

Annette Wiegand · Doreen Vollmer ·
Magdalena Foitzik · Rengin Attin · Thomas Attin

Efficacy of different whitening modalities on bovine enamel and dentin

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Abstract Previous studies have shown that bleaching treatment may be efficient in both enamel and dentin, but it is still unknown how much the subsurface dentin contributes to the color change of teeth. This in vitro study evaluated the whitening effect of different external bleaching agents on enamel-dentin slabs and subsurface dentin. Ninety bovine teeth were distributed among six groups (A, Opalescence 10%; B, Opalescence PF 15%; C, Opalescence Quick; D, Opalescence Extra Boost; E, Rapid White; F, Whitestrips). Two enamel-dentin specimens were prepared from the labial surface of each teeth. In one of the specimens enamel was removed, resulting in a dentin (CD) disc of 1 mm high. The labial and the pulpal sides of the second specimen were ground until the remaining enamel and dentin layers of the enamel-dentin sample (ED) were 1 mm each. Whitening treatment of the ED specimens was performed according to manufacturers' instructions. Pre- and posttreatment Lab values of ED samples were analyzed using CIE-Lab. Baseline Lab values of dentin were analyzed by evaluation of the CD specimen. Finally, enamel of the ED specimens was removed and color change of the exposed dentin (D) was recorded. For all treatment agents significant color changes (ΔE) were observed for enamel-dentin samples and subsurface dentin specimens compared to controls. In groups A–D ΔE was significantly higher in dentin than enamel-dentin. Furthermore, L and b values of bleached enamel-dentin and subsurface dentin samples differed significantly from baseline. Treatment with the tested external whitening bleaching agents resulted in color change of both enamel-dentin and subsurface dentin samples. The results indicate that color change of treated

teeth might be highly influenced by color change of the subsurface dentin.

Keywords External bleaching agents · Color change · Enamel-dentin samples · Subsurface dentin · Nightguard vital bleaching · In-office bleaching

Introduction

External bleaching treatment is an effective method for restoring the color of discolored vital teeth. Both in-office and home bleaching methods have become popular in dental practices for removing intrinsic stains from the tooth for esthetic purposes. The current bleaching mechanism is based on the ability of hydrogen peroxide to penetrate tooth structure and produce free radicals that oxidize organic stains within the tooth [18]. Several in vitro and in vivo studies have demonstrated the efficacy of external bleaching solutions with various concentrations of either hydrogen peroxide or carbamide peroxide when used as the primary active ingredient [4, 14, 15, 16]. Most in vivo studies show an increase in tooth lightness by either increasing concentration of the applied gels [5, 12, 16] or with longer duration of bleaching treatment [4]. As bleaching of vital teeth involves direct contact of the whitening agent to the outer enamel surface for an extensive period of time, most studies have evaluated whitening effects of the superficial enamel layer only [4, 12, 14].

Although previous studies reported that both enamel and dentin demonstrate permeability to hydrogen peroxide and carbamide peroxide [3, 6, 10, 24], very little information is available about the color changes of subsurface dentin that can be expected from a whitening procedure. McCaslin et al. [17] assessed color changes of enamel and dentin after bleaching treatment with 10% carbamide peroxide gel. They found an increase in lightness but no differences for color changes in inner or outer dentinal areas. However, the methodology of their color analysis allowed no information about the effect of

A. Wiegand (✉) · D. Vollmer · M. Foitzik · R. Attin · T. Attin
Department of Operative and Preventive Dentistry
and Periodontology,
University of Göttingen,
Robert-Koch-Strasse 40, 37070 Göttingen, Germany
e-mail: Annette.Wiegand@med.uni-goettingen.de
Tel.: +49-551-392844
Fax: +49-551-392037

Fig. 1 Study designs (whitening treatment and color assessment) of previous studies compared to the experimental design of the present study

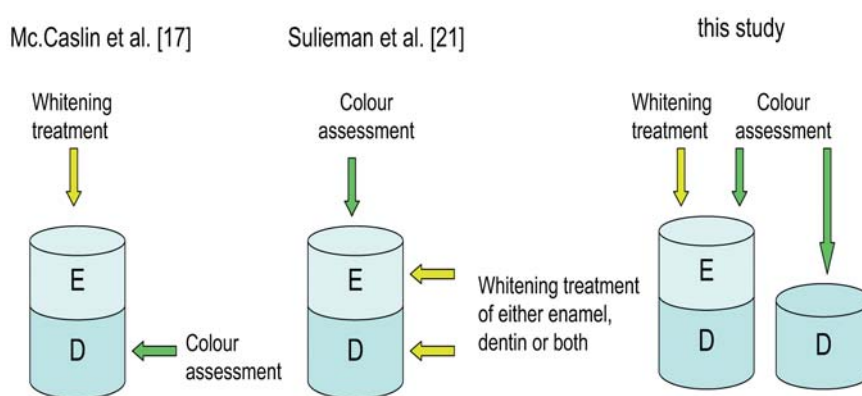


Table 1 Active whitening solutions' composition and pH value used in the study according to information given by the manufacturers

Group	Product	Active bleaching substance	Composition	pH value
A	Opalescence 10%	10% carbamide peroxide ($\cong 3.4\% \text{H}_2\text{O}_2$)	Fillers, flavor	7.8
B	Opalescence PF 15%	15% carbamide peroxide ($\cong 5.1\% \text{H}_2\text{O}_2$)	Potassium nitrate, fluoride	7.9
C	Opalescence Quick	35% carbamide peroxide ($\cong 11.9\% \text{H}_2\text{O}_2$)	No information	7.0
D	Opalescence Extra Boost	35% hydrogen peroxide	No information	5.5
E	Rapid White	Sodium chloride	Accelerator: sodium chloride. Whitening gel: aqua, glycerin, carbomer 974P, aroma, sodium hydroxide, citric acid, methylparaben	3.7
F	Whitestrips	6.0% hydrogen peroxide	Purified water, glycerin, hydrogen peroxide, carbopol 956, sodium hydroxide, sodium acid pyrophosphate, sodium stannate	6.4

subsurface dentin color change on color change of the enamel-dentin unit (Fig. 1). Sulieman et al. [21] evaluated bleaching effects of 35% hydrogen peroxide applied on enamel, dentin, or both enamel and dentin of human third molars (Fig. 1). This does not simulate clinical situation very well since in most cases when bleaching is performed, the whitening agent must penetrate the enamel before reaching subsurface dentin. In this study a significant increase in enamel lightness was observed for all treatments, but ΔE values of the samples did not differ significantly with regard to bleaching treatment of enamel, dentin, or both enamel and dentin. Color change of subsurface dentin was not assessed by Sulieman et al. [21]. Furthermore, in these studies bleaching treatment did not well simulate the intraoral situation. Whitening agents were not applied according to the manufacturer's information, and bleaching treatment was performed without the impact of saliva on the samples.

The purpose of the present in vitro study was to determine the effect of different external whitening agents on the color change of both enamel-dentin samples and subsurface dentin. Moreover, this study intended to determine how much the subsurface dentin contributes to the color change of the tooth. Since the enamel-dentin unit could not be separated into enamel and dentin slabs, careful removal of the enamel surface by grinding and polishing was necessary to expose subsurface dentin for assessment of subsurface dentin color (Fig. 1). The methodology of bleaching treatment and color assessment

of this in vitro study allowed us to analyze the possible effect of subsurface dentin color change on color change of the enamel-dentin unit.

Materials and method

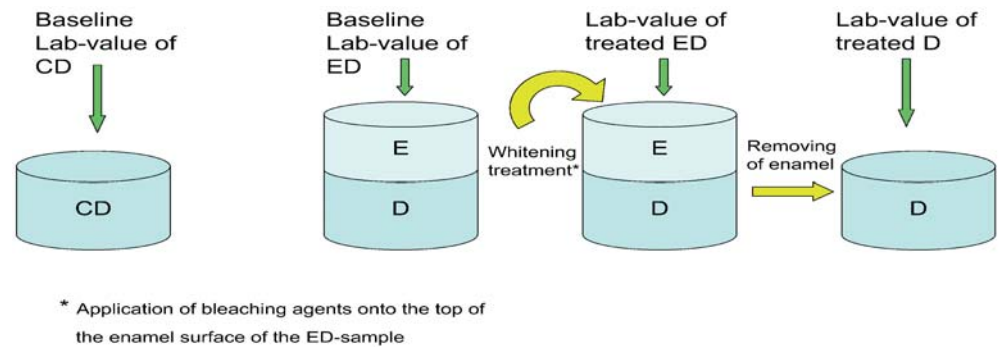
Ninety freshly extracted bovine intact incisors were stored in 0.1% thymol solution at room temperature until required. The teeth were divided into six groups (A–F, $n=15$ each; Table 1) according to the individual bleaching treatment (Table 2). From each crown two enamel-dentin specimens (5 mm in diameter) were prepared from the labial surface with a trephine mill (Komet, Lemgo, Germany). In one of these specimens enamel was removed with water-cooled carborundum discs (400 and 800 grit; Water Proof Silicon Carbide Paper, Stuers, Erkrat, Germany) resulting in dentin specimens (CD) with a height of 1 mm (Fig. 2). The thickness of the dentin disc was determined with a micrometer (Digimatic, Micrometer, Mitutoyo, Tokyo, Japan). In the second specimen the labial and pulpal sides were ground flat and polished with water-cooled carborundum discs (400 and 800 grit; Water Proof Silicon Carbide Paper) until the remaining enamel and dentin layers of the enamel-dentin (ED) specimen were 1 mm each. The enamel and dentin thickness of the enamel-dentin specimen was determined by a microscope (Zeiss, Wetzlar, Germany). The attempt to separate the enamel-dentin slabs into enamel and dentin samples by splitting at the enamel-dentin junction after NO_2 freezing did not result in predictable, standardized dentin samples. Therefore removal of the enamel layer by grinding and polishing was necessary to expose subsurface dentin and allow color assessment of subsurface dentin.

The labial surface of the enamel was cleaned and polished with a prophylaxis paste (RDA 40, CCS, Borlänge, Sweden) using a polishing brush (Hawe Neos, Biggio, Switzerland) which was mounted in a contra angle (1200 rpm). After preparation the

Table 2 Daily whitening treatment and total duration of treatment during the experiment in groups A–F

Group	Product	Whitening treatment	Total duration of treatment
A	Opalescence 10%	8 h, daily	80 h
B	Opalescence PF 15%	4 h, daily	40 h
C	Opalescence Quick	1 h, days 1 and 5	2 h
D	Opalescence Extra Boost	30 min, twice daily, days 1 and 5	2 h
E	Rapid White	20 min, daily	200 min
F	Whitestrips	30 min, twice daily	10 h

Fig. 2 CIE-Lab measurement of dentin control disc, enamel-dentin (ED) and subsurface dentin samples (D) in the different experimental groups A–F



specimens were stored in thymol solution to avoid dehydration. Prior to treatment the color of each specimen was assessed at standardized ambient conditions according to the CIE-Lab system using a dental colorimeter (Shade Eye, Shofu, Kyoto, Japan). Preparing two specimens from each tooth allowed a homogeneous distribution among the experimental groups with respect to baseline color values of the ED specimens.

The samples were carefully dried (not desiccated) and placed on a dark paper. The colorimeter's light sensor was set at right angles to the samples' surfaces and fixed directly on the enamel surface. The color of each sample was measured ten times, and mean values were calculated. The results of color measurement were quantified in terms of three coordinate values (L, a, b) which locate color of an object in a three dimensional color space. After each individual whitening treatment laboratory measurements of treated ED and untreated CD samples were repeated. Lab values of the CD samples served as baseline control. Finally, enamel of the ED specimens was removed by grinding and color change of the exposed dentin (D) samples was assessed (Fig. 2).

To summarize, the experimental groups were as follows: ED specimens were used for color determination of the enamel-dentin unit, D samples were used for color assessment of the subsurface dentin, and CD specimens served as baseline control of subsurface dentin color. To compare tooth color before and after treatment color changes (ΔE) and differences (ΔL , Δa , Δb) were calculated, with the following color definitions of the respective positive (+) and negative (–) values: ΔL =(+) white; (–) black; Δa =(+) red; (–) green; Δb =(+) yellow; (–) blue [7]. For determination of ΔE the following formula was used: $\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{1/2}$.

Whitening treatment

Over a period of 10 days the experimental ED samples were treated with six different home bleaching and in-office bleaching agents (Table 1) according to manufacturers' instructions as described below:

- Group A: Opalescence 10% (Ultradent Products, South Jordan, Utah, USA). Each day the samples were covered with a layer of 1 mm of the bleaching gel for 8 h in a humid atmosphere at 37°C.

- Group B: Opalescence PF 15% (Ultradent Products). Each day the specimens were covered with a layer of 1 mm of the whitening gel for 4 h in a humid atmosphere at 37°C.
- Group C: Opalescence Quick (Ultradent Products). The enamel surfaces were covered with a 1-mm-thick layer of the treatment agent for 1 h. The bleaching treatment was performed on days 1 and 5 at room temperature.
- Group D: Opalescence Extra Boost (Ultradent Products). The samples were treated twice daily on days 1 and 5 and stored in artificial saliva for 7 h between treatments. The samples were covered with a 1-mm-thick layer of the whitening agent for 30 min at room temperature.
- Group E: Rapid White (Natural White, Tonawanda, N.Y., USA). Rapid White accelerator was applied to the specimens followed by coverage with a 1-mm-thick layer of Rapid White gel in a humid atmosphere at 37°C. Treatment was conducted for 20 min once daily.
- Group F: Whitestrips (Procter & Gamble Technical Centres, Egham, UK). The bleaching treatments were carried out according to manufacturer's instructions. The enamel surfaces were covered with a strip twice a day for 30 min each in a humid atmosphere at 37°C with storage time in saliva of 7 h between treatments.

Bleaching with A, B, E, and F was conducted each day; systems C and D were applied on days 1 and 5 (Table 2). The ED samples were embedded in silicone (Silaplast, Detax, Germany) in such a way that the labial surface of the specimen was not covered. The samples ran through their individual treatment cycle on 10 successive days. Additional information about the whitening agents are given in Table 1. At intervals between treatment the samples were transferred into 500 ml artificial saliva formulated by Klimek et al. [13] which was renewed daily. Prior to whitening the enamel surfaces were dried with cotton wool pellets. After treatment and before retransferring to the artificial saliva the whitening agents were carefully removed with tap water and cotton wool pellets.

Statistical analysis

The data were statistically analyzed with the Wilcoxon ranking test using the software package statistica 6.0 (statsoft, Tulsa, Okla., USA). The level of statistical significance was set at $P \leq 0.05$.

Fig. 3 ΔE values (mean \pm SD) of enamel-dentin (ED) and subsurface dentin (D) samples in all experimental groups after 10 treatment days

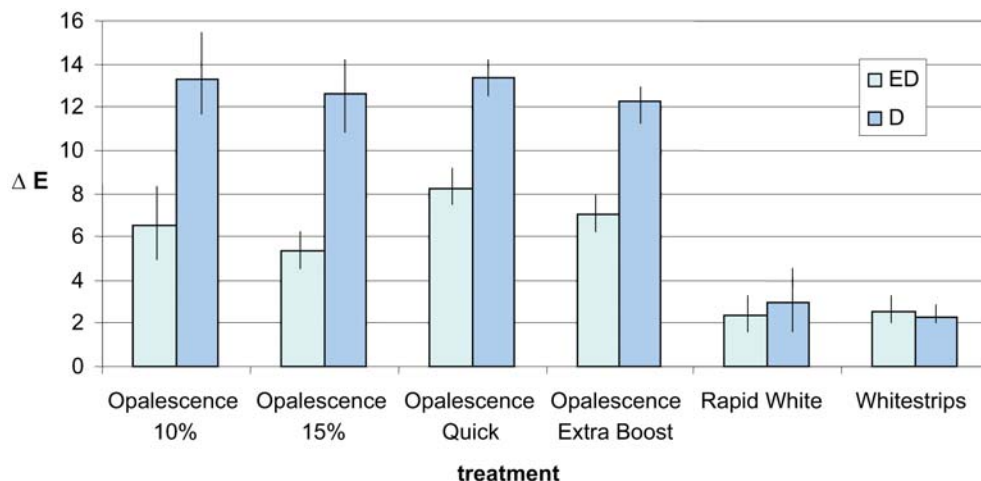
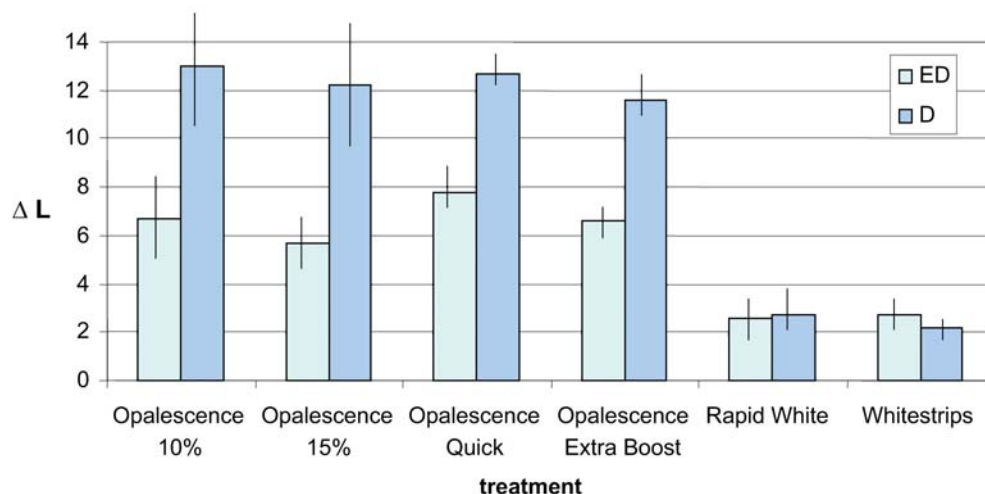


Fig. 4 ΔL values (mean \pm SD) of enamel-dentin (ED) and subsurface dentin (D) samples in all experimental groups after 10 treatment days



Results

Figure 3 presents the color changes (ΔE) of enamel-dentin samples and subsurface dentin specimens in all groups after 10 treatment days. Statistical analysis revealed significant differences ($P \leq 0.05$) between baseline values and the respective test specimen for all whitening agents in both enamel-dentin slabs and subsurface dentin. Furthermore, the color of the bleached ED and D specimens of groups A–D differed significantly from samples of groups E and F. In groups A–D comparison of enamel-dentin and subsurface dentin samples showed significantly higher ΔE values for dentin. No significant differences were observed between ΔE values of ED and D specimens of groups E and F.

Figure 4 presents ΔL values of the experimental groups in enamel-dentin and dentin samples. In both ED and D specimens treatment with all tested materials led to significantly higher ΔL values than in controls. Comparison of the L values of the bleached specimens revealed significant differences between groups A–D and groups E and F in both enamel-dentin and dentin samples. Treatment agents of groups A–D exhibited significantly higher

ΔL values for dentin than for enamel-dentin, whereas ΔL values of groups E and F did not differ significantly between ED and D samples.

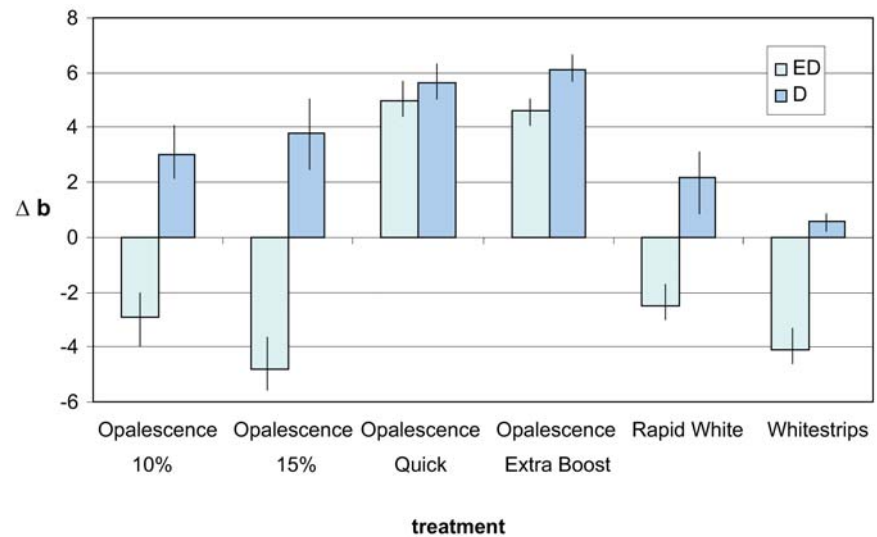
Figure 5 illustrates Δb values of the treated enamel-dentin and subsurface dentin specimens. In ED samples bleaching agents of group A, B, E, and F led to significantly lower b values than baseline, whereas groups C and D showed higher Δb ($P \leq 0.05$). In all subsurface dentin samples (D), whitening treatment led to significantly higher Δb values than baseline (CD; $P \leq 0.05$).

Discussion

Technique

The bleaching agents analyzed in the present study included both home bleaching and in-office bleaching agents. The home bleaching gels of groups A, B, E, and F were applied according to manufacturers' instructions to the enamel surfaces in a humid atmosphere at 37°C to simulate the clinical situation during home bleaching treatment. The in-office bleaching agents (C and D) are

Fig. 5 Δb values (mean \pm SD) of enamel-dentin (ED) and subsurface dentin (D) samples in all experimental groups after 10 treatment days



designed for chairside application under isolation of the teeth with rubber dam; therefore they were applied at room temperature without simulation of a humid atmosphere. To simulate clinical situations and to standardize the experimental conditions the samples were stored in artificial saliva between the treatments. This procedure ensured hydration of the samples throughout the study and prevented color change due to dehydration effects. Furthermore, saliva is known to be an important factor for the remineralization of bleached enamel [1] and is thought to influence the formation of tooth staining [2, 8].

As in other in vitro investigations evaluating color changes after bleaching treatment, bovine teeth were used for specimen preparation [2, 5, 14]. Enamel samples were 1 mm thick, according to the average enamel thickness in human maxillary incisors [11]. As a result of the size of bovine teeth it was possible to prepare two specimens each 5 mm in diameter, which guarantees a homogeneous distribution of the samples among the experimental groups with respect to baseline color values. Diameter of the samples and the light sensor of the colorimeter was 5 mm, ensuring reproducible measuring of Lab values.

Kwon et al. [14] found a difference in absolute surface reflectance in human and bovine teeth which could be explained by differences in human and bovine diet and age. However, only L values were found to be higher in bovine than in human teeth [14]. Nevertheless, chemical and physical properties such as composition, density, diameter of enamel, heat capacity and Vicker's hardness are very similar to human enamel [9, 23]. Moreover, the permeability of coronal incisor bovine dentin is similar to that of human root dentin [22].

Color changes were evaluated using the CIE-Lab system. This method presents a reliable and objective tool for determination of tooth color. Color changes of ΔE values less than 1 U are considered as a color change which cannot be identified by independent observers [19]. Therefore the colorimetric method is a predictable and

accurate method for evaluation of tooth shades in vitro allowing quantitative comparisons [20].

Material-related results

Application of the different external bleaching gels resulted in a lightening of the enamel-dentin and subsurface dentin samples in all groups. With the exception of groups E (Rapid White) and F (Whitestrips) the color change was distinctly greater in subsurface dentin than in compound enamel-dentin specimens. It might be assumed that dentin is more susceptible to whitening than enamel, despite the fact that the bleaching product must penetrate through the enamel layer before perfuse the subsurface dentin.

An increase in ΔE values of both enamel-dentin and subsurface dentin were very pronounced after application of Opalescence 10%, Opalescence 15%, Opalescence Quick and Opalescence Extra Boost. Total duration of bleaching (Table 2) with Opalescence 10% (80 h) and Opalescence 15% (40 h) was considerably longer than for Whitestrips (10 h), Rapid White (200 min), Opalescence Quick (2 h), and Opalescence Extra Boost (2 h). In contrast, the effective concentration of the active bleaching component (H_2O_2) is higher for Opalescence Quick and Opalescence Extra Boost than for the home bleaching agents Opalescence 10%, Opalescence 15% and Whitestrips (Table 1). Rapid White does not contain H_2O_2 as the active bleaching agent. It may be conceived that application time and concentration of the active bleaching ingredient H_2O_2 support degradation of complex organic molecules to less complex molecules. As a result of reduction or elimination of discoloration there was an increase in ΔE values.

In the present study L values demonstrated a shift to positive values in all experimental groups for both enamel-dentin and subsurface dentin. These positive L values are indicators for a perceptible lightening of treated teeth. The increase in L values was very pro-

nounced in groups A–D emphasizing the relationship of concentration and duration of application of the treatment agents. Comparison of b values in enamel-dentin samples showed a decrease for treatment with Opalescence 10%, Opalescence 15%, Rapid White, and Whitestrips, whereas whitening treatment with the in-office agents resulted in a slight increase in b values. In subsurface dentin samples bleaching treatment led to a increase in b values (=more yellow and less blue) in all groups. It may be speculated that the prolonged contact to artificial saliva of the samples treated with these in-office bleaching agents (238 h) influences the outcome of b values. The impact of different intervals of remineralization in artificial saliva to L values of bleached enamel has also been observed by Attin et al. [2].

The results of the present study were confirmed by McCaslin et al. [17]. In their study overnight bleaching treatment using 10% carbamide peroxide for 10 days led to a significant increase in dentin lightness, but no differences were found for inner and outer dentinal areas. McCaslin et al. [17] believed that the uniform color change in dentin was a result of the rapid ingress and easy penetration of the bleaching material into the dental hard tissues. However, in their study dentin color was assessed by digitized and converted photographs taken from the cut surface of the incisogingivally sectioned teeth. This procedure did not allow for measuring of the dentin color change separately (Fig. 1). Therefore information about the influence of the dentin color change on the color change of the enamel-dentin unit was not available.

Sulieman et al. [21] assessed effectiveness of bleaching treatment after application of 35% hydrogen peroxide gel in vertically sectioned tooth halves. Bleaching gel was applied to enamel, dentin, or both at three 10-min intervals. Change in color measured from the enamel surface revealed an improvement in all specimens (Fig. 1). In contrast to the results of McCaslin et al. [15], they observed that bleaching through enamel did not penetrate the entire thickness of the dentin. As a sequela dentin partly remained unchanged by the bleaching agents. Therefore Sulieman et al. [15] concluded that the color change in the dentin area immediately below the enamel layer produce the apparent whitening effect of the compound enamel-dentin specimens. However, color assessments of the teeth were performed on the outer enamel surface only, and information about the subsurface dentin color before and after bleaching was not available.

In contrast to these studies, the study design of the present study provided information about the color change of the subsurface dentin separately. The results indicate that color change of bleached teeth could highly be influenced by color change of the subsurface dentin. However, it is not known whether color regression in bleached teeth would also be influenced by color reversal of subsurface dentin.

Conclusion

Within the limitations of an in vitro study the results of the present investigation indicate that bleaching treatment may result in increased lightness of both enamel-dentin samples and subsurface dentin specimens. This was especially true for bleaching treatment with highly concentrated active bleaching ingredients or bleaching regimens applied for long time periods. Due to the fact that subsurface dentin samples mostly exhibited greater lightness than enamel-dentin samples, it might be concluded that color of bleached teeth is highly influenced by color change of the subsurface dentin. Color change caused by bleaching agents used in the present evaluation seems to be clinically relevant since ΔE values exceeded the clinically detectable threshold of 2 Cie-Lab units [19] in all groups.

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