

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 suitability of a modified image analysis system for measuring 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 hours daily through a tea solution. CIE L* values were obtained after each treatment step using a customised image analysis system and a reflectance spectrophotometer. 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 systematically higher using image analysis compared with spectrophotometry; 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 and +2.07, with the 95% confidence of the difference calculated as -14.26 to -11.84. The combined results for all treatment steps showed a significant difference in CIE L* values ($p < 0.05$).

Conclusion: The image system is a valid method with very high reproducibility for assessing stain on extracted teeth when compared with reflectance spectrophotometry. It could be further adapted for *in vivo* studies.

Keywords: Image analysis; spectrophotometry; staining; extracted teeth; lightness

Introduction

The discoloration of teeth is of primary concern to many people who desire an aesthetically pleasing appearance. The majority of tooth discolourations on permanent erupted teeth are of extrinsic origin (1), and are predominantly caused by stain build up on the tooth surface. Many extrinsic stains are pigments embedded in the calculus or plaque which have become coloured from interactions with food, drink and tobacco smoke. As the use of whitening dentifrices to remove tooth stain is becoming more popular there is a pressing need for accurate and reproducible quantitative instrumental assessment of tooth stain.

Numerous longitudinal studies have been carried on extrinsic stain removal using the Lobene (2) and Shaw & Murray indices (3), and although these indices are quick and easy to use to apply, the subjective nature of this approach can give rise to inter and intra operator variations in measurement. Reflectance spectrophotometry has been utilised for colour assessment of extracted teeth (4). Although spectrophotometry has been shown to give reproducible results and is able to measure small changes in colour, it has a major disadvantage when used on teeth. Although spectrophotometry gives precise colour assessment on flat materials, problems arise when measuring tooth colour, because teeth have curved surfaces and are also translucent which can lead to systematic errors (5).

As an alternative objective approach to spectrophotometry, digital image analysis for tooth colour measurement has been investigated. A customised digital image analysis system was previously used in a study to assess stain build up and removal on acrylic blocks and was found to be a reliable alternative measurement method when compared to absorbance spectrophotometry (6).

The aim of this study was to assess the suitability of a further developed image analysis system for measuring stain build up and removal on extracted teeth and to compare it with reflectance spectrophotometry using a modified staining protocol utilised in previous *in vitro* staining studies (7-9) together 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 patient consent. The teeth were scrubbed with deionised water and a hard toothbrush to remove any soft tissue or debris. The teeth were then autoclaved at 121°C for 30 min. Then each extracted tooth was stored in phosphate buffered saline with 1% thymol crystals (to avoid any bacterial growth) until required. Each extracted tooth was mounted on a glass microscope slide using a glass and ceramic glue. Each slide was numbered and left to dry.

Artificial saliva soaking

Each extracted tooth was soaked in a sterilised and filtered artificial saliva 24 hours before the study and during the study period except for treatment and measurement periods. The artificial saliva contained 2mmol/L CaCl₂, 4.2mmol/L MgCl₂, 4mmol/L NaCl, 1.3mmol/L NaSCN, 15µmol/L NaF, 8nmol/L KI, 5mmol/L KHCO₃, 6mmol/L KH₂PO₄, 10mmol/L KCl, 3.2 mmol/L urea, 0.55 mmol/L glucose, 5.4 mg/L lactoferrin, 0.264 g/L lysozyme, 0.38 g/L α-amylase, 2 mg/L lactoperoxidase, 2.2 g/L

serum albumin made up to 1 litre with deionised water. The artificial saliva was warmed to 37°C using a water bath one hour before each treatment step to simulate the temperature of the human oral cavity. The artificial saliva was replaced daily to avoid bacterial growth. A batch of artificial saliva was freshly made up at the beginning of each day.

Standard toothpaste brushing

Each extracted tooth was 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 deionised water administered with a 5-ml pipette.

Bleaching step

After the brushing step, the teeth were soaked for 1hr in 13.25% Urea hydrogen peroxide (Sigma-Aldrich company Ltd, Gillingham, UK) equivalent to 5.3% hydrogen peroxide. After this step each tooth was rinsed with 5ml deionised water.

Tea cycling

The tea staining solution was made up by adding 5g 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 baths containing the tea solution and left to soak for 6 hrs on a daily basis. After the soaking

step, each tooth was rinsed with 5ml of deionised water to remove any residue. A total of 5 tea cycling 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 obtain CIE lightness (L^*) values (10) of the extracted teeth, with L^* values ranging from 0 (black) to 100 (white). The settings used were a D-65 standard illuminant, 6mm 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 developed 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 sec), mounted on a purpose built frame (constructed within the Department of Oral Health and Development, University of Sheffield, Fig. 2) rotated around a

cephalometric head positioning apparatus (Fig. 3). The lighting array was developed to match daylight conditions and comprised four 50W Solux halogen lamps (Outside-in Ltd, Cambridge, UK) and eight 4W 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 was placed vertically and attached with adhesive tape to the cephalometric head holder.

Image acquisition

A system calibration was carried out using a standard white tile (Avian technologies, Ohio, USA) before each set of measurements. 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 statistical analysis.

System validation

The reliability and reproducibility of the modified image system was tested by taking an image of an untreated extracted tooth 5 times 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 calculate the differences between the 25 repeat measurements of the untreated extracted teeth to assess intra-operator repeatability of the image system and the spectrophotometer. 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, Illinois 60606). 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 7 treatment steps for the 20 extracted teeth (N=160). A two tailed paired t-test was also carried for all 160 measurements.

Results

Fleiss' coefficient of reliability for the 25 repeat measurements of the untreated extracted tooth using the image 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 ($p < 0.05$).

Discussion

There has been numerous longitudinal studies using reflectance spectrophotometry to quantify changes in tooth colour after treatments to remove extrinsic stain. Consequently in this study the reliability and suitability of the image analysis system was compared with that of reflectance spectrophotometry. The custom made frame for the digital camera was designed to allow accurate and reproducible lightness measurements of extracted teeth, and the lighting incorporated into the image analysis system was developed to closely reflect standard daylight conditions. CIE L* values were used to indicate the changes in lightness of the extracted teeth and thus the level of staining on the tooth surface, e.g. an increase in L* indicates a decrease in tooth stain and vice versa. CIE L* values are widely used in industry and have been used in

previous tooth colour studies (14,15). The bleaching step used a 5.3% hydrogen peroxide solution which reflects the strength of a number of clinical whitening treatments. The tea staining cycles did not represent a realistic daily build up of extrinsic stain on vital teeth but was highly exaggerated to test if the two measurement methods could sensitively quantify the changes in lightness of the teeth after extreme staining.

As the same operator carried out the measurements using both instrumental method inter-operator variability could not be assessed.

The intra-operator result for the image analysis system (0.996) demonstrates the very high level of reproducibility this approach achieves, and supports the results from a previous study of lightness measurements on acrylic blocks (6). The reproducibility 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, introducing random error. Even a small change in contact of the tooth surface could lead to an underestimation of lightness values.

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 difference in 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 27.95 CIE L* units below and 2.07 units above those of spectrophotometry, this disagreement would be unacceptable for research and clinical purposes.

The reproducibility 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 simpler than 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 and review at any time; (d) the imaging approach can analyse the whole surface of an extracted tooth whereas a spectrophotometer can only assess a small measurement area of a specimen at one time, this is important in quantifying stain as it would be heterogeneously laid 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 a 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 an small element of random error.

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 compared to spectrophotometry, and can be used for *in vivo* stain assessment.

Conclusion

In conclusion the results show that the customised digital image analysis system is a valid method for assessing stain build up and removal on extracted teeth. Further adaptation for *in vivo* studies on vital teeth is possible.

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Figure legends

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 Cephalometric had positioning apparatus showing specimen position.

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

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

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

		N	Mean	Std. Dev	Std. Error Mean	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 1 st stain/brush cycle	Spec	20	50.22	8.27	1.85	0.00
	IA	20	72.05	8.45	1.89	
After 2 nd stain/brush cycle	Spec	20	50.56	6.40	1.43	0.00
	IA	20	64.85	8.44	1.89	
After 3 rd stain/brush cycle	Spec	20	49.74	5.25	1.17	0.00
	IA	20	62.39	7.84	1.75	
After 4 th stain/brush cycle	Spec	20	43.32	8.53	1.90	0.00
	IA	20	56.26	8.65	1.93	
After 5 th stain/brush cycle	Spec	20	44.65	6.42	1.43	0.00
	IA	20	50.01	8.18	1.83	