

# Tooth colour change with Ozicure Oxygen Activator: a comparative *in vitro* tooth bleaching study

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## SUMMARY

**Introduction:** This *in vitro* study compared a new tooth bleaching product, Ozicure Oxygen Activator (O<sub>3</sub>, RSA) with Opalescence Quick (Ultradent, USA) using a randomised block design to assess tooth colour change. Aim: Colour change, stability and relapse in canine, incisor and premolar teeth was assessed following three bleach treatments and subsequent tooth colour assessment.

**Methods:** Ninety nine teeth (canines, incisors and premolars), which were caries free, had no surface defects and were within the colour range 1M2 and 5M3 were selected. Teeth were randomly divided into the three experimental groups: Opalescence Quick, Ozicure Oxygen Activator and control. The three experimental groups received three treatments of one hour each over three consecutive days. Tooth colour was assessed using the Vitapan 3D Master Tooth Guide (VITA, Germany). A General Linear Models test for analysis of variance for a fractional design with significance set at  $P < 0.05$  was used to test for significance.

**Results:** Both bleaching methods significantly lightened the teeth ( $P < 0.0001$ ). Tooth colour change was mainly after the first hour of tooth bleaching. The tooth type was significant in tooth colour change ( $P = 0.0416$ ). Tooth colour relapse and resistance to colour change were observed.

**Conclusions:** Ozicure Oxygen Activator bleached teeth in a manner and to an extent similar to Opalescence Quick.

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**Key words:** tooth bleaching, Ozicure Oxygen Activator, Opalescence Quick, tooth colour change

## INTRODUCTION

Bleaching discoloured teeth is a conservative, non-invasive treatment to create a "bright white smile". It is an efficient and relatively safe procedure<sup>1</sup> which is attracting an increasing number of patients who seek this comparatively inexpensive option for perceived tooth discolouration. Tooth bleaching may pose side-effects for some patients, such as tooth sensitivity and gingival irritation. These side-effects can be present during or after vital tooth bleaching, and can deter the patient from completing the bleaching treatment. A bleaching product has recently come onto the market that specifically addresses a reduction of sensitivity and irritation. It is called Ozicure Oxygen Activator (O<sub>3</sub>, RSA), and as its name suggests, this product relies on ozone to achieve bleaching by facilitating the production of hydrogen peroxide, with the claim that this procedure is as efficient as conventional bleaching products. To date no studies have been published on the bleaching capacity of this product, which this investigation attempts to address. In addition, three variables were specifically incorporated into the study because the literature was ambivalent on these matters, i.e. effectiveness of the product on different tooth types, application frequency and colour stability. The literature on the three variables is summarised below.

**Different tooth types.** Bleaching agents are usually applied to the anterior teeth that are visible when one smiles. Only one study has specifically compared the response of these three tooth types to bleaching,<sup>2</sup> where it was found that canines responded better to certain bleaching solutions than did incisors. Unfortunately, there were insufficient premolars in the sample to draw conclusions for this tooth type.

**Application frequency.** Colour change following repeated tooth bleaching is less definitive. Two studies<sup>3,4</sup> have shown little benefit resulting from repeated *in vitro* bleaching beyond

the tooth colour obtained from the first bleach treatment. In contrast, de Silva Gottardi<sup>5</sup> demonstrated an average colour change of 2.1-3.7 units per single and up to four in-office bleaching regimens in a clinical study. In yet a third permutation,<sup>6</sup> observed tooth colour change was observed in patients only after an average of two-and-a-half nights using a night guard bleaching system. In summary, there is no agreement on the optimal bleaching time to obtain effective and efficient tooth colour change.

**Colour stability.** Unfortunately tooth colour following bleaching is not maintained. Usually staining continues and the teeth darken; a phenomenon referred to as colour fall-back or relapse. A darkening of the teeth by two units during the first six months after a bleaching treatment has been previously indicated using digital images<sup>7</sup> or a colorimeter.<sup>8</sup> On the other hand, Kihn<sup>9</sup> reports on a half-shade colour lightening, directly following treatment, which he ascribes to an immediate rehydration of the enamel. For this reason, it has been argued that a twenty-four-hour follow-up tooth colour assessment should be undertaken to compensate for any dehydration that may have resulted from the bleaching procedure.<sup>7</sup> This is concurred with by others,<sup>4</sup> who maintain that the colour reading taken 24 hours after bleaching is the most stable. Consequently timing of tooth colour assessment is critical in tooth bleaching colour determination studies, especially where variables need to be controlled. Additionally, the literature shows that little attention has been devoted to short-term tooth colour stability during bleaching treatment: most studies reviewed report only a baseline and final colour reading taken at termination of the research.<sup>10</sup> No studies have examined sequential changes in tooth colour following repeated bleachings within a single study.

Hand-in-hand with tooth colour change is the colour assessment method. Traditionally, tooth colour assessment is done visually using tooth shade guides and charts. The use of shade guides is controversial: Watts and Addy<sup>11</sup> maintain their use is highly subjective, but concede that visual colour perception assessment can be improved with training. Others admit that while dental shade guides have been improved, they do not cover the colour distribution of natural teeth.<sup>12</sup> Despite the limitations, tooth shade guides have been used successfully in several tooth whitening studies<sup>9</sup> and such guides are both quick and cost effective. All agree that consistent, accurate tooth colour readings require standard light conditions under which such readings can be uniformly made.

Consequently this *in vitro* study was conceived to compare the bleaching effect of Ozicure Oxygen Activator (Ozicure) with a clinical bleaching gold standard. Opalescence Quick (Ultradent, USA) was selected as it has been extensively studied and compared with many other bleaching products.<sup>6,13-19</sup> Included within the study was an investigation of continuous colour change occurring in human incisors, canines and premolars during the bleaching treatment and a reporting of tooth colour relapse. The hypotheses which were to be tested were:

1. Tooth colour change with Ozicure is superior and more stable to that obtained by Opalescence Quick (Opalescence).
2. The three tooth types (canine, incisor and premolar) respond differently to bleaching.

3. Teeth will become progressively lighter with ongoing bleaching.

The objectives of this *in vitro* study were formulated to test the hypotheses.

1. Tooth colour obtained from Ozicure bleach was compared to that of the gold standard Opalescence and a control (distilled water). Three tooth types (canine, incisor and premolar) were bleached three times with tooth colour assessments done at three intervals (baseline, one hour and 24 hours after each bleach) using visual tooth colour assessment methods.
2. Examination of colour stability between bleaching treatments and colour relapse noted between readings.

## MATERIALS AND METHODS

Permission to use human extracted teeth in this study was obtained through the Human Research Ethics Committee (Medical) at the University of the Witwatersrand, Johannesburg, which issued an ethics clearance certificate (M050760). Teeth used in the study were obtained from those accumulated by the Dental Research Institute, University of the Witwatersrand. A pool of two-hundred-and-fifty teeth was initially selected for the study according to the following criteria: incisor teeth, canine teeth, and premolar teeth with a tooth colour darker than a 1M2 Vitapan 3D Master Tooth Guide (Vita, Germany) reading. The external debris and stains on the teeth were removed with a Cavit-Jet (CavitorDentsply, USA) scaler. A polishing cup [Kerr Hawe Optishine™ (Switzerland)] and polishing paste [Nupro prophylaxis paste with fluoride (Dentsply, USA)] was used to polish the tooth surface for one minute. The teeth were re-examined and only those with no caries, no surface defects and within the colour ranges 1M2 to 5M3 (the colours most commonly requested for bleaching) were included in the study, to give 99 teeth. The colour of each tooth was recorded as the experimental baseline colour. Thereafter teeth were divided by tooth type and then randomly subdivided into three groups of 33 teeth each, according to the experimental treatment, Ozicure, Opalescence and the Control group. Each tooth was coded and placed into a corresponding individually numbered bottle containing distilled water and a few thymol crystals (Merck, Germany) and stored at six degrees Celsius.

The 33 teeth were further divided into three replicates of 11 teeth apiece consisting of four incisors, two canines and five premolars. Nine custom made, numbered acrylic base plates were made, one for each replicate, to hold the teeth and facilitate bleaching (Figure 1). The precise manufacture of each base plate ensured that every individual tooth had its own specific socket and there could be no accidental mixing of teeth. In addition, a bleaching guard was made for each tooth-containing base plate to mimic the clinical situation. In the case of the Ozicure bleaching guards, each had three nozzles, one anterior to serve as an ozone gas inlet and two posterior for gas exit. Before each bleach application, the teeth were removed from the bottles and the individual teeth placed in their own specific socket of the custom made acrylic base plate precise for the



**Figure1:** One of the replicate Ozicure teeth sets in position in the custom-made base plate prior to bleaching.

replicate and experimental group. Each tooth in the three groups received three experimental treatments.

### OZICURE GROUP

The Ozicure bleaching procedure consisted of two phases which were carried out in strict accordance with the manufacturer's instructions. During the first phase, the teeth were positioned in the base plate, wetted with the activated water included with the product and covered with the bleaching guard. The



**Figure 2:** Ozone gas is sucked into the bleach guard through tube on the left and extracted via the two tubes on the right. Extracted ozone gas went through the flow meter to the suction pump in the Ozicure treatments. The flow meter regulated the removal of the ozone gas from the guard that covered the teeth in the base model. The ozone gas was extracted at a rate of 5l/min.

Ozicure base plate was attached via a small diameter tube to the bleaching guard inlet. This tube transferred the ozone gas (3l/min) from the Ozicure Oxygen Activator into the bleaching guard to react with the wetted teeth (Figure 2). Ozone gas was extracted by the dental suction pump at a rate of 5l/min. After 15 minutes, the bleaching guard was removed, the teeth were again wetted with activated water and the bleaching guard was immediately repositioned over the teeth for another 15 minutes of ozone gas flow. Thus teeth were exposed to ozone for a total of 30 minutes at each treatment. The second phase followed directly after the first phase and involved sculpting the teeth with Trèswwhite (Ultradent, USA) strips containing nine percent hydrogen peroxide. The strips were removed after 15 minutes and the teeth moistened with distilled water. The strips were then repositioned on the teeth and left for another 15 minutes giving a total exposure time of 30 minutes. After the procedure, the teeth were rinsed with distilled water and cleaned with paper towel. The colour of each tooth was determined immediately following the treatment and 24 hours later. Prior to successive bleaching thereafter, a baseline colour was recorded before the next treatment occurred.

### OPAESCENCE GROUP

Following placement of teeth in the base plates, Opalescence gel containing 35% carbamide peroxide was used to bleach the teeth in this group. Copious gel was inserted into the bleaching guard using a syringe. The bleaching guard containing the bleaching agent was placed in position over the teeth ensuring full contact of the bleaching agent on the coronal aspect of the teeth. Bleaching took place over four 15-minute intervals for a total bleaching time of one hour. At the end of each interval the bleaching guard was removed and the teeth wetted with distilled water using a spray bottle. New bleaching agent was added to the bleaching guard and agitated prior to placement over the teeth. Tooth colour

**Table 1:** Comparison of F- and P- values for independent and dependant variables of the study using baseline and 24 hour colour readings following each of the three experimental treatments.

Independent variables	Visual tooth colour assessment method		Number of observations
	F value	P value	
Treatments: n=3 (Control, Opalescence, Ozicure)	9.72	<0.0001	396
Number of tooth bleaches: n=3	3.41	0.0176	396
Tooth type: n=3 (canine, incisor, premolar)	3.21	0.0416	396
Tooth type and treatment	2.75	0.0280	396

**Table 2:** Summary of tooth colour relapse occurring at two stages in the study.

Treatment group	Tooth colour relapse %
(a) Colour relapse: comparing 24-hour tooth colour after first bleaching with baseline tooth colour before second bleaching treatment.	
Ozicure Oxygen Activator	9%
Opalescence Quick	6%
(b) Colour relapse: comparing 24-hour tooth colour after first bleaching with final tooth colour.	
Ozicure Oxygen Activator	9%
Opalescence Quick	9%

was determined immediately following the treatment and 24 hours later. For each sequential bleach thereafter, a baseline colour was recorded before the next treatment occurred.

### CONTROL GROUP

The treatment of control teeth was exactly the same as the Opalescence group with four 15 minute intervals and using distilled water instead of an active bleaching ingredient. As with the other two experimental groups, tooth colour assessments were determined immediately following the treatment and 24 hours later. Also, a baseline colour was recorded before the next treatment occurred.

Internal validity was ensured using the following protocols:

- Tooth-colour assessment was recorded at the same spot within the dental surgery. The walls of the surgery were painted a light grey matt finish and day light globes were the only light source used. External light sources were blocked out by closing the blinds in front of all the windows and the surgery door was kept closed. A black matt background was used during tooth-colour assessment.
- Consistent colour assessment was ensured by performing a Cochran-Mantel-Haenzel test on the colour readings of 30 teeth assessed on four consecutive days by an investigator blinded to previous readings. The test was performed in the same surgery under identical light conditions as for the experiment. At  $P=0.1841$  it was established that there was no significant difference between the colour assessments, indicating that tooth colour was consistently read.
- The researcher (AAG) was blinded during the entire study to the previous tooth colour readings.

Tooth colour shades were captured on a Microsoft Office Excel spreadsheet. For the statistical evaluation of the results, the shade tabs which record shades alpha-numerically, were numbered consecutively from the lightest (0M3) to the darkest (5M3) colour. This gave a ranked numerical series from one (0M3) to 29 (5M3). These ranked numbers were used for the statistical analysis using SAS (SAS for Windows Version 9.1, SAS Institute Inc., Cary, NC: USA). A randomised block design was used to examine and analyse tooth colour change. Statistical analysis was done using the General Linear Models (GLM) test for Analysis of Variance for a Fractional Design set at a significance of  $P<0.05$ . The bleaching methods, number of tooth bleaches and tooth type were the independent variables of the study and the tooth colour the dependent variable. Data was analysed in selected subsets to further explore the interactions within the study.

Descriptive statistics using the rank number to obtain mean values, standard deviations and maximum and minimum readings were used to further investigate the dynamics of the study.

**Table 3: Teeth showing no visual colour change after three bleaching treatments.**

Treatment Groups	Canine n=18	Premolar n=45	Incisor n=36	% of teeth
Opalescence Quick	2	3	0	15%
Ozicure Oxygen Activator	2	5	1	24%
Percentage of teeth	22% of canines	17% of premolars	2.7% of incisors	
Control	11	11	10	97%

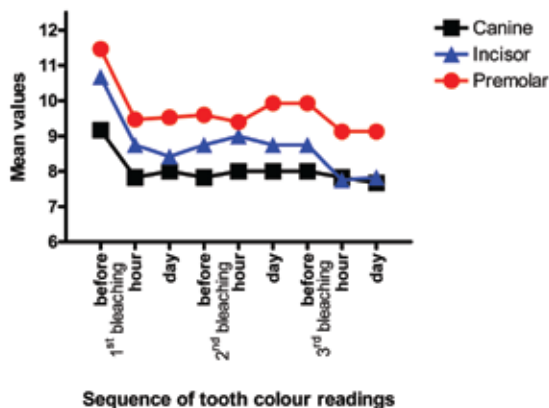


Figure 3: Mean values of visual tooth colour for Ozicure Oxygen Activator over the three bleach treatments.

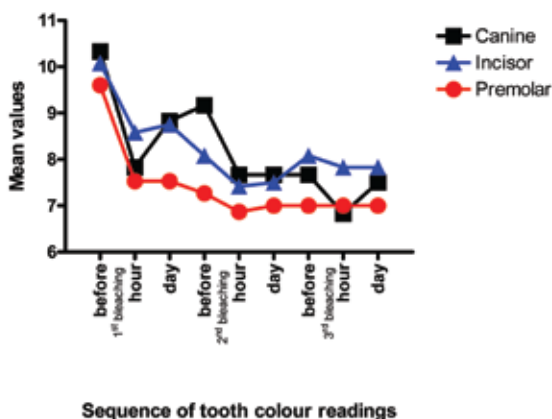


Figure 4: Mean values of visual tooth colour for the Opalescence Quick over the three bleach treatments.

**RESULTS**

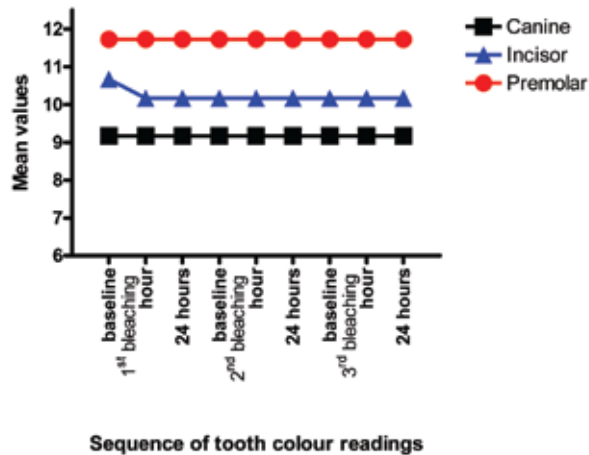
During the treatment phase, the bleaching guards effectively brought the bleaching agents in contact with the teeth. This was confirmed by gel adhering to the teeth in the case of Opalescence and by constantly monitoring the flow meter to regulate the ozone gas flow at 5l/min for Ozicure. Additionally the absence of leaks around the guards confirmed an optimum seal. Statistical data analysis to establish overall interactions between the variables was confined to the initial baseline readings and each 24 hour tooth colour assessment reading following bleaching as these are the most accurate and stable tooth colour readings to use in these studies. This data set of 396 readings was used to determine the influence of treatment, number of treatments, tooth type and the combination tooth type and treatment on tooth colour change. Table 1 shows the F and P values for the three test groups (Ozicure, Opalescence and Con-

tro). Tooth colour showed significance for independent variables treatment (F=9.72; P< 0.0001); number of treatments (F=3.41; P=0.0176); tooth type (F=3.21; P=0.0416) and the combination tooth type and treatment (F=5.92; P=0.0001). Other combinations of treatment\*number of treatments and tooth type\*number of treatments proved insignificant. Further analysis showed both bleaching treatments were equally effective in changing tooth colour, there being no significant difference between tooth colour change when the two products were compared (F=2.45; P=0.1193). However, a significant difference was apparent when Ozicure was compared with the control (F=9.78; P=0.0021) and when Opalescence was compared with the control (F=24.94; P<0.0001). To explore the response of individual tooth types to bleaching, the GLM test was run selectively by tooth type against the pooled treatment sample (i.e. the combined Ozicure and Opalescence colour readings totalling 594 data points). In each case the baseline colour value (first reading) was run individually against each sequential reading (i.e. reading 1 against 2; reading 1 against 3; reading 1 against 4 and so on) until a significant difference in colour was obtained. Incisor teeth lightened significantly from the sixth colour assessment onwards (F= 4.31; P= 0.0147), this being the second bleach, 24 hour assessment. Premolar teeth showed a significant tooth colour change from the second tooth colour assessment (F=5.37; P=0.0064) onwards, one hour after the first bleach. Canine tooth colour did not change significantly throughout this analysis. These results show that the different tooth types studied respond differently to bleaching.

Mean values and standard deviations show that tooth colour lightened by approximately two and a half shades overall for each product: Opalescence tooth colour for the baseline observation (1) was 9.91± 3.14 and for the last observation (9) was 7.39± 3.53. Likewise, Ozicure went from 10.76± 3.23 to 8.39± 3.20.

The statistical programme using repeated measurements on the same specimen cannot arrange variables from best to worst. This can only be deduced by comparing mean values and standard deviations based on the ranked numerical colour shade values. Graphs have been used to show tooth bleaching trends using the sequential numerical ranking of colour. The mean values for tooth colours bleached by Ozicure per tooth type are represented in Figure 3. All teeth lightened following bleaching, but little or no further lightening was achieved after the first bleach treatment. Mean values for first and final colour readings are as follows: canine (from 9.17 ± 2.32 to 7.67 ± 3.27); incisors (from 10.67 ± 3.42 to 7.83 ± 2.82); premolars (from 11.47 ± 3.34 to 9.13 ± 3.50). A similar trend is apparent in the Opalescence treatment (Figure 4) with mean values for first and final colour readings being: canine (from 10.33 ± 4.27 to 7.50 ± 5.01); incisors (from 10.08 ± 3.03 to 7.83 ± 4.34); premolars (from 9.60 ± 2.92 to 7.00 ± 2.07). Control treatment (Figure 5) showed that the mean values for canine and premolar tooth colour stayed the same throughout the study, with incisors lightening by half a shade between baseline and first treatment, one hour colour reading. Mean values for first and final colour readings are as follows: canine (from 9.17 ± 1.17 to 9.17 ± 1.17); incisors (from 10.17 ± 3.35 to 10.17 ± 3.35) premolars (from 11.73 ± 4.51 to 11.73 ± 4.51). Colour relapse (Table 2) was deemed to have occurred if a tooth was one shade darker than the previous reading. Two intervals in the study were selected to best represent relapse: (a) between the 24 hour first bleach and baseline colour before the start of the second bleaching treatment (i.e. readings 3 and 4) and (b) between the 24 hours tooth colour after the first





**Figure 5:** Mean values of visual tooth colour for the Control over the three "bleach" treatments..

bleaching and final tooth colour, 24 hours after third bleaching (readings 3 and 9). In the case of Ozicure, 9% of teeth showed a colour relapse of one or more units between both measuring intervals. Opalescence treated teeth showed a 6% relapse between readings 3 and 4 and a 9% relapse between readings 3 and 9. Teeth resistant to colour change represent 19.6% of the sample. These were mainly canines in the Ozicure treatment group (Table 3).

## DISCUSSION

Both bleaching methods resulted in a statistically significant lighter final tooth colour, although the statistical programme using repeated measurements on the same specimen cannot indicate which bleaching treatment method was "best". However, this could be deduced by comparing mean values and standard deviations based on the ranked numerical colour shade values. These numbers indicated that Opalescence performed slightly better than the Ozicure in bleaching the teeth. Furthermore, greater colour relapse and a greater number of teeth resistant to colour change were found in the Ozicure group. This does not mean that Ozicure is an inferior bleach, as statistically both bleaches significantly lightened the teeth in the study.

The slight difference in bleaching performance of the two products could lie in the differing type and concentration of the bleaching agent. Ozicure TrèswHITE strips contain 9% hydrogen peroxide, a strong oxidizing agent, which splits the long-chained, dark-coloured chromophore molecules, responsible for the stain, into smaller, less coloured and more diffusible molecules, thereby lightening the tooth. The gel of Opalescence contains 35% carbamide peroxide, but pointers in the literature indicate that it is unclear how much active agent is produced by this compound. When carbamide peroxide reacts with water, urea forms as a by-product which can be broken down to carbon dioxide and ammonia, but the amount of ammonia formed during tooth bleaching with carbamide peroxide is unclear.<sup>20</sup> Kihn *et al.*<sup>21</sup> claim that bleaching agents based on carbamide peroxide produce less of the active agent than hydrogen peroxide based bleaching agents, thereby producing a less effective bleach. These product differences could possibly account for the changes in bleaching performance of the two materials examined.

The General Linear Models test for Analysis of Variance for a Fractional Design indicated that colour showed significance

with the number of bleach treatments. This is supported by interactive statistics which indicated that significant tooth colour change occurred in a range measured one hour after the first bleach to the final colour reading, depending on what tooth type was examined. However, the descriptive results are conflicting and appear to indicate that after the first tooth bleaching treatment, no, or little, benefit was achieved with repetitive bleaching. Thus the results of the present study are unable to confirm the findings of others<sup>3,4</sup> that multiple bleaching treatments are of limited value. What our study does confirm is that the colour reading taken 24 hours after bleaching is the most stable.<sup>4</sup>

We found that the three tooth types responded differently to the bleaching treatments. Linked variables "tooth type\*treatment" indicated that treatment type plays a role in tooth-type response. The interactive statistics show that visually, premolars change colour significantly one hour after the first bleach and confirm the findings of others<sup>2</sup> that tooth type plays a significant role in tooth colour change and that the different tooth types responded differently to the bleaching treatments. However, in the present investigation there were unequal numbers of the different tooth types (canines=18; premolars=45; incisors=36) and this may have played a role in the statistical outcome of this part of the investigation.

Tooth colour relapse is a normal complication found during a bleaching treatment. Evaluating colour relapse is confounded by three issues: determining a time interval between which to measure relapse; the threshold number of shades beyond which relapse is considered a problem and the acceptable number of teeth relapsing within a specific treatment regimen. Although Gerlach *et al.*<sup>7</sup> reported tooth colour relapse after tooth bleaching, they failed to specify the amount of tooth colour relapse recorded. Therefore our findings of a 6-9% tooth colour relapse appear to be a first reporting on actual numbers of teeth relapsing after bleaching.

Linked to relapse is the phenomenon of teeth resistant to bleaching. We have found no reports in the literature which mention teeth resistant to bleaching, thus one can only speculate as to why certain teeth in the present study did not show any tooth colour change. It could be that these specific teeth were not bleached long enough. Alternatively the bleach did not reach the stain or thirdly the stain type of the tooth was resistant to the bleach. The causes of tooth staining are varied and complex.<sup>11</sup> Little is available on the response of different stains to bleaching. Furthermore, stain type, which is heavily reliant on clinical history, could not be determined with any accuracy for the teeth in this study. It is interesting to note that a greater colour relapse and a greater number of teeth resistant to colour change were found in the Ozicure group and we have no explanation for this observation. Further studies linking stain type to bleach efficacy are called for.

## CONCLUSIONS

The conclusions of this study are best realised by reflecting on the initial hypotheses

1. Tooth colour change with Opalescence is superior and more stable to that obtained by Ozicure.
2. The three anterior tooth types (canine, incisor and premolar) respond differently to bleaching.
3. Teeth will become progressively lighter with ongoing bleaching.

**Declaration:** No conflict of interest declared

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