Research

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Tooth-whitening activity of a novel home-bleaching system utilising thermal diffusion: a multifactorial simultaneous evaluation of efficacy at cervical, body and incisal tooth sites

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Monitors the intensities of different tooth shade parameters simultaneously at three different regions of stained 'smile-zone' teeth.

Studies the differential response of the cervical, body and incisal tooth areas to tooth-whitening treatment.

Analyses the highly significant reduction observed for the tooth shade parameter a* by the treatment applied, which is difficult to achieve in clinical practice.

Abstract

Introduction The ability of a thermal diffusion system (TDS) to promote the tooth-whitening actions of a bleaching gel/bleaching activator combination product (containing a final hydrogen peroxide (H_2O_2) content of 10.0% (w/v)) towards discoloured 'smile-zone' teeth was examined.

Methods Fifty teeth in 15 participants aged 18-62 years were investigated. The CIE tooth shade parameters L*, a* and b*, together with Vitapan shade scores (VSSs), were simultaneously recorded at three separate tooth areas (cervical, body and incisal sites) with a novel spectrophotometric monitoring system before treatment, and also at 14 days after completion of a 10-day treatment period in which the product was applied 'at-home' (twice daily).

Results The tooth-whitening treatment administered gave rise to extremely significant increases in L*, and decreases in the a* and b* shade parameters for each of the tooth areas investigated ($p < 10^{-10}$). Post-treatment mean decreases in the VSS values were 8.26, 9.70 and 9.14 for the cervical, body and incisal areas respectively ($p < 10^{-8}$ in each case). Mean ΔE values determined post-treatment were also very highly significant for each tooth region examined ($p < 10^{-10}$ in each case).

Conclusions The tooth-whitening system tested exerted extremely powerful bleaching actions in all tooth areas investigated. The order of tooth-whitening effectiveness was body > incisal > cervical for Δb* and ΔE, and incisal > body > cervical for Δa* and ΔL*, and this may reflect the TDS's ability to promote the penetration of H_2O_2 to intrinsic stain sites.

Introduction

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Introduction

Currently, there are numerous clinical studies documented in the scientific literature regarding the application of a series of differing tooth-whitening systems for application at home. These studies include investigations involving trays1, 2 (dentist-prescribed, customised and pre-formed), strips3, 4 and 'paint-on' materials.4, 7 Such investigations range from those which evaluate the safety of hydrogen and carbamide peroxides5, 6, 7 (H_2O_2 and CP respectively, the active ingredients involved in the bleaching process), and related peroxo-adducts such as peroxoborate, to the activities of such products both in vivo and in vitro.8 All of these investigations have demonstrated the efficacies of the bleaching effects of these agents.9, 10, 11, 12 However, the majority of them have involved measurement of the shades of the teeth by a subjective visual method, typically using the Vitapan Classical shade guide system (Vitapan Classical, Vita Zahnfabrik, Bad Säckingen, Germany) which consists of 16 different shades (Fig. 1).1, 2, 3, 4, 5, 6, 7, 9, 10 The Vitapan Classical shade guide system is highly subjective and dependent on many factors, such as surgery lighting and clinical evaluator fatigue. Moreover, it yields only an overall classification of tooth discolouration since only one shade classification (intensity) value is obtainable for each tooth examined or investigated.

Figure 1: Subjective Vitapan classical shade guide system (Vitaplan Classical, Vita Zahnfabrik, Bad Säkingen, Germany)

Full size image (31 KB)

Clearly, this subjective shade guide system is complicated by the phenomenon of metamerism, which is a psychophysical phenomenon that relates to two colours and their relationship under different light sources. It also defines the capability of the naked eyes of humans to recognise different colours and their relationships under different lighting systems, such as those generated by light bulbs, neon and fluorescent lights, and the differing intensities of daylight. For example, two different coloured items may appear 'matched' and similar under certain light(s) (such as fluorescent light), but may appear quite different and 'unmatched' under strong sunlight. The fundamental reason for this difference is that colour is a sensation rather than a property of an object. Consequently, human eye cones can register the same sensation from an essentially infinite variety of combinations of differing light frequencies.

The efficacies of teeth-whitening processes depend on the diffusion of the active ingredients to the source of chromophoric staining molecules residing within the enamel and dentine structures.11, 12, 13, 14 Diffusion, by definition, is the physical movement of molecules/biomolecules together with particulate matter, from a higher chemical/thermal potential to a lower one, which is, of course, a time-dependent process. Hence, the rate of this diffusion depends on three known factors:

The concentration (amount) of the bleaching agent placed on teeth surfaces

The contact time

The temperature gradient between the bleaching agent and thetooth surfaces.

In this investigation, we have employed a newly-developed, novel and objective digital spectrophotometric system to monitor tooth discolouration, and also investigate the capacity of an established 'at-home' tooth-whitening product (containing H_2O_2) to remove such tooth stains. Of particular interest is the ability of the above spectrophotometric facility to monitor tooth stain intensities (and also the bleaching effects of the tooth-whitening product involved) simultaneously at three different regions of each tooth (specifically, the cervical, body and incisal areas). Hence, we have also explored the ability of this unique monitoring system to provide valuable information regarding the relative extent of the product's bleaching activities at each tooth site investigated. To the best of our knowledge, this is the first time that this particular spectrophotometric facility has been employed to monitor the actions and effectiveness of a tooth-whitening product simultaneously at the above three sites in vivo.

This facility also has the capacity to determine the CIE tooth stain parameters L* (the level of brightness, which ranges from 0 (black) to 100 (white)), and a* and b* (representing the intensities of red and yellow colourations, that is, transmissions of red and yellow light in the visible region of the electromagnetic spectrum, respectively), for each tooth area, tooth and participant (patient) selected.15, 16, 17 Thus, the degree of tooth whitening achieved by products or formulations tested is represented by a combination of an increase in the intensity of L*, coupled with decreases in those of a* and b*. Moreover, the spectrophotometric system can also display tooth-whitening efficacies in terms of ΔE and VSS values (the former composite measure is described in our section on clinical trial participants and tooth shade determinations).

Chen et al.18 evaluated the reliability and accuracy of this spectrophotometric system and concluded that it exhibited a high level of reliability for different shade parameters and tooth areas, an observation suggesting predictable repeated determinations (although accuracies in the cervical and incisal regions were somewhat lower and more variable than that of the body region). Moreover, Sun et al.19 employed this device to investigate the whitening of extracted teeth with H_2O_2, a process which was facilitated by a direct-current cold atmospheric-pressure air plasma microjet.

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Materials and methods

Clinical trial participants and tooth shade determinations

Invitations to participants to take part in this study were promoted at the investigators' dental practice and the local press. Participants recruited to the study were required to possess discoloured teeth from the six anterior teeth darker than A3.0 according to the Vitapan Classical shade guide. Approval to conduct this investigation was obtained from the University of Bolton's Research Ethics

Committee. Informed consent of the participants was sought via their signatures on a University of Bolton Research Ethics Committee consent form.

Fifty 'smile-zone' teeth from 15 human study participants aged between 18 to 62 years were selected for this study. The shades of the upper anterior teeth of these subjects were recorded with a Crystaleye digital dental spectrophotometer (Olympus Corporation, Tokyo, Japan; Fig. 2), which also served as a digital camera. This facility permitted colour classification within a three-dimensional space, and was also calibrated to the Vitapan Classical shade mode system.

Figure 2a: Olympus Crystaleye spectrophotometric tooth shade intensity monitoring system

Full size image (22 KB)

For each study participant, teeth shades were objectively determined in three different tooth areas (specifically the cervical, body and incisal areas) using the above digital spectrophotometric device. Shades were determined as a*, b* and L* CIE classifications, where a* and b* represent the (regional) tooth shade intensities in the green-red and blue-yellow regions of the visible region of the electromagnetic spectrum respectively, and L* the level of brightness.

Moreover, ΔE values (where ΔE2 = (Δa*2 + Δb*2 + ΔL*2)1/2), which represent composite measures of overall colour modifications, were also employed to determine modifications in tooth shade intensities following tooth-whitening treatment for each of the three specified tooth regions. In the above equation, Δa* represents the colour change in the green-red region, Δb* that in the blue-yellow region, and ΔL* the brightness level modification.

Primarily, the Crystaleye spectrophotometric system was employed to simultaneously determine the baseline shades (A) of selected teeth of each participant in each of the three specified areas. These values were recorded and given a numerical score value, that is, a*, b* and L*, together with a corresponding Vitapan shade score. For the latter, the A3 shade has a score of 9, and the A4 shade a score of 15 (Fig. 1).

Since the areas encompassed by the Crystaleye spectrophotometer were those present in a 'window' of tooth surface for each region, measurement of the areas of the tooth surface for these regions was increased three-fold by moving the original (central) areas in four directions (north, east, south and west) by half a 'window' (Fig. 2d) in order to evaluate any 'clustering' effects for regional tooth stain intensities both before and after tooth-whitening treatment. In this manner, any

detectable 'clustering' effects between the VSS scores of the central cervical, body and incisal tooth areas, and those immediately north, east, south or west of them (that is, tooth area sub-regions) were determined. The statistical analyses of these datasets are described in our experimental design and statistical analysis section.

Figure 2d: Investigation of tooth shade 'clustering'effects in sub-regions located one-half of a spectrophotometric 'window' (either north, east, south and west from central tooth stain monitoring areas) from the central monitoring point; in this example, the area of the body region for measurement was relocated one-half of a 'window' west of the centroid

Full size image (38 KB)

The mean of these three areas was then computed as the (combined) overall shade of that particular tooth.

Application of tooth-whitening treatment

The thermal diffusion home-whitening system selected for this investigation was the get2smile Home-Whitening System (Wyten Technology Ltd, UK). This consisted of a S2Power Thermal Diffuser (Fig. 3) and two opaque 4:1 double-barrelled syringes (each of a 5.00 ml capacity), the larger barrel containing the wy10 home-bleaching gel with 12.5% (w/w) H_2O_2, the smaller barrel an aqueous solution containing an 'activating' amino-alcohol agent (Wyten Technology Ltd, UK; Fig. 4a). The bleaching and activating components were mixed with a mixing unit; the final H_2O_2 content of this gel admixture (consisting of a 4:1 (v/v) mixture of bleaching gel:activator solution) was 10.0% (w/v), and its final pH value was 10.0. A specially-designed mouth retractor was employed to retract the lips away from the teeth, and then the wy10 bleaching gel/activator mixture was painted onto tooth surface enamel as a thin coat varnish (Fig. 4b) in order to ensure that the bleaching agent did not gain access to areas outside the tooth surface. Adequate further steps were taken to ensure that the bleaching agent components were isolated from all exposed dentine and soft tissues. Participants had all teeth located in the 'smile-zone' treated (eight upper and eight lower teeth). Two or more of those with a baseline (untreated control) Vitapan shade scale value darker than A3.0 were monitored throughout the trial (a minimum of two and a maximum of six per participant). The S2Power thermal diffuser, which served to elevate the temperature of the bleaching gel on the enamel surfaces up to 38°C12, 13, 20 was mounted over the retractor as shown in Figures 3b and c.

Figure 3: The get2smile S2 power thermal diffusion system

Full size image (25 KB)

Figure 4a: Double-barrelled opaque syringe with mixing tip, Dappen's dish and brush

Full size image (13 KB)

Figure 4b: A thin coat of bleaching gel (similar to varnish) is painted onto the tooth enamel surface

Full size image (11 KB)

The bleaching procedure involved a 20 minute primary 'jump-start' treatment in which participants were shown how to perform the required bleaching procedure by one of the investigators, followed by a 10-day period of once-daily home-whitening treatment (40 minutes in length, involving a fresh bleaching episode after 20 minutes with a repeated application (replenished) volume of the 4:1 tooth-whitening gel:activator solution mixture).

Post-treatment determination of tooth shade parameters

The objective Crystaleye spectrophotometric system was then again employed to determine the bleaching effects of the tooth-whitening product applied 14 days after completion of the above 10-day treatment regimen. The shade A (before treatment), shade B (after treatment) and the parameter [A-B] (improvement level measure) was then determined for each of the three tooth areas specified above for both ΔE and Vitaplan Classical shade scores. Similarly, the post-treatment values of a*, b* and L* were also determined 14 days after completion of the10-day trial phase. [A-B] tooth stain improvement levels for each of these parameters (Δa*, Δb* and ΔL* respectively) were also determined during this second (final) visit of the participants to the dental surgery.

Experimental design and statistical analysis

For the a*, b* and L* tooth shade parameters, the experimental design for this investigation was classified as a mixed-model, 4-factor analysis of variance (ANOVA) system with treatments (specifically, each tooth shade intensity measurement performed prior and subsequent to the above

tooth-whitening treatment) and tooth areas (cervical, body and incisal) in which these determinations were made being fixed effects at 2 and 3 classifications respectively. Both the 'between-participants' (n = 15) and 'between-teeth-within-participants' (n = 2-6 per participant) effects were classified as random effects, although it should be noted that the latter selection was not strictly random since those incorporated into the study had to have a minimum Vitapan shade guide value of ≥3.0; however, those teeth with such shades greater than or equivalent to this value were randomly selected from those available. A mixed-model component analysis for data acquired on each tooth shade intensity parameter monitored (a*, b* and L*) comprising five sources of variation (two fixed and two random effects, plus one first-order (treatment × tooth area) interaction), and the variance components estimated by each, are delineated in Table 1a.

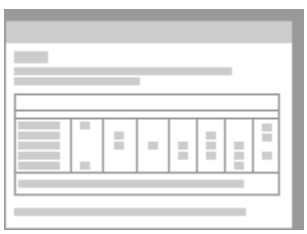
Table 1a: Experimental design for the analysis of each dataset of the tooth stain parameters L, a, and b, representing a combination of a completely randomised with a randomised block design: mixed model with teeth (n = 2-6 per group) 'nested' within participants

Full table

All datasets were untransformed before statistical analysis in this manner by ANOVA since in this format they gave a higher level of fit to a normal distribution than that achieved with a log10-tranformation (Kolmogorov-Smirnoff test).

However, for the ΔE values observed, the experimental design comprised only three sources of variation ('between-tooth areas' (fixed effect), 'between-participants' (random effect), and 'between-teeth-within-participants' (selectively random effect)), and the levels, degrees of freedom, nature of the factors involved, and parameters estimated are given in Table 1b.

Table 1b: Experimental design for the analysis of each dataset of the tooth stain parameter ΔE, also representing a combination of completely randomised with a randomised block design: mixed model with teeth (n = 2-6 per group) 'nested' within participants



Full table

The overall statistical significance of the individual post-treatment improvements in shade scores (that is, pre- versus post-treatment values for a*, b*, L* and Vitapan shade scores, together with the ΔE values) were determined by tooth-dependent paired sample Student's t-tests, performed on untransformed data for each of the three tooth areas examined.

For the investigation of 'clustering' effects at each of the three tooth regions, spectrophotometrically determined VSS measurements were separated into a total of six datasets (cervical, body and incisal tooth regions both before and after treatment), and each of these sets was also analysed by (type I) ANOVA in an experimental design which incorporated 'between-participants' (random effect), 'between-specified-teeth-within-participants' (L1, L2, L3, R1, R2, R3; fixed effect) and 'between-tooth-sub-regions' (central, north, east, south and west; fixed effect) as components of variance. Subsequent to determination of the statistical significance of each of these factors, post hoc analysis of the mean tooth sub-region VSS values was performed with Tukey's honestly significant difference (HSD) test for pairwise comparisons, and Dunnett's test for determining the significance of differences between the mean values of this parameter at the north, east, south and west sub-regions and that of the central control mean value. Again, untransformed data were subjected to this form of statistical analysis.

The number of teeth involved was 50. Indeed, statistical power calculations conducted on data derived from a large number of previously published tooth-whitening investigations involving either H_2O_2 or CP as bleaching agents revealed that sample sizes of n = 20 teeth are sufficient to achieve statistically significant differences for matched comparisons of the shade intensities of teeth before versus after tooth-whitening treatment.

Moreover, independent studies conducted on untreated teeth in the investigators' surgery revealed that this sample size is also stastistically sufficient to detect differences in tooth stain intensities (discolouration levels) between each of the three tooth areas monitored in this study (each tooth shade was also monitored non-invasively with the Crystaleye digital dental spectrophotometer).

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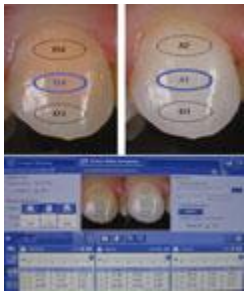
Results

The Crystaleye spectrophotometric monitoring system provided data regarding the shades of each tooth examined in the cervical, body and incisal regions (Fig. 5). A preliminary ANOVA performed on untransformed tooth stain intensity data (that is, those acquired before the whitening treatment applied in this study) revealed highly significant differences between the mean shade intensity values of each of these three areas for a*, b*, and L*, together with those for the Vitapan Classical shade scores ($p < 0.0001$ in each case), information which confirmed a substantial 'within-teeth' component of variance for each of these parameters.

Figure 5: Crystaleye digital dental spectrophotometric data showing clear differences in tooth shade intensities for the body (central), cervical (top) and incisal (bottom) tooth areas.



a) Before; b) subsequent to tooth-whitening treatment (typical data are shown); c) digital spectrophotmetric data showing clear and substantial reductions in tooth shade intensities for the body, cervical and incisal tooth areas for the parameters a*, b* and the Vitapan shade score values, and increases in that of L*: ΔE values are also computed (typical data are shown)

Full size image (72 KB)

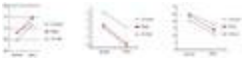
Multifactorial analysis of variance (ANOVA) of the untransformed L*, a* and b* tooth shade parameters according to the component analysis outlined in Table 1a revealed that the differences observed between each of the main effects (tooth-whitening treatment (fixed), tooth areas (fixed and 'nested' within treatments), participants (random), and teeth-within-participants (conditionally random)) were all very highly significant for each one monitored (Table 2). The p values for each of the components of variance tested for each experimental tooth shade parameter (L*, a*, b* and ΔE) are listed in Table 2.

Table 2: List of p values for each of the components of variance tested for each experimental tooth shade parameter (L*, a*, b* and ΔE). With the exception of the treatment × tooth area interaction effect for parameter a*, all other factors/components of variance were substantially significant for each tooth stain parameter investigated. In addition to highly significant differences between treatments (for L*, a* and b*), these analyses also revealed very highly significant differences 'between-participants' and 'between teeth-within-participants' (for ΔE in addition to L*, a* and b*)

Full table

However, the first-order, two-factor interaction component of variance tested was also found to be significant for the L* and b* parameters monitored ($p = 1.40 \times 10{-}3$ and $2.61 \times 10{-}5$ respectively), an observation providing strong evidence for differences between the manner in which each tooth area responds to the applied tooth-whitening treatment. This observation confirms that these interaction effects are not ascribable to sampling variation. Such differences in this response are clearly visualised in Figure 6, which plots the tooth-whitening treatment response for each of the tooth areas examined and each of the three shade parameters (L*, a* and b*). The nature of this significant interaction effect for L* and b* appears to arise from the lower level of treatment response for the cervical region of teeth, and also perhaps the greater responsiveness of the body area to the tooth-whitening treatment employed for shade parameter b*.

Figure 6: Plots of overall mean tooth shade parameter values for a) L*, b) a* and c) b* before and following treatment with the tooth-whitening product investigated and its associated thermal diffusion system, for each of the incisal, body and cervical tooth areas.



The significance of the treatment × tooth area interaction component of variance is readily visible in the plots shown in a) and c)

Full size image (21 KB)

Despite this significant interaction effect observed for the shade parameters L* and b*, substantial shade improvements for all of these shade scores were observed following the tooth-whitening treatment period of only 10 days (Fig. 6). Indeed, overall tooth shades improved by mean values of 2.38, 9.02, 6.01 and 11.21 for Δa*, Δb*, ΔL* and ΔE respectively, with a 9.03 decrease observed in the Vitapan shade score. The decreases in tooth shade intensities observed were highest in the body region, and lowest in the cervical area when tooth-bleaching effects were expressed as Vitapan shade and ΔE scores. When monitored as the parameters a* and L*, the greatest decrease and increase, respectively, observed was in the incisal region, and, as noted above for L*, the smallest increase observed was that in the cervical area.

Mean differences (decreases) between the VSS values were 8.26, 9.70 and 9.14 for the cervical, body and incisal areas respectively ($p < 10{-}8$ in each case). The (overall) mean Vitapan shade values of teeth prior and subsequent to tooth-whitening treatment were 12.48 (A3.5) and 3.45 (B2) respectively (Table 3).

Table 3: Tooth-whitening treatment-induced decreases in the mean Vitapan shade score (VSS) values for the cervical, body and incisal areas of teeth. Each decrease in shade intensity was highly statistically significant ($p < 10{-}8$)

Full table

We monitored the VSS scores determined both before and subsequent to tooth-whitening treatment in order to investigate the nature of any 'clustering' effects within each of the three tooth regions examined. Statistical analyses of these sub-regional data, that is, comparisons of the mean VSS values (specifically, central versus north versus east versus south versus west) for each tooth area demonstrated that there were no significant differences between these sub-regions (each located one-half of a 'window' away from the centre) and the centre for either the cervical, body or incisal regions before tooth-whitening treatment. However, for the datasets acquired following this treatment, though there were no such sub-regional differences between these mean VSS values for the body and incisal areas, there were highly significant differences between these sub-regions for the cervical area ($p < 10-4$). Further analysis of these data by Tukey's HSD and Dunnett's tests revealed that these differences were primarily ascribable to a significantly greater mean VSS value in the northern sub-region over that of the centroid ($p < 10-4$). Post hoc comparisons of these mean values between the east, south and west sub-regions and that of the centroid were not significant for this area after tooth-whitening treatment (Table 4).

Table 4: Statistical significance of differences observed between the cervical area sub-region mean VSS stain intensities (ie those located one-half of a 'window' north, east, south and west of the centroid) and that of the control (centroid) value subsequent to tooth-whitening treatment (this analysis was conducted by Dunnett's test following ANOVA performed as described in our experimental design and statistical analysis section). The mean differences were obtained by subtraction of the north, east, south or west values from that of the centroid

Full table

As expected for this form of ANOVA, the 'between-participants' and 'between-specified-teeth-within-participants' components of variance were all highly significant for all tooth areas and their sub-regions both before and subsequent to tooth-whitening treatment ($p < 0.0001$, and < 0.0001 to 0.021 respectively); information which again confirms a high level of heterogeneity in tooth stain intensities both between subjects and their 'smile-zone' teeth.

Mean Δa^*, Δb^*, ΔL^* and ΔE values for the cervical, body and incisal tooth regions are listed in Table 5. All of these mean difference values were very highly significant for each of these tooth areas ($p < 10-10$ for all comparisons, Student's t-test performed on untransformed data).

Table 5: Tooth-whitening treatment-induced decreases in the spectrophotometrically determined mean tooth shades (Δa^*, Δb^*, and ΔE), and increases in that of ΔL^*. All these values were highly statistically significant ($p < 10-10$)

Full table

Mean ΔE tooth-whitening score values were 9.23, 12.42 and 11.98 for the cervical, body and incisal areas respectively. The (overall) mean shade decrease observed following tooth-whitening treatment was 11.21. ANOVA performed on the complete ΔE dataset (Table 2) revealed marked differences 'between-tooth-areas', 'between-participants' and 'between-teeth-within-participants' ($p = 2.19 \times 10-20$, $4.00 \times 10-4$ and $1.30 \times 10-7$ respectively). Therefore, the differences in ΔE observed between the three tooth areas examined here provide a high level of evidence that the order of tooth-whitening effectiveness is in the order body area > incisal area > cervical area.

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Discussion

The determination of tooth shades is a highly complex process and represents a significant challenge to dental professionals.21, 22 However, the recent development of digital spectrophotometric systems for tooth colour monitoring such as the one employed here markedly facilitate such measurements.22, 23, 24 Indeed, results acquired from these devices have an extremely high level of accuracy and reproducibility25 and such spectrophotometric data are unaffected by ambient light. Moreover, the level of light reflected from teeth is monitored throughout the entire spectral range, and this monitoring system acquires two– or three-dimensional spectrophotometric images coupled to the analysis of colour intensity data.

Major factors involved in determinations of the tooth-whitening capacities of H_2O_2-containing formulations available for this purpose are:

The concentration of H_2O_2/peroxide adduct therein (and therefore that applied on the tooth surface)

The frequency of the product's application

The duration of such applications.

Sulieman et al. (2004)25 compared the tooth-whitening efficacies of gels with H_2O_2 contents ranging from 5-35% (w/w), and as expected, found that the higher the concentration of H_2O_2, the smaller the number of gel applications required to produce uniform bleaching. Notwithstanding, a further investigation26 revealed that the tooth-bleaching capacities of 5% (w/w) H_2O_2 gels were of a standard similar to those achievable by higher H_2O_2 concentration gels when the period of treatment time was increased.

In this study, the H_2O_2-containing tooth-whitening formulation was employed for a 10-day period and the second, post-treatment tooth colour shade determination was made 14 days following the completion of this treatment regimen. This particular treatment period is somewhat shorter than that of most clinical studies, which generally have a duration of H_2O_2 or CP application of between 2 to 4 weeks.

The experimental work performed here represents the first tooth-whitening study involving concomitant measurements of the shades of teeth in three different, pre-specified areas. Indeed, tooth shade data derived therefrom served to provide valuable information regarding the 'global' distribution of tooth stains (that is, their relative discolouration intensities) in teeth.

Data acquired here demonstrates that the tooth-whitening system tested exerted extremely effective bleaching properties in all tooth areas examined. Moreover, the spectrophotometric system employed revealed that the values determined following the treatment regimen were all very highly statistically significant, the order of this tooth-whitening effectiveness being $\Delta b^* > \Delta L^* > \Delta a^*$. The order of tooth-whitening effectiveness for Δb^* and ΔE in these tooth areas was body > incisal > cervical, and for Δa^* and ΔL^* it was incisal > body > cervical, an observation reflecting the applied thermal diffusion system's ability to facilitate the penetration of H_2O_2 to intrinsically-stained enamel and dentine sites. This study also demonstrated that the most effective stains removable from teeth were those of a yellow colouration, whereas the related red-coloured stains were the most difficult to bleach. Indeed, previous investigations such as that conducted by Westland et al.15 have found that the a* value of stained teeth was relatively invariant to tooth-whitening treatment with a CP-containing product. Despite this, this study demonstrated extremely highly significant differences between the mean a* values before and after application of the tooth-whitening product tested in all tooth areas examined.

The cervical areas of the teeth exhibited the least bleaching agent-induced modification in terms of the overall score measurements acquired. This phenomenon may be ascribable to the thin and translucent enamel layer present in the cervical areas, which allows a greater visibility of the more highly-discoloured underlying dentine. Hence, the dentine colour in this area is required to be

bleached to a greater level so that the overall discoloured appearance of the teeth is significantly diminished.

The statistically significant greater mean VSS value in the northern sub-region over that of its centroid observed in the cervical area post-treatment is readily explicable by the consideration that diffusion of the bleaching agent will be expected to be much less at the cervical margin in view of the study protocol, which instructed participants to 'paint' the bleaching agent >2 mm away from the gum/cervical margins. A further consideration is the secretion of crevicular fluid from gingival tissues, and this biofluid may dilute and/or neutralise the H_2O_2 bleaching agent in the cervical areas, a phenomenon giving rise to poor bleaching results in this area. Moreover, the enamel thickness is much thinner in the cervical area, especially at the cervical margin where it merges with dentine at the amelo-dentinal junction, and this thin layer of enamel is therefore more translucent, and hence more of the underlying dentine is visible and hence responsive to the digital spectrophotometric device employed.

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Conclusions

The spectrophotometric shade determination system readily detected very highly significant differences between the three tooth regions investigated for each of the shade parameters monitored ($p < 10^{-10}$ for a*, b* and L*), data which confirms a highly heterogeneous distribution of tooth stains 'between-tooth-areas-within-teeth'.

The tooth-whitening treatment administered gave rise to extremely significant decreases in a* and b* ($p < 10^{-10}$ in each case) and VSS values ($p < 10^{-8}$), together with increases in L* ($p < 10^{-10}$) for each of the tooth areas investigated, an observation of much significance to cosmetic dentistry, especially since treatment-induced reductions in the shade parameter a* (Δa*) are extremely difficult to achieve in practice. These modifications arising from tooth-whitening treatment are fully consistent with the teeth becoming lighter (increases in L*), and less yellow and red (reductions in b* and a* respectively), and are linked to an elevation of the perceptual whiteness of teeth. Post-treatment mean decreases in the VSS values were 8.26, 9.70 and 9.14 for the cervical, body and incisal areas respectively. Similarly, mean ΔE values determined post-treatment were also very highly significant for each tooth region examined ($p < 10^{-10}$ in each case); the 'between-tooth-area' component of variance for ΔE values was also very highly significant ($p = 2.19 \times 10^{-20}$).

The experimental design employed permitted an evaluation of the differing responses of stains present in the cervical, body and incisal tooth regions towards the product applied. Indeed, it was found that for shade parameters b* and L* (but not a*), there was a non-parallel response of the three tooth regions to treatment, with stains present in the cervical region responding less well to this form of whitening treatment than the incisal and body areas. This phenomenon was demonstrated by the statistical significance of the treatment × tooth area interaction component of variance for these particular shade parameters.

The tooth-whitening system tested exerted extremely powerful bleaching actions in all tooth areas investigated. The order of tooth-whitening effectiveness was body > incisal > cervical for Δb* and ΔE,

and incisal > body > cervical for Δa* and ΔL*, and this observation may arise from the TDS's ability to promote the penetration of bleaching-active H_2O_2 to intrinsic stain sites.

'Clustering' of tooth stain intensities were investigated via a statistical analysis of VSS values in sub-regions located one-half of a spectrophotometric 'window' north, east, south and west from the central cervical, body and incisal tooth stain monitoring areas. The only detectable clustering was that observed in the cervical area subsequent to treatment, in this case a significantly greater stain intensity in the northern sub-region than that at the regional centre. This observation is ascribable to the diffusion of much less H_2O_2 at the cervical margin (participants were instructed to apply the bleaching agent >2 mm away from the gum/cervical margins), the secretion of crevicular fluid from gingival tissues, together with the diminished enamel thickness in the cervical area.

Competing interests statement

The authors declare no competing financial interests.

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