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RESEARCH AND EDUCATION

Effect of carbamide peroxide bleaching on enamel characteristics and susceptibility to further discoloration

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ABSTRACT

Statement of problem. Whether tooth whitening alters the surface topography of enamel causing an increase in surface roughness that could increase susceptibility to restaining is unclear.

Purpose. The purpose of this in vitro study was to evaluate whether immersing enamel in common solutions produces a color change of ΔE greater than 2; whether the highest concentration carbamide peroxide bleaching agent produces the greatest ΔE; whether bleaching increases the susceptibility to further staining by common solutions; and whether morphologic changes to the enamel surface are observed after staining and bleaching as evidenced by scanning electron microscopy (SEM) analysis and energy-dispersive X-ray spectroscopy (EDS).

Material and methods. Forty-five extracted human teeth were immersed in 5 solutions (wine, coffee, tea, soda, and water) for 15 days at 80°C, and the change in ΔE was assessed with a colorimeter. The teeth were bleached using different concentrations of carbamide peroxide (20%, 35%, and 44%) and ΔE was measured at different time intervals. The teeth were then restained with the same solutions. The ΔE after initial staining was compared with the ΔE after bleaching and restaining of the same teeth. SEM was performed at baseline, after staining, bleaching, and restaining to evaluate the changes in the enamel surface topography. EDS was used to determine the elemental composition of tooth surfaces after restaining.

Results. All liquids caused a ΔE greater than 2 after 15 days. The concentration of bleaching agent was not significantly associated with ΔE for any stain types. No significant difference was found in the rate of staining between initial staining and restaining after bleaching. However, a significant effect of time was found for the staining, where the overall ΔE increased by 0.34 for each day in the solution ($P<.001$). SEM images showed no major changes to enamel topography after bleaching. However, a coating was noted on teeth stained with wine and tea, which had different elemental compositions when compared with the tooth surface.

Conclusions. Based on SEM observation, bleaching teeth with carbamide peroxide does not increase the susceptibility of enamel to staining and does not alter the topography of the enamel. Using higher bleaching concentrations did not increase tooth whitening as a function of time. (J Prosthet Dent 2019;121:340-6)

Tooth bleaching is a popular option for patients displaying intrinsic or extrinsic tooth discoloration. Chromogen deposits produce extrinsic stains on the enamel surface and the pellicle.[1] Color changes on a surface are due to the accumulation of organic molecules made of conjugated chains with single or double bonds.[2,3] Bleaching agents cleave these chains by oxidation and destroy the double bonds in the conjugated chain.[4]

Color changes after tooth bleaching can be measured with different methods. The most common is a standard shade guide,[5] which can be subjective and is affected by the observer's age and experience and room lighting and décor, among other factors.[6] A colorimeter measures color according to the Commission Internationale de l'Eclairage

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Clinical Implications

Since lower bleaching concentrations were as effective as higher concentrations, dentists can prescribe bleaching agents at lower concentrations to remove stains effectively. This will also minimize possible tooth sensitivity.

(CIE), which expresses color in a color space[2] with 3 axes, L*, a*, and b*. L* or lightness is a value from 0, representing perfect black, to 100, representing a perfect reflecting diffuser. The a* value represents redness (positive values) and greenness (negative values).[7] The b* value measures yellowness (positive values) and blueness (negative values).[7] Neutral colors (white and grey) coordinate with a* and b* values approaching zero and increase in magnitude for more saturated colors.[6]

Changes in color are measured by calculating ΔE*, the difference between 2 colors measured by the distance between 2 points in the CIELab color space. An equation[8] developed in 1976 was used to determine the color of a sample $E^{*}=\sqrt{(a^{*})^{2}+(b^{*})^{2}+(L^{*})^{2}}$. To assess color differences between 2 samples, a color difference equation was used (ΔE*), where $(\Delta E^{*})=\sqrt{(L_{1}^{*}-L_{2}^{*})^{2}+(a_{1}^{*}-a_{2}^{*})^{2}+(b_{1}^{*}-b_{2}^{*})^{2}}$. The most current version, developed in 2000, compensates for issues with saturation and lightness in earlier formulas (1976 and 1994)[9,10] and is important in the estimation of human perception of color change.[10,11]

Scanning electron microscopy (SEM) and profilometric analysis have been used to assess morphologic changes of the enamel surface. No significant microsurface alterations have been demonstrated by the whitening process with 10% carbamide peroxide,[12] 20% carbamide peroxide,[13] or even 35% hydrogen peroxide.[14] In contrast, 35% hydrogen peroxide has demonstrated morphological changes in enamel with significant decreases in Ca and P content, with carbamide peroxide having no effect.[15] However, other studies have shown morphologic changes of the enamel surface after tooth whitening, with increased surface roughness[16] and a decreased surface hardness with adverse changes to the elastic modulus of enamel,[17] random fragmentation of the protein matrix of enamel,[18] deeper clefts,[19] and a rise in surface corrosiveness after bleaching with 10% carbamide peroxide.[20] Teeth with increased roughness from bleaching may be more susceptible to discoloration[21] and may be affected by bleaching time and concentration.[22]

The conflicting results among these studies can be attributed to differences in methodology, such as time of exposure, storage medium, solution pH, and composition of the bleaching agents.[12] Acidic bleaching gels may decrease the microhardness of the enamel resulting in surface morphology alteration.[23] However, the remineralizing potential of saliva may counteract the effects bleaching products have on enamel and dentin.[24]

Therefore, whether tooth whitening alters the enamel surface topography by increasing surface roughness and thereby the susceptibility to restaining is unclear. The null hypotheses of the current study were that immersing enamel in common solutions does not produce a color change of ΔE greater than 2; that different concentrations of carbamide peroxide bleaching agent produce a similar change in ΔE; that bleaching does not increase the susceptibility to further staining by common solutions; and that morphologic changes to the enamel are not noticeable after staining and bleaching as evidenced by SEM and energy-dispersive X-ray spectroscopy (EDS) analysis.

MATERIAL AND METHODS

Institutional Review Board approval was obtained prior to the use of all human-derived materials. A power analysis showed that 45 teeth were sufficient to detect a 94% significance. Forty-five extracted human teeth were sectioned perpendicular to the long axis to obtain dental crowns of 10 mm without roots to match the inner diameter of the colorimeter (Chroma meter CR-300; Konica Minolta). The pulp chambers of the teeth were sealed apically with a flowable composite resin (Natural Elegance Flowable Tip A2 Refill Composite; Henry Schein Inc) after etching and rinsing to prevent ingress of the staining liquid through the roots. The teeth were mounted on a plastic holder, and a marker was placed on the plastic holders to match the tripod socket on the colorimeter to standardize the color reading position. Colorimeter readings were made 3 times and averaged before staining. The CR-300 has a repeatability (short-term) of ±0.0002 for chromaticity (x, y), with a measured color difference (ΔE^{*}_{ab}) within a standard deviation of ±0.07. Studies show the validity to be 0.42 for redness and 0.23 to 0.24 for pigmentation and the reliability to be very high.[25,26]

The teeth were divided into 5 groups, with 9 teeth per group in wine (Oak Leaf Merlot wine; The Wine Group LLC), tea (Great Value black tea bags; Walmart Inc), coffee (Great Value Classic Roast Instant Coffee; Walmart Inc), soda (Coca-Cola; The Coca Cola Co), and water (Crystal Springs bottled water; Crystal Springs). SEM analysis (Nova NanoSEM 230; FEI Co) was performed on the coronal and apical positions of 1 tooth from each group (n=5) for baseline.

Each tooth was washed 3 times in ethyl alcohol, dried, and placed in 15-mL polyethylene corrosion jars (Thermo Scientific Nalgene Oak Ridge High-Speed Centrifuge Tubes; Fisher Scientific). The teeth were immersed in 1

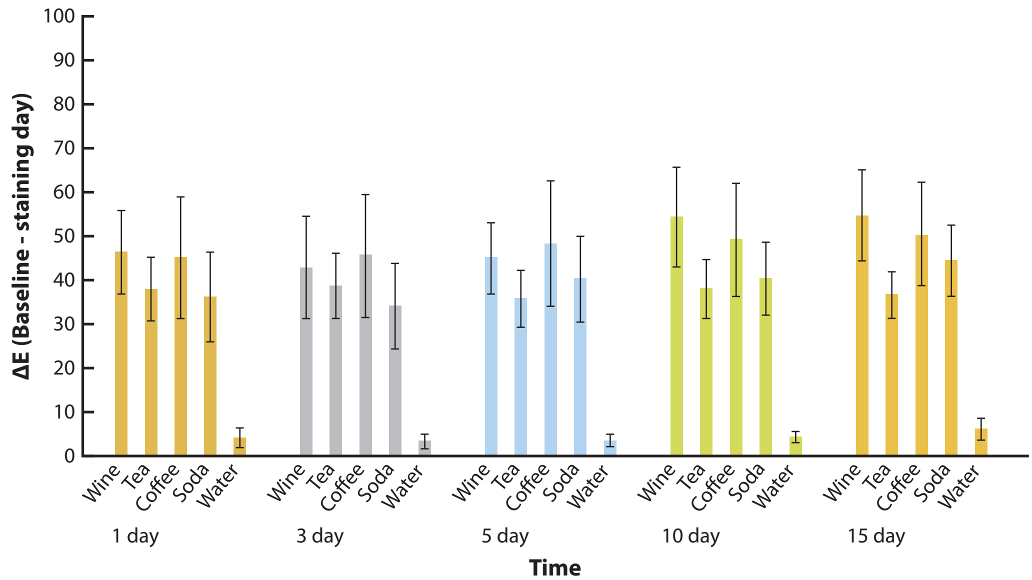

Figure 1. ΔE after immersion in different liquids at different time intervals.

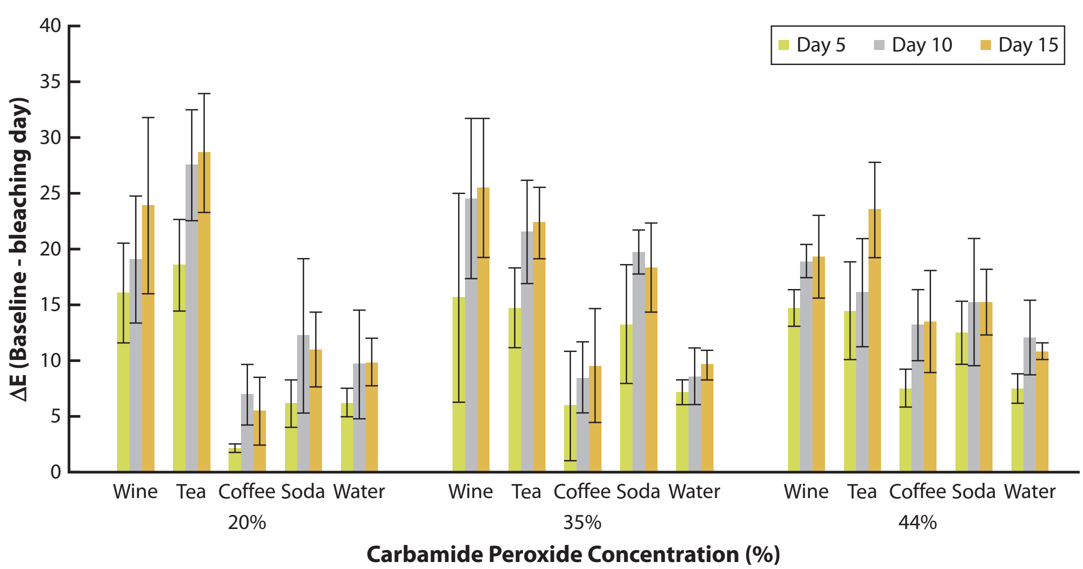

Figure 2. ΔE after bleaching in different concentrations of carbamide peroxide at different time intervals.

of the 5 solutions in corrosion jars. The pH of the solutions was measured (S47 SevenMulti dual meter pH/Conductivity; Mettler Toledo) before and after the experiment. Each specimen was sealed using Teflon tape and placed in a shaker bath (TSBS40; Techne USA) containing de-ionized-distilled water at 80°C with a vibrating speed of 50 oscillations per minute to promote accelerated aging. Based on the Arrenhius equation,[27] 15 days at 80°C is equivalent to 183 days or 6 months at 37°C. The teeth were left for 15 days in the shaker bath, and the colorimeter values (L*a*b*) were obtained for each tooth at baseline and at time intervals of 1, 3, 5, 10, and 15 days during the staining process. After immersion, SEM images were made to compare with baseline.

The teeth were divided into 3 groups (15 teeth per group) of bleaching agents: 20% (Opalescence PF; Ultradent Products, Inc), 35% (Rapideffects Teeth Bleaching Whitening, gel syringes; WhitenSwiftly), and 44% carbamide peroxide gels (Dr. Song's home professional whitening kit 44%; Dr. Song). The teeth were bleached per manufacturer instructions for 15 days. The teeth were stored in distilled water during bleaching to prevent enamel desiccation. L*a*b* values for each tooth were obtained at 5, 10, and 15 days and compared with baseline. SEM images were made on the representative teeth to determine changes to the enamel surface. After bleaching, the teeth were restained following the same procedure described to determine

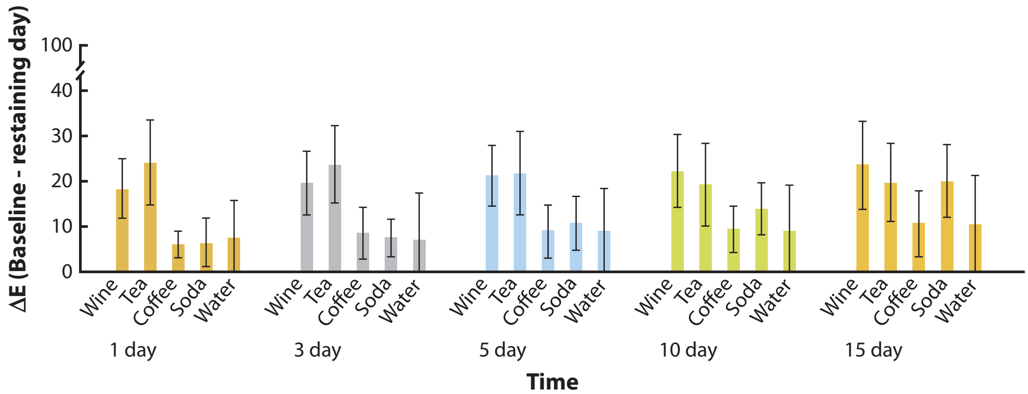

Figure 3. ΔE after restaining in different liquids at different time intervals.

whether the teeth stained more rapidly after bleaching. L*a*b* values, EDS, and SEM analyses were conducted again after restaining.

Statistical analysis was performed using the Mann-Whitney test to detect differences in ΔE before and after staining. One-way ANOVA was used to determine which liquid stained the most and to compare the outcomes of different concentrations of bleaching solutions. A mixed-effects linear model with a random factor for 'tooth' to account for the multiple observations on each tooth was used to determine whether staining occurred faster during the restaining process (α=.005 with Bonferroni correction).

RESULTS

The pH values for all liquids remained constant before and after the immersion process with water at pH 7.562, wine at pH 3.268, tea at pH 5.278, coffee at pH 4.935, and soda at pH 2.457. All liquids produced a ΔE greater than 2, which is the ADA Standard 80 threshold for detecting a perceptible difference in color (Fig. 1, P=.004). Liquid was significantly associated with ΔE (df=4, 40; F=48.6; overall P<.001) with wine staining teeth the most. Using a significance of P<.005 for the Bonferroni correction for multiple comparisons, the following conclusions were derived from pairwise comparisons of solutions. Coffee, soda, tea, and wine caused significantly more staining than water (all P<.001). Coffee (ΔE 13.9 units larger) and wine (ΔE 18 units larger) caused significantly more staining than tea but were not significantly different from one another (P=.301). Soda was not significantly different from tea (P=.054), coffee (P=.126), and wine (P=.013).

The ΔE for the different concentrations of whitening solutions are shown in Figure 2. One-way ANOVA shows no significant differences among the concentration groups (df=2, 42; F=.101; P=.905). This test was repeated for each liquid separately. All P values were >.15, showing no evidence that bleach concentration acts differently on different stains. Even at 20% concentration, similar changes in color were observed as with higher concentrations.

The ΔE to determine whether the bleaching process made the teeth susceptible to restaining is shown in Figure 3. A mixed-effects linear model with a random factor for 'tooth' to account for the multiple observations on each tooth was used. The data were grouped as first stain and second stain, and the trajectories of all teeth during each staining were compared. No significant difference was found in the rate of staining between the 2 time periods, with P=.569 for the interaction between stain group and time, meaning the slope of the trajectories across time did not differ among stain groups (Fig. 4). The ΔE after the second staining was considerably lower than for the first stain. However, a significant effect of time was found where the overall ΔE was estimated to increase by 0.34 for each day in solution (P<.001). A significant effect of the staining group was found where the ΔE across all time points was estimated to be 22.1 higher in the first staining process than in the second (P<.001). This analysis was repeated for each type of liquid to determine whether this effect was the same for all solution types.

SEM analysis revealed no increased roughness or irregularities in the enamel topography in all specimens after staining or bleaching. However, wine and tea left a residual coating layer on the surface of the enamel (Figs. 5, 6). EDS was used for the elemental analysis of the surfaces. The soda and coffee groups showed elemental composition similar to that of the water group.

DISCUSSION

This in vitro study demonstrated that immersing enamel in common solutions produced a color change of ΔE greater than 2; hence, teeth are susceptible to extrinsic staining using common liquids and the first null hypothesis was rejected. The staining potential of foods is theorized to be a result of the presence of chromogenic polyphenols. These compounds are believed to bind to proteins, such as the pellicle or bacteria on the surface of the teeth.[3] For this in vitro study, the teeth were immersed in the liquids for 15

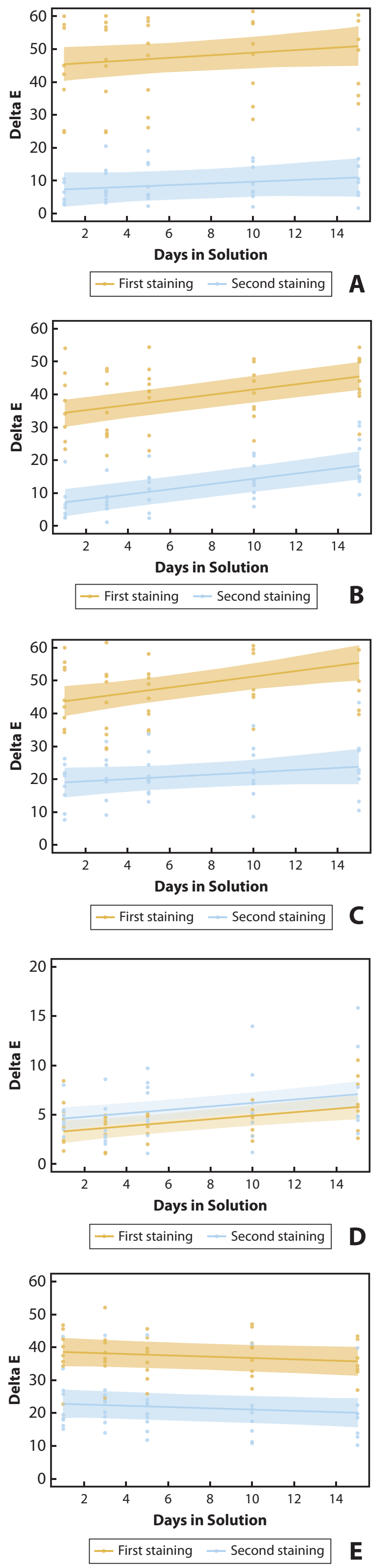

Figure 4. Trajectories of ΔE comparing first stain (*red slope*) and second stain (*blue slope*). Slopes remained constant for both staining processes. A, Coffee. B, Soda. C, Wine. D, Water. E, Tea.

days at an elevated temperature to mimic the effects of an extended period of consumption. However, in reality, teeth are not soaked in these liquids. The absence of saliva, tooth brushing, and the motions of the tongue and cheeks are limitations of the study because of the role they play in cleaning the surfaces of teeth. The effect of continuous immersion in vitro can translate into longer exposure in vivo. A different composition coating was noted in the wine and the tea groups as evidenced by SEM and EDS. Wine and tea both have high concentrations of polyphenols, so the assumption can be made that these attached to residual proteins on the surface of the extracted teeth. Since the teeth are nonvital and not in a nutrient environment, the proteins eventually peeled off, taking the coating with them.

This study also found that the highest concentration of carbamide peroxide did not produce the greatest change in ΔE; hence, there is no difference in the efficacy of the different concentrations and the second null hypothesis was accepted. Manufacturers typically recommend a regimen of 7 to 10 days for effective removal of stains when teeth are restored to their original color or bleached lighter than the original. This study showed that stains were minimized after 5 days by the lowest concentration bleaching agent as evidenced by the color change (Fig. 2).

This study also examined the effect of bleaching on the susceptibility of the enamel for increased or faster restaining when re-exposed to solution. Previous studies have shown bleaching to cause enamel roughness, which can make the tooth susceptible to restaining.[16,19,20] However, results from SEM analysis of teeth from this study revealed no topographical changes after bleaching, in agreement with other studies,[12-14] leading to acceptance of the fourth null hypothesis. Possible explanations for the conflicting results are differences in methodology; 1 study[16] used a pretreatment solution which contained either citric or phosphoric acid, which was not used in studies that reported no changes to enamel after bleaching. Other studies[19,20] used hydrogen peroxide, known to etch enamel, as a bleaching agent. Reimmersion of the bleached teeth also did not show any increased staining susceptibility, leading to the acceptance of the third null hypothesis.

Another limitation of the study is that the bleaching process did not include the effect of saliva as a remineralizing agent. This protocol would again represent several cycles of bleaching in vivo. Thus, within the limitations of the study and the bleaching solutions which were used, bleaching does not seem to affect the restaining potential of teeth. The results from this study demonstrated a lower ΔE for the restain than for the initial stain, which can be explained by the fact that the baseline used for the ΔE for the restain was the L*a*b* values after bleaching for 15 days. When the ΔE value

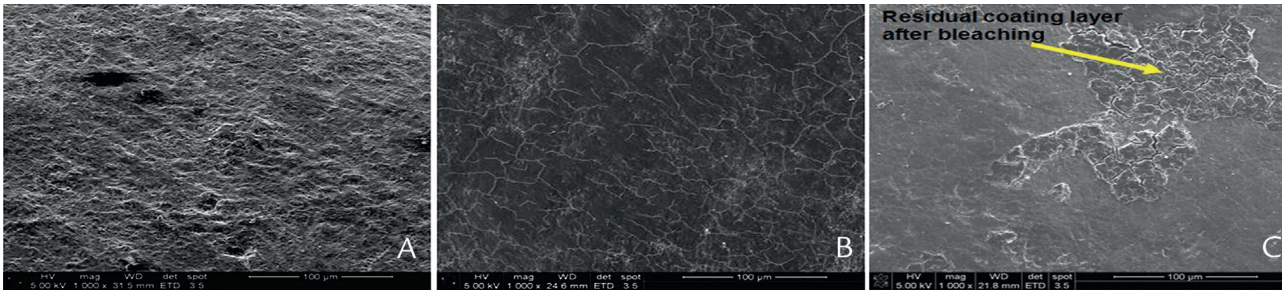

Figure 5. Scanning electron micrographs of wine-stained specimen (original magnification ×1000). A, Baseline. B, After staining. C, After bleaching. Remaining coating layer denoted by *yellow arrow*.

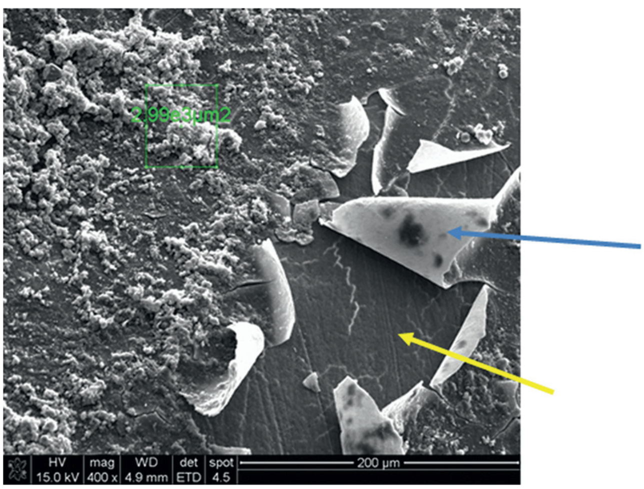

Figure 6. Scanning electron micrographs of wine-stained specimen (original magnification ×400). *Blue arrow*, coating layer of polyphenols starting to peel; *yellow arrow*, tooth structure underneath.

between the teeth bleached at 15 days and the teeth before any treatment was done was calculated, ΔE showed differences ranging from 34 to 42 for wine, coffee, and soda (Fig. 7). The water, which was the control,

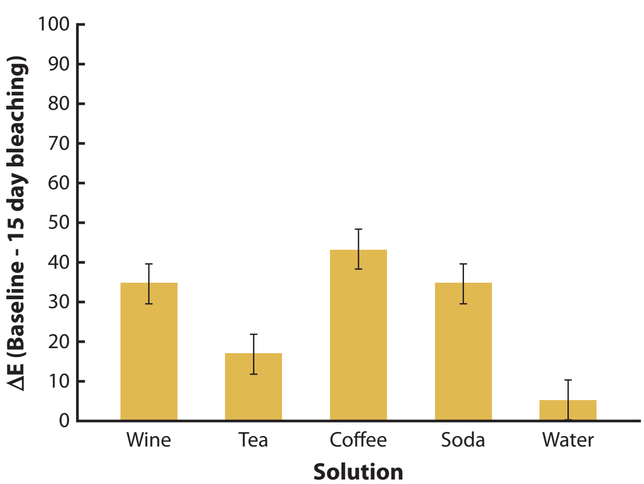

Figure 7. ΔE after 15 days bleaching and untreated teeth.

had a ΔE of only 5. The L* values after the 15-day bleaching were generally lower (L* of 0 is equivalent to black). This would indicate that the bleaching process did not return the teeth to their original color. The restain ΔE values reflected a move from an already dark color to a darker one. Another possible explanation is that whatever proteins or reaction occurred on the surface with the chromogenic phenols had already been saturated with the initial stain and were no longer available for restain. Future studies should be performed on 2 groups, with 1 just stained and the other bleached and stained to isolate the effect of bleaching before staining. Profilometric analysis can also be conducted on the teeth before and after bleaching as another method of measuring surface roughness.

CONCLUSIONS

Within the limitations of this in vitro study, the following conclusions were drawn:

1. Common liquids produce a color change in enamel that is perceptible to the human eye with a ΔE greater than 2. Wine, which contains high amounts of polyphenols, stained the teeth the most.
2. Different concentrations of carbamide peroxide did not show a difference in their effectiveness in reducing the stain on enamel.
3. The process of bleaching with carbamide peroxide used in this study did not increase susceptibility to further staining.
4. Bleaching with the carbamide peroxide products used in this study did not produce changes to the enamel topography, further supporting the conclusion that bleaching does not increase susceptibility to restaining.

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