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Development and evaluation of a method in vitro to study the effectiveness of tooth bleaching

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Summary Aim. To develop and evaluate a reproducible intrinsic discolouration model in vitro, based on tea, which would allow the effectiveness of bleaching to be evaluated.

Method. The crown portions of extracted human third molars were sectioned buccolingually in half. Colour assessments were made at baseline, post staining and post whitening using a standard clinical shade guide (SG), a shade vision clinical colorimeter system (SVS) and a reflectance chromometer. Internal staining employed a standard tea solution into which groups of five specimens were placed from 1 to 6 days. All assessments demonstrated maximum staining within one day. Groups of stained specimens were exposed to 1. Water (placebo control) 2. Enamel polished 3. Enamel polished and bleached through enamel 4. Bleached through enamel 5. Bleached through dentine 6. Bleached through enamel and dentine 7. Exposed to the bleach vehicle (minus active control). Control and bleach gel treatments were for 30 min. Comparisons of treatment effects were made using unpaired *t*-test on groups selected a priori for analysis.

Results. SG and SVS revealed that control and polish treatments had no or little effect respectively on tooth shade but all bleach treatments produced marked and statistically significant whitening effects and to a similar magnitude. Bleaching treatments returned the majority of specimens to the original shade or beyond representing a SG mean change of 13.8-15 shade guide units (SGU). Chromometer readings were consistent except that polishing alone increased tooth lightness slightly.

Conclusions. Teeth were reproducibly stained internally, to provide a model in vitro by which to evaluate bleaching. The model, could be used to study many aspects of vital tooth bleaching, but has the limitation, without in vivo or in situ data, of cautiously extrapolating the effects in vitro to outcome clinically.

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Introduction

The causes of tooth discolouration are varied and complex but are usually classified as being either

intrinsic or extrinsic in nature. Extrinsic discolouration arises when external chromogens are deposited on the tooth surface or within the pellicle layer. Intrinsic discolouration occurs, when, the chromogens are deposited within the bulk of the tooth, usually in the dentine and are often of systemic or pulpal origin.^{1,2} A third category of 'stain internalisation' has recently been

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described to include those circumstances where extrinsic stain enters the tooth through defects in the tooth structure.¹

Tooth discolouration creates a wide range of cosmetic problems and the dental profession and the public expend considerable amounts of time and money in attempts to improve the appearance of discoloured teeth. The methods available to manage discoloured teeth range from removal of surface stain, bleaching or tooth whitening techniques and operative techniques to camouflage the underlying discolouration, such as veneers and crowns.

The use of a variety of bleaching techniques has attracted much interest from the profession, as they are non-invasive and relatively simple to carry out. Contemporary bleaching systems are based primarily on hydrogen peroxide or one of its precursors, notably carbamide peroxide, and these are often used in combination with an activating agent such as heat or light. Bleaching agents can be applied externally to the teeth (vital bleaching), or internally within the pulp chamber (non-vital bleaching).^{3,4} Both techniques aim to bleach the chromogens within the dentine, thereby changing the body colour of the tooth. A variety of case reports and small clinical studies have shown that a 10% carbamide peroxide gel used in a bleaching tray at night, the so called night guard vital bleaching technique, produces predictable results⁵⁻¹³ as do hydrogen peroxide strips.¹⁴ Similarly, 'power bleaching' using 35% hydrogen peroxide with or without light and/or heat activation has also been shown to be effective.¹⁵

Although randomised controlled clinical studies provide the ultimate proof of effectiveness, it would be useful to develop a laboratory model to evaluate tooth-bleaching techniques prior to the expensive clinical trial stage. Sharif et al.¹⁶ developed an extrinsic stain method to assess the stain removing ability of whitening toothpastes, which was based on the original model to study the aetiology of chlorhexidine staining.¹⁷ The technique involved reciprocal immersion of clear acrylic blocks in tea and chlorhexidine to produce extrinsic strain. Stain removal from these blocks by the toothpaste was then assessed using spectrophotometric analysis. Pretty et al.¹⁸ employed a similar method to stain extracted human molars using a combination of human saliva, chlorhexidine and tea.

The aim of this study was to develop and evaluate a reproducible intrinsic discolouration model, also based on tea, which would allow the effectiveness of bleaching to be evaluated.

Materials and methods

The plan was to develop a model, which was as standardised and reproducible as possible. Thus, extracted human third molars were stained internally with tea and then subjected to a single, clinically effective, bleaching regime applied to enamel, dentine or both using a randomised placebo controlled parallel group study design. Three systems of colour evaluation were employed.

Sample production

In an attempt to standardise specimens in terms of age, development and maturation, Human permanent third molar teeth, extracted from subjects aged between 18 and 30 years, were obtained from the Oral Surgery Department of Bristol Dental School. It is likely that the enamel of the teeth had matured, since apical closure of the teeth was complete or at an advanced stage. Prior to experimental use the teeth were examined for the absence of disease, cracks or other surface defects. The roots were removed and the crowns were sectioned vertically in half using a diamond saw with copious water irrigation beginning at the level of the occlusal fissure. The specimens produced had a naturally curved outer enamel surface with a flat dentine surface beneath. The exposed dentine surface was polished using a 1000 grit silicon carbide paper in a lapping and polishing unit with water irrigation. Following polishing, the dentine was etched with 35% phosphoric acid etching gel for 60 s. The etching gel was then removed by rinsing in water for a further 30 s. This was carried out to remove the smear layer, expose the tubule system and thereby encourage stain uptake into the tooth.

Following tooth preparation, there were three phases of the experiment

- Stain assessment
- Stain development
- Tooth whitening

Throughout the study all specimens were maintained in a moist environment provided by damp gauze in a sealed container.

Stain assessment

Prior to stain development the baseline shade of each specimen was determined using three different methods:

1. Tooth colour was determined subjectively using value-orientated tooth shade guide tabs

(SG)(Vita, Zahnfabrik, Germany). The shade tabs were arranged in a sequence suggested by the manufacturer and each shade tab was assigned a numerical value ranging from 1 to 16 (B1, A1, B2, D2, A2, C1, C2, D4, A3, D3, B3, A3.5, B4, C3, A4, C4). One investigator (MS) performed all the shade comparisons and was blinded to the allocation of specimens. SG assessments were made with specimens lying on a black background.

2. Shade Vision System (SVS, X-rite, 3100 44th St SW, Grandville, Missouri, USA). This is a commercially available shade taking system that provides an accurate coloured 'contour map' image of the tooth. It is essentially a colorimeter that utilises image-grabbing technology. It comprises a hand-held measuring device that is used to scan the tooth surface together with a docking station linked to a computer and associated software. SVS was used to give a mean Vita shade for each of the specimens. As with SG, SVS recordings were made with specimens lying on a black background.

3. Electronic chromometer (Minolta CR 221, Minolta, UK, 1-3, Blakelands North, Milton Keynes, UK). This is a compact tristimulus colour analyser that electronically measures the reflective colours of surfaces. It has a 3 mm diameter measuring area with a 45° illumination angle and a zero degree, viewing angle. An internal pulsed xenon arc lamp in a mixing chamber provided diffuse, even illumination of the sample surface. Six high-sensitivity silicon photocells, filtered to match the Commission Internationale de l'Éclairage standard observer response, were used by the meter's double-beam feedback system to measure both incident and reflected light. The chromometer detected any slight deviation in the spectral power distribution of the pulsed xenon arc lamp, and compensated for this automatically. The image of the tooth specimen is automatically transformed to derive a set of numerical values in terms of the $L^*a^*b^*$ system.¹⁹ The $L^*a^*b^*$ system allows colour specification within a three dimensional space. The L^* axis represents the degree of lightness within a sample and ranges from 0 (black) to 100 (white). The a^* plane represents the degree of green/red colour, while the b^* plane represents the degree of blue/yellow colour in the sample. The chromometer was applied to the outer surface of the enamel and the shade of each specimen was recorded from nine separate locations taken across the full width of the specimen working from right to left. The system operates in a similar manner to a reflectance spectrophotometer collecting light from the outer surface and is independent of background.

Stain development

Stain development was monitored daily over a six day period by immersion of specimens in a standard tea solution at room temperature ($22 \pm 2^\circ\text{C}$) in screw capped plastic universal containers. Thus, once the baseline shade of each of the specimens had been determined, the samples were immersed in a standard tea solution. The tea solution was produced by boiling 2 g of tea (Marks and Spencer's Extra Strong tea, Marks and Spencer, London, UK) in 100 ml of distilled water, for 5 min and filtered through gauze to remove the tea from the infusion. Thirty-five samples were randomly divided into seven groups with five tooth samples per group using a random allocation table. The first group of five samples were immersed in the tea solution for one day, the second group for two days and so on until the final group was immersed for six days. The tooth shade was assessed using the three methods described above at the end of the immersion time for each group. A control group of specimens were placed in water for six days and the shade of these specimens were also assessed at the end using the same measurements systems. Tea and water solutions were renewed each day for the appropriate groups. After taking the respective readings, the groups of tooth specimens were then standardised for exposure to tea. Thus, the specimens from each group were placed in the tea solution for time periods until all specimens had been soaked in tea for 6 days i.e., the water group were placed in tea for six days, the one-day group for five days, the two-day group for four days, etc.

Tooth whitening

This project had the prime aim of developing and evaluating a model in vitro for eventual application to study tooth bleaching. In the first instance therefore, the model was tested with a single bleaching system, which was already in clinical use by one of the authors and judged effective. For the tooth-whitening phase of the study, the tea stained samples were randomly allocated to seven groups using a random allocation table. All groups were maintained at room temperature ($22 \pm 2^\circ\text{C}$).

Group 1. Water placebo control.

Group 2. Specimens polished with a mixture of pumice and water for 30 s using a bristle brush in a slow handpiece (extrinsic staining control).

Group 3. Specimens polished and a dentine-bonding agent (Gluma, Bayer, Germany), applied to the dentine of the cut face of the specimen, following the manufacturers instructions.

The bleaching agent (Quick white bleaching powder, Quickwhite, Lombard House, 12-17, Upper Bridge St., Canterbury) was prepared by mixing together 0.1 g of the quick white bleaching powder, containing fumed silica, photoactive colorants and dyes, with 0.5 ml of 35% hydrogen peroxide gel. The mixture was then applied to the enamel surface of the specimen to produce a uniform layer 2-3 mm thick. The mixture was then activated with a plasma arc lamp (Apolite II, DMDS UK Lombard House, 12-17, Upper Bridge St., Canterbury) placed just above the surface of the gel. The gel was illuminated for a period of 6 s using the whitening mode of the lamp and the gel was left on the tooth surface for a period of 10 min. The gel was then removed with a damp piece of gauze, refreshed using a fresh mix and illuminated for a further 6 s and left for a further 10 min. This cycle was repeated once more, so that each specimen was bleached using three 10 min passes, as suggested by the manufacturer for the clinical use of the product.

Group 4. Specimens were not polished, but the dentine of the cut surface was sealed and the enamel surface was bleached, as in Group 3.

Group 5. No dentine-bonding agent was applied to the cut surface and the bleaching agent was applied to both the enamel and dentine surfaces and the specimens bleached as in Group 3.

Group 6. No dentine-bonding agent was applied to the cut dentine surface and the bleaching agent was applied to the dentine surface only and bleached as in Group 3.

Group 7. The dentine-bonding agent was applied and the gel carrier vehicle without hydrogen peroxide was applied to enamel and illuminated exactly as for Group 3 (minus active control).

The change in the colour of the specimens was assessed using the three methods above. For the Vita SG and SVS the mean change in shade was recorded and converted to the numerical scale of shade guide unit (SGU). For the chromometer the mean change in the values of L^* , a^* and b^* between baseline and following treatment was recorded. In addition to this, the overall colour difference of the specimens in each group (ΔE^*) were calculated following the following expression:

$$\Delta E^* = \sqrt{[(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]}$$

Statistical methods

For the tooth staining experiments and to avoid multiple paired comparisons a priori it was decided to first compare the 1 and 6 day tea treatment

groups using unpaired t -tests. If significant differences were found, groups 1 and 2 day, 2 and 3 day, etc. would be compared. For the bleaching treatment groups a priori paired treatment comparisons of most interest were made using unpaired t -tests. The pre-study selected pairs were the control groups (water and control gel) with the polish group and the polish group with the polish/bleach group. All of the bleach treatments were compared by analysis of variance and if a significant difference were found, pairs of treatments would be compared using unpaired t -tests. Again to avoid multiple statistical tests, analysis of the chromometer results only used E^* data.

Results

Tooth staining

The results for the SG and SVS assessments are given in Table 1 and for the chromometer in Table 2 and expressed as SGU changes from baseline recordings. The water group showed no change and therefore data are not included in the tables. It is apparent that by all three colour assessment methods most of the colour change occurred after 24 h in the tea solution. The SG and SVS assessments scored the majority of teeth in each day group as C4. The SG assessment gave C3 as the next most frequent shade followed by an equal frequency for shades D3, A3.5 and C1. The SVS gave the following order in decreasing frequency; D2, D3, C3 and C1. The chromometer showed L^* values were in the direction of black, i.e. the teeth had darkened, a^* and b^* values were in the red and yellow direction, respectively. Statistical comparisons of differences between day 1 and day 6 data were only analysed for the three systems and none reached significance ($p > 0.05$).

Table 1 Tooth staining: mean change in SG and SVS values (SGU) after 1-6 days tea soaking.

Day	SG			SVS		
	Mean	SD	Range	Mean	SD	Range
1	9.2	2.2	6-12	9.8	4.1	2-13
2	8.8	1.8	7-12	11.0	2.7	7-14
3	9.6	2.3	7-14	12.8	1.6	10-14
4	9.8	1.3	8-11	11.8	2.1	9-15
5	8.2	1.6	7-11	13.2	0.4	13-14
6	9.8	2.3	7-14	12.8	1.6	10-14

Table 2 Tooth staining: mean (standard deviation) change in chromometer readings after 1-6 days soaking in tea.

Day	ΔL^*	Δa^*	Δb^*	ΔE^*
1	22.08 (2.66)	-2.82 (1.40)	0.31 (3.97)	22.58 (2.95)
2	18.84 (17.95)	-2.98 (1.77)	0.21 (2.08)	24.96 (8.48)
3	16.46 (4.04)	-3.27 (1.73)	-1.98 (2.32)	17.15 (4.21)
4	21.16 (3.40)	-3.05 (1.64)	-0.79 (1.56)	21.53 (3.43)
5	20.08 (2.78)	-2.22 (0.40)	-0.20 (1.09)	20.28 (2.78)
6	24.66 (3.23)	-2.46 (1.04)	-0.010 (3.21)	25.01 (3.34)

Tooth whitening

The SGU change in colour after the different treatment regimens measured from the enamel surface using SG and SVS methods are shown in Table 3 and by chromometer in Table 4. Overall by all three measurement methods the order for increasing colour change was water = placebo gel < polish < polish/bleach with the four bleaching regimens essentially similar. For SG and SVS, in mean terms, water and the control gel produced no SGU changes by SG or SVS and formal statistical comparisons with polishing were not performed. Polishing of enamel alone produced small SGU changes by SG and SVS. However, these effects were much less than recorded for the polish/bleach treatment and differences were significant ($p < 0.001$). The SGU changes determined by SG and SVS for the remaining treatments, polish/bleach, bleach enamel, bleach enamel and dentine and bleach dentine, were all very similar and analysis of variance showed no significant differences between these treatments ($p > 0.05$). As an observation, it was apparent that bleaching through enamel did not penetrate the entire thickness of the dentine and the cut surface remained stained. Bleaching through dentine however, returned the cut surface to a similar colour to that prior to tea staining.

The changes recorded by chromometer for water and placebo gel were small and significantly

less than polishing ($p < 0.01$). Polishing was less than polishing/bleach, and the difference was significant ($p < 0.05$). The four bleach treatments were not significantly different by analysis of variance ($p > 0.05$).

Discussion

Stain development

The brand of tea was chosen as it had been used in a number of previous extrinsic stain experiments, where it reproducibly brought about stain development as measured by spectrophotometry.¹⁶ The choice of tea as the chromogen may be questioned on the basis that intrinsic discolouration of teeth is caused by other chromogens,² except when dentine becomes exposed. Tea did reliably produce however, an intrinsic stain, which was consistent with tooth discolouration observed clinically and assessed by SG systems employed clinically. Also it would have been difficult to mimic true intrinsic staining because of the multi-factorial aetiologies,² some of which merely reflect age changes in teeth. Other attempts have been made to model intrinsic staining and bleaching in vitro, using, for example, blood, but appears to have only been referred to in a text book.³ Following immersion in tea, stain development assessed by the two shade systems, SG or SVS, gave broadly similar results over the six-day immersion period. Stain developed quickly, with a mean change of just over nine units for both assessment systems after only day 1. By SG staining after one day in tea showed no consistent pattern to increase, indeed in mean terms was slightly less on days 2 and 5. By SVS there was a pattern for increased scores but colour change at day 6 was not significantly different from day 1. Similarly, the chromometer revealed considerable colour changes in all $L^*a^*b^*$ values and within 24 h with the most marked value change in L^* indicating tooth

Table 3 Tooth whitening: mean change in SG and SVS scores (SGU) after control, polishing and bleaching treatments.

Group	SG			SVS		
	Mean	SD	Range	Mean	SD	Range
Water	0.0	0.0	0.0	0.0	0.0	0.0
Polish	0.40	0.80	0-2	0.66	1.15	0-2
Polish + bleach enamel	13.80	1.17	12-15	13.80	1.47	12-15
Bleach enamel	14.00	1.26	12-15	14.00	1.26	13-15
Bleach enamel + dentine	15.00	0.0	15-15	13.80	2.40	9-15
Bleach dentine	14.40	1.20	12-15	12.60	3.88	5-15
Gel	0.00	0	0	0.00	0	0

Table 4 Tooth whitening: mean (standard deviation) change in chromometer readings after control, polishing and bleaching treatments.

Group	ΔL^*	Δa^*	Δb^*	ΔE^*
Water	3.58 (3.17)	-0.25 (0.52)	-0.40 (0.98)	4.17 (2.72)
Polish	11.54 (9.88)	1.32 (1.24)	5.67 (5.66)	13.00 (11.81)
Polish + bleach enamel	17.81 (4.18)	1.74 (1.76)	7.16 (1.39)	19.44 (4.15)
Bleach enamel	20.66 (8.20)	2.57 (1.80)	6.70 (2.20)	22.25 (7.66)
Bleach enamel + dentine	22.71 (5.50)	1.85 (1.74)	4.31 (3.06)	23.49 (5.54)
Bleach dentine	23.67 (3.67)	1.86 (0.94)	1.58 (2.36)	23.93 (3.83)
Gel	3.22 (2.22)	-0.07 (0.84)	0.56 (1.65)	3.90 (2.04)

darkening. The changes in a^* and b^* were of interest since these moved in the direction of red and yellow, this would be consistent with the polyphenolic chromogens found in tea namely, the theorubigins and theoflavins, which are red and yellow, respectively.^{20,21} The time to maximum staining of 24 h suggests that the chromogens diffuse rapidly into dentine to saturate binding sites. Presumably the diffusion occurs mainly through the dentinal tubule system, although diffusion through inter-tubular dentine is possible²² and must occur to produce staining in the body of the dentine. The primary aim of the study thus appeared to have been achieved.

Of the two shade systems the SVS system would seem to be more sensitive with the shade change always greater by SVS compared to SG. This is not too surprising, since the SG system of assessment is limited by its subjective nature. Moreover, inter and intra examiner variability has been reported as quite poor with SG assessments.²³

Tooth whitening

The visual SG and SVS assessment of the control groups stored in water showed no SGU changes in overall colour as was expected. However, polishing improved the specimen colour slightly as assessed by both shade methods. The change was probably due to polishing removing the surface (extrinsic) stain from the enamel surface. This supports the purely subjective observation that soaking the specimens in tea caused little staining of the enamel surface and most of the SGU changes were due to intrinsic staining of the dentine. Application of the gel only, minus active control, with light activation, to the tooth specimens provided confirmatory evidence that the carrier vehicle for the hydrogen peroxide did not cause any bleaching effects.

The remaining treatments, that included bleaching enamel, dentine or both, showed a much more profound improvement in specimen colour. Indeed by SG and SVS there was little difference in colour

between the bleaching treatments applied to enamel only, the dentine only or both. This was an important finding for several reasons. Foremost, the data prove that bleach applied to enamel does penetrate this layer to the dentine to bring about a 'whitening' affect, and would be consistent with the published case report and randomised, controlled clinical trial data for tooth bleaching in vivo.¹¹ The depth of dentine penetration by the bleach, after crossing the enamel layer, could not be assessed in the present study. As an observation however penetration was not complete and the cut dentine surface appeared unchanged: the brown tea discolouration was still evident. On the other hand and again observationally, bleaching from the dentine surface appeared to completely remove the tea colour to return the cut surface to its natural colour. Also, the fact that the SG and SVS graded SGU changes taken from the enamel surfaces were similar to those produced by bleaching through enamel suggest the dentine was bleached through the entire depth. This would be consistent with observations concerning internal non-vital tooth bleaching.⁴ Finally, the lack of enhanced whitening also by bleaching both tissues, suggests that bleaching through enamel only needs to alter the colour of the dentine immediately below the enamel.

The results obtained by chromometer were essentially confirmatory of the SG and SVS data. Water had a very slight effect but polishing the teeth showed a smaller change than that for the bleaching treatments, which may have resulted from a small change in the reflective properties of the enamel surface.²⁴ The overall changes (E^*) for the bleaching treatments were similar. By the nature of instrument analysing reflected light, the findings support the previous conclusion that it is the colour change in the dentine immediately below the enamel that produces the apparent whitening effect. The individual values indicate that the bleaching has increased lightness (L^*), greenness

(a^*) and blueness (b^*), which would be consistent with changes to the tea chromogens.

There are clearly limitations in comparing the bleaching results from the present model with clinical reports, not only because of the differing environments but also the variation in bleaching agents used and the protocols employed. Despite these confounding influences, there are interesting similarities in results from studies in vivo and those reported here in vitro. Thus, visual assessment using the SG system found that the shade improved by similar amounts in each of the treatment groups (13–14.8 SGU). Although this technique was a fairly crude and subjective method for assessing changes in tooth colour, it did confirm that the bleaching agent used was effective in improving colour. The change in tooth colour was also broadly similar to previous clinical studies. Heymann et al.¹² reported a mean change of 7 SGU on the Vita SG after bleaching with a 10% carbamide peroxide gel over seven days. Interestingly, they also reported a wide range of responses ranging from 3 to 13 SGU, which was found in this study. This further highlights the unpredictable nature of the biological substrate. Thus, Papathanasiou et al.¹³ reported a mean change of 8 SGU when using a 15% in office hydrogen peroxide system, whereas Gerlach and Zhou¹⁴ reported a mean change of 5.5 SGU when using a whitening strip, but a quarter of their sample had a shade change in excess of 8 SGU.

The SVS system provided very similar results. It is also interesting to note that the difference between the polish only group and the other groups that involved bleaching of enamel, dentine or both, was 8 SGU. This seems to be an average value found in previous clinical studies^{12,13} and presumably the teeth included in these studies had a prophylaxis carried out prior to bleaching. One rather paradoxical finding, was that the change in tooth colour for each of the active groups (polish and bleach enamel/bleach enamel/bleach enamel and dentine/bleach dentine) were remarkably similar at 12.6–14.0 SGU. One possible explanation for this could be due to the use of a particularly high concentration of hydrogen peroxide in this study. This could potentially bleach the specimens to a point where the tooth colour could be altered no further. This effect could be assisted, in this non-vital model, by the lack of a positive outward pressure along the dentinal tubules,²⁵ which in vivo might retard the penetration of any bleaching agent clinically with a vital tooth.

In respect of the chromometer results, ΔL^* is often used in tooth whitening studies as this assesses the lightness of a tooth. This study found that the various bleaching regimes produced

changes of around 20 units. Previous clinical and laboratory studies have reported smaller changes. For example, an in vitro study on dentine by White et al.²⁶ reported an improvement of ΔL^* of seven units when a 10.5 carbamide peroxide gel was used for 30 h. Similarly, Gerlach and Zhou¹⁴ reported an improvement of ΔL^* of two units with a whitening strip product. The larger changes in ΔL^* values observed in this study are probably due to the much higher concentration of hydrogen peroxide used. The use of extracted teeth that were devoid of dentinal fluid also probably allowed the bleaching agent to permeate the tooth more quickly than would be the case clinically. The change in Δa^* values represents a reduction in redness an effect reported by others.²⁷ The change in Δb^* values showed a reduction in yellowness of around 4–7 units that is broadly similar to the mean change of 2–4 units reported by Gerlach and Zhou.¹⁴ As the values for ΔL^* were numerically much larger than Δa^* and Δb^* , the values for ΔE^* being a composite of these first three parameters, followed the trends of ΔL^* .

In conclusion this study describes the development of a tooth model in vitro by which to study bleaching. Tooth specimens could be reproducibly stained internally and bleached back to baseline levels. The findings for the model were not inconsistent with clinical reports. The model could be used to study many potential agents, variables, protocols and side effects of tooth whitening procedures. The model could also be used as a predictor of clinical outcome but would need parallel studies in vivo or in situ to determine to what extent extrapolation of the data in vitro is possible.

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