

## The Effect of Toothpaste Containing Bioactive Glass Treatment on Surface Staining Susceptibility of Bleached Teeth (*In Vitro* Study)

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### Abstract

The present study investigated the effects on enamel of a toothpaste containing bioactive glasses added at different time periods of in-office bleaching with 40 % hydrogen peroxide (HP) gel by evaluating the effectiveness of bleaching and staining susceptibility, using a spectrophotometer, non-contact profilometer and scanning electron microscope (SEM). At baseline, luminosity (L1) and surface roughness (Ra0) of enamel were measured. Samples (n=48) were allocated into four groups according to the treatments: 1) bleached with 40 % HP gel (Control); 2) applied toothpaste containing bioactive glasses for 5 min prior to bleaching with 40 % HP gel (Bio\_Bleach); 3) bleached with a mixture of 40 % HP gel and the toothpaste in a 1:1 proportion (Mix); 4) bleached with 40 % HP gel and immediately applied the toothpaste for 5 min (Bleach\_Bio). After treatment, luminosity (L2) and roughness (Ra1) measurements and also SEM examination were performed. Samples were immersed in the staining solution (red wine) every day for 14 days. Luminosity was measured on day 7 (L3) and day 14 (L4). At baseline, L1 values of all groups were not significantly different. After bleaching, L values (L2) in all groups increased significantly and Mix group significantly showed the lowest L values comparing to the others. After 7-day staining, L values (L3) in all groups decreased but only the control and Bio\_Bleach groups showed significant differences compared to the after bleached values. After 14 days of staining, all groups, except the Mix group, significantly demonstrated lower L values relatively to the values at 7-day staining. Despite the fact that surface roughness in all groups increased after the completion of the bleaching process, only Bio\_Bleach and Bleach\_Bio groups were statistically significant relatively to the baseline. SEM analysis presented morphological alterations characterized by depressions, porosities and superficial irregularities in different degrees. A treated enamel surface with toothpaste containing bioactive glass either during or after 40 % HP in-office bleaching process reduced red wine staining.

**Keywords:** Bioactive glasses, Novamin, Red wine staining, Remineralizing agents, Vital tooth bleaching

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## Introduction

After tooth bleaching with hydrogen peroxide (HP), coloring pigments can adhere to the enamel surface and cause more discoloration.<sup>1-8</sup> It is conceivable that dietary components from coffee, tea, juices, red wine and cola-based soft drinks consumed during or just after the completion of bleaching increase staining susceptibility and demineralization<sup>9,10</sup> of the bleached enamel surface, manifested by the shift of brightness or luminosity parameter towards the negative (darker) direction.<sup>3</sup> Indeed, the bleached enamel particularly with 35 % HP is more susceptible to red-wine staining than the other beverages.<sup>2,5-7,11</sup> It was suggested that increased surface roughness with pores or superficial defects caused by the bleaching treatment makes the surface more prone to staining.<sup>12</sup> Therefore, post-bleaching roughness<sup>3,10,13,14</sup> of the enamel surface is considered a predisposed factors for stain absorption. Thus, the damaged enamel surface should be recovered or protected after bleaching for a long-lasting whitening effect.

Remineralizing agents have been investigated to improve the deleterious effects of the bleaching procedure. An application of toothpastes containing bioactive glasses (5.5 % NovaMin<sup>®</sup>) can reverse and repair the significant loss of sodium and magnesium that occur during tooth-bleaching with 40 % HP.<sup>10</sup> Moreover, the application of desensitizing toothpastes on enamel surface prior to bleaching with 35 % HP resulted in a decrease of enamel roughness and a significant loss of enamel micro-hardness.<sup>9</sup> Furthermore, bioactive glasses promote an enhancement of the micro-hardness values in bleached enamel compared with the unbleached area, demonstrating a potential benefit for bleaching therapy.<sup>9</sup>

It has been documented that the relative degradation and roughness of the enamel surface after tooth bleaching have a direct influence on their staining susceptibility.<sup>2,5-7</sup> If the enamel of a freshly bleached

tooth was surface treated by remineralizing agents, it may reduce the absorption of stains and therefore maintain the effect of bleaching for a longer time.<sup>15</sup> It seems that the damage done by bleaching procedures can be repaired by either a subsequent use of toothpaste containing bioactive glasses remineralizing agents or an application of these agents prior to dental bleaching. The sequence of bioactive glass surface treatment to prevent the destructive bleaching effects on enamel is yet to be evaluated and optimized. There has been no study done that assessed the effect of bioactive glasses incorporated in toothpastes on the staining behavior of enamel after in-office bleaching with 40 % HP. Therefore, the present study investigated the effects on enamel of a toothpaste containing bioactive glasses added at different time periods of in-office bleaching with 40 % HP gel by evaluating the lightness, morphology and staining susceptibility of enamel. The tested null hypothesis was that enamel staining susceptibility post in-office bleaching to red wine is not influenced by surface treatment procedures regardless of different time periods used.

## Materials and Methods

This study was reviewed and approved by the Human Research Ethics Committee, Faculty of Dentistry, Chulalongkorn University under study code HREC-DCU 2017-092 and carried out in the Dental Material Science Research Center at the Faculty of Dentistry, Chulalongkorn University, Thailand. The bleaching agent used in this study was Opalescence<sup>®</sup> Boost PF 40 % (Ultradent Products, Inc., South Jordan, USA) and the toothpaste containing bioactive glasses was Sensodyne<sup>®</sup> Repair & Protect NovaMin<sup>®</sup> (SmithKline Beecham Consumer Healthcare, Berkshire, United Kingdom). A schematic outline of the experiment is shown in Figure 1.

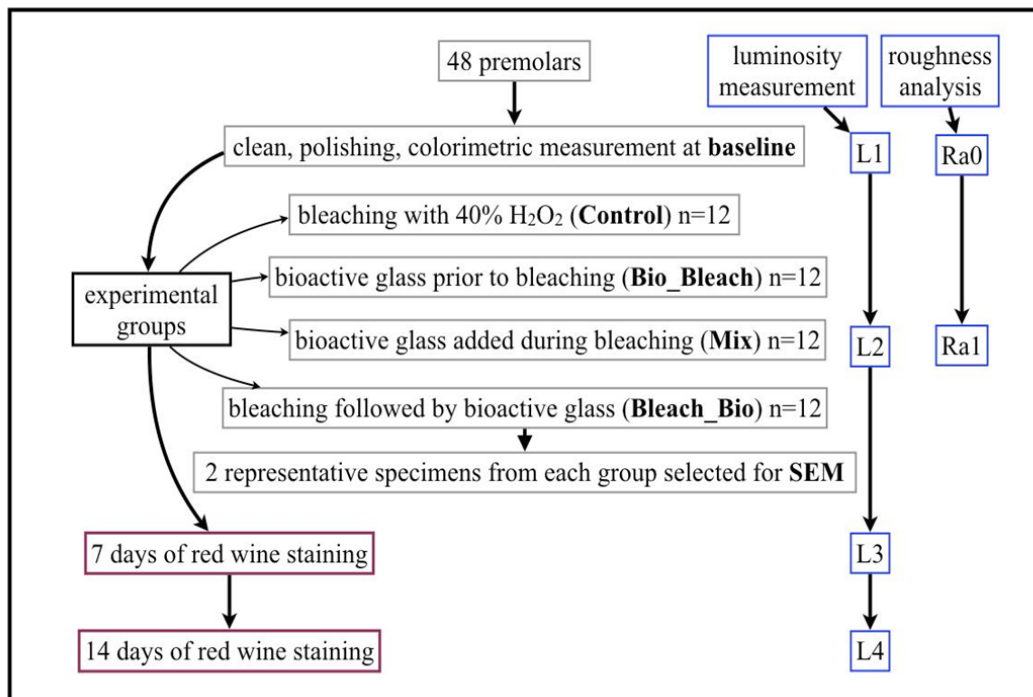


Figure 1 Schematic outline of study's design and experimental groups.

### Inclusion & Exclusion Criteria and Sample Preparation

Forty-eight human extracted maxillary premolars were used. The buccal surfaces of the teeth were examined. Teeth extracted for an orthodontic purpose with no visible caries or structural defects or significant discoloration on the enamel surface were selected while teeth with obvious visible defects were excluded from the study. The teeth were examined under 4.5x magnification (MEIJI EMZ, MEIJI TECHNO CO.LTD, Tokyo, Japan). The teeth were ultrasonically cleaned and polished with non-fluoride pumice paste, stored in a 0.1 % thymol solution at 4°C and used within 6 months after extraction. To ensure standardized repositioning of the tooth surface for tested measurements, the crown of the tooth sample was embedded in putty silicone (Silagum® Putty Soft, DMG Hamburg, Germany) with approximately 10 mm thickness and a window, diameter of 5 mm, was created in the middle of the buccal surface of the tooth. All tests and measurements were done on enamel surface at the prepared window.

### Study design

All samples (n=48) were measured for the

baseline luminosity (L1) using a spectrophotometer (VITA Easyshade® V, Vivadent, Brea, CA, USA) and surface roughness (Ra0) using non-contacted profilometer ( Alicona, Infinite Focus SL, Austria). The initial luminosity (L value) of each sample was used to stratify and allocate samples into all four groups. The evaluation methods of the L coordinate and surface roughness were described below. The samples were divided into four groups according to the treatment categories: 1) bleached with 40 % HP gel (Control); 2) applied the toothpaste for 5 min prior to bleaching (Bio\_Bleach)<sup>9</sup>; 3) bleached with a mixture of the bleaching gel and the toothpaste in a 1:1 proportion (Mix)<sup>15</sup>; 4) bleached with HP gel and immediately followed by applying the toothpaste for 5 min<sup>16,17</sup> (Bleach\_Bio). The samples were then washed for 1 min with water-spray and stored for 24 hr in non-fluoridated artificial saliva at 37°C. The components of artificial saliva were described elsewhere.<sup>18</sup> A second luminosity measurement (L2) and roughness analysis (Ra1) were carried out. Following the bleaching and surface treatment processes, two representative samples from each group were randomly selected for scanning electron microscope (SEM) analysis

(JSM-5410LV, JEOL, Tokyo, Japan). The other 10 samples in each group were then immersed in staining solution (red wine, Montepulciano D'abruzzo, Velenosi, Italy, pH 4.5) for 15 min at room temperature and washed for 1 min with a water-spray and then stored for 24 hrs in freshly prepared artificial saliva at 37°C.<sup>5</sup> The staining process was repeated daily in which at day 7 and at the end day 14, the third (L3) and the fourth (L4) luminosity were recorded, respectively. Since determining appropriate lightness or a correct value of the teeth is the key success in the clinical situation<sup>19</sup>, the present study utilized luminosity (L values) as the primary outcome to monitor any changes following bleaching and staining procedures.

#### Luminosity measurement<sup>20</sup>

Before measuring the L values, the tested samples, which were always soaked in artificial saliva, were quickly dried with blotting paper and then immediately evaluated by a spectrophotometer. The 5-mm diameter at buccal surface of each sample was recorded in terms of the Commission Internationale De L'Eclairage (CIE) or CIE L\*a\*b\* system, providing numeric three-dimensional color space with L\* representing luminosity, a\* green-to-red and b\* blue-to-yellow. L\* represents the value from 0 - 100 (darkness to brightness) and a\* and b\* represent the shade. Each sample was measured in triplicate and rotated by 120° between two consecutive measurements.<sup>20</sup>

#### Surface roughness<sup>9</sup> & SEM analysis<sup>20</sup>

Each sample was rinsed with distilled water and air-dried prior to analyzation with a non-contact profilometer which performs three measurements in different directions on the enamel surface using the Ra parameter ( $\mu\text{m}$ ) and a cut-off of 0.25 mm to determine the average surface roughness (Ra) of each sample.<sup>21</sup> Before SEM analysis, the 2 representative samples from each group were ultrasonically cleaned for 5 min and dehydrated by immersion in increasing alcohol concentrations and air-dried and then gold sputter-coated (JFC-1200 Fine Coater, JEOL, Tokyo, Japan).<sup>20</sup>

#### Surface treatment & Bleaching procedure

For surface treatment in Bio\_Bleach and

Bleach\_Bio groups, the buccal surface of the sample was applied with 1 mm thickness of the toothpaste using a disposable applicator (Micro-brush/Vigodent) and left in artificial saliva at 37°C for 5 min<sup>16</sup> and then water-sprayed for 1 min and stored in artificial saliva 37°C until further evaluation. The bleaching agents used for samples in Control, Bio\_Bleach and Bleach\_Bio groups were mixed according to the manufacturer's recommendation and dispensed to cover all the buccal surface of the samples at a 2 mm in thickness twice for 20 min each. To ensure an equal amount of bleaching agent was applied on each sample treatment, an individual polyacetate tray in which a 4x4x2 mm blocking out area was placed on the buccal surface of the tooth. For samples in Mix group, the bleaching agent comprised of a mixture of the bleaching gel and the toothpaste in a 1:1 proportion by weight<sup>15</sup> was applied. After each bleaching cycle completion, all of the teeth were cleaned by water-spray for 1 min then stored in artificial saliva 37°C in the dark for 24 hr before further evaluation.

## Statistical analysis

The statistical analysis was performed using Statistical Package for the Social Sciences (SPSS Inc, Version 16.0, Chicago, ILL) at 0.05 significant level. Normal distribution of the data and homogeneity of variances were confirmed by Shapiro-wilk test. A one-way ANOVA and a repeated measures ANOVA were used to compare the mean of luminosity (L values), followed by Bonferroni/Games-Howell post hoc analysis for pairwise comparisons. A non-parametric Kruskal-Wallis H test and a pair-t-test were used to compare the mean of surface roughness (Ra).

## Results

### Luminosity analysis

Table 1. shows the mean and standard deviation ( $\pm$ SD) values for luminosity (L) of treatments at five different time points and the data are also plotted in Figure 2. After surface treatment and bleaching, L values in all groups increased significantly compared to the

baseline and Mix group which showed significantly the lowest values. Following 7-days of staining, L values in all groups decreased although only the Control and Bio\_Bleach groups showed significant differences compared to the after bleached values. After 14 days of staining, all treatment groups except for the Mix group significantly demonstrated lower L values relatively to the values at 7-day staining. In Mix group, the L values did not show significant differences at either 7-day or 14-day staining periods.

### Surface roughness

Table 2 shows the mean values and standard deviation ( $\pm$ SD) of surface roughness (Ra). Despite the fact that surface roughness in all groups increased after completion of the bleaching process, significant differences were found only in the Bio\_Bleach and Bleach\_Bio groups compared to the baseline. Comparing groups at

either baseline or after bleaching, there was no significant difference observed.

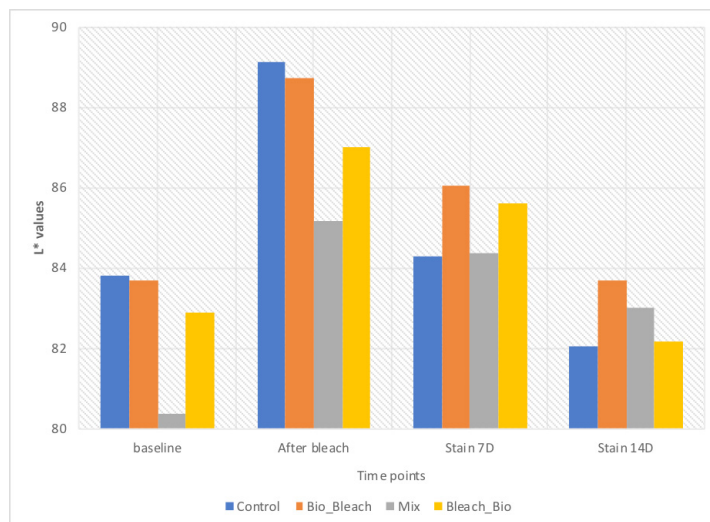
### Changes in the surface microstructure

Representative SEM micrographs of enamel morphology are shown in Figure 3. All the bleached specimens demonstrated the noticeable loss of integrity of the enamel surface with morphological alterations characterized by depressions, porosities and superficial irregularities in different degrees. The control group treated with only 40 % HP gel showed surface damage and noticeably eroded regions although some areas remained as preserved normal enamel. Unlike the control group, the treated enamel surfaces (Bio\_bleach, Mix, Bleach\_bio) were covered by amorphous deposits on the surface with disorderly packed of unidentified particles at a variety of sizes and the most distinguished and dispersed particles were found in the Mix group.

**Table 1** Mean values and standard deviation ( $\pm$ SD) of Luminosity

TREATMENT	TIME				P-value
	baseline	After bleach	Stain 7D	Stain 14D	
CONTROL	83.81 ( $\pm$ 2.77) <sup>AB</sup>	89.14 ( $\pm$ 1.77) <sup>bc</sup>	84.28 ( $\pm$ 0.74) <sup>B</sup>	82.06 ( $\pm$ 1.55) <sup>A</sup>	<0.001
BIO_BLEACH	83.69 ( $\pm$ 2.80) <sup>AC</sup>	88.73 ( $\pm$ 2.30) <sup>bb</sup>	86.04 ( $\pm$ 2.94) <sup>C</sup>	83.68 ( $\pm$ 3.66) <sup>A</sup>	<0.001
MIX	80.38 ( $\pm$ 3.23) <sup>A</sup>	85.15 ( $\pm$ 3.44) <sup>abc</sup>	84.38 ( $\pm$ 4.01) <sup>AC</sup>	82.99 ( $\pm$ 3.95) <sup>AB</sup>	<0.001
BLEACH_BIO	82.88 ( $\pm$ 2.69) <sup>A</sup>	87.02 ( $\pm$ 2.17) <sup>bc</sup>	85.59 ( $\pm$ 2.94) <sup>B</sup>	82.17 ( $\pm$ 1.88) <sup>A</sup>	<0.001
P-VALUE	0.79	0.018	0.245	0.607	

Means followed by different letters (UPPERCASE letters in the lines and lowercase letters in the columns) indicate statistical differences ( $p \leq 0.05$ ). a,b The same letters were not significant by one-way ANOVA and Bonferroni multiple comparison of  $p$ -value  $>0.05$ . ABCD Same letters were not significant by repeated-measures ANOVA and Pairwise comparisons of  $p$ -value  $>0.05$ .



**Figure 2** Mean values of luminosity (L)

Table 2 Mean values ( $\pm$ SD) of surface roughness (Ra)

TREATMENT	ROUGHNESS (RA)		
	baseline	After bleach	P-value
CONTROL	510.8 ( $\pm$ 144.1)	590.7 ( $\pm$ 204.4)	0.258
BIO_BLEACH	474.0 ( $\pm$ 230.1)	672.3 ( $\pm$ 178.8)	0.021
MIX	536.7 ( $\pm$ 178.6)	545.3 ( $\pm$ 315.6)	0.980
BLEACH_BIO	466.1( $\pm$ 190.8)	560.7 ( $\pm$ 214.4)	0.021

A non-parametric Kruskal-Wallis H test was used to compare the mean of Ra among groups. The mean surface roughness (Ra) and Sa at baseline and after bleaching in each group was compared using a pair-t-test at a significant level <0.05.

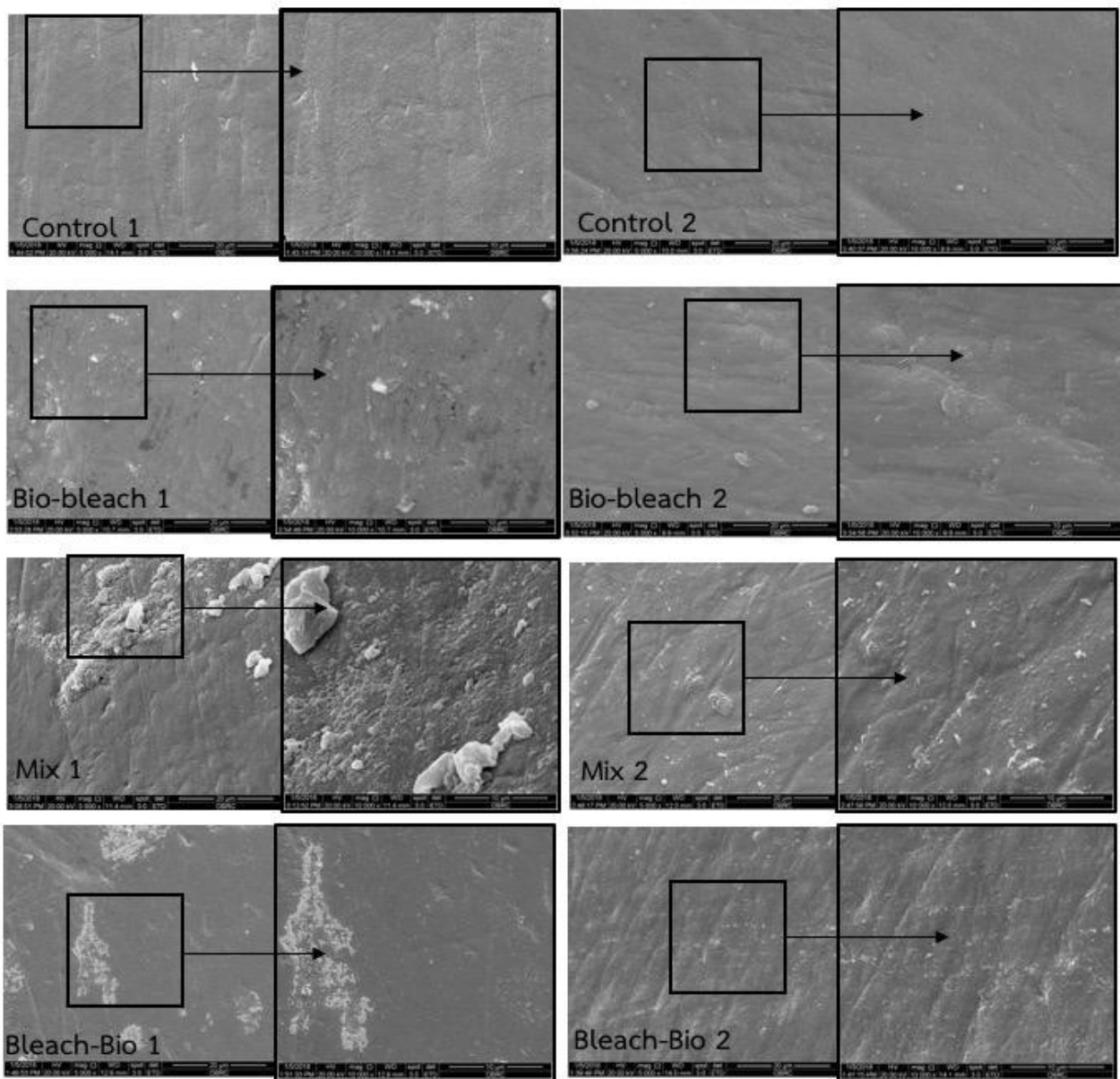


Figure 3 The SEM images of x5000 and x10000 magnifications of enamel surfaces according to different treatments

## Discussion

The results in the present study showed that all bleaching procedures in the test and control groups were efficient and that the enamel lightness improved relatively to the baseline values. Among all groups, the Mix group significantly demonstrated the lowest improvement. This may be due to the concentrations of HP were diluted by the toothpaste. A previous study, evaluated the effect of synthetic 45S5 bioactive glasses and demonstrated that the combination of pure bioactive glasses and HP gel did not alter the whitening efficacy and using bioactive glasses alone could not whiten the enamel.<sup>20</sup> In addition, another study<sup>15</sup> presented that addition of desensitizing toothpaste containing bioactive glasses (NovaMin<sup>®</sup>) with 15 % carbamide peroxide (CP) in a 1:1 proportion during home-bleaching procedure could occlude the dentinal tubules and it did not affect the bleaching outcome. However, the bleaching outcome of that study was assessed by visual shade-guide analysis.

After 7-day staining, the lightness (L values) in all groups of the present study decreased. Only the Control and Bio\_Bleach groups showed significant differences whereas the values in Mix and Bleach\_Bio groups were fairly similar to that of the after bleaching group. Interestingly, this effect continued in the Mix group until the 14-day immersion. It may be speculated that the addition of the toothpaste during (Mix group) and after (Bleach\_Bio group) bleaching procedures may influence staining susceptibility of the bleached enamel. Indeed, as found in the results of the Mix group, red-wine staining could be prevented up to 14 days. Red-wine<sup>11</sup>, which is an acidic, colored and alcoholic beverage, is more potent/efficacious staining for teeth bleached with highly concentrated 35 % HP gel than the other beverages.<sup>2,5-7</sup> The acidity of red wine is one of the factors possibly influencing the surface roughness and the staining outcome after bleaching and thus monitoring the pH of the staining solution during the testing procedure is noteworthy for further studies. The actual mechanisms of dietary pigments

affecting the discoloration of bleached teeth remain unclear. The color-producing stains within tooth structures are often organic compounds that contain conjugated double bonds.<sup>22</sup> In addition, anionic polyphenols found in highly pigmented foods/beverages such as red wine and black tea, react with cationic salivary pellicles, forming thickened layers of stained material that adheres to the tooth surface.<sup>4</sup>

The present study utilized toothpaste containing 5 % bioactive glasses (Novamin or calcium sodium phosphosilicate), which is an inorganic compound that reacts in aqueous environments to release calcium, sodium, and phosphate ions over time. It was suggested that bioactive glasses as alkaline salts, might buffer the acidity of HP and reduce the demineralization of enamel surface after being mixed with HP gel.<sup>23</sup> The alkalinity and accelerated ionic releasing of bioactive glasses in HP make bioactive glasses a promising biomimetic adjunct for bleaching therapy to ensure the lifelong integrity of a tooth.<sup>20</sup> In principle, it was suggested that bioactive glasses may form a protective layer on the enamel surface to inhibit demineralization when used before HP gel bleaching, or enhance remineralization when used after peroxide bleaching.<sup>20</sup> However, it is arbitrary not to mention that not only the bioactive glass but also other components in the toothpaste could also influence the staining outcome of the present study. Therefore, the effects of an individual component in the toothpaste on the prevention of staining post-bleaching are subjected to be further evaluated.

Although some studies indicated that peroxide has no effect on surface topography<sup>24-26</sup>, there are as many studies in which significant changes in surface roughness have been documented.<sup>22,27,28</sup> Our study showed that post-bleaching surface roughness of the enamel in all groups increased although only the Bio\_Bleach and Bleach\_Bio groups were found significant differences. There are no significant differences observed

in Control and Mix groups which may possibly be due to limited numbers of samples and a wide range of standard deviations of the data, suggesting an increase of the sample size in further studies. Indeed, the present study revealed that the enamel treated with a mixture of the bleaching gel and the toothpaste did not significantly increase surface roughness post-bleaching. Another study also found that combination of 7.5 % bioactive glass (Bioglass 45S5/ NovaBone<sup>®</sup>) with 38 % HP bleaching gel during the bleaching process restored enamel fracture toughness and surface micro-hardness.<sup>29</sup> Bioactive glasses were found to be the most effective in decreasing enamel surface roughness<sup>30</sup> and increasing micro-hardness<sup>30</sup> subsequent to in-office bleaching technique with 40 % HP gel, compared with casein phosphopeptide amorphous calcium phosphate (CPP-ACP) and nano-hydroxyapatite. When used immediately after bleaching, bioactive glasses can reduce the demineralization effect of bleaching products and prevent the exposure of dentin tubules.<sup>31</sup> Other studies have stated that the application of dentifrices containing bioactive glasses (NovaMin<sup>®</sup>) after bleaching with 16 % CP also caused increases in the calcium and phosphate content of the enamel layer, returning it to that of pristine enamel.<sup>17</sup> Compared to pre/post-bleaching use of the toothpaste, the application of toothpaste mixed with bleaching gel (Mix) in the present study seems to be the optimal way to reduce the surface roughness of enamel and retaining the enamel surface integrity.

Many research studies in teeth bleaching have stated that morphological changes, such as erosion, craters and porosity<sup>10,23,28,30,32,33</sup>, were observed in the enamel surface. Our SEM evaluation of the enamel surfaces treated with only 40 % HP gel bleaching suggested slightly irregular and eroded surfaces. These changes indicate the loss of enamel integrity which is consistent with findings from the above studies. Although the bleached enamel treated with the toothpaste revealed a partly similar enamel pattern as the Control group, there are some isolated and aggregated deposits of

angular components of supposedly bioactive glass fragments on the enamel surface. The presence of a precipitated layer of bioactive glass is seen distinctively in Mix group. This phenomenon has also been observed in a previous study of tooth bleaching with remineralization.<sup>10</sup>

Overall, the present study demonstrated that tooth bleaching with 40 % hydrogen peroxide damage the enamel surface integrities; however, using the bleaching gel incorporated with the toothpaste containing bioactive glasses potentially diminished its adverse effects. Therefore, the tested null hypothesis that enamel staining susceptibility post in-office bleaching to red wine is not influenced by surface treatment procedures regardless of different time periods used was rejected. On the other hand, the results of surface characteristics were inconsistent with its staining behavior implying that surface roughness may not solely influence enamel surface-staining post-bleaching. It was, however, speculated that staining susceptibility of the bleached enamel may be lessened by the application of the mixture of toothpaste with bleaching gel or immediately after bleaching. Due to limitations of the present *in vitro* study, further clinical trials of remineralizing agents including bioactive glasses for prevention of post-bleaching staining are yet to be conducted.

## Conclusions

In-office tooth bleaching procedures can potentially damage the enamel surface integrities. Staining behavior post-bleaching could possibly be prevented by applying a mixture of the toothpaste containing bioactive glasses with the bleaching gel or immediately after bleaching. Roughness of enamel surface may not solely influence post-bleaching surface staining.

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