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IN VITRO EVALUATION OF MICROHARDNESS, SURFACE ROUGHNESS, AND COLOR CHANGES IN BLEACHED ENAMEL AFTER BISCOVER LV APPLICATION

Ö. Erçin[a,1] and E. Güler[b] UDC 539.4

The purpose of this study was to investigate the effect of the BisCover LV application on the microhardness, surface roughness, and color change after bleaching treatment application. 120 bovine teeth were collected for this study. Spectrophotometric color analysis of the samples was performed as an initial step and after bleaching. The samples were randomly divided into 8 groups (n=15) as BisCover LV and Biscover LV-free samples, submerged in discoloring solutions (coffee, wine, cola, water) for 15 min, 7 h, and 3.5 days. Spectrophotometric color analysis was performed and recorded at each evaluation period. ΔE color change was calculated. BisCover LV applied samples were significantly less colored than BisCover LV-free samples regardless of solution type and evaluation period, after bleaching ($p<0.001$). BisCover LV applied water-submerged samples showed the least ΔE value (2.35±1.13) and this difference was statistically significant ($p<0.001$). BisCover LV-free samples kept in wine showed the maximum ΔE (25.60 ± 7.28) and this difference was shown to be statistically significant ($p<0.001$). Teeth come to the fore in providing esthetics therefore bleaching has become more common. It is important to maintain tooth color after bleaching treatment. Biscover LV can protect the teeth after bleaching.

***Keywords*:** bleaching, discoloration, esthetic, BisCover LV, spectrophotometer.

Introduction. Color disorders are upon the most commonly encountered problems in dental practice that can ruin esthetic appearance [1]. Vital tooth bleaching is one of the most commonly applied procedures in dental practice as it provides a more conservative and economical treatment option when compared to other approaches like composite veneers, ceramic veneers or ceramic crowns. Several studies have been introduced investigating the effectiveness of bleaching materials and techniques on discolorations [2]. The effectiveness of bleaching can be determined in many ways. The most commonly used methods for determining color changes in clinical trials are; color scales, computer aided methods and spectrophotometers [3]. In the evaluation process of color changes, color scales are used to describe subjective results, while spectrophotometers show objective results [4].

Generally, hydrogen peroxide, carbamide peroxide and sodium perborate are used in the bleaching process [5, 6]. Most of the office-type bleaching materials contain 30–40% hydrogen peroxide or 35% carbamide peroxide [7]. The success of bleaching treatment is directly related to penetration of bleaching agent into the enamel tissue.

Bleaching materials are effective in whitening but they have some side-effects such as surface changes (microhardness, surface roughness, mineral content decrease), microleakage in restoration, external root resorption and pulp irritation [8]. Many studies have evaluated the effect of peroxide-containing bleaching materials on dental hard tissue, however, there is limited data that evaluate the effectiveness, continuity and morphological effects of different

[a]Biruni University, Faculty of Dentistry, Department of Restorative Dentistry, Istanbul, Turkey ([1]ozlem.ercin@kent.edu.tr). [b]Ondokuz Mayis University, Faculty of Dentistry, Department of Restorative Dentistry, Samsun, Turkey. Translated from Problemy Mitsnosti, No. 6, p. 118, November – December, 2022. Original article submitted December 10, 2021.

bleaching techniques on enamel surface. In the majority of these studies, significant changes were not detected on the enamel surface [9, 10].

To the authors knowledge, there was no study about preventing the relapse of the desired effect of bleaching agent on enamel with BisCover LV application. Thus, the aim of this in vitro study was to evaluate the color stabilizing, effect of BisCover LV application on bleached enamel after being stored in different coloring solutions. The tested null hypothesis was that the application of BisCover LV after bleaching, will increase the resistance of enamel to discoloration in the long and short term.

Materials and Methods. A total of 120 freshly extracted bovine incisor teeth were included in this study. Calculus, debris and soft tissues on the teeth were removed with a handpiece. The teeth were examined with a loop at ×2.5 magnification and those with cracks, caries and fractures were excluded. The teeth were debrided by rinsing under running water followed by prophylaxis and polished with pumice rubber cups and kept in isotonic saline for days. The roots of the teeth were removed away from their crowns by the help of diamond burs. The teeth were then embedded in acrylic blocks, with buccal surfaces exposed (Fig. 1). Table 1 shows the materials used throughout the study.

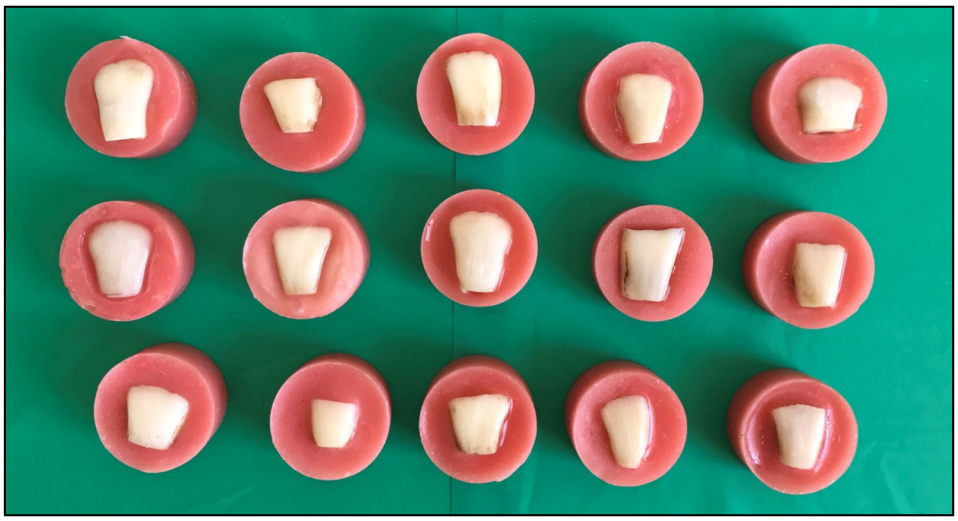

Fig. 1. Samples embedded into the acrylic blocks.

TABLE 1. Materials Used in the Present Study

Material	Content	Manufacturer	Batch Number
Opalescence Boost	40% Hydrogen Peroxide	Ultradent Products (South Jordan, Ut, USA)	Q108
Biscover LV	Dipentaerythritol Pentaacrylate Esters and Ethanol	Bisco (Schaumburg, Il, USA)	1600001672
Uni-Etch	32% Phosphoric Acid	Bisco (Schaumburg, Il, USA)	1600001677

The first color measurement of the teeth was performed with SpectroShade MICRO (MHT Optic Research, Niederhassen, Switzerland) according to the manufacturer's instructions. All color measurements were performed by a single operator in the dark room. Measurements were made according to the CIE Lab color system and recorded. After the first color measurement of teeth, teeth were divided randomly into 8 groups with 15 samples in each group (Table 2).

TABLE 2. Separation of Groups

	Biscover LV (+)				Biscover LV (–)			
Groups	1. Group	2. Group	3. Group	4. Group	5. Group	6. Group	7. Group	8. Group
Solutions	Coffee	Wine	Cola	Water	Coffee	Wine	Cola	Water

The bleaching agent was applied to the specimens in a thickness of 2 mm, mixed according to the instructions of the manufacturer and kept for 15 min and then washed away with distilled water. This process was repeated 3 times in the same day. Bleached samples were stored in an incubator for 1 day at 37ºC. The second color measurement was also performed with SpectroShade MICRO and recorded (Fig. 2). For 4 groups, BisCover LV applied for surface protection, the other 4 groups were left without protection. 32% phosphoric acid (Uni-etch, Bisco Inc, Schaumburg, IL, USA) was applied to the specimens for 30 s prior to application of the BisCover LV (Bisco Inc, Schaumburg, IL, USA). The acid was removed from the samples by washing with water for 20 s and the surfaces were dried. Prior its use, BisCover LV was thoroughly shaken and poured into a thin layer of soft motion using a brush and left untouched for 15 s to allow solvent evaporation, then it was polymerized for 30 s with a LED light curing device. All samples were immersed in coffee (Nescafe, Nestlé, São Paulo, SP, India), red wine (Doluca, Turkey), cola (Coca Cola, Turkey) and water. Coffee was prepared by adding 2 teaspoons of coffee to 100 ml of boiled water at 100°C.

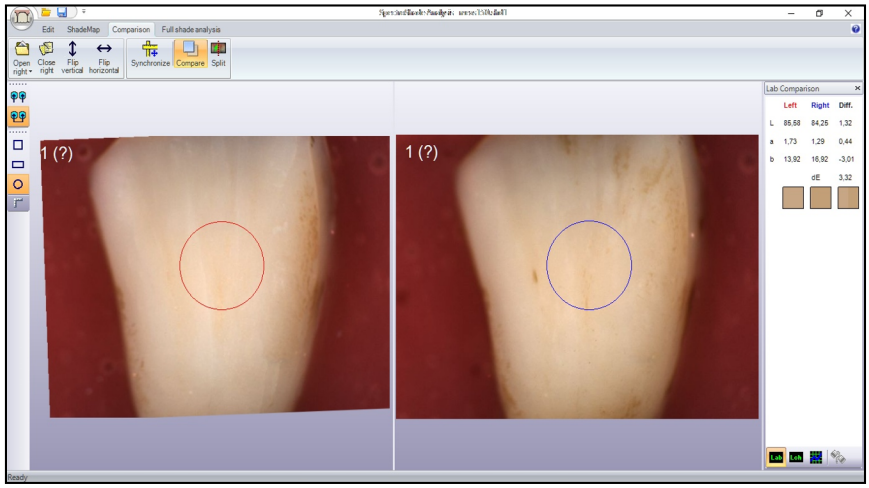

Fig. 2. Determining the discoloration.

The samples were left in the solutions for 15 min, then removed and washed with running water and dried. Color analysis was then performed with a SpectroShade MICRO. The samples were synchronized before the color difference was determined then the samples were re-immersed in the solutions.

After 7 h, the samples were removed from the solutions and washed throughly and color measurement was repeated. Solutions were renewed, and the specimens were immersed again and placed in the incubator. After 3.5 days the third color measurement was performed, and the values were recorded. The first color measurement was performed to simulate 1 day (15 min), the second color measurement to 1 month (15 min + 7 h) and the third color measurement to one year (15 min + 7 h + 3.5 d).

The results were compared with the L^*, a^*, and b^* values obtained after bleaching. Color values were obtained from the middle trio of teeth. As shown in Table 3, the ΔE values are calculated by the following formula: $\Delta E = [(L_1 - L_2)^2 + (a_1 - a_2)^2 + (b_1 - b_2)^2]^{1/2}$.

One-way ANOVA, post hoc Dunn, Kruskal–Wallis and Tukey multiple comparison tests were used to evaluate the ΔE values of the samples. The statistical significance level is set to 5%. The results were considered significant for p <0.05.

Results. The averages and standard deviations of the ΔE values of the tested samples as 4 subgroups for 2 groups based on 2 different surface finish shapes and 4 different coloring solutions are shown in Table 4.

First ΔE measurements showed that, bleaching agent effectively bleached the samples. Wine-stained samples without BisCover LV showed the highest ΔE measurement in all groups for third ΔE measurement. The lowest value was recorded for third ΔE measurement of the water-immersed samples after application of BisCover LV.

TABLE 3. Calculation of ΔE Values

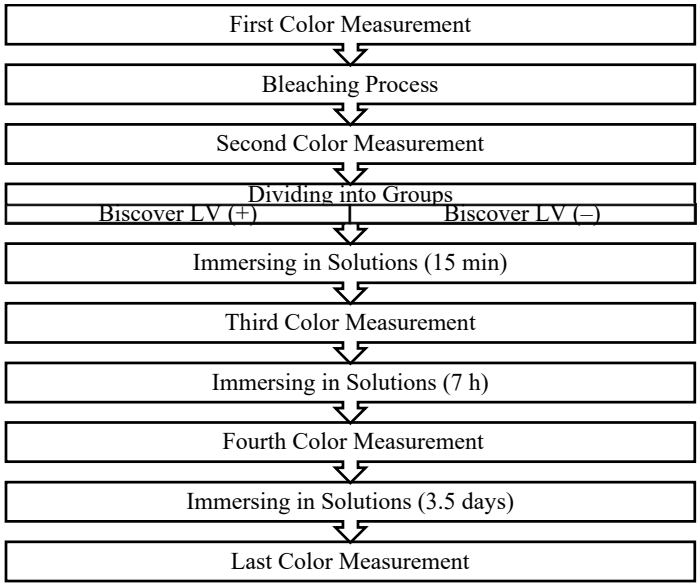

TABLE 4. ΔE Values

	$\Delta E_1\ (T_0 - T_1)$ *Mean ± Sd*	$\Delta E_2\ (T_1 - T_2)$ *Mean ± Sd*	$\Delta E_3\ (T_1 - T_3)$ *Mean ± Sd*	$\Delta E_4\ (T_1 - T_4)$ *Mean ± Sd*
Water	8.53 ± 2.71 $^{a\,x}$	2.55 ± 1.71 $^{a\,y}$	2.53 ± 1.62 $^{a\,y}$	6.05 ± 3.88 $^{a\,x}$
Wine	5.03 ± 2.50 $^{b\,x}$	2.87 ± 1.26 $^{a\,x}$	4.90 ± 3.16 $^{ab\,x}$	**25.60 ±7.28** $^{\mathbf{b\,y}}$
Cola	7.20 ± 3.51 $^{ab\,x}$	3.58 ±1.25 $^{a\,y}$	6.32 ± 7.34 $^{bc\,y}$	12.57 ± 8.53 $^{c\,x}$
Coffee	7.22 ± 3.64 $^{ab\,x}$	2.43 ± 0.92 $^{a\,y}$	4.29 ± 3.51 $^{ab\,xy}$	11.00 ± 4.54 $^{c\,z}$
Biscover LV+Water	7.34 ± 2.99 $^{ab\,x}$	3.41 ± 1.70 $^{a\,yz}$	3.03 ± 1.50 $^{a\,yz}$	**2.35 ± 1.13** $^{\mathbf{d\,z}}$
Biscover LV+Wine	7.49 ± 2.45 $^{ab\,x}$	2.40 ± 0.97 $^{a\,y}$	7.66 ± 3.55 $^{c\,x}$	17.63 ± 4.80 $^{e\,z}$
Biscover LV+Cola	6.22 ± 3.52 $^{ab\,x}$	3.05 ± 1.41 $^{a\,y}$	5.49 ± 4.18 $^{ab\,xy}$	9.18 ± 5.92 $^{ac\,x}$
Biscover LV+Coffee	5.26 ± 2.15 $^{ab\,x}$	2.54 ± 0.86 $^{a\,y}$	4.43 ± 1.71 $^{ab\,x}$	5.04 ± 3.37 $^{a\,x}$
P value	0.022	0.150	< 0.001	< 0.001

**Different letters indicate statistical difference (^{abcde} Column, ^{xyz} Row)*

$\boldsymbol{T_0}$: Initial color determination, $\boldsymbol{T_1}$: color difference after bleaching, $\boldsymbol{T_2}$: color difference after 15 min immersion in solutions, $\boldsymbol{T_3}$: color difference after 15 min+7 h immersion in solutions, $\boldsymbol{T_4}$: color difference after 15 min+7 h+3.5 days immersion in solutions

Discussion. Tooth discolorations are one of the most commonly encountered problems that adversely affects the beauty of a smile. Today, the esthetic expectations of patients have increased and studies about this issue have gained importance [11]. Bleaching is one of the economical and conservative treatment options available when compared to other options for the provision of this expectancy [12].

Office type bleaching provides an immediate solution for patients who seek whiter teeth in a short period of time [13]. It is generally carried out with the help of high concentrations of bleaching products containing hydrogen peroxide [7, 14].

Peroxide-containing bleaching agents at high concentrations have been widely investigated in many studies as they can increase the enamel's porosity [15]. Examinations under atomic force microscopy (AFM) revealed an irregular and rough enamel surface with deep grooves, both after bleaching treatments containing 10% carbamide peroxide and 30% hydrogen peroxide [15, 16].

Some researchers have attributed the demineralization that occurs after bleaching to the loss of Ca and PO_4 ions. Bistey et al. [17] reported that, both organic and inorganic changes may increase enamel sensitivity to discoloration. According to their studies; the use of hydrogen peroxide, even at low concentration (10%), can cause noticeable changes in the superficial enamel. They also mentioned that not only the hydrogen peroxide concentration, but also the duration of its application can make such changes.

Discoloration is not only related to surface roughness, but also the composition of the enamel and the water sorption rate caused by the irregularity and permeability of the surface after bleaching [15].

Bleaching is a time-consuming treatment for the patient and the dentist. After frequent repeated bleaching treatment, the teeth can become sensitive. In addition to that, bleaching is a costly process for patients. For such reasons, it is of a great importance to maintain the results obtained by bleaching for as long as possible. Concerns about color stability after bleaching are common and need to be resolved [18]. Although there are many studies on the effectiveness of bleaching techniques and the different bleaching materials on the enamel surface, studies on the persistence of bleaching are rare [19].

Several studies have investigated the effectiveness of BisCover in protecting the dental enamel and prolonging the effects of bleaching. In these studies, BisCover was applied on composite resin to evaluate its effects on discoloration, microleakage and surface roughness [20–22].

BisCover LV manufacturers claim that application of BisCover LV on the enamel surface after bleaching prevents discolorations. In our study BisCover LV was applied to the enamel surfaces as a preservative agent in order to prolong the obtained desired effects of bleaching. The results of our experiment showed that the application of BisCover LV on enamel minimizes discoloration.

All mammalian teeth exhibit histochemical and anatomical similarities. Attia et al. [23] reported that human and bovine enamels gave similar responses to bleaching and coloring. Bovine teeth are easy to find and are deprived of decay. For these reasons bovine teeth were preferred for the present study.

Color scales are the most commonly used method for color determination in dentistry. However, this method of color detection is not objective and may also show differences due to experience, age, fatigue, light conditions and physiological changes [24]. Spectrophotometers are also widely used for surface color measurement. The fact that spectrophotometers give accurate and standardized results for a long time leads to the preference of these instruments for color measurement [25].

Khurana et al. [26] reported that SpectroShade Micro shows more repeatable and reliable results when compared to the other two devices (Vita Easyshade and X-Rite Shadevision). Aka et al. [27] performed color measurements using Spectroshade to compare two different home bleaching agents. Color analysis was performed using Spectroshade's own light, in a dark room. Based on these studies, our color measurements were performed in a dark room by same operator.

Rough surfaces tend to accumulate bacteria and plaques. Studies show that BisCover increases the surface smoothness of composites [28, 29]. Catelan et al. [28] reported that all surface finishing protocols evaluated in their studies resulted in less discoloration than the control group and that BisCover was the most resistant to discoloration among them. Küçükesmen et al. [30] have shown that the highest surface contact angle is in BisCover, among the various polishing systems they applied to composite resin. In this study, it is possible that the BisCover applied enamel surfaces are more resistant to coloring than the BisCover-free enamel surfaces, which may be related to the high surface contact angle.

Cola, black tea, coffee and wine are the most commonly used discoloring solutions. These solutions have the potency to stain or discolor the surface of bleached enamel. Some of these are acidic solutions that can increase demineralization, while others contain ethanol and pigments [31]. The pH of the solutions used in our study; 3 for red wine, 3.2 for red wine and 5 for coffee. Although the lowest pH is in cola, the most discoloration was seen in red wine. This may be due to the fact that the wine contains alcohol and red pigments. Although the wine has resulted in the most discoloration, it has been shown to significantly prevent discoloration in final color measurements of samples placed in the wine after the application of BisCover LV.

Similar to the present study, Pirolo et al. [32] showed that, cola caused more discoloration than coffee. This result can be attributed to the phosphoric acid content. When the ΔE values of the specimens kept in cola were examined, the specimens were obviously more resistant to coloring in the BisCover LV applied samples, regardless of the time.

It was shown that BisCover prevents discoloring when the groups immersed in coffee are evaluated and compared within themselves. When the ΔE values of the control groups were examined, the ΔE_4 values of the water-immersed samples were calculated as 6.05 ± 3.88. That is, in the samples which were not applied any protective agent after bleaching, some discoloration could be seen after water absorption. After applying BisCover, the ΔE_4 values of the water-immersed samples was calculated to be 2.35 ± 1.13. This demonstrates that the BisCover application can protect the samples from discoloration due to water sorption and are within clinically acceptable limits.

There are many studies that evaluated color stability after the application of BisCover on the composite resin as a protective agent, however, none have investigated its protective effects when applied onto the enamel surface. In this study it has been showed that applying BisCover on the enamel surface could protect it from discoloration.

According to the results of our study; BisCover increased the resistance to discoloration after office bleaching of enamel at a statistically significant level against various coloring solutions. Within the limitation of present study, our hypothesis is accepted.

Rehydration of teeth after bleaching treatment and their subsequent recoloration due to food-beverages habits is inevitable. Thereby, the use of protective materials like BisCover will delay the re-coloring period when applied to tooth enamel after bleaching. The application of BisCover can make a positive contribution to get a longer-term color stability which is of a great benefit for both the dentist and the patient as it helps save time, effort and money. This study should be further supported by in vivo studies.

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