

# Effects of Three Commonly Used Mouthwashes on Color Stability of Bleached Teeth

MR. Maleki Pour <sup>1</sup>, F. Shirani <sup>2✉</sup>, H. Mazaheri <sup>3</sup>, M. Moghtadaei <sup>4</sup>, M. Ghasemi <sup>5</sup>.

<sup>1</sup> Associate Professor, Department of Operative Dentistry, School of Dentistry, Islamic Azad University Isfahan (Khorasgan) Branch, Isfahan, Iran

<sup>2</sup> Associate Professor, Dental Material Research Center, Department of Operative Dentistry, School of Dentistry, Isfahan University of Medical Sciences, Isfahan, Iran

<sup>3</sup> Assistant Professor, Department of Operative Dentistry, School of Dentistry, Isfahan University of Medical Sciences, Isfahan, Iran

<sup>4</sup> Dentist, Private Office, Isfahan, Iran

<sup>5</sup> Postgraduate Student of Operative Dentistry, School of Dentistry, Isfahan University of Medical Sciences, Isfahan, Iran

## Abstract

**Background and Aim:** Bleached teeth are susceptible to discoloration. The aim of this study was to evaluate discoloration of bleached teeth following short-term use of three mouthwashes.

**Materials and Methods:** In this In-vitro experimental study, 48 extracted maxillary central incisors were selected and divided into four groups of 12. All the teeth were subjected to external bleaching with 38% hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). After bleaching, the teeth were immersed in distilled water (control group or group 1), chlorhexidine (CHX; group 2), Irsha (group 3) or Persica (group 4) for three, seven and 14 days. The color change was assessed by a spectrophotometer at each time point and reported as ΔE1, ΔE2 and ΔE3. The results were analyzed by paired t-test, repeated measures ANOVA, one-way ANOVA and LSD test.

**Results:** Different mouthwashes and immersion times caused significantly different color change in bleached teeth. After 14 days of immersion, the color change of samples in Persica group had significant differences with that of other groups (P<0.001). The results of repeated measures ANOVA was only significant for the Persica group indicating significant color change over time. Thus, paired t-test was applied for pairwise comparison of the time points, which revealed significant differences between ΔE1 and ΔE3 and also ΔE2 and ΔE3 (P<0.05).

**Conclusion:** Persica mouthwash would cause significant discoloration in recently bleached teeth.

**Key Words:** Tooth Bleaching, Mouthwash, Color Stability

✉ Corresponding author:  
F. Shirani, Associate Professor,  
Dental Material Research  
Center, Department of  
Operative Dentistry, School of  
Dentistry, Isfahan University of  
Medical Sciences, Isfahan, Iran

f\_shirani@dnt.mui.ac.ir

Received: 24 Feb 2015  
Accepted: 13 June 2015

➤ **Cite this article as:** Maleki Pour MR, Shirani F, Mazaheri H, Moghtadaei M, Ghasemi M. Effects of Three Commonly Used Mouthwashes on Color Stability of Bleached Teeth. J Islam Dent Assoc Iran. 2015; 27(3):144-149.

## Introduction

The demand for esthetic dental procedures and tooth whitening has greatly increased in the recent years. Tooth whitening is the first recommended technique for treatment of tooth discolorations due to maximum benefits and minimum side effects [1]. Hydrogen peroxide and carbamide peroxide are the two most commonly used materials for this purpose. Evidence shows that these products have

side effects such as tooth hypersensitivity and irritation of oral mucosa [2]. Carbamide peroxide causes enamel demineralization and surface roughness. Hydrogen peroxide decreases microhardness, concentration of calcium and enamel toughness and increases its porosities and permeability. All these factors decrease the physical properties of teeth and increase their staining susceptibility in the first couple of days

after tooth bleaching [3,4]. The side effects include increased tooth hypersensitivity, mucosal irritation, the consequent need for the use of mouthwashes and increased susceptibility to staining. Mouthwashes are prescribed to relieve tooth hypersensitivity and mucosal irritation following tooth bleaching and also for anti-plaque, anti-inflammatory, anti-septic, analgesic and periodontal purposes. However, a question arises that whether these mouthwashes affect the color of freshly bleached teeth. On the other hand, the adverse effects of CHX gluconate on tooth color have been previously confirmed [5,6], but no study has evaluated its effects on bleached teeth. Thus, the current study aimed to assess the effects of short-term use of three mouthwashes namely CHX, Irsha and Persica on teeth bleached with 38% hydrogen peroxide gel (Opalescence Extra Boost, Ultradent) via the vital bleaching technique using the CIE  $L^*a^*b^*$  system [7]. The null hypothesis was that these mouthwashes would have no effect on the color of bleached teeth at different time points.

### Materials and Methods

In this in vitro, experimental study, the effects of three different mouthwashes namely CHX (Behsa, Tehran, Iran), Irsha (Shafa Pharmaceuticals, Tehran, Iran) and Persica (Poorsina Laboratories, Tehran, Iran) on the color of freshly bleached teeth were assessed. A total of 48 sound human maxillary central incisors with completely formed roots extracted due to periodontal reasons in young patients with periodontitis were collected from the clinics of Isfahan Province using convenience sampling. The teeth had to be sound with no caries, hypoplasia, fracture or coronal deformity. To match the teeth in terms of dimensions (for their equal distribution in the groups), the greatest mesiodistal width and occlusogingival height of each tooth were measured with a caliper and small teeth, which were not suitable for colorimetry by a spectrophotometer were excluded. The teeth were removed from thymol solution, tissue residues were removed with a scalpel and debris and calculus were removed by a scaler. Next, the teeth were cleaned using non-fluoridated pumice paste and prophylaxis brush with low-speed handpiece. The teeth were randomly divided into four groups of 12 and stored in distilled water. Color shade of

each tooth was recorded prior to bleaching using a spectrophotometer (Specitra Flash 600, Data Color International, USA). External bleaching was carried out for all teeth using 38% hydrogen peroxide (Opalescence Xtra Boost, Ultradent, USA). The entire tooth surface was covered in rose wax and then the wax on the buccal surface was removed by a scalpel to create a window on the buccal surface. Syringes containing the whitening agent and the activator were connected and compressed 20 times in back and forth motion to achieve 38% hydrogen peroxide in one syringe. It was then applied through the window in 0.5 to 1mm thickness on the teeth. For greater efficacy, it was agitated on the tooth surface every five minutes. After 20 minutes, it was rinsed and this process was repeated twice. After bleaching, the wax was removed from the tooth surface and the teeth were immersed in distilled water for 24 hours. Their color shade was measured again by a spectrophotometer and  $b^*$ ,  $a^*$  and  $L^*$  values were obtained. It should be mentioned that  $L^*$  parameter indicates lightness;  $a^*$  and  $b^*$  indicate red, green and yellow-blue axes, respectively ranging from -100 (green or blue) to +100 (red or yellow). Overall color change ( $\Delta E$ ) was calculated using the formula below: [8]

$$\Delta E_{lab} = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$$

Color shade of teeth after bleaching was measured in the four groups. Next, the teeth in group one were immersed in distilled water as control. The teeth in group two were immersed in CHX, the teeth in group three were immersed in Irsha and the teeth in group four were immersed in Persica. Duration of immersion was three, seven and 14 days. The teeth were immersed in coloring agents (mouthwashes) twice a day (in the morning and in the evening) as recommended by the manufacturers; CHX was used for one minute each time, Irsha was used for 30 seconds each time and Persica was used for 20 seconds each time. During the intervals, the teeth were stored in distilled water. The three, seven and 14-day time points were chosen to simulate the clinical setting because in the clinical setting, mouthwashes are prescribed for use for different time periods depending on the condition for which, they have been prescribed. After each time period, the color change was measured by a spectrophotometer

(Specitra Flash 600, Data Color International, USA). The samples were placed in the middle of a window with 6mm diameter.

After spectrophotometric measurements, the  $a^*$ ,  $b^*$  and  $L^*$  values were obtained and color change were calculated. The data were analyzed using SPSS version 22. To assess the color change of teeth due to tooth bleaching, paired t-test was used while one-way ANOVA was applied to compare the mean color values after bleaching among the groups.

Repeated measures ANOVA was used to compare the mouthwashes at different time points with the type of mouthwash serving as the "between subject factor". Repeated measures ANOVA was also used for intragroup comparisons at the three time points. Paired t-test was also applied whenever appropriate. One-way ANOVA was used to assess presence/absence of a statistically significant difference in color change among the groups at specific time points. LSD test was applied to determine the presence of statistically significant differences among the groups at a specific time point.

## Results

A total of 48 extracted sound human maxillary central incisors were evaluated. Color of each tooth was measured before (T before) and after bleaching (T0). To assess discoloration due to bleaching, the mean  $L^*$  parameter before and after bleaching was compared using paired t-test and a significant change was noted in this regard ( $P<0.001$ ). This shows that the process of bleaching increased the lightness of teeth. Also,  $\Delta E$  of each sample was calculated and the mean value of  $\Delta E$  was calculated. Using one-way ANOVA, the mean  $\Delta E$  was compared among the three groups. Since the result was not significant ( $P=0.4$ ), it was found that the process of bleaching similarly affected the teeth in the four groups. In other words, the  $\Delta E$  was the same in all groups and thus, the four groups had normal distribution of teeth in terms of color.

Next, color of teeth following their immersion in coloring agents for three (T1), seven (T2) and 14 days (T3) was measured. Using the calculated  $b^*$ ,  $a^*$  and  $L^*$  values at these time points,  $\Delta E$  of each sample was calculated during the intervals of (T0-

T3), (T0-T2) and (T0-T1) and was reported as  $\Delta E_1$ ,  $\Delta E_2$  and  $\Delta E_3$ . The mean  $\Delta E$  in each group was also calculated at different time points and is presented in Table 1.

Repeated measures ANOVA was used for comparison of the effect of three mouthwashes at different time points and showed that the interaction effect of type of mouthwash and time point was significant ( $P=0.007$ ). Thus, comparisons were made between different time points for each mouthwash and different mouthwashes at each time point.

Table 1 shows the  $\Delta E$  over time caused by each mouthwash. It also shows the comparison of  $\Delta E$  of teeth in the four groups at the same time points.

Repeated measures ANOVA was applied to assess the changes in  $\Delta E$  of teeth in each group over time. Pvalues are presented in Table 1. This analysis showed that the difference in  $\Delta E$  over time was only significant in Persica group. Thus, paired t-test was applied for pairwise comparison of time points, which showed that the difference between the three and seven-day time points was not significant ( $P=0.83$ ) while the difference between three and 14-day ( $P=0.01$ ) and seven and 14-day ( $P<0.001$ ) time points was statistically significant. One-way ANOVA was then applied to compare  $\Delta E$  among the four groups at each time point. The results are shown in Table 1. This analysis revealed that  $\Delta E$  was only significant after 14 days of immersion. LSD test was then used (Table 1), which revealed that at 14 days, the difference in  $\Delta E$  of Persica and that of other groups was statistically significant ( $P=0.001$ ).

## Discussion

Tooth bleaching is a conservative method of correcting tooth discolorations, which does not require removal of tooth structure [9]. Irrespective of the method of bleaching, some concerns exist regarding the unfavorable consequences of bleaching such as tooth hypersensitivity as well as gingival and mucosal irritation [3,4,10]. Enamel surface roughness also increases during the process of bleaching, which increases enamel susceptibility to discoloration after tooth whitening [4,11,12] and this is another drawback of tooth bleaching, responsible color instability over time. Decreased lightness of tooth

**Table 1.** Comparison of the mean  $\Delta E^*$  at different time points in the four groups

Groups	$\Delta E1$	$\Delta E2$	$\Delta E3$	P value
	Mean( SD)	Mean( SD)	Mean( SD)	
<b>Distilled water</b>	2.07 <sup>1</sup> (1.06)	2.14 <sup>1</sup> (1.20)	2.72 <sup>1</sup> (1.18)	0.19
<b>Chlorhexidine</b>	2.40 <sup>1</sup> (1.36)	2.14 <sup>1</sup> (1.14)	1.84 <sup>1</sup> (1.49)	0.45
<b>Irsha</b>	1.90 <sup>1</sup> (0.81)	1.79 <sup>1</sup> (1.54)	2.35 <sup>1</sup> (1.80)	0.37
<b>Persica</b>	3.07 <sup>1a</sup> (1.54)	2.96 <sup>1a</sup> (1.08)	4.93 <sup>2b</sup> (1.77)	0.001*
<b>P value</b>	0.11	0.15	0.001*	

\*The color changes ( $\Delta E$ ) are calculated from the  $L^*$ ,  $a^*$ , and  $b^*$  values for each specimen according to the following formula which determines the 3-dimensional color space:  $\Delta E_{lab} = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$ , while luminosity values ( $\Delta L^*$ ), were reached using  $\Delta L^* = L^*(tx) - L^*(t0)$ , where (tx) represents immersion time and (t0) baseline

\*Different superscripted numbers on each column indicate significant differences among the groups

\*Different superscripted letters in group four indicate significant differences in  $\Delta E$  following different immersion periods

color is due to the rehydration process, which occurs in the first weeks after tooth bleaching [13,14]. Several methods such as spectrophotometry, colorimetry and analysis of computerized images are used for assessment of tooth color [15]. Gehrke et al. measured the accuracy of spectrophotometry to be 82%, which was higher than that of other methods [16]. The CIE-Lab system enables assessment of the effect of whitening agents on tooth color using spectrophotometry and it has been used in many previous studies such as the one by Braun et al [17]. The advantage of this system is assessment of  $\Delta E$  by measuring three color parameters of  $L^*$ ,  $a^*$  and  $b^*$ . One concern in this regard is standardization of the entire process and care must be taken in tooth selection, bleaching process and reading the color. Selection of middle third of the crown is appropriate for color measurement since the translucent and worn incisal third and polychromatic nature of crown are among the confounding factors in color analysis [1]. Many patients even with dental-gingival problems demand dental bleaching and sometimes, mouthwashes need to be used after bleaching treatments or patients may use them without prescription. Studies on the effects of mouthwashes on tooth color after bleaching are scarce.

Tooth discoloration is a side effect of some mouthrinses, although they may be beneficial for inflammatory gingival conditions [18]. Several types of mouthwashes are available in the market. Irsha and Persica are herbal mouthwashes

manufactured in Iran. Persica mouthwash contains hydroalcoholic extract of *Salvadora persica*, *Mentha spicata* and *Achillea millefolium* and its effective ingredients include calcium, fluoride, chloride, isothiocyanate, tannins, volatile oils and acylolnalkaloid. Irsha is a yellow antiseptic mouthwash. It has a composition similar to that of oil-based Listerine mouthwash. It is also herbal and beneficial for gingival conditions.

Effects of mouthwashes on tooth color have been evaluated in some previous studies. Ernst et al, and Nordbo et al, also mentioned the discoloration of teeth and enamel pellicles as a side effect of CHX use [19,20]. Thus, the current study was designed to assess the discoloration caused by the use of three mouthwashes on bleached teeth using a precise color measurement method.

Statistical analysis after immersion of samples in the mouthwashes and assessment of tooth color at three, seven and 14 days after immersion showed that the null hypothesis was accepted for Irsha and CHX and refuted for Persica mouthwash since the discoloration caused by Persica was not acceptable. In the control group, the mean color change increased after bleaching over time. However, color change was not significant, showing that water immersion had no effect on tooth color. This finding was in line with that of Attin et al, in 2003 on no discoloration of samples in distilled water [21].

In the CHX group, the greatest discoloration occurred in the first three days after bleaching. Despite the color change, the change after seven days was less than that after three days. The color

change at 14 days was less than that after seven days as well but this decrease was not statistically significant. In other words, discoloration did not have a cumulative effect and was the highest at three days. This finding was in line with the results of Marinone and Savoldi [22] and Leard and Addy [23], who stated that the effect of CHX alone was not sufficient to cause discoloration. Although the discoloration caused by CHX at three days was not significantly different from that in the control group, it was the highest indicating that use of CHX can affect the lightness of teeth following bleaching. Since this effect was the greatest in the first three days, patients should be advised not to use it in the first three days following bleaching. No previous study has evaluated the effect of CHX on the color of bleached teeth to compare our results with.

The least color change was noted in Irsha group and the difference in color change at different time points was not significant. However, color change was higher at 14 days compared to three and seven days, which shows the cumulative effect of this mouthwash on tooth color. Thus, it may be recommended for short-term use following bleaching. The yellow hue of this mouthwash matches natural color of teeth, which probably explains its lack of significant effect on teeth. [7,13].

In the Persica group, the mean color change indicated equal discoloration after three and seven days while the mean color change at 14 days was higher than that at three and seven days. Color change over time was significant in this group. Also, color change at 14 days in this group was significantly greater than that in other groups. According to the manufacturer, the hydroalcoholic extract of Miswak in Persica prevents tooth discoloration due to high chloride content, which is in contrast to our findings. This difference may be due to the pH of this mouthwash or its constituents such as micro-molecules. Its pH may enhance its penetration into bleached tooth structure or its molecular dimension may allow penetration of stains into enamel microporosities caused by bleaching. Our findings in this respect were confirming that this mouthwash can cause discoloration in non-bleached and bleached teeth.

The current study assessed the effects of mouthwashes on tooth color in short-term and did not provide any information on their long-term effects but can be a start for similar studies in this respect. Also, sound human maxillary central incisors were used in this study, which is an advantage because in order to use spectrophotometer, tooth samples must be sound and the surface must be smooth and large. However, this study had an in vitro design and thus, cannot completely simulate the clinical setting. Therefore, future studies are required to better simulate the clinical setting. Also, other commonly used mouthwashes and different techniques of bleaching must be investigated in future studies.

### Conclusion

Persica significantly affected the color of bleached teeth after seven and 14 days of use compared to Irsha and CHX. Irsha did not cause significant discoloration in bleached teeth.

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