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Laboratory Research

Effects of Bleaching Agents on Human Enamel Light Reflectance

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Clinical Relevance

In dental bleaching, the micromorphological alterations of hard tissues have been well investigated, but little is known about the optical changes involved. The results of the current study showed that enamel, irrespective of maturation, is subjected to a shift in reflectance towards blue within the color space and enhanced reflection. This effect was demonstrated in different bleaching agent concentrations and protocols.

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SUMMARY

Objectives: Tooth whitening has been associated with splitting-up chromogenic molecules by hydrogen peroxides. Though micromorphological alterations are well documented, little is known about optical changes as a function of shifting in wavelengths. Therefore, the aim of the current study was to measure reflectance changes after bleaching *in vitro* by using a spectrometer.

Methods: Forty-eight enamel slabs (diameter = 5 mm) were prepared from the sound enamel of extracted human teeth that were: 1) fully impacted, 2) from juveniles ages 10 to 16 years, 3) from adults 35 to 45 years of age and 4) from seniors older than age 65. In all specimens, the baseline total reflectance measurement was performed with a computer-assisted spectrometer

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(Ocean Optics, Dunedin, FL, USA) within wavelengths (*wl*) from 430 nm to 800 nm. Four enamel samples of each age group were exposed to either 10% or 15% carbamide peroxide (Illuminé Home, Dentsply, Konstanz, Germany) or 35% hydrogen peroxide (Pola Office, SDI Limited, Victoria, Australia). After surface treatment, all slabs underwent total reflectance measurement again. Statistical analysis was calculated at *wl* 450, 500 and 750 nm using the Student's paired *t*-test and one-way variance analysis.

Results: Total reflectance significantly increased after bleaching at all enamel maturation stages, irrespective of the bleaching agent concentration, for *wl* 450 nm (blue) and 500 nm (green) with $p<0.0001$. At 750 nm (red), significant changes only occurred in enamel from adults and seniors ($p<0.04$). However, the efficacy of bleaching was significantly increased in the blue and green light spectra as compared to the red spectra ($p<0.0001$).

Conclusions: The results of the current study showed that the exclusive assumption of the "chromophore effect" in dental bleaching could not be sustained, because whitening of the dental enamel works at different maturation stages, even in impacted teeth. This effect is irrespective of the bleaching protocol used and the bleaching agent concentration.

INTRODUCTION

The color of teeth is determined by optical properties of mineralized tissues and the combined effects of intrinsic and extrinsic pigmentation.[1-2] Intrinsic discoloration emerges because of the presence of chromatogenic material influencing light scattering and adsorption properties of enamel and dentin. Fluorosis, haematologic disorders, tetracycline administration, malformation of dental tissues, etc are described as sources of intrinsic discoloration.[3]

Extrinsic discoloration tends to form on enamel areas that are difficult to clean and are often promoted by smoking, dietary intake of tannin-rich foods (red wine, tea), chlorhexidine and iron salts.[4]

The wear of tooth structure, deposition of secondary dentin due to aging and dentin sclerosis affects the light properties of teeth, resulting in a gradual darkening and an impact on tooth appearance.[2] Hydroxyapatite crystals surrounded by a protein/lipid/water matrix contribute to light scattering; whereas in dentin, the anisotropic structure and tubules are the most important scattering factors.[5-7]

Brightness may be improved by several approaches and methods, including abrasive toothpastes, microabrasion, scaling and polishing to remove extrinsic stain and calculus, and different bleaching approaches that reduce intrinsic discoloration, for example, internal bleaching of non-vital teeth and external bleaching of vital teeth.

An *in-vitro* study showed that, where the enamel was removed, the color of teeth correlated strongly with the color of the complete tooth.[8]

Clinical experience after bleaching seems to indicate that the main effect of color change does not result from the enamel but reflects alterations in the color of dentin.[9] It was hypothesized that the bleaching effect is a result of the degradation of complex organic molecules being responsible for the color of teeth to less complex molecules and results in a reduction or complete elimination of discoloration.[3]

Studies assessing color change after bleaching, depending on the maturation stage of the teeth and the role of optical impact of enamel on the whitening effect, are lacking. Therefore, it was the aim of the current study to assess visible light reflectance in human enamel before and after the bleaching procedures of different maturation stages.

METHODS AND MATERIALS

Preparation of Specimens

Forty-eight human teeth of different maturation stages were extracted for orthodontic, surgical and/or periodontal reasons. The teeth were stored in saline containing 0.1% thymol. Teeth exhibiting visible cracks or hypoplastic defects or restorations within the examination areas were excluded. The specimens were cleaned using a fluoride-free prophylaxis paste (Miraclean, Hager und Werken, Duisburg, Germany). They were attributed to four age groups: 12 teeth each were: 1) fully impacted teeth, 2) erupted teeth from 10-16 year olds, 3) teeth from adults between 35-45 years of age and 4) teeth from seniors over 65 years of age. All samples were prepared from sound enamel and dentin with a diamond-coated trepan (diameter = 5.0 mm, custom-made product, Komet/Brasseler, Lemgo, Germany) under water-cooling (60 ml/minute). Each specimen was embedded in standardized templates (diameter = 25.0 mm) containing light curing resin (Supertec, DMG Chemisch-Pharmazeutische Fabrik, Hamburg, Germany) without coating the enamel surface. After five-minute polymerization with a 350-500 nm light-curing unit (Polylux PT, Dreve Dentamid GmbH, Unna, Germany), the specimens were removed from the template and flattened from the dentin-surface side by removing the resin with an abrasive wheel (edge 6847 KR, Komet/Gebr Brasseler GmbH & Co KG, Lemgo, Germany) (Figure 1). The dentin was then milled out completely using a rose head bur (H1SE.204.010, Komet/Gebr Brasseler GmbH & Co KG) until the dentin-enamel junction was reached.

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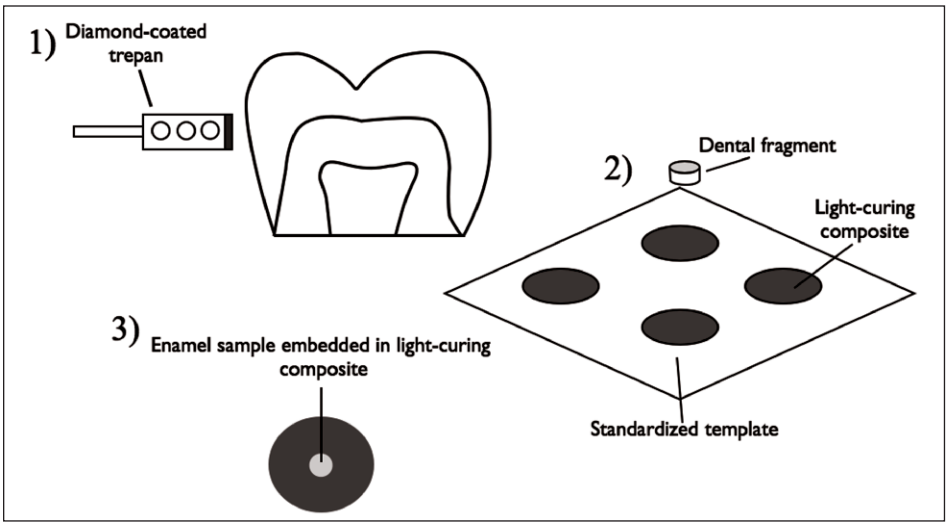

Figure 1. *Preparation of enamel samples.*

This was checked by visual inspection under UV-light (UVGL-15, LTF Labortechnik GmbH & Co KG, Wasserburg/Bodensee, Germany). The enamel slabs were then standardized to a 0.6 mm +/- 0.3 mm thickness assessed by calipers at different measurement points of the specimen.

Spectroscopic Test Arrangement

In all specimens, the baseline total reflectance measurement was performed with a computer-assisted spectrometer (Ocean Optics, Dunedin, FL, USA) within wavelengths (*wl*) from 430 to 800 nm. A halogen light (HL-2000-FSHA, Ocean Optics) and fiber optic cable (QR400-7-VIS/BX, Ocean Optics) with six optical fibers in circular arrangement were used for light emission at a distance of 4.4 mm from the enamel slabs, generating a circular measurement field (diameter = 3.0 mm) in the specimen center. Reflected light was collected by a centrally located optical fiber and transmitted to the spectrometer (Red Tide USB 650, Ocean Optics), which was connected to a computer. The collected light was spectrally enlarged and imaged in a silicium-CCD with 650 pixels (Red Tide USB 650, Ocean Optics). Each pixel represents a certain wavelength corresponding to a band of approximately 1 nm (+/- 0.5 nm). The irradiating light produces an electric charge proportional to the measured light intensity, which is read out as a voltage signal at the CCD. Therefore, the measured voltage signal of each pixel is proportional to the reflected light intensity of a specific wavelength. The wavelength-dependent light reflection intensity was calculated by the Spectra Suite software (Spectra Suite, Ocean Optics).

Baseline Measurement

Measurement was performed in a darkened room with standardized air-conditioning. A white standard plate (WS-1-SS, Ocean Optics) was used to calibrate the measurement unit. The specimens were attached to a holder in such a way that the light always hit the same place throughout repeated measurements (light focus diameter = 3.0 mm). The holder also served as an integrating sphere that maximizes the collection of diffused light inside the sphere. To validate the measurement system, total reflectance assessment was performed five times in untreated specimens, serving as negative controls. Reflectance was equalized arbitrarily at a wavelength of 600 nm (reflectance = 1 at 600 nm), indicating that numerical values did not represent absolute reflectance; instead, they reflected arbitrary units. The measurement of test samples was repeated four times.

Treatment of Specimens

After the initial measurement, the samples were treated as follows: four enamel samples from each age group were exposed to either 10% or 15% carbamide peroxide formulation (Illuminé Home, Dentsply, Konstanz, Germany) or 35% hydrogen peroxide formulation (Pola Office, SDI Limited, Victoria, Australia). In the carbamide peroxide groups of 10% and 15% concentration (pH = 6.0), 14 application cycles of eight hours were executed at intervals of 16 hours. The hydrogen peroxide group was treated three times for eight minutes. This procedure was repeated three times at 12-hour intervals. A final treatment of 3 x 8 minutes of bleaching was performed, of which the last five minutes were activated using a light-curing unit (Polylux PT, Dreve Dentamid GmbH, Unna, Germany). The bleaching agents were removed by rinsing the specimens under running tap water for 30 seconds (flow rate 50 ml/second). During non-treatment times, all the teeth were placed in artificial saliva containing 150 mmol/l KCl, 1.5 mmol/l $CaCl_2$ and 0.9 mmol/l KH_2PO_4 in 100 ml aqua bidest, pH 6.9 to 7.0.

Post-intervention Measurement

After the bleaching treatment, all the slabs underwent a second total reflectance measurement. *A priori* power calculation was performed using the software AXUM 6.0 (Adept Scientific, Frankfurt, Germany) with the following parameters: adjusted *p*-value 0.05, power setting 0.8 and sigma value 0.075. The power calculation computed a sample size of minimum 11. Therefore, samples for statistical analysis were pooled according to maturation stages and bleaching agent concentrations. Each maturation group consisted of 12 teeth composed from four teeth bleached with 10%, four teeth bleached with 15% carbamide peroxide formula-

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tion and four teeth bleached with 35% hydrogen peroxide formulation. The bleaching concentration groups consisted of four impacted teeth, four juvenile teeth, four adult teeth and four senior teeth, totaling 16 teeth per group.

Statistical analysis was calculated at *wl* 450 nm, 500 nm and 750 nm and was performed using the paired Student's *t*-test and one-way analysis of variance in combination with the Bonferroni's multiple comparison test (Prism Version 4.0C, GraphPad Software Inc, La Jolla, CA, USA).

RESULTS

Focus on Maturation Stages

Total reflectance increased after bleaching in all teeth of all maturation stages, regardless of the bleaching agent concentration for *wl* 450 nm (blue) and 500 nm (green) with $p<0.0001$. Significant changes for *wl* 750 nm (red) occurred in the enamel samples of adults and seniors only ($p<0.04$). The total reflectance is presented in Table 1.

Focus on Bleaching Agent Concentrations

A comparison of the change in total reflectance (T_1-T_0) among all three bleaching agent concentration groups did not show any significant differences ($p>0.05$), regardless of the maturation stages of dental enamel (Figure 2a-c).

Focus on Wavelengths

All samples were pooled to analyze statistical differences in the changes of total reflectance, depending on the different wavelengths. Efficacy of bleaching was significantly increased in the blue spectrum when compared with the green and red spectra ($p<0.001$), as well as in the green spectra compared with the red spectra ($p<0.001$). These results are shown in Figure 3.

No statistically significant differences in the negative controls were found for all three wavelengths at any time of measurement ($p>0.9$)

DISCUSSION

Perception of color is a psychophysiological response to the physical interaction of optical waves with an object and the subjective experience of the individual observer. Three factors may influence the perception of color: 1) light source, 2) the object being viewed and 3) the observer viewing the object. The spectral reflectance and transmittance of the viewed object characterize the color makeup of the object.[1] The spectral reflectance and transmission of teeth are determined by a combination of their complex optical properties. These may be categorized as four phenomena associated with the tooth light flux interaction: 1) specular transmission of light through the tooth, 2) specular reflection at the surface, 3) diffuse light reflection at the surface according to Lambert and Beer and 4) absorption and scattering of light within the dental tissues.[10] In general, electromagnetic waves change their propagation direction and reflection at the interface between two different media; that is, tooth and air, which are known as the refractive index $n=c_0/c$ ($n=1.62$ for enamel). Spitzer

Table 1: *Changes in Light Reflectance ($T_1 - T_0$) of All Enamel Specimens According to Different Wavelengths*

Reflectance at		Mean	Min	Max	SD	p T_0 vs T_1
Teeth *wl* 450 nm						
impacted						
	T_0	1.03	0.92	1.13	0.06	0.0001
	T_1	1.13	1.01	1.25	0.07	
juvenile						
	T_0	0.99	0.92	1.05	0.04	0.0001
	T_1	1.08	0.99	1.15	0.04	
adult						
	T_0	0.87	0.71	1.00	0.08	0.0001
	T_1	1.06	0.88	1.17	0.08	
senior						
	T_0	0.76	0.46	1.02	0.19	0.0001
	T_1	0.93	0.67	1.16	0.15	
Teeth *wl* 500 nm		**Mean**	**Min**	**Max**	**SD**	**p T_0 vs T_1**
impacted						
	T_0	1.07	1.00	1.14	0.04	0.0001
	T_1	1.12	1.04	1.19	0.04	
juvenile						
	T_0	1.04	1.01	1.08	0.02	0.0001
	T_1	1.08	1.04	1.14	0.03	
adult						
	T_0	0.97	0.86	1.05	0.05	0.0001
	T_1	1.08	1.00	1.14	0.04	
senior						
	T_0	0.91	0.77	0.8	0.11	0.0001
	T_1	1.01	0.85	1.07	0.09	
Teeth *wl* 750 nm		**Mean**	**Min**	**Max**	**SD**	**p T_0 vs T_1**
impacted						
	T_0	0.82	0.75	0.91	0.04	0.69
	T_1	0.82	0.75	0.91	0.05	
juvenile						
	T_0	0.86	0.80	0.91	0.03	0.34
	T_1	0.86	0.79	0.93	0.04	
adult						
	T_0	0.87	0.82	0.96	0.04	0.0006
	T_1	0.83	0.79	0.88	0.03	
senior						
	T_0	0.98	0.82	1.44	0.18	0.04
	T_1	0.89	0.77	1.03	0.08	

T_0 = baseline: T_1 = post-intervention

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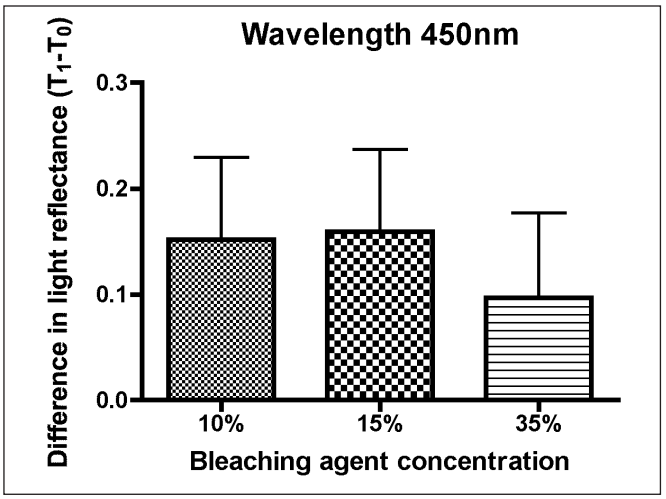

Figure 2a. *Effectiveness (T_1-T_0) of bleaching depending on bleaching agent concentrations at wavelenghth 450 nm (*p*>0.05).*

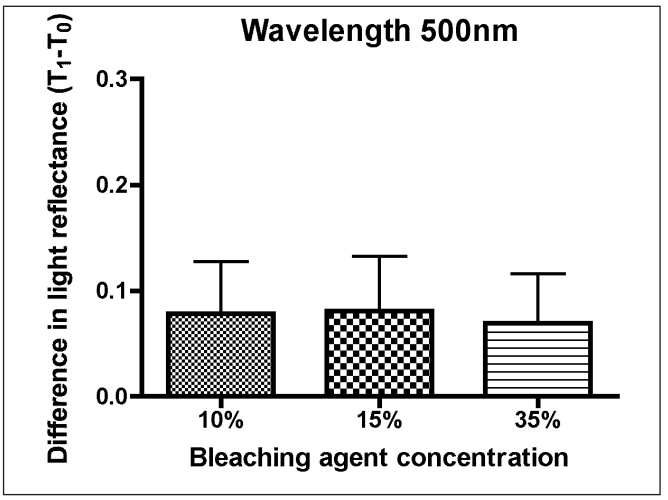

Figure 2b. *Effectiveness (T_1-T_0) of bleaching depending on bleaching agent concentrations at wavelenghth 500 nm (*p*>0.7).*

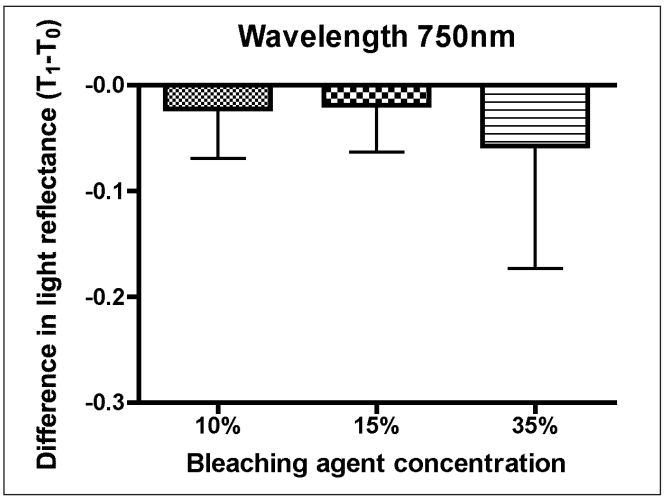

Figure 2c. *Effectiveness (T_1-T_0) of bleaching depending on bleaching agent concentrations at wavelength 750 nm (*p*>0.2). Note: The red light reflectance at wavelength 750 nm in arbitrary units is decreasing, mainly due to the effect of the adult and senior age group (see Table 1).*

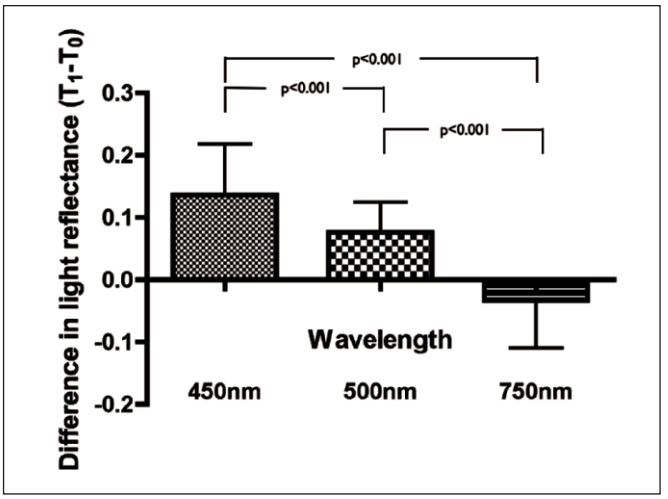

Figure 3. *Changes in light reflectance of all enamel specimens according to different wavelengths. Note: Difference of light reflectance of wavelengths 450 nm, 500 nm and 750 nm in arbitrary units and their statistical values: so the three reflectance-mean values are statistically different (*p*<0.0001).*

and Ten Bosch showed that this phenomenon remains nearly constant between blue and red light for the refractive index of enamel.[11] For this reason, the refractive index may be neglected in the current study, because all evaluated measurements of reflectance were performed within these wavelengths.

The coloring pigments within an object will absorb various wavelengths of light, allowing other wavelengths to scatter out of the object.[12] This selective wavelength absorption is the primary source of color.[13] Since blue-green lights are absorbed by yellowish chromogens inside the enamel as a result of the complementary color relationship, the reflectance in these wavelengths is lower at unbleached teeth. In accordance with the current results, Kwon and others[3] showed a gradually increased reflectance in the 400 to 500 nm (blue-green) wavelengths for bovine enamel after the bleaching process.

According to recently published results, different concentrations of bleaching agents do not show visible differences *in vivo* regarding the whitening effect; the current results confirmed these findings physically by indicating that there is no statistical difference in light reflectance after different bleaching procedures.[14-15]

Lenhardt demonstrated a shift towards the blue direction within the color space and improved lightness of the tooth after bleaching, which corresponds to the current study when all samples were pooled, regardless of their maturation stage and concentration of the bleaching agent.[16] This may be explained by two phe-

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nomena: first, the tooth surface morphology affects the amount and type of reflection—a rough or coarse surface results in more diffuse reflection, turning the object brighter, while a smooth surface leads to more specular reflection; secondly, increased back scattering of short wavelengths that are reflected as bluish-white due to opalescent effects at small structures (known as the Rayleigh scattering) plays a considerable role in the light-scattering process of teeth.[1,17-18]

Some researchers assume that the major mechanism of bleaching consists of a splitting up of chromophore molecules that are supposed to be mostly in a range of red visible light spectra, for example, tanin.[19] It was concluded that the organic component was responsible for most or all of the observed optical absorption.[11] The mechanism is believed to result from the degradation of complex organic molecules that are responsible for the discoloration of teeth to less complex molecules, resulting in a reduction of discoloration.[3] This "chromophore effect" of dental bleaching would not be expected in younger teeth, especially not in impacted teeth, because no incorporation of organic chromogens would be expected. On the other hand, studies dealing with bleaching efficacy in deciduous and juvenile permanent teeth demonstrated brightening.[20-21]

A significant light reflectance decrease in wavelengths of 750 nm—representing red visible light—was found only in aged enamel (in adults and seniors), where a considerable incorporation of chromogens may be expected but not in impacted and freshly-erupted teeth. On the other hand, significant changes in light reflectance in the blue and green spectra (450-500 nm) were evident in all groups of teeth tested, including impacted ones. These findings indicate that tooth whitening is not only related to the "chromophore effect" but also to additional changes in the optical properties of human enamel. Furthermore, reflectance changes in dental enamel played an additional role in the whitening effect over all maturation stages tested. Further studies should investigate the underlying mechanism.

CONCLUSIONS

The results of the current study showed that the exclusive assumption of the "chromophore effect" in dental bleaching could not be sustained, because the whitening of dental enamel works at different maturation stages, even in impacted teeth. This effect is irrespective of the bleaching protocol used and the bleaching agent concentration.

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