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Measurement of stain on extracted teeth using spectrophotometry and digital image analysis

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Abstract: *Aim:* The aim of this study was to assess the reliability and validate a customized image analysis system, designed for use within clinical trials of general dental hygiene and whitening products, for the measurement of stain levels on extracted teeth and to compare it with reflectance spectrophotometry. *Method:* Twenty non-carious extracted teeth were soaked in an artificial saliva, brushed for 1 min using an electric toothbrush and a standard toothpaste, bleached using a 5.3% hydrogen peroxide solution and cycled for 6 h daily through a tea solution. CIE L^* values were obtained after each treatment step using the customized image analysis system and a reflectance spectrophotometer. A statistical analysis was carried out in SPSS. *Results:* Fleiss' coefficient of reliability for intra-operator repeatability of the image analysis system and spectrophotometry was 0.996 and 0.946 respectively. CIE L^* values were consistently higher using the image analysis compared with spectrophotometry, and *t*-tests for each treatment step showed significant differences ($P < 0.05$) for the two methods. Limits of agreement between the methods were −27.95 to +2.07, with a 95% confidence of the difference calculated as −14.26 to −11.84. The combined results for all treatment steps showed a significant difference between the methods for the CIE L^* values ($P < 0.05$). *Conclusion:* The image analysis system has proven to be a reliable method for assessment of changes in stain level on extracted teeth. The method has been validated against reflectance spectrophotometry. This method may be used for pilot *in vitro* studies/trials of oral hygiene and whitening products, before expensive *in vivo* tests are carried out.

Key words: extracted teeth; image analysis; lightness; spectrophotometry; staining

Introduction

The discolouration of teeth is a major concern to many people. The majority of tooth discolourations on permanent teeth are of extrinsic origin (1), caused by stain build-up on the tooth surface. Many extrinsic stains are pigments embedded in the plaque or calculus arising from interactions with food, drink and tobacco smoke. As one of the uses of general and whitening dentifrices is to remove tooth stain, there is a pressing need for quantitative objective measurement of tooth stain to further improve the sensitivity and selectivity possible when assessing commercial dentrifices.

The indices of Lobene (2) and Shaw and Murray (3) have been widely used for stain measurement, but, although these indices are quick and easy to use, the subjective nature of these approaches gives rise to reliability problems. Reflectance spectrophotometry has been utilized for colour assessment of extracted teeth (4) and has been shown to give reproducible results measuring small changes in tooth colour. However, this approach has limitations when measuring tooth colour, because teeth have curved surfaces and are translucent, both of which can lead to systematic errors (5).

As an alternative objective approach to spectrophotometry, we have used a customized digital image analysis system to assess stain build-up and removal on acrylic blocks and found it to be a reliable alternative measurement method when compared with absorbance spectrophotometry (6).

The aim of the present study was to assess the reliability of a further modified image analysis system, designed for use within clinical trials of general dental hygiene and whitening products, for measuring stain build-up and removal on extracted teeth and to compare it and validate it against a reflectance spectrophotometer using a modified staining protocol utilized in previous *in vitro* staining studies (7–9) along with a brushing and bleaching step.

Materials and methods

Preparation of extracted teeth

Twenty extracted permanent non-carious posterior teeth were collected (Charles Clifford Dental Hospital, Sheffield, UK) with the consent of the patients. The teeth were scrubbed with deionised water and a hard toothbrush to remove any tissue or debris. The teeth were then autoclaved at 121°C for 30 min and stored in phosphate-buffered saline with 1% thymol crystals to avoid any bacterial growth. Before the study, each extracted tooth was mounted on a glass microscope slide using a glass and ceramic glue. Each slide was numbered and left to dry before soaking in an artificial saliva.

Artificial saliva soaking

The extracted teeth were soaked in sterilized and filtered artificial saliva for 24 h and again during the study period except for treatment and measurement periods. The artificial saliva contained 2 mmol l^{-1} $CaCl_2$, 4.2 mmol l^{-1} $MgCl_2$, 4 mmol l^{-1} NaCl, 1.3 mmol l^{-1} NaSCN, 15 μmol l^{-1} NaF, 8 nmol l^{-1} KI, 5 mmol l^{-1} $KHCO_3$, 6 mmol l^{-1} KH_2PO_4, 10 mmol l^{-1} KCl, 3.2 mmol l^{-1} urea, 0.55 mmol l^{-1} glucose, 5.4 mg l^{-1} lactoferrin, 0.264 g l^{-1} lysozyme, 0.38 g l^{-1} α-amylase, 2 mg l^{-1} lactoperoxidase, 2.2 g l^{-1} serum albumin (all from Sigma-Aldrich, Poole, UK) made up to 1 l with deionized water. The artificial saliva was warmed to 37°C using a water bath 1 h before each treatment step to simulate the temperature of the human oral cavity. The artificial saliva was replaced daily to avoid bacterial growth.

Standard toothpaste brushing

The extracted teeth were brushed with Boots Essentials toothpaste (Boots PLC, Nottingham, UK) to provide a baseline lightness value. A pea-sized amount of the paste was squeezed onto the head of an Oral B plaque remover toothbrush (Gillettec, Galashields, UK). Each tooth was brushed for 1 min. After the brushing treatment any excess paste was rinsed off using 5 ml of deionized water administered with a 5-ml pipette.

Bleaching step

Next, the teeth were soaked for 1 h in 13.25% Urea hydrogen peroxide (Sigma-Aldrich company Ltd, Gillingham, UK) equivalent to 5.3% hydrogen peroxide. They were then rinsed with 5 ml of deionized water.

Tea cycling

The tea staining solution was made up by adding 5 g of extra strong tea leaves (Marks and Spencers Group PLC, London, UK) to 500 ml of boiling water, and left to brew for 3 min. The tea was then filtered through a fine mesh into Perspex beakers and left to cool to 50°C. The mounted extracted teeth were placed in microscope slide baths containing the tea solution and left to soak for 6 h on a daily basis. After the soaking step, each tooth was rinsed with 5 ml of deionized water to remove any residue. A total of five tea cyling steps were carried out within a 5-day period.

Brushing step

A brushing step was carried out directly before and after the tea cycling step using the above standard toothbrushing method.

Measurement methods

Each extracted tooth was measured before any treatment (baseline) and after each treatment step using image analysis and spectrophotometry.

Spectrophotometry

A Minolta CM-2600d reflectance spectrophotometer (Minolta Uk Ltd, Rooksley, UK, Fig. 1) was used to measure tooth stain on extracted teeth. The spectrophotometer calculates Commission International de l'Eclairage (International Commission on Illumination, CIE) lightness (L^*) values (10), ranging from 0 (black) to 100 (white). The settings used were a D-65 standard illuminant, 6-mm measurement window, a 10° observer angle and the specular excluded option. Before each set of measurements, the spectrophotometer was calibrated using the standard white tile provided.

The image analysis system

The image analysis system used in a previous study (6) was modified further to incorporate simulated daylight conditions using a carefully selected lighting array.

The system consisted of a Kodak DCS 410 digital camera (aperture F11, shutter speed 1/10 s), mounted on a purpose-built frame (Fig. 2) rotated around cephalometric head-positioning apparatus. The lighting array was developed to closely match daylight conditions and comprised four 50-W Solux halogen lamps (Outside-in Ltd, Cambridge, UK) and eight 4-W UV fluorescent tubes (Lighting Technology, Manchester, UK). The Solux lamps were arranged in a ring structure, with the UV lamps at 30° to each Solux lamp. Each extracted tooth to be measured was placed vertically and attached with adhesive tape to the cephalometric head holder (Fig. 3).

Fig. 1. Lightness assessment of an extracted tooth using a Minolta CM2600-d spectrophotometer.

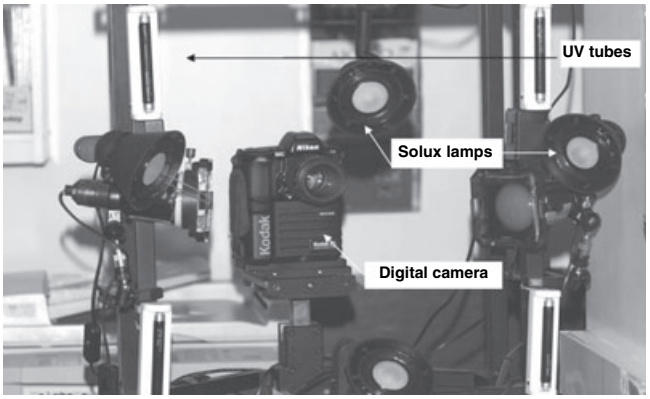

Fig. 2. The custom made frame showing digital camera and modified lighting.

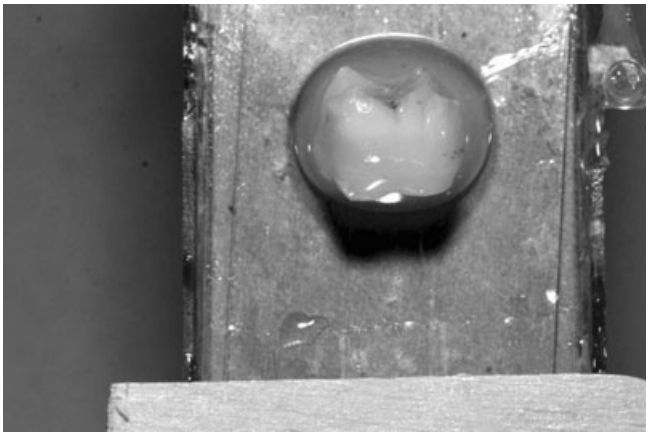

Fig. 3. A mounted extracted tooth after staining.

Image acquisition

Before each set of measurements, a system calibration was carried out using a standard white tile (Avian Technologies, Wilmington, OH, USA). An image was taken of each extracted tooth and transferred from the camera using a twain driver and displayed using Adobe Photoshop (version 5, Adobe Systems, Uxbridge, UK). Images were saved as tagged image format files (TIFFs).

Image analysis

Each TIFF file image was examined using Adobe Photoshop software. The extracted tooth crowns were highlighted using the freestyle drawing tool within the drawing toolbar of Adobe Photoshop. The mean Adobe *L* value of each tooth crown was obtained and then converted to a CIE *L** value using the following equation: CIE *L** = 100* Adobe L/255. All values were recorded on a Microsoft Excel spreadsheet for a statistical analysis.

System validation

The repeatability of the image system was tested by taking images of five untreated extracted teeth once a day over 5 days ($N = 25$) and obtaining CIE *L** values. Twenty five CIE *L** measurements were also obtained using the spectrophotometer.

Analysis of data

Fleiss' coefficient of reliability (11) was used to assess intra-operator repeatability of the image analysis system and the spectrophotometer by assessment of the differences between the 25 repeat measurements of the untreated extracted teeth. The mean, standard deviation, standard error of the mean and a two-tailed paired *t*-test (95% confidence level) were calculated for the 20 extracted teeth after each treatment step using the SPSS statistical package (version 14.0.1, Chicago, IL, USA). The 95% limits of agreement (12) and confidence intervals were calculated for the two measurement methods by combining CIE *L** values for the baseline and all seven treatment steps for the 20 extracted teeth ($N = 160$). A two tailed paired *t*-test was also carried out for all 160 measurements.

Results

Fleiss' coefficient of reliability for the 25 repeat measurements of the untreated extracted teeth using the image analysis system and spectrophotometry was 0.996 and 0.946 respectively. Both of these results were in the excellent range according to the benchmarks for Fleiss' (13).

Descriptive statistics for each treatment step (Table 1) show that the CIE *L** values obtained from image analysis were systematically higher than those obtained from spectrophotometry, although both methods show a similar trend (Fig. 4). *P* values obtained from *t*-tests show that the CIE *L** values were significantly different ($P < 0.05$) for the two measurement methods for all treatment steps. When all the measurement step results for the 20 teeth were combined ($N = 160$), the mean difference, standard deviation of the difference, and standard error of the mean difference for the two measurement methods were −12.98, 7.67 and 0.61 respectively. The 95% limits of agreement between methods (Fig. 5) were −27.95 to +2.07. The 95% confidence of the difference was calculated as −14.26 to −11.84. The combined CIE *L** values ($N = 160$) showed significant differences between the measurement methods ($P < 0.05$).

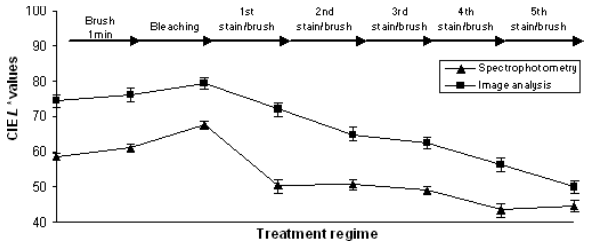

Fig. 4. Change in CIE *L** values for all treatment steps using image analysis and spectrophotometry. Also shown are standard error bars.

Table 1. **Descriptive statistics of the 20 extracted teeth for all treatment steps using image analysis and spectrophotometry (all values for mean, SD and SEM are in terms of CIE *L****)

		N	Mean	SD	SEM	*P* value
Before brushing	Spec	20	58.68	4.38	0.98	0.00
	IA	20	74.34	8.80	1.96	
After brushing	Spec	20	61.01	5.03	1.13	0.00
	IA	20	76.04	8.87	1.98	
After bleaching	Spec	20	67.43	4.84	1.02	0.00
	IA	20	79.37	7.65	1.71	
After 1st stain/brush cycle	Spec	20	50.22	8.27	1.85	0.00
	IA	20	72.05	8.45	1.89	
After 2nd stain/brush cycle	Spec	20	50.56	6.40	1.43	0.00
	IA	20	64.85	8.44	1.89	
After 3rd stain/brush cycle	Spec	20	49.74	5.25	1.17	0.00
	IA	20	62.39	7.84	1.75	
After 4th stain/brush cycle	Spec	20	43.32	8.53	1.90	0.00
	IA	20	56.26	8.65	1.93	
After 5th stain/brush cycle	Spec	20	44.65	6.42	1.43	0.00
	IA	20	50.01	8.18	1.83	

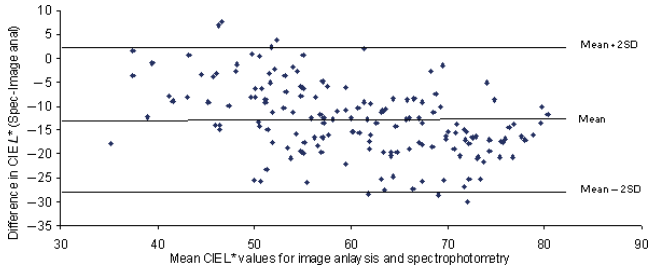

Fig. 5. Mean and Mean difference in CIE L^* values for the two measurement methods (N = 160).

Discussion

In this study the reliability and suitability of the image analysis system was compared with that of reflectance spectrophotometry, a technique used in a number of previous studies. The custom made frame for the digital camera was designed to allow standardized, accurate and reproducible lightness measurements of extracted teeth. The standardized lighting incorporated into the image analysis system closely reflects standard daylight conditions and provides a consistent lighting source. CIE L^* values were used to indicate the changes in lightness of the extracted teeth and thus changes in the level of staining on the tooth surface, as an increase in L^* indicates a decrease in tooth stain and vice versa. CIE L^* values were used in this study as they are recommended by the CIE, which has been recognized as the source of internationally agreed information on subject matters relating to light and lighting. CIE L^* values are regularly used in industry and have been widely used in previous tooth colour studies (14, 15).

The bleaching step used a 5.3% hydrogen peroxide solution which reflects the strength of a typical in-house clinical whitening treatment. The tea staining cycles did not represent a realistic daily build-up of extrinsic stain on vital teeth but was exaggerated to test if the two measurement methods could sensitively quantify the changes in stain level of the teeth after extreme staining. One operator carried out the measurements using both instrumental methods.

The intra-operator result for the image analysis system (0.996) demonstrates the high level of repeatability this approach achieves, and supports the results from a previous study of measurements on acrylic blocks (6). The repeatability of the spectrophotometrical approach was slightly lower (0.946) probably due in part to the difficulty in repositioning the spectrophotometer on the same area of the tooth surface for each repeat measurement. Even a small change in contact of the tooth surface could lead to an underestimation of lightness values. This is not a problem for the image analysis technique as the whole surface is included in the measurement.

Both of the measurement methods were able to pick up the reduction in CIE L^* values after each staining cycle, although there were systematic differences in CIE L^* values for the two measurement approaches for all of the treatment steps. These differences could be due in part to the 'edge loss effect' (16, 17) which occurs when spectrophotometers are used to measure curved and translucent objects such as teeth, leading to significant measurement errors and an underestimation of lightness values.

Also a difference in lighting of the two methods could have contributed to the disparate sets of results.

The combined CIE L^* values for all the treatment steps (Fig. 4) also highlighted the differing results between methods. The limits of agreement show that the image analysis system may be up to 27.95 CIE L^* units below and up to 2.07 units above those of spectrophotometry.

The reliability results show that the image system is a more accurate method of measuring the lightness of extracted teeth than spectrophotometry and thus would be more suitable for quantifying extrinsic tooth staining *in vitro*. The advantages of the image system over spectrophotometry are: (a) the image analysis system can provide highly accurate repeat measurements as precise repositioning of a specimen is simple to achieve compared with spectrophotometry; (b) the imaging method does not require surface contact with a specimen as spectrophotometry does; (c) a permanent database of images can be obtained by image analysis allowing analysis at any time; (d) the imaging approach can analyse the whole surface of an extracted tooth whereas a spectrophotometer can assess only a small measurement area of a specimen at one time. This is important in quantifying stain as it would be heterogeneously layed down on a tooth surface; (e) as well as assessing tooth lightness in relation to tooth stain, the image system is able to make spatial measurements such as area and perimeter of a stained area.

Although the image analysis approach is of an objective nature, there is an element of subjectivity relating to the drawing by hand of an area of interest around the tooth crown. This procedure may introduce a small element of random error, although as part of the method is included in the reliability measurements which have shown little effect.

Considering all the above points, the image system is a more accurate and suitable measurement method for assessing stain build-up and removal on extracted teeth than spectrophotometry.

The mobile version of the image system has been developed to make the system more practical for use in *in vivo* clinical trials. This measurement method could be used as a stand alone objective approach or to verify results obtained from the current subjective assessments such as the Lobene or Shaw and Murray indices.

Conclusion

In conclusion the results show that the modified digital image analysis system is a valid and reliable method for assessing stain build-up and removal on extracted teeth.

This objective method should provide more sensitivity and therefore selectivity between dental dentrifices within a clinical trial environment. The development of a mobile version for *in vivo* studies provides further flexibility of the system.

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