



Research article

Insights into blue light accelerated tooth whitening

Bart Gottenbos^{*}, Christiane de Witz, Sophie Heintzmann, Matthias Born, Sandra Hötzl

Philips Research, Department Oral Healthcare, High Tech Campus 11, 5656 AE, Eindhoven, the Netherlands



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ABSTRACT

Objective: To test the hypotheses that blue light accelerates whitening through either (1) direct photobleaching or (2) photon-assisted oxidation using sequential longitudinal bleaching.

Methods: Thirty extracted human tooth samples having natural life accumulated color were divided over five groups: A. 9h light + 10h 6% H₂O₂ gel + 6h light & 6% H₂O₂ combined; B. 9h 6% H₂O₂ gel + 10h light + 6h light & 6% H₂O₂ combined; C. 11 h light & 6% H₂O₂ combined; D. 8.45h 25 %H₂O₂ gel + 10h of light only + 6h light & 25% H₂O₂ combined E. 10.45 h light & 25 %H₂O₂ combined. Blue light (456nm) was used at 190 mW/cm². Color change (ΔE) was measured over time, and reported after 48h color stabilization.

Results: Groups A, B and D reached saturation in the first phase (at 9h) at a ΔE of 4.3 ± 0.7 , 4.9 ± 1.3 and 10.9 ± 2.2 , respectively. Groups C and E achieved in the same time a significantly higher ΔE of 14.2 ± 1.7 and 15.6 ± 1.9 , respectively. Subsequently adding the opposite single modality to groups A, B and D for 10h did reach an end stage at 8.1 ± 1.3 , 8.8 ± 1.8 and 10.8 ± 1.4 ΔE , respectively. The final 6h treatment combining light and H₂O₂ showed in these groups a statistically significant step in ΔE reaching 12.9 ± 1.4 , 10.7 ± 2.5 and 15.3 ± 1.7 , respectively.

Conclusions: Blue light significantly increases bleaching rate and final achievable ΔE .

This sequential whitening study provides a first indication that this enhanced bleaching is the result of the hypothesized light mechanisms acting in parallel to hydrogen peroxide bleaching.

Clinical significance: This study shows that blue light can accelerate whitening, within the limits of an *in-vitro* model. The findings help the clinician explain to their patients that in light accelerated whitening the light not merely accelerates the bleaching process, but that it attacks more stain compounds than peroxide alone does.

1. Introduction

Despite light accelerated whitening (LAW) being on the market for decades, there is an ongoing scientific debate on the benefit of adding light irradiation to the hydrogen peroxide driven bleaching process. This ongoing uncertainty can have several reasons:

1. Clinical bleaching shows a high variance and standard deviations of over 3 VITA shade units are not uncommon [1, 2, 3]. Therefore, studies need to be sufficiently powered to find differences between light and dark treatments, if existing. Studies using 19–20 subjects find no statistically significant difference in shade changes between light and no-light groups [2, 4], although the trend is positive for light treatment, while studies having similar treatment protocols using 25–49 subjects do find the difference to be statistically significant [1, 5, 6]. Additionally, heterogeneity is present in the available LAW studies with respect to the average starting shade. While most studies use relatively dark teeth with average starting shade numbers between 9 (A3) and 13 (A3,5) on the

VITA shade guide [3, 4, 5, 6] there are studies using an average starting shade down to 5 (A2) [7]. As average shade changes (ΔS_{GU}) up to 8 shades for the dark control are reported [3, 4], it cannot be expected light adds any benefit if the average starting point is at relatively light colored teeth.

2. There is a large heterogeneity in LAW products reported, varying in wavelengths, intensities and light dose used. As for any photochemical process these factors are of high importance for the reaction efficiency, some LAW products may be effective, while others are not. Hydrogen peroxide formulations also differ between products, and especially higher peroxide concentrations (>30%) may reduce the benefit of an addition of light, since strong bleaching results are obtained from the dark control group, minimizing any benefit light could add.

3. The mechanism of LAW is not well understood. It is well known that hydrogen peroxide can be directly activated using UV light. As current LAW products usually work with visible light only, which is not absorbed by H₂O₂, this explanation is not satisfactory, and other

^{*} Corresponding author.

E-mail address: bart.gottenbos@philips.com (B. Gottenbos).

mechanisms should be in place for visible LAW to work. It has been postulated that heat generated from light absorption is enhancing the bleaching effect through increased reaction and diffusion rates [8]. As the allowable temperature elevation is quite limited on teeth due to pulpal safety, it is questionable whether a few degrees Celsius can bring a significant enhancement.

In a review article on the chemistry of LAW [8] photochemical mechanisms potentially occurring in bleaching are explained. For visible light irradiation these can be summarized in two main hypotheses of enhanced bleaching:

1. Direct photobleaching: chromophores in the teeth absorb visible light, entering into an excited state. In this excited state they can lose an electron and become oxidized: a process called photooxidation. Alternatively, absorption of photons can also result in breaking chemical bonds, called photolysis. Therefore, LAW could simply be the addition of H₂O₂ reactions and light reactions, leading to an increased bleaching rate.
2. Photon-assisted oxidation: oxidation of the stain molecule bonds requires a certain activation energy. When the stain molecules absorb light (photons), their electrons may be excited into a different energy state. In this excited state the needed activation energy to oxidize the bonds is decreased. Therefore more reactions are occurring during LAW, and also bonds that would normally require a too high activation energy for H₂O₂ to oxidize may be oxidized in the excited state. Additionally chromophores in an excited state can donate their electron to H₂O₂, forming OH· radicals, one of the most potent oxidizers. Alternatively, singlet oxygen ¹O₂ may be formed out of normal O₂ by photosensitizing chromophores, which is next to OH· radicals a very potent oxidizing agent easily oxidizing tooth chromophores, a process called photodynamic oxidation.

Controlled *in vitro* studies are most suitable for investigating the mechanism of LAW. Bleaching of extrinsic colorants, such as tea solutions, shows a light dose dependent peroxide bleaching rate [9]. However, dietary stains employed in such studies represent only a part of the chromophores that accumulate in human teeth over time, and miss the natural intrinsic chromophores.

The aim of this study is to determine whether visible light enhances hydrogen peroxide mediated tooth bleaching in a controlled laboratory study using extracted human teeth with natural life accumulated chromophores. Additionally, to test the hypotheses on the LAW mechanism, sequential treatments are performed separating three different treatment modalities, being hydrogen peroxide, light in phases 1 or 2 and their combination in phase 3. This phased bleaching treatment has the potential to show the light accelerated bleaching mechanisms in the following ways:

1. Photobleaching can be proven from the phase where only light is used as treatment. If a significant bleaching effect is seen in this phase this was the result of photo-bleaching. As light can also cause a drying effect of tooth enamel, resulting in a whiter appearance, the color change should be measured after full re-hydration of the samples.
2. Photon-assisted oxidation can be proven if the combination of hydrogen peroxide and light can still significantly bleach samples in the third phase, after one phase with extensive light only treatment and another phase with extensive hydrogen peroxide treatment. In pilot experiments it was found that both single treatments cannot bleach significantly anymore after 7 h of treatment. Therefore around 9 h of treatment is used in this investigation for the phases where single modalities are employed. Using these extensive treatment times makes sure that all chromophores sensitive to light only and hydrogen peroxide only have been bleached. Only chromophores sensitive to the combined treatment can remain in the samples, which can be identified through their bleaching in the third phase, again measuring after full rehydration.

2. Materials and methods

2.1. Extracted human tooth samples

The use of human tooth material was approved by an internal ethical review board. A power analysis was performed (Minitab 18) for determining the required sample size for this 5 group study at a 95% confidence and 80% power level. Historical standard deviations from similar studies showed a sample size of 6 would be required for finding significant differences at a clinically significant color change (ΔE) difference of 2. Extracted human tooth samples (n = 30) were supplied by Intertek (UK). The samples were enamel-dentin blocks cut from premolars, sized approximately 4.2 × 4.2mm and 3–4mm thick, embedded in acrylic resin in a 10 × 10 × 10mm cuvette. The enamel surface was left untreated. Samples were stored at 4 °C in a moist closed jar to keep the samples hydrated. They were placed in a 0.01M phosphate buffered saline (PBS) solution before use and between treatments.

2.2. Color measurements

Color measurements were taken using a spectrophotometer (CM-5, Konica Minolta, Japan) to obtain L*, a* and b* values as an average of the four different directions for each tooth. Each time the spectrophotometer was used also a white reference tile (calibration plate no14033001 CR-200/-300/-400/-40, Konica Minolta, Japan) was measured. Fluid covering the sample was removed using a tissue immediately before the measurement and after the measurement the sample was placed back in PBS. Before the treatments a baseline color was measured, after each 15 minute (min) treatment slot and during rehydration after each treatment session at 2, 14 and 48 hours (h) rehydration color measurements were done.

2.3. Treatments

As a light source the Philips Zoom! Whitespeed LED accelerator (Philips Oral Healthcare, USA) was used at the high setting specified at 190 mW/cm² intensity of which the output was verified using a laser power meter (Field max II + Power max PS10 sensor, Coherent, USA). Philips Zoom! whitening kits (Philips Oral Healthcare, USA) were applied, using commercial bleaching gels in the concentrations of 6% H₂O₂ and 25% H₂O₂. The samples were randomly divided over the five treatment groups shown in Table 1. Groups C and E only received the light and H₂O₂ gel combination, while groups A, B and D received in the subsequent phases different treatments:

1. Light only: the samples were covered with 0.2ml of demineralized water, and subsequently placed under the light source at the right distance to obtain 190 mW/cm².
2. Hydrogen peroxide gel only: the samples were briefly dried with a tissue and covered with a 1mm gel layer using a poly(methyl) methacrylate (PMMA) 10 × 10mm frame of 1mm thickness. Subsequently, the samples were placed in the dark.
3. Combination of hydrogen peroxide gel and light: the samples were briefly dried with a tissue and covered with a 1mm gel layer. Subsequently, the samples were placed under the LED accelerator at the correct distance to obtain 190 mW/cm² of light power.

The treatments were divided in 15 min slots to mimic clinical practice. After each 15 min treatment samples were washed with double deionized water, placed in PBS and L*, a* and b* measured. To obtain insights at the recommended clinical treatment time of 45min for 25% H₂O₂ and 60min for 6% H₂O₂, following the first three (for groups D and E) or four (for groups A, B and C) 15 min treatments the samples were placed in PBS to stabilize the color through rehydration. Color was measured at 2, 24 and 48h post treatment, to obtain the rehydration curve. After rehydration in PBS for 48–72h the treatments were

Table 1. Five different treatment regimen groups were investigated. Sample groups A, B and D received a sequence of three treatments divided over three phases.

Group	Phase 1	Phase 2	Phase 3
A	9h light only	10h 6% H ₂ O ₂ gel only	6h light & 6% H ₂ O ₂
B	9h 6% H ₂ O ₂ gel only	10h light only	6h light & 6% H ₂ O ₂
C	11h light & 6% H ₂ O ₂		
D	8.75h 25% H ₂ O ₂ gel only	10h light only	6h light & 25% H ₂ O ₂
E	10.75h light & 25% H ₂ O ₂		

continued, applying subsequent treatments were in blocks of 8×15 min also followed by storage in PBS and measuring color at 2, 24 and 48h post the 8×15 min treatment block. These 2h treatment sessions were repeated until the required treatment time was achieved. After each 2h treatment block rehydration color curves were measured at 2, 24 and 48h post treatment to verify full hydration was achieved. Between the 2h treatment blocks the samples were in PBS for 48h–72h. ΔE was calculated over time as the square root of $(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2$.

2.4. Statistics

One-way ANOVA was performed for comparisons between the groups using Minitab 18 software, assuming equal variances and post-hoc Tukey testing for comparisons. Paired Student t-testing was used to compare different time points within groups.

3. Results

Figure 1 shows the ΔE results for the first $3 \times$ or 4×15 min treatment, similar to clinical in-office treatment times, and for the post treatment 48 h color stabilization during rehydration. In group A ΔE jumped to high levels immediately after treatment. Post bleaching stabilization showed this could be attributed mainly to dehydration. For all groups ΔE stabilized at 24h post bleaching, with no significant change in the following

24h, which showed rehydration was close to complete after 24h in PBS. At 48h post bleaching groups A and B had a similar bleaching effect. Group D showed a statistically significantly higher ΔE than groups A and B ($p < 0.05$). Groups C and E had significantly increased bleaching outcomes over B and D, respectively ($p < 0.001$), and also the difference between groups C and E was statistically significant ($p = 0.001$).

For the full time series the bleaching result is depicted in Figure 2, for each time point after 48h color stabilization during rehydration. Again the 24h and 48h rehydration results were not significantly different, showing also after longer treatment times the samples were fully hydrated after 48h in PBS. After the full treatment time of each phase the bleaching results saturated to a maximum level achievable with the used bleaching component, indicating longer treatment times would not have achieved additional bleaching. In phase 1 groups A and B had a similar bleaching rate, while group C showed an approximately 3 fold higher bleaching rate. The subsequent phase 2 of groups A & B, achieved a similar pattern as in phase 1, though at a slightly decreased rate. Group D showed a significantly higher ΔE compared to group B in phase 1, but significantly lower than group E. Subsequent phase 2 treatments in group D did not change ΔE anymore. The final stage of groups A, B & D led to another statistically significant increase in ΔE . At the end point the ΔE from group B was statistically significantly lower than groups D and E ($p < 0.05$).

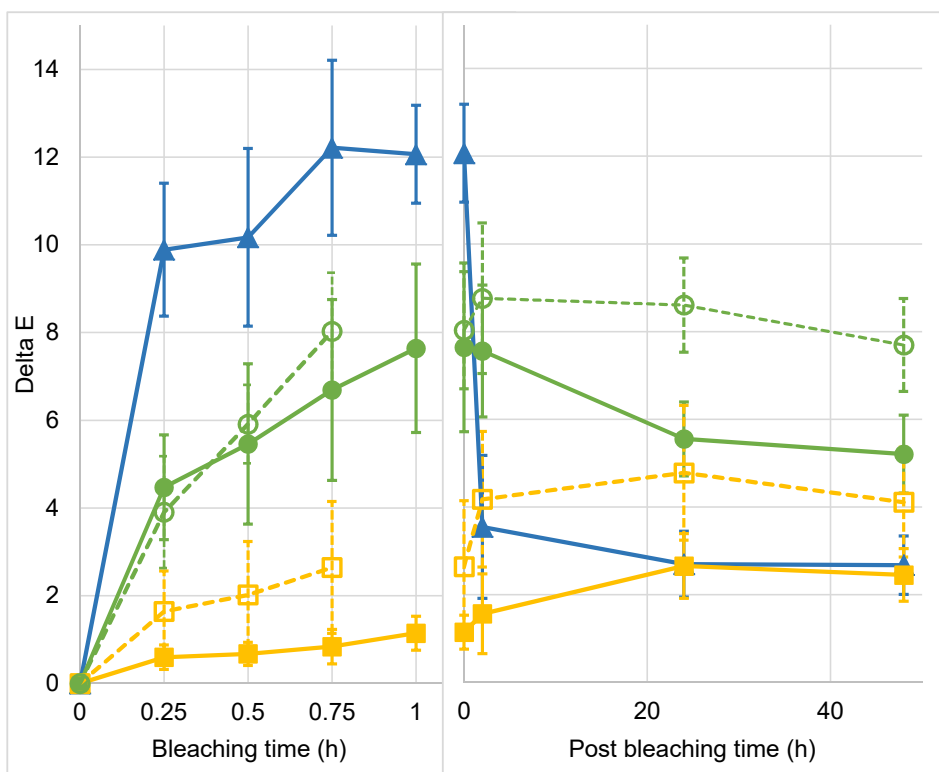


Figure 1. Mean color change (ΔE) in the first 4×15 min (6% H₂O₂) or 3×15 min (25% H₂O₂) treatment +48h color stabilization for groups A (solid triangles), B (solid squares), C (solid circles), D (open squares) and E (open circles). Error bars show standard deviations ($n = 6$).

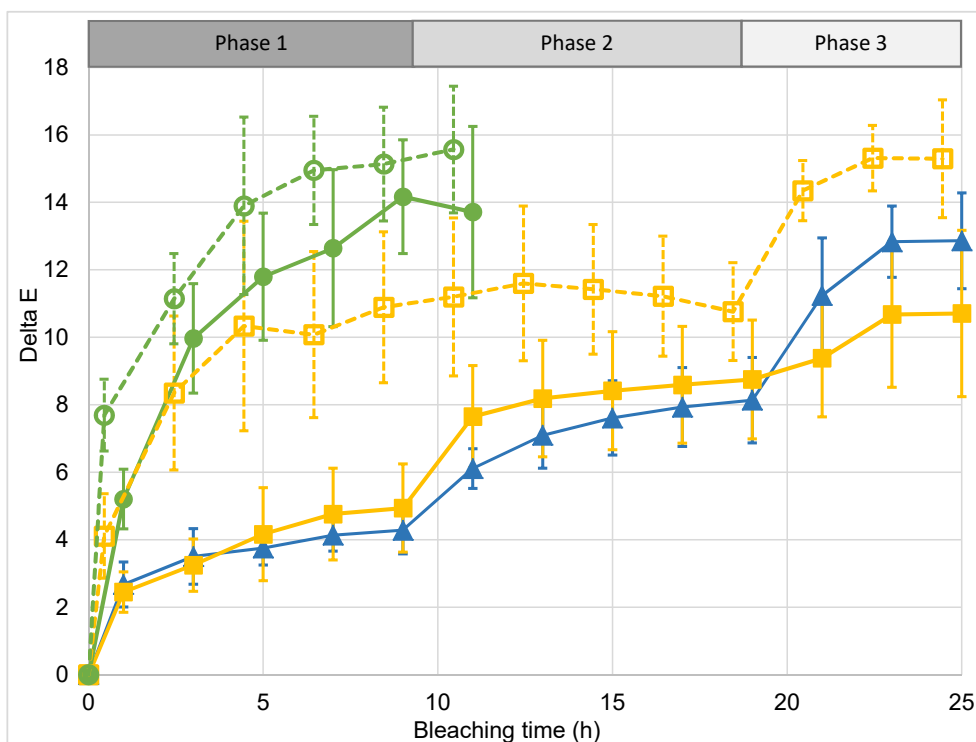


Figure 2. Mean color change (ΔE) over the full time series measured each time point after 48h color stabilization for groups A (solid triangles), B (solid squares), C (solid circles), D (open squares) and E (open circles). The three phases are indicated in time: phase 1 = first modality; phase 2 = second modality; phase 3 = combined peroxide gel & light. Error bars show standard deviations ($n = 6$).

Table 2 shows the L^* , a^* and b^* values at the beginning and end of each phase, and the delta in ΔE ($\Delta\Delta E$) over each phase. Each individual phase $\Delta\Delta E$ in groups A, B and D was a statistically significant step up ($p < 0.05$, paired Student-t-test), except for phase 2 in group D. Lightness L^* increased over all the phases, again with the exception of group D where

it significantly decreased. Yellowness (b^*) reduction had the largest contribution to ΔE , and b^* also decreased in every phase, even in group D. Redness (a^*) reduction showed an increasing trend (non-significant) during light alone treatment, and a decreasing trend during other treatments. Because the Δa^* is only small, it had little effect on ΔE .

Table 2. Color variables at the different stages and the delta in ΔE ($\Delta\Delta E$) over each single phase (mean \pm standard deviation, measured after 48h color stabilization). Phase 1 = first modality; phase 2 = second modality; phase 3 = combined peroxide gel & light. The white reference tile in the used measurement system obtained $L^* = 71.3$; $a^* = -0.04$; $b^* = 1.14$.

Group	Outcome	Baseline	Phase 1	Phase 2	Phase 3
A	L^*	60.9 \pm 3.5	63 \pm 3.7	64.4 \pm 3.4	67.3 \pm 3.8
	a^*	1.3 \pm 0.6	1.7 \pm 0.5	0.6 \pm 0.6	0.3 \pm 0.6
	b^*	12.0 \pm 2.5	8.4 \pm 2.7	2.5 \pm 2.5	1.0 \pm 2.3
	$\Delta\Delta E$		4.3 \pm 0.6	3.9 \pm 1.2	4.7 \pm 0.3
B	L^*	61.0 \pm 2.6	63.6 \pm 3	64.3 \pm 3.3	64.8 \pm 3.6
	a^*	1.3 \pm 0.6	0.6 \pm 0.7	1.0 \pm 0.7	0.8 \pm 0.8
	b^*	12.1 \pm 1.8	8.0 \pm 2.0	4.0 \pm 2.0	2.2 \pm 2.4
	$\Delta\Delta E$		4.9 \pm 1.3	3.8 \pm 0.9	2.0 \pm 1.4
C	L^*	61.4 \pm 2.6	69.5 \pm 2.7		
	a^*	1.3 \pm 0.3	0.0 \pm 0.4		
	b^*	12.8 \pm 1.4	1.9 \pm 1.1		
	$\Delta\Delta E$		13.7 \pm 2.5		
D	L^*	60.1 \pm 2.4	67.7 \pm 3.3	63.8 \pm 1.8	70.4 \pm 3.0
	a^*	1.2 \pm 0.4	-0.3 \pm 0.5	0.5 \pm 0.5	-0.5 \pm 0.4
	b^*	12.8 \pm 1.0	6.4 \pm 1.5	2.7 \pm 0.9	1.6 \pm 1.0
	$\Delta\Delta E$		10.9 \pm 2.2	-0.1 \pm 2.6	4.5 \pm 1.4
E	L^*	61.5 \pm 1.8	72.8 \pm 2.0		
	a^*	0.9 \pm 0.7	-0.5 \pm 0.4		
	b^*	12.5 \pm 1.2	2.0 \pm 1.1		
	$\Delta\Delta E$		15.6 \pm 1.9		

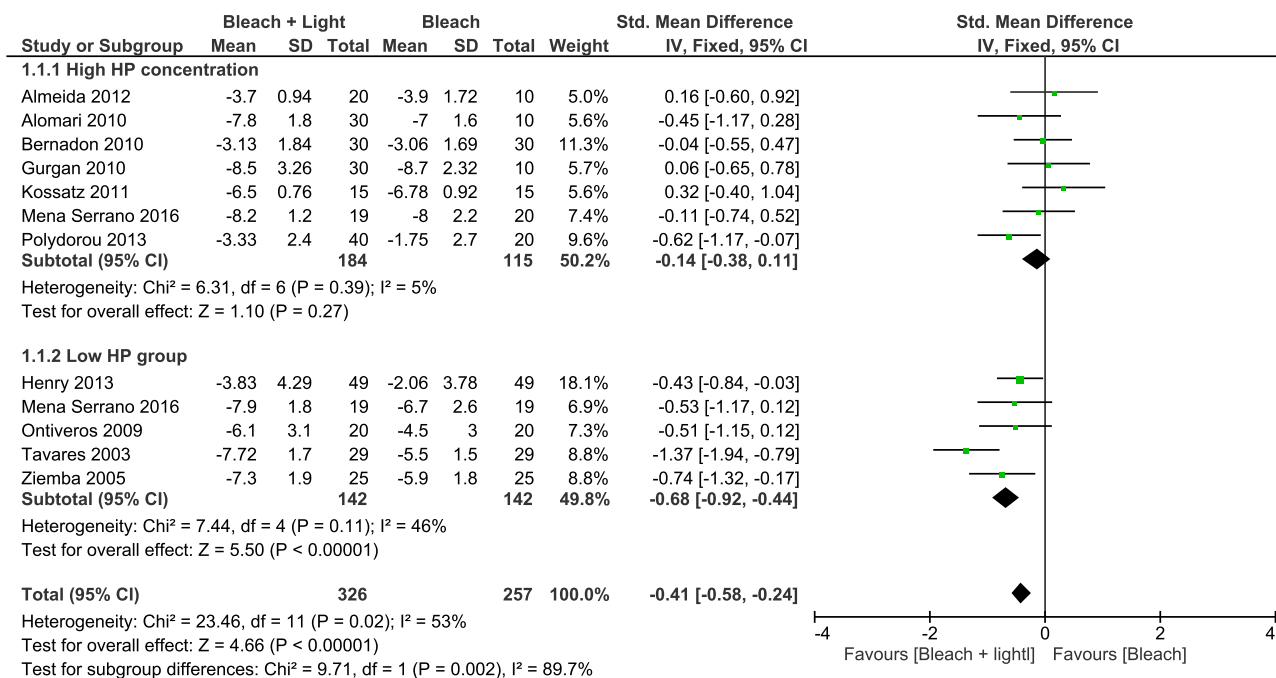


Figure 3. Forrest plot of the re-analysis of the meta-review data from Maran *et al.* with the correct Δ S_{GU} and standard deviation data taken over from the original papers.

4. Discussion

To the best of our knowledge, this is the first study to apply longitudinal whitening treatments using the different treatment components sequentially. The sequential analysis provides insights into the mechanism of the increased bleaching efficacy of the light and peroxide combination. While the long 9–10h treatments are of less clinical significance, given the typical 45–60 min treatment time in-office, they were in a pilot study found to be needed to reach the achievable end stage color change for each separate modality. After the applied long treatment times with gel or light alone also currently reported results show that the color of the teeth cannot be expected to significantly change anymore, even when applying infinite treatment times. This proves that neither the peroxide gel nor light alone bleach the full range of chromophores present in the teeth. The sequential results of groups A, B and D further show that the two individual bleaching components are complementary: chromophores not bleached by one single modality can be bleached still by the other modality. The pattern found in the redness component of the color (a^*), even though not statistically significant, is another indicator that peroxide and light react with different components: the blue light does not bleach these red compounds, and actually resulted in an increased trend of a^* , indicating that blue light may convert some yellow compounds into red compounds. However, in the presence of hydrogen peroxide both a^* and b^* are reduced. For the sequential result in group D (25% H₂O₂ gel) the pattern in b^* and a^* was similar to group B, but was offset by the pattern in L*, which dropped during the light only treatment in phase 2, resulting in no ΔE change. Possibly the 25% H₂O₂ gel increased the enamel scattering leading to a strong increase in L* in phase 1 of group D, which was gradually reduced in phase 2 using light only treatment, causing the drop in L*. The last phase 3 shows there were chromophores left in the teeth, which could not be bleached by the separate components, and needed the combination of light and peroxide. The results show that the tooth chromophores consist of three distinct fractions, a first fraction sensitive to light only bleaching, a second fraction sensitive to hydrogen peroxide only bleaching and a third fraction that can only be bleached using the combination of peroxide and light. Combining light with hydrogen peroxide from the start of the

bleaching treatment therefore attacks all these chromophores with different sensitivities simultaneously, explaining the accelerated and elevated color change. Combining all observations shows that two distinct types of photonic bleaching reactions are both occurring during light accelerated whitening: direct photobleaching and photon-assisted oxidation.

Direct photobleaching is evident from a significant ΔE found during the light only phases. The light only effect cannot be explained from dehydration, as the samples were fully rehydrated after the 48h in PBS, evident from the rehydration curves. Photobleaching was already utilized centuries ago in bleaching fabrics using sunlight, and is still used in bleaching paper. Many chromophores are sensitive to photobleaching, since by absorbing light they get in a higher energy level. In the excited state (electron in a higher energy state) after photon absorption, bonds may become unstable [8]. Since most organic chromophores derive their color from extended conjugated chemical bonds, oxidizing or lysing one of the bonds may already yield a color-less product. The current study results show however not all chromophores in the teeth are sensitive to photobleaching nor to hydrogen peroxide bleaching, as more than half of the tooth color remained after treating with a single modality.

Photon-assisted oxidation is indicated by the significant increase of ΔE when using light combined with hydrogen peroxide, following the extensive light only and gel only phases. The combination treatment was able to whiten even the hardest to whiten chromophores, not sensitive to both single treatment modalities, reaching close to white tile L*, a^* and b^* values. These observations can only be explained by the occurrence of different types of bleach reactions when light and hydrogen peroxide are combined. Photon-assisted oxidation can be explained similarly to photobleaching. The very stable stain molecules where bonds are not being oxidized by H₂O₂ in the ground state, become less stable in the higher energy state by absorbing one or more photons. In the excited state H₂O₂ becomes capable of oxidizing at least one of the double bonds, rendering the molecule colorless [8].

For clinical significance the initial color change after 45 and 60 min were separately reported, showing both a statistically significant and a clinically significant benefit of light on the color change using in-office bleaching times, approximately doubling the end result after color

stabilizing compared to peroxide alone. A number of clinical studies compare in-office bleaching with and without the use of light. Clinical studies report either in final absolute shade numbers or in the delta in shade grade units (Δ SGU). Several attempts of meta-reviewing [10, 11, 12] the clinical data have failed in recognizing this difference, and they either report absolute numbers but wrongly use original Δ SGU data or report Δ SGU but erroneously have taken over absolute numbers. Since about half the papers report in Δ SGU and the others in absolute numbers such errors in meta-analyses will find no significant contribution of light, even if there would be one. Indeed all three erroneous meta-analyses conclude there would be no significant benefit of light. Figure 3 shows a corrected meta-analysis based on the papers reported in Maran et al. [11] for the first fade-back point in time, usually at 1 week after whitening. The Δ SGU was extracted from the original papers [1, 2, 3, 4, 5, 6, 7, 13, 14, 15, 16], converting reported absolute SGU data to Δ SGU. If not available, standard deviations were calculated from absolute SGU standard deviations. Note that for Δ SGU negative numbers are used, as shade numbers are going down. Some papers falling in the high HP group included in the review by Maran et al. needed to be removed from the analysis, as they did not report all data needed. The data were analyzed using the same method as Maran et al. [11]. The meta-review shows over the total analysis a significant improvement when using light, but when specifically looking at the different H_2O_2 concentration groups, at high H_2O_2 concentrations there is no significant benefit of using light, while a significant effect is found at 25% H_2O_2 or lower. This result can be explained from the fact that high concentrations of H_2O_2 alone achieve a strong bleaching result, leaving less room for improvement using the light. In current *ex vivo* results this effect was also seen for the longer treatment times, where after 9h of treatment the percentage color change difference of light + gel vs gel only was with the 25% gel 39% while for the 6% gel it was 187%. At typical in-office treatment times this difference was not so clear with 87 and 112%, respectively.

The reported so-called low HP group from Figure 3 includes HP concentrations of 15–25%. Clinical data from 6% HP was recently published [17], using the same bleaching product as in our *ex vivo* study, showing a Δ SGU of -4.4 with light and -3.6 without light, a significant difference ($p < 0.01$). This adds clinical confirmation that when using low HP concentrations light adds a significant benefit.

5. Conclusions

Within the limits of this *in vitro* study, blue light significantly enhances both the bleaching rate and the final whiteness level that can be achieved, when using the tested commercially available peroxide products on human teeth. Blue light alone can bleach chromophores directly through photobleaching, which are partly a different chromophore fraction as those bleached by the hydrogen peroxide products used. Human teeth additionally contain chromophores which can neither be bleached using the hydrogen peroxide products alone, nor by light alone. Only the combination of hydrogen peroxide and light can cause bleaching of these chromophores. The total efficacy of LAW is therefore driven by the sum of the activities of three different bleaching modalities, H_2O_2 alone, light alone and the combined action of light and H_2O_2 .

Declarations

Author contribution statement

B. Gottenbos: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

C. de Witz and S. Heintzmann: Performed the experiments; Analyzed and interpreted the data.

M. Born: Analyzed and interpreted the data.

S. Hötzl: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

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Data availability statement

Data will be made available on request.

Declaration of interests statement

The authors were employed by Philips Electronics N.V. at the time of the study. The bleaching products used were from Philips Oral Healthcare.

Additional information

No additional information is available for this paper.

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