleaching agents creates a more uniform appearance in the white spot (WS) [3].

Application of a large amount of calcified agents results in remineralization of demineralized areas [1]. CPP-ACP is a nano-complex containing calcium, phosphate, and hydroxide ions stabilized with CPP. The calcium, phosphate and hydroxide ions are released on the tooth surface and remineralize the demineralized surface. CPP-ACP remineralizes the WS lesions and prevents the progression of caries. By mineralizing the tooth structure. **CPP-ACP** decreases tooth hypersensitivity and improves esthetics. translucency and luminosity of the enamel [4].

Application of remineralizing agents like CPP-ACP or fluoride to recently bleached teeth decreases the risk of stain absorption [5]. CPP-ACP is used for treatment of WS lesions [6] and improves the translucency and brightness of teeth [7,8].

Torres et al, and White et al. reported that remineralizing agents mostly had limited potential for treatment of WS lesions. These lesions remained clinically and radiographically visible after the application of remineralizing agents and thus, these agents have limited applications for treatment of WS lesions in the clinical setting [9, 10]. However, the efficacy of RI for masking the WS lesions has been confirmed [11]. The resin fills the inter-crystalline gaps and prevents the progression of caries. RI application is a definite treatment and a preventive measure for non-cavitated caries [9, 11]. Spectrophotometry (Shade Pilot, DeguDent; Hanau, Germany) is a type of digital photography that measures the tooth color parameters on its entire surface [12, 13] and reports the results in the CIE lab system.

The International Commission on Illumination - abbreviated as CIE  $L^*a^*b^*$  system, defines color in the form of three parameters of  $L^*$ ,  $a^*$  and  $b^*$ .

The overall color change is calculated using the equation below:

# $\Delta E = \sqrt{\left[ (\Delta a)^2 + (\Delta L)^2 + (\Delta b)^2 \right]}$

Where L\* indicates lightness and its value changes from 0 to 100 and a\* and b\* are both numerical parameters for hue and chroma; a\* indicates greenness to redness of color and b\* indicates blueness to yellowness of color, changing from-120 to+120 [2, 14].

The current approach to improve the unesthetic appearance of BS lesions is far from being conservative. Considering the available literature on the applications of CPP-ACP and RI, this study aimed to assess the effect of application of RI and CPP-ACP after using 20% CP on BS parameters and compare their effect on the visual appearance of these lesions in the esthetic zone.

### **Materials and Methods**

This in vitro experimental study was conducted on 70 extracted sound human teeth with smooth surfaces and no caries or previous restorations. All teeth had BS lesions due to previous caries. The teeth had been collected from several dental clinics. Soft tissue residues and debris were removed using prophylactic pumice paste and rubber cup in order to be able to accurately observe the tooth color. Specimens were immersed in 0.2% thymol solution for one week and were then kept in water until the experiment. The teeth were mounted in prefabricated plastic dental arches and digital X ray radiographs (CS2200 intraoral X ray system, Carestream Dental, Stuttgart, Germany) were obtained from them to differentiate carious teeth from sound and remineralized teeth. The teeth were mounted in an acrylic cast and digital bitewing radiographs (Carestream Kodak RVG 6200 intraoral sensors, Stuttgart, Germany) were obtained to exclude carious teeth. Using bitewing radiography, degree of mineralization in BS lesions was assessed and in case of presence of a radiolucency exceeding half the distance from the outer surface of dentinoenamel junction to pulp at the respective location (BS lesion), the tooth was excluded from the study. Teeth with sound structure at the BS area were included (n=70).

The teeth were prepared for color analysis by spectrophotometry (Shade Pilot<sup>TM</sup>, Degu Dent GmbH, Italy). The roots were cut in order for the teeth to fit in the spectrophotometer and prevent entry of external light into the device. Each specimen was mounted in dental red wax with dimensions similar to those of the device orifice. The tooth shape was recorded in heat-softened wax to make color measurements reproducible and decrease errors in the following steps of the experiment. By doing so, changes in the position of teeth in the wax and also changes in the radiation angle were prevented and minimized.

The spectrophotometer was calibrated for each tooth. When the green light appeared on the display monitor, image was captured by pressing the respective button. Next, each image was compared with its duplicate using the comparison feature of the device and the differences in color parameters were reported using the CIE lab system.

Each specimen was wrapped in dental red wax (Bilkim Chemical Company, Izmir, Turkey) with 2mm thickness and a window corresponding to the size of BS was created to limit the application of 20% CP (Tooth whitening gel PF, Opalescence Products Inc., South Jordan, Utah, USA) to the BS lesion only and prevent its leakage to the adjacent areas. The tooth surface at the window was dried and the bleaching gel was applied in 2mm thickness to the area. The tooth was then wrapped in a plastic wrap to ensure the contact of gel with the BS; 20% carbamide peroxide was applied to the area 8 hours a day for 14 days and the specimens were stored in an incubator (01154, Behdad Digital Incubator, Tehran, Iran) at 37°C and 100% humidity [15]. Color parameters were measured again by a spectrophotometer and the L\*, a\* and b\* parameters of the BS and the adjacent sound enamel were measured. The teeth were then randomly divided into two groups. Group one specimens were subjected to CPP-ACP (Tooth Mousse, GC Corp., Tokyo, Japan) and group two samples were subjected to RI (Icon ®, DMG, Hamburg, Germany). Random allocation was done by choosing random numbers of specimens. Odd numbers were subjected to the application of CPP-ACP and even numbers were subjected to the application of RI.

In CPP-ACP group (group A), the teeth were subjected to pH cycling to simulate oral conditions. Despite in-vitro limitations, pH cycling highly simulates the oral clinical setting [15].

The demineralizing solution contained 2.2 m mol calcium chloride, 1mol potassium hydroxide, 2.2mmol potassium di-hydrogen phosphate and 0.05mol acetic acid. Acetic acid was used to adjust the pH to 4. The remineralizing agent contained 1.5 m mol calcium chloride, 0.15 mol potassium chloride and 0.9 m mol sodium di-hydrogen phosphate. Acetic acid was used to adjust the pH to 7. In this cycle, specimens were immersed in demineralizing solution twice daily for 3 hours each time. In-between the two immersions, specimens were placed in remineralizing solution for 2 hours. Before and after immersion of specimens in the demineralizing solution, they were subjected to application of CPP-ACP for 15 minutes to the respective area and eventually, the teeth were immersed in remineralizing agent for 14 hours until the next phase. After each phase, the teeth were rinsed with water and dried with gauze [16, 17].

In RI group (group B), RI kit containing three components was used according to the manufacturer's instructions. The specimens were first cleaned and the etchant (Icon-Etch, Icon ®, DMG, Hamburg, Germany) was applied for 2 minutes to the whitened brown spot and was then rinsed for 30 seconds and dried. The drying agent (Icon-Dry, Icon ®, DMG, Hamburg, Germany) was then applied to the respective area for 30 seconds and dried. RI (Icon-Infiltrant, Icon ®, DMG, Hamburg, Germany) was then applied to the area for 3 minutes. Excess material was removed and light curing was done for 40 seconds (Blue phase® style LED curing light, Ivoclar Vivadent,

Mississauga, Canada). Resin was re-applied to the area for one minute and cured for 40 seconds [18].

Colorimetry was done again in the two groups. The L\*, a\* and b\* parameters were measured again for the BS and the adjacent enamel and  $\Delta E$  of the BS was calculated and compared at the three time points (baseline, after bleaching, and after using CPP-ACP or RI). Also,  $\Delta E$  of the BS and the adjacent enamel was calculated in each tooth and the mean value was obtained. The mean  $\Delta E$  and

Winter 2015; Vol. 27, No. 1

the mean value for each color parameter were compared in the two groups of RI and CPP-ACP alone and in combination with CP and the efficacy of CPP-ACP and RI was compared.

Data were analyzed using SPSS version 20 and paired t-test was applied to compare changes in color parameters during each phase separately in groups A and B. Repeated measures ANOVA was used to compare changes in color parameters at different time points i.e. before the intervention, after bleaching and after the application of RI or CPP-ACP in groups A and B together and separately. Independent t-test was used to compare the effects of CPP-ACP and RI on color parameters at different time points. p<0.05 was considered statistically significant.

### Results

The mean and standard deviation (SD) of  $a^*$ ,  $b^*$ and  $L^*$  color parameters of BS before the intervention (phase 1), after bleaching (phase 2) and after the application of RI or CPP-ACP (phase 3) in the two groups of RI and CPP-ACP are shown in Table 1. The mean and SD of  $a^*$ ,  $b^*$  and  $L^*$  color parameters of adjacent sound enamel before the intervention (phase 1), after bleaching (phase 2) and after the application of RI or CPP-ACP (phase 3) in the two groups of RI and CPP-ACP are shown in Table 2.

Paired t-test revealed that the mean L\* and a\* parameters of BS increased and decreased, respectively at phase 2 compared to the baseline values at phase 1 (p<0.001). The mean b\* parameter of BS significantly increased in phase 2 compared to its baseline value in phase 1 (p=0.03). The changes in a\* and b\* parameters of BS at the third phase were not significant for CPP-ACP group (p>0.05). But, L\* parameter significantly increased (p<0.001). In RI group, a\* significantly decreased (p<0.001); but changes in L\* and b\* due to the application of RI were not significant (p>0.05) (Table 1).

Paired t-test in sound enamel adjacent to the BS showed that during the process of bleaching, the mean L\* and b\* parameters significantly increased while a\* significantly decreased (p<0.001).

In CPP-ACP group, L\* significantly increased (p<0.001); but in RI group, changes in L\* parameter were not significant (p>0.05). Changes

in the mean  $a^*$  and  $b^*$  parameters in the two groups of A and B during the third phase were not statistically significant (p>0.05) (Table 2).

Comparison of the mean and SD of  $\Delta E$  of the BS and the adjacent enamel before the intervention, after bleaching and after the application of RI and CPP-ACP in the two groups by repeated measures ANOVA is shown in Table 3.

The between subjects factors showed that the interaction effect was not significant (p=0.57). In general, no significant difference was found between the two groups (p=0.27) and comparisons showed that data in the two groups of A and B at the three time points (at the beginning and at the end of each phase) were not significantly different (p=0.18). Comparison of the mean and SD of  $\Delta E$  of the BS between the two groups of A and B before the intervention, after bleaching and after the application of RI or CPP-ACP with independent t-test along with the P values are shown in Table 4.

### Discussion

Considering the importance of smile esthetics, BSs on teeth surfaces in the esthetic zone can be extremely annoying for patients. The currently recommended treatment approach for these lesions, based on textbooks, is tooth preparation and application of tooth-colored restorative materials. However, this treatment results in loss of sound tooth structure; which contradicts the principles of conservative dentistry. Thus, attempts were made to find a conservative treatment modality for BS lesions. In the current study, clinical examination and radiography were used for selection of specimens to simulate the in vivo conditions as much as possible in order to be able to generalize the results to the clinical setting.

Bitewing radiography is a valuable tool for detection of proximal caries before they manifest clinically [19].

Thus, this method was used for detection of caries. This study aimed to compare the effect of RI and CPP-ACP after the application of 20% CP on color parameters of BSs in the esthetic zone. Based on the results, application of CP along with CPP-ACP or RI was more effective than the application of each of them alone. The appearance of BS improved with the application of these agents; however, it did not vanish completely. Color and appearance of teeth are complex concepts and many factors such as the lighting of the environment, translucency and opacity of tooth, reflection and transmission of light, visual parameters of the observer and the brain's processing capabilities affect the overall perception of tooth color. Several techniques such as the use of shade guides, spectroradiometry, spectrophotometry, digital camera and color analysis by a computer are used for color assessment [20].

Spectrophotometer is the most reliable tool for color assessment both in-vivo and in-vitro [21].

Table 1. The mean and SD of L*, a* and b* parameters of B	3S before the intervention (phase 1), after bleaching
(phase 2) and after the application of RI and CPP-ACP (	phase 3) in the two groups of RI and CPP-ACP

С		<b>CPP-ACE</b>			RI	
Colorimetry phase	b*	a*	L*	b*	a*	L*
Phase 1	30.7 <sup>(A)</sup>	8.9 <sup>(C)</sup>	61.9 <sup>(E)</sup>	31.7 <sup>(G)</sup>	8.5 <sup>(J)</sup>	63.2 <sup>(M)</sup>
r nase 1	$\pm 5.5$	±3.2	±6.3	±5.4	±3.9	±5.7
Dhaga 7	39.1 <sup>(B)</sup>	-3.3 <sup>(D)</sup>	74.0 <sup>(F)</sup>	41.4 <sup>(H)</sup>	-4.5 <sup>(K)</sup>	74.2 <sup>(N)</sup>
Phase 2	±16.0	±7.4	±3.9	±16.6	±7.2	±6.3
Phase 3	34.5 <sup>(B)</sup>	-4.0 <sup>(D)</sup>	76.6 <sup>(0)</sup>	44.5 <sup>(H)</sup>	-8.6 <sup>(L)</sup>	72.5 <sup>(N)</sup>
	$\pm 16.0$	±7.4	$\pm 4.8$	±17.2	$\pm 8.5$	$\pm 6.0$

\*CPP-ACP: Casein phosphopeptide amorphous calcium phosphate

\*In each column, similar letters indicate non-significant (p>0.05) and dissimilar letters indicate significant differences (p<0.05)

<b>Table 2.</b> The mean and SD of b*, a* and L* parameters of the enamel adjacent to the BS before the intervention (phase 1),
after bleaching (phase 2) and after the application of RI and CPP-ACP (phase 3) in the two groups of RI and CPP-ACP

С		CPP-ACP	)		RI	
Colorimetry phase	b*	<b>a</b> *	L*	b*	<b>a</b> *	L*
Dhasa 1	25.7 <sup>(A)</sup>	5.5 <sup>(C)</sup>	67.8 <sup>(E)</sup>	32.4 <sup>(H)</sup>	3.2 <sup>(J)</sup>	68.0 <sup>(L)</sup>
Phase 1	±12.0	$\pm 4.0$	±5.5	±15.5	±6.7	$\pm 4.8$
Phase 2	43.0 <sup>(B)</sup>	-8.3 <sup>(D)</sup>	72.5 <sup>(F)</sup>	43.3 <sup>(I)</sup>	-11.6 <sup>(K)</sup>	71.0 <sup>(M)</sup>
Flase 2	±15.7	$\pm 7.8$	$\pm 6.0$	$\pm 14.8$	±9.4	±8.7
Dhaga 2	36.1 <sup>(B)</sup>	-8.6 <sup>(D)</sup>	75.8 <sup>(G)</sup>	42.8 <sup>(I)</sup>	-15.7 <sup>(K)</sup>	68.3 <sup>(M)</sup>
Phase 3	$\pm 17.0$	±9.7	±6.1	±12.6	$\pm 10.0$	±9.1

\*CPP-ACP: Casein phosphopeptide amorphous calcium phosphate

\*In each column, similar letters indicate non-significant (p>0.05) and dissimilar letters indicate significant differences (p<0.05)

Shade  $Pilot^{TM}$  (by DeguDent) spectrophotometer has higher objective value than other spectrophotometers [12, 13]. The results of color assessment by this tool can be reported using different systems. The most commonly used systems to describe color are the Munsell system and the CIE lab system. The CIE lab system is more commonly used for perception and assessment of tooth color because it is intended to be a perceptually uniform color space [21]. The CIE lab space is among the most famous color systems. This system highly resembles the human vision system and is extensively used to compare two different colors [22]. In the CIE lab system, L\* indicates lightness: 0 indicates complete blackness and 100 indicates whiteness. Thus, by increasing the L\* parameter the color becomes lighter; a\* positive indicates redness and a\* negative indicates greenness; b\* positive shows yellow and b\* negative indicates blue color. Such three-dimensional color space highly resembles the human vision [18].

In this study, 20% CP, CPP-ACP and RI were used in accord with similar studies. Following the application of CPP-ACP, considering its saturated state, the concentration of free calcium ions increases over time as 95% of ions are released within 15 minutes following the application of CPP-ACP to the tooth surface. Thus, following each application, CPP-ACP remained at the respective area for 15 minutes in our study [23]. After the application of CP, L\* parameter of BS and the adjacent enamel increased and the tooth became significantly lighter. In studies by Singh et al, Manton et al and Kim et al, the bleaching agent increased the lightness ( $\uparrow L^*$ ), which is in accord with our study results [4, 5, 15]. Due to the application of bleaching agent, the BS turns into a WS. The WS has a decreased translucency and confers an opaque appearance to the tooth surface. This is because by the loss of minerals and increased roughness and surface porosities, light emission increases and decalcified enamel shows a chalky appearance [18].

During the third phase, in the CPP-ACP group, specimens became significantly lighter. In a study by Kim et al, application of CPP-ACP increased the lightness of specimens [15]. In a study by Manton et al, application of this material improved

the opaque, non-vital appearance of teeth after bleaching. This appearance was due to the significant reduction of b\*, significant increase of L\* and decreased brightness and translucency of tooth that occurred during the process of tooth bleaching [5]. These results confirm the changes in L\* parameter in group A specimens in the current study. However, further whitening of the teeth after bleaching and also following the application of CPP-ACP conferred a lighter-than-normal appearance to teeth in our study. In the current study, the teeth in RI group became darker during the phase 3; however, these changes were not significant. In a study by Bak et al, L\* parameter of the WS phase was the highest and decreased following the application of RI. Its value further decreased as the application frequency increased [18]. The results of Bak et al. confirmed our findings. The dark, opaque appearance of teeth in group B was attributed to the fact that the RI had a darker color than the teeth, and decreased light scattering.

In a study by Xiaoyi et al. RI had greater efficacy than fluoride in masking the WS appearance; however, the efficacy of RI for decreasing the increased L\* parameter due to demineralization and masking of the WS had a significant correlation with the degree of demineralization [24]. Thus, insignificant changes in L\* parameter in the current study may be due to the heterogeneity of specimens in terms of the degree of demineralization.

Comparison of the phases 1 and 3 in group A shows that application of bleaching gel in conjunction with CPP-ACP leads to greater tooth lightening compared to the single application of each of them and results in excessively white and artificial appearance of teeth. Therefore, application of whitening gel with RI is preferred because it confers a more natural appearance to teeth.After the application of whitening agent, a\* parameter of BS and the adjacent enamel significantly decreased and the hue significantly shifted toward green (from red). In a study by Kim et al, application of whitening agent shifted the tooth hue towards green; which confirms the current study results [15].

During the third phase, a\* parameter in the CPP-ACP group decreased, but not significantly.

Table 3. The mean and SD of $\Delta E$ of the BS and adjacent enamel before the interventio	n (phase 1), after bleaching
(phase 2) and after the application of RI and CPP-ACP (phase 3) in the two group	s of RI and CPP-ACP

ΔΕ	CPP-ACP	RI
The mean $\Delta E$ of BS and the adjacent	<sup>(A)</sup> 13.1	<sup>(B)</sup> 15.7
enamel before the intervention	$\pm 6.7$	±9.4
The mean $\Delta E$ of BS and the adjacent	<sup>(A)</sup> 16.1	<sup>(B)</sup> 16.9
enamel after bleaching	$\pm 10.1$	±9.7
The mean $\Delta E$ of BS and the adjacent	<sup>(A)</sup> 13.7	<sup>(B)</sup> 17.0
enamel after the application of remineralizing agent and RI	±9.8	±9.5
P value	0.28	0.77

\*CPP-ACP: Casein phosphopeptide amorphous calcium phosphate

\*In each column, similar letters indicate non-significant (p>0.05) and dissimilar letters indicate significant differences (p<0.05)

**Table 4.** The mean and SD of  $\Delta E$  of BS before the intervention (phase 1), after bleaching (phase 2) and after the application of RI and CPP-ACP (phase 3) in the two groups of RI and CPP-ACP

ΔΕ	CPP-ACP	RI	P value	
<b>ΔE of BS between</b>	25.8	7.29	*0.04	
phases 1 and 3	$\pm 9.8$	±9.3	10.04	
$\Delta E$ of BS between	24.2	26.8	*<0.001	
phases 1 and 2	$\pm 9.9$	±9.5	•<0.001	
$\Delta E$ of BS between	17.8	21.7	0.12	
phases 2 and 3	$\pm 12.8$	±15.9	0.13	

\*CPP-ACP: Casein phosphopeptide amorphous calcium phosphate

\*Indicates statistically significant changes (p<0.05)

In the study by Kim et al, application of CPP-ACP increased the redness ( $\uparrow a^*$ ) of specimens [15].

The remineralizing agent tint is the possible reason behind the reduction in a\* parameter in the current study.

However, considering the fact that changes in a\* parameter in the third phase in group A were not significant, a definite conclusion cannot be drawn.

The a\* parameter in the RI group significantly decreased in the third phase. In a study by Bak et al, a\* parameter was the highest in the WS phase; however, this value decreased by the use of RI. This reduction further continued as the frequency of applications increased [18]. These results confirm our findings regarding the changes in a\* parameter due to the application of RI to the BS.

Thus, applications of CPP-ACP or RI along with CP were more effective in decreasing the red hue of the BS and the adjacent intact enamel than the application of each of them alone. Moreover, application of RI had a greater efficacy for decreasing this parameter.

After the application of CP, b\* parameter of the BS and the adjacent sound enamel increased and the tooth color became significantly more yellow. In studies by Singh et al, and Manton et al, application of whitening agent decreased the yellowness of  $(\downarrow b^*)$  of teeth [4, 5]. In a study by Kim et al, application of whitening agent shifted the tooth color towards blue  $(\downarrow b^*)$  [15].

These results are not in line with the current study findings regarding the b\* parameter. Such controversial results regarding the b\* parameter may be due to the excessive application of bleaching agents. Since specimens were obtained from different individuals, the composition of teeth was variable. However, all teeth were subjected to the same bleaching protocol. Bleaching in some teeth caused their oxidation and conferred a yellow appearance to specimens. After bleaching, the b\* parameter decreased compared to its value before the intervention in half the specimens and increased in the other half; this finding confirms the possibility of oxidation of some teeth during the process of bleaching. The b\* parameter in the current study decreased with the application of CPP-ACP; but this change was not significant. In a study by Kim et al, application of CPP-ACP increased the yellowness of specimens  $(\uparrow b^*)$  [15]. The color of CPP-ACP can explain the reduction in the yellowness of the BS and the adjacent enamel; however, since these changes were not significant, a definite conclusion cannot be drawn.

In group B, the color of BS became slightly yellower following the application of RI. In a study by Bak et al, the b\* parameter was the lowest in the WS phase and then reached its maximum value after the first application of resin. Frequent application of resin decreased this value again [15]. The results of Bak et al. were in accord with ours and confirmed that higher frequency of application of RI to the WS further decreased this parameter.

Comparison of  $\Delta E$  of BSs between phases 1 and 3 with that between phases 1 and 2 reveals that simultaneous applications of CP and RI or CP and

CPP-ACP are superior to the application of each of them alone. Considering the fact that  $\Delta E$  of BS was significantly different between the first and second phases (before dividing the specimens into two groups), the significant difference in  $\Delta E$  of BS between phases 1 and 3 cannot be attributed to the different effects of RI and CPP-ACP.

Comparison of  $\Delta E$  of the BS with the adjacent enamel during the three phases revealed that the interaction effect was not significant, indicating similar changes in the two groups. Overall, no significant difference was noted between the two groups of A and B during the three phases namely before the intervention, after bleaching and after the application of CPP-ACP or RI. Data showed that the results in the two groups were similar at each time point.  $\Delta E$  increased by the application of CPP-ACP; however, since the changes were not significant, a definite conclusion was not drawn.

In a study by Kim et al, application of bleaching agent caused no significant change in  $\Delta E$  [15]. In a study by Bak et al, application of RI to the WS caused a reduction in  $\Delta E$ , this means that the WS could not be easily distinguished from the adjacent tooth structure after resin application [18]. Paris et al. stated that the color match of WS and the adjacent enamel improved by the application of RI [25].

After the application of CP,  $\Delta E$  of the BS and the adjacent enamel did not change significantly. Specimens were evaluated by three observers and since the appearance of BS against a milky background was more prominent than that of a WS against a milky background, the color match of BS with its adjacent enamel significantly improved visually after bleaching. However, considering the high sensitivity of Shade Pilot (by DeguDent) spectrophotometer, the changes in  $\Delta E$  were found to be not significant. This study had some limitations including the short duration of treatment with RI and that the specimens were not matched in terms of the degree of demineralization of BSs (this factor can significantly affect the efficacy of RI and CP). Future studies are recommended to use hydrogen peroxide instead of CP to assess the efficacy of in-office bleaching as well. The effect of more frequent applications of RI must be investigated as well.

Colorimetry kits can also be used for evaluation of changes in color parameters in conjunction with spectrophotometer; and last but not list, more homogenous specimens, in terms of the degree of demineralization, must be chosen for future studies.

#### Conclusion

Based on the results, the CP bleaching agent along with RI or CPP-ACP can be used for treatment of BS lesions in the esthetic zone and their appearance can be significantly improved as such.

RI decreased the chalky white appearance, which was created due to the application of CP. Thus, the tooth surface became more translucent. However, none of the understudy materials could completely vanish the BSs. In conclusion, RI had higher efficacy than CPP-ACP in increasing the translucency and improving the visual appearance of teeth with BS lesions.

### References

1. Fontana M, Young DA, Wolff MS, Pitts NB, Longbottom C. Defining dental caries for 2010 and beyond. Dent Clin North Am. 2010 Jul;54(3):423-40.

2. Joiner A. The bleaching of teeth: A review of the literature. J of Dent. 2006 Aug;34(7):412-9.

3. Donly KJ, Sasa IS, Potential remineralization of postorthodontic demineralized enamel and the use of enamel microabrasion and bleaching for esthetics. 2008 Sep; 14(3):220-225.

4. Manton D, Bhide R, Hopcraft M, Reynolds E. Effect of ozone and tooth mousse<sup>TM</sup> on the efficacy of peroxide bleaching. Aust Dent J. 2008 Jun;53(2):128-32.

5. Singh RD, Ram SM, Shetty O, Chand P, Yadav R. Efficacy of casein phosphopeptide-amorphous calcium phosphate to prevent stain absorption on freshly bleached enamel: An in vitro study. J Conserv Dent. 2010 Apr-Jun; 13 (2):76-79.

6. Westerman GH, Hicks MJ, Flaitz CM, Powell GL. In vitro caries formation in primary tooth enamel: role of argon laser irradiation and remineralizing solution treatment. J Am Dent Assoc. 2006 May;137(5):638-44.

7. Pulido MT, Wefel JS, Hernandez MM, Denehy G, Guzman-Armstrong S, Chalmers JM, et al. The inhibitory effect of MI paste, fluoride and a

combination of both on the progression of artificial caries-like lesions in enamel. Oper Dent. 2008 Sept-Oct;33(5):550-5.

8. Cochrane NJ, Saranathan S, Cai F, Cross KJ, Reynolds EC. Enamel subsurface lesion remineralisation with casein phosphopeptide stabilised solutions of calcium, phosphate and fluoride. Caries Res. 2008 Jan;42(2):88-97.

9. Torres CRG, Borges AB, Torres LM, Gomes IS, de Oliveira RS. Effect of caries infiltration technique and fluoride therapy on the colour masking of white spot lesions. J Dent. 2011 Mar; 39(3):202-7.

10. White D. The application of in vitro models to research on demineralization and remineralization of the teeth. Adv Dent Res. 1995 Nov;9(3):175-93.

11. Paris S, Meyer-Lueckel H. Masking of labial enamel white spot lesions by resin infiltration-a clinical report. Quintessence Int. 2009 Oct; 40 (9):713-8.

12. Raoufi S, Birkhed D. Effect of whitening toothpastes on tooth staining using two different colour measuring devices-a 12 week clinical trial. Int Dent J. 2010 Dec; 60(6):419-23.

13. Kim Y, Son HH, Yi K, Kim HY, Ahn J, Chang J. The color change in artificial white spot lesions measured using a spectroradiometer. Clin oral investig. 2013 Jan; 17(1):139-46.

14. Dietschi D, Rossier S, Krejci I. In vitro colorimetric evaluation of the efficacy of various bleaching methods and products. Quintessence Int. 2006 Jul-Aug;37(7):515-26.

15. Kim YS, Kwon HK, Kim BL. Effect of nano-carbonate apatite to prevent re-stain after dental bleaching (in vitro). J Dent. 2011 Sept; 39 (9):636-42.

16. Agnihotri Y, Pragada NL, Patri G, Thajuraj P. The Effect of CPP-ACP on Remineralization of Artificial Caries like lesions: An Invitro study. Indian J Multidiscip Dent. 2011 Nov;2(1); 366-369.

17. Uysal T, Amasyali M, Koyuturk AE, Sagdic D. Efficiency of amorphous calcium phosphate– containing orthodontic composite and resin modified glass ionomer on demineralization

evaluated by a new laser fluorescence device. Eur J Dent. 2009 Apr; 3(2): 127-134.

18. Bak SY, Kim YJ, Hyun HK. Color change of white spot lesions after resin infiltration. Color Res

and Appens. 2014 Oct;39(5):506-10.

19. Akdeniz BG, Gröndahl HG, Magnusson B. Accuracy of proximal caries depth measurements: comparison between limited cone beam computed tomography, storage phosphor and film radiography. Caries Res. 2006 Aug;40(3):202-7.

20. Joiner A. Tooth colour: a review of the literature. J Dent. 2004 Mar;32:3-12.

21. Dozić A, Kleverlaan CJ, El Zohairy A, Feilzer AJ, Khashayar G. Performance of Five Commercially Available Tooth Color Measuring Devices. J Prosthodont. 2007 Mar-Apr;16(2):93-100.

22. Zeng P, Chen Z, editors. Perceptual quality measure using JND model of the human visual

system. Electric Information and Control Engineering (ICEICE), 2011 Inter Confer on; 2011 Apr: IEEE.

23. Walsh LJ. Evidence that demands a verdict: latest developments in remineralization therapies. Aust Dent Prac. 2009 Mar-Apr:48-59.

24. Zhao X, Gao X. Effect of resin infiltration treatment on the colour of white spot lesions. Hua Xi Kou Qiang Yi Xue Za Zhi. 2014 Jun; 32(3): 306-9.

25. Paris S, Schwendicke F, Keltsch J, Dörfer C, Meyer-Lueckel H. Masking of white spot lesions by resin infiltration in vitro. J Dent. 2013 Nov; 41 (suppl 5):e28-34.