The evaluation of a novel method comparing Quantitative Light-induced Fluorescence (QLF) with spectrophotometry to assess staining and bleaching of teeth.

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Abstract

Title: The evaluation of a novel method comparing Quantitative Light-induced Fluorescence (QLF) with spectrophotometry to assess staining and bleaching of teeth.

Objectives: This study reports the development and evaluation of a novel method using QLF which enables its use for quantifying and assessing whole tooth surface staining and tooth whitening. The method was compared with a spectrophotometer to assess reliability.

Methods: Two experimental phases, intrinsic stain formation and tooth whitening were conducted *in vitro* on sixteen extracted bovine teeth. Intrinsic stains were developed via access through lingual surfaces and root canals of these teeth using tea solution (2 g/100 ml, Marks and Spencer Extra Strong Tea, Marks and Spencer, London, U.K) for six days. Stains were removed using 33 % hydrogen peroxide (VWR Prolab, Leicestershire, U.K) in cycles over 150 min. Stain development / whitening was monitored with QLF (Inspektor Research systems, Amsterdam, Netherlands) and Spectrophotometry (Easy shade, Vita Zahnfabrik, Bad Säckingen, Germany). Parameters Delta F for QLF and Delta E for the Spectrophotometer were obtained. The progression of stain intensity and removal observed by the methods were tested for correlation using Pearson's correlation coefficient. Intra examiner reliability for each method was tested.

Results: QLF showed a high correlation with spectrophotometry for detecting and monitoring intrinsic tooth stain progression (Pearson coefficient r was -0.987 with correlation significant $p < 0.0001$. For stain removal the Pearson coefficient (r) between both methods was -0.906 with no significance $p = 0.094$.

Conclusion: The use of an external reference material in combination with the inner patch QLF analysis technique had the ability to detect and measure whole tooth surface staining and its removal longitudinally. The reliability of the method shows a potential clinical application.

Introduction

Tooth colour is determined by a combination of the different optical properties of the enamel, dentine and pulp [1, 12]. Colour can be described using a number of colour order systems. The CIELAB colour system is a 3-dimensional uniform colour space with equal distances corresponding to equal perceived colour differences. This system has three axes L*, a*, b*. The L* axis represent lightness and extends from 0 (black) to 100 (white). Both a* and b* represent the redness-greenness and yellowness-blueness axis respectively. When a* and b* coordinates approach zero the colour become neutral. They also (a*and b* values) can be used to derive metric chroma and hue angle. A colour difference (ΔE) between two objects can be calculated within the CIELAB colour system [5].

Tooth discolouration and bleaching assessments / measurements can range from subjective comparison methods, to the use of objective instrumentation. There are limitations to the use of subjective measurement methods using porcelain or acrylic resin shade guides; these include the effect of variables such as light conditions, experience, age, fatigue of the human eye, and conditions such as colour blindness [24]. There is also a limitation with communication of visually assessed colour characteristics as there may be inconsistencies and bias when reporting among observers [7, 9, 16, 17, 23]. This is complicated by the lack of similarity in the commercially available shade guides [17].

Objective evaluation of discolouration and colour change undergone during tooth whitening procedures is possible by a number of methods including spectrophotometry, colourmetry and computer analysis of digital imaging [12, 13]. The use of quantitative light-induced fluorescence (QLF) *in vitro* has also been

described in the literature [1, 2, 18]. Spectrophotometers measure one wave length at a time from the reflectance or transmittance of an object and have been used to measure the visible spectra of extracted and vital teeth [14, 17]. Digital imaging uses the CCD sensor to capture an image. It uses millions of pixels to record RGB colours (red, green and blue) [1, 10].

QLF measures the fluorescence loss of the captured tooth image [1]. Whilst, each method has limitations, advantages and disadvantages [1, 12], spectrophotometers can measure relatively large areas and they are sensitive to positioning, tooth size and curvature [3, 6, 15]. Digital imaging is capable of measuring small areas across the tooth surface, however, large and accurate positioning devices are needed to control distance, angle and polarised diffused light to eliminate highlights. Currently QLF can only be used *in vitro* to measure extrinsically stained areas because it requires sound or clean enamel around the stained area to complete the analysis of fluorescence loss. This standard method of analysis will be described in the materials and method section.

Various treatment methods have been suggested to improve tooth colour, these include the use of whitening paste, over the counter products, professional cleaning by scaling and polishing to remove extrinsic stains and tarter, internal bleaching of non vital teeth, external bleaching of vital teeth, microabrasion of enamel and placement of veneers and crowns to mask the discolouration [4, 11, 13, 21, 25].

In vitro models used to assess tooth whitening

Joiner [13] in a review of the literature on tooth bleaching mentioned the importance of using *in vitro* models for the evaluation and optimisation of treatment conditions.

Important information on safety of products, effect on hard tissues and an understanding of the bleaching process are gained. Various models utilise either human or bovine enamel with or without increasing the levels of intrinsic tooth colour by staining with tea or blood components [13]. These models generally tend to be measured with instruments. 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 an expensive clinical trial stage [22]. It is also important that such objective methods be transferable and applicable to clinical situations with a similar reliability.

In a previous study, QLF was used to quantify extrinsic tooth discolouration and bleaching *in vitro* [1]. One limitation of the method was the need to compare the stained tissue with a clean, unstained tissue as a reference point. This limited the potential use of this technique clinically since bleaching teeth *in vivo* results in whitening of the whole tooth surface.

The aims of this study were twofold. Firstly, to develop and evaluate a new method using QLF to assess reliably, whole tooth surface staining and tooth whitening without the need to compare to unstained tissue in the same tooth. Secondly to compare this method with a spectrophotometer. The null hypothesis is that there is no correlation between QLF and spectrophotometry in measuring stain progression and whitening.

Materials and Methods

Intrinsic stain formation

Sixteen extracted bovine teeth previously stored in thymol solution (Sigma-Aldrich, Dublin, Ireland) were cleaned and gently pumiced. The roots of the teeth were removed using a diamond disc (Skilldenta; Skillbond, Slough, U.K). Access cavities were created through the lingual surface of these teeth and the exposed root canal. The Labial surfaces of the teeth were coated with acid resistant clear nail varnish (Maxfactor; Procter and Gamble, Weybridge, U.K) and left to air dry for 24 h. This was to encourage the development of intrinsic stain instead of extrinsic stains. The teeth were attached to lengths of cotton and suspended in pots containing 30 ml of tea solution (Marks and Spencer Extra Strong Tea; Marks and Spencer, London, U.K) which was prepared using 2 g of tea bags in 100 ml of boiling water. The infusion was allowed to cool over a period of 3 h. The tea bags were discarded. The teeth were left in the tea solution which was changed daily and gently stirred for 6 d.

QLF (Inspektor Research systems, Amsterdam, Netherlands) and Spectrophotometer (Easy shade; Vita Zahnfabrik, Bad Säckingen, Germany) readings were taken at baseline and once daily.

Prior to taking the readings, the varnished surfaces of the teeth were wiped to remove any extrinsic stains using a wet cotton swab stick and left to bench dry for 10 minutes. QLF images and spectrophotometer readings were then recorded.

Tooth whitening

After 6 da, the teeth were removed from the tea solution and immersed in pots containing 30 ml solutions of 33 % hydrogen peroxide (VWR Prolab, Leicestershire,

U.K). In order to determine levels of stain removal or whitening, the teeth were rinsed in deionised water, bench dried for 10 min and readings taken using QLF and spectrophotometry after 30, 60, 120 and 150 min immersion in hydrogen peroxide respectively.

Spectrophotometer and QLF recording process

Spectrophotometer readings

Easy shade spectrophotometer (Easy shade; Vita Zahnfabrik, Bad Säckingen, Germany) was used in this study. Prior to each use, the unit was calibrated against an inbuilt ceramic block, according to manufacturer instruction. A composite block (SpectrumTPH; shade A4, Dentsply De-Trey, Konstanz, Germany) was constructed and used as a measuring block at the start of each set of readings to ensure accuracy and consistency. The samples were measured using the same background in a room with controlled lighting conditions.

L*, a*, b* readings were obtained and 2 consecutive readings were taken and recorded for each tooth by a single examiner. The colour difference (ΔE) was calculated for the baseline and subsequent staining and removal cycles.

QLF readings

A QLF-Clin system, QLF (Inspektor Research systems, Amsterdam, Netherlands) was used. The system consisted of a special camera connected to a personal computer containing QLF software. In order to visualise and capture the tooth image, the QLF device utilised visual white light from an arc lamp based on xenon technology which

is filtered through a blue- transmitting band pass filter with peak intensity of λ= 410 nm and a band width of 80 nm (to ensure that only fluorescent light is detected) to illuminate the tooth with blue-violet light with the aid of a CCD sensor which had a yellow transmitting filter ($\lambda \geq 520$ nm) positioned in front of it to filter out reflected and backscattered light. QLF images were captured and stored using Inspektor Pro software (version 2.00.37, Inspektor Research systems, Amsterdam, Netherlands) and analysed by a single examiner using QLF software (version 2.00c, Inspektor Research systems, Amsterdam, Netherlands).

The QLF camera handpiece was mounted in a fixed position over a laboratory jack. A square shaped composite grid (SpectrumTPH; shade A4, Dentsply De-Trey, Konstanz, Germany) measuring 18 mm × 16 mm was constructed. This was used as a repositioning grid to aid reproducible placement of the teeth on each occasion in addition to serving as a reference area which would be later used in the image analysis. Each tooth was placed and aligned in the composite grid on a black plastic cap (figure 1) which was placed on a laboratory jack, ensuring that the labial surface of the tooth was perpendicular to the direction of blue light from the camera. A video repositioning software [20] was also employed to ensure accurate repositioning of the teeth when recording the baseline and subsequent images. Fine focussing of the QLF image was obtained by adjustment of the jack. Two consecutive readings were taken and recorded for each tooth by a single examiner.

QLF Analysis

The standard method of QLF image analysis

The standard *in vitro* QLF software quantifies fluorescence loss parameters ΔF and ΔQ (the integrated value of ΔF over the lesion area per pixel). This involves the reconstruction of the fluorescence of enamel at the site of the lesion or stain from the fluorescence of the surrounding sound enamel. The decrease in fluorescence is determined by calculating the percentage difference between the actual and reconstructed fluorescence surface at each image pixel. The standard analysis of the image involved the placement of an analysis patch over the stained area ensuring that the borders of the patch were placed on clean (unstained) enamel. The image was analysed and the reconstructed stained area checked to ensure that it mimicked the original stained area morphology.

New QLF image analysis method*: The development of whole tooth surface QLF image analysis (Inner patch technique)*

This new method involved the introduction of two additional components to the standard QLF analysis method:

Firstly, the construction and use of an external composite grid (SpectrumTPH; shade A4, Dentsply De-Trey, Konstanz, Germany) having similar fluorescence intensity as sound clean tooth tissue. This was placed around the tooth sample prior to recording the image (described in section on QLF readings).

Secondly, the use of the 'inner patch' technique. The images obtained were analysed using a modified version of the QLF software which utilised the placement of two rectangular analysis patches (instead of one) over the composite grid and stained tooth surface, respectively while ensuring that the borders of the outer patch fell on the

reference composite grid area (serving as unstained/sound enamel). The outer rectangular patch positioned on the reference composite grid was used to calculate a reconstructed image extrapolating the gray values of each pixel at the border of the rectangle. While the inner patch was placed on the tooth surface.The QLF software then quantified the percentage fluorescence loss (ΔF) by reconstructing fluorescence of the enamel at the site of the stain lesion from that of the surrounding reference area (instead an area of sound enamel). The decrease in fluorescence was determined by calculating the percentage difference between the actual and reconstructed fluorescence surface at each image pixel. Figure 2 shows an example of a tooth analysed using this technique.

Statistical methods

The data obtained were analysed statistically using SPSS (Version 13, SPSS, Chicago, USA). The stain values (ΔF from QLF and ΔE from the spectrophotometer) for the progression of stain intensity and its removal obtained by the two methods were tested for correlation using Pearson's correlation coefficient with $\alpha < 0.05$. The intra-examiner reliability for each method was tested using the intraclass correlation coefficient of reliability (ICC) with $\alpha < 0.05$ to compare the repeated measurement of the same phenomenon for QLF and the spectrophotometer.

Results

The range of stain intensity as measured by QLF and the spectrophotometer showed a linear reverse correlation. The Pearson coefficient r was –0.987 with correlation significant at p = 0.0001. For stain removal the Pearson coefficient (r) between both methods was -0.906 with no significance at 0.01 levels (p = 0.094). There was an overall (staining and stain removal) significant correlation r = 0.65 at p = 0.02 for both staining and its removal.

Mean ΔE and ΔF values with standard deviations (SD) are shown in tables 1 and 2. The ability of both methods to monitor the progression of staining and stain removal longitudinally and their trend is shown in tables 1 and 2.

Intra-examiner reliability as measured by Intraclass Correlation Coefficient (ICC) between the two attempts at analysing the images for QLF was 0.825 whereas with the Spectrophotometer it was 0.9969 ($p < 0.001$ for both methods).

Discussion

The optical method employed by QLF using fluorescence measurements to detect caries / demineralisation / remineralisation, monitor and distinguish between sound enamel and extrinsically stained enamel has been described in previous studies [1, 2, 18]. Adeyemi et al [1] described the longitudinal monitoring of extrinsic stains and its removal *in vitro* but there have been no published reports for the present application *in vivo* for both intrinsic and extrinsic staining.

One limitation of the standard QLF *in vitro* method previously used [1], was the need to compare the stained tissue with a clean, unstained tissue as a reference. QLF analysis for stained lesions was limited to *in vitro* situations as an area of sound enamel around the lesion was required as a reference area for the extrapolation of fluorescence loss measurement. It was not possible to measure intrinsic stains or stains on whole tooth surfaces. This limited the potential use of this technique clinically since bleaching teeth *in vivo* results in whitening of the whole tooth surface. Changes to the methodology was required so that stain intensity could be recorded and longitudinally monitored *in* vivo without the possibility of having a reference area of sound and clean enamel around the lesion. The need to protect a sound area of enamel for use in later analysis with QLF is no longer necessary following the modifications reported in this study.

In this study, QLF demonstrated the ability to monitor intrinsic staining and removal over whole tooth surfaces longitudinally in this study. The results obtained using this modified analysis, correlated well for both staining and the whitening process when compared with spectrophotometry. The reverse correlation observed between QLF

and the spectrophotometer is similar to that described in a previous study comparing QLF with digital imaging [1]. This reverse in correlation is due to the different analysis parameters utilised by both techniques. With spectrophotometry, ΔE is the colour difference relative to zero (black) and calculated from the L, a, b values obtained from the spectrophotometer, as the intensity of staining increases, ΔE values decrease and increases as whitening occurs. For QLF, an increase in fluorescence loss is observed as stain intensity increases while a decrease is observed with whitening.

The ideal reference material needs to have fluorescing properties similar to unstained enamel. The composite material used as a standard reference area in this study was appropriate for this purpose when compared with unstained enamel. As several composite materials from different manufacturers and shades had to be tested under QLF conditions as they all had different fluorescing properties. Some materials fluoresced too brightly others did not. This paper describes only one material which was used as an external reference. There is still a need to further explore the suitability of other materials in terms of their fluorescing properties with minimal variable effects such as shade and manufacturer variation.

There was a strong correlation for both methods for staining and whitening. The non significant p value for the whitening phase might be due to the number of removal cycles being limited to four. With staining it was interesting to note that the assessment of stain progression showed a continuous increase in intensity (6 d) when compared with results from a study carried out by Sulieman et al [22], in which tea staining intensity reached its maximum within one day. The colour change observed by both QLF and spectrophotometer after the sixth day of staining was considerably

different from day one. This observation may be explained by the differences in the porosity of bovine teeth when compared with human teeth [22] which could lead to increased diffusion of the tea solution into the dentinal tubules. The slight differences in the staining procedures in comparison with that of Sulieman et al [22] may also be a factor giving rise to these differences.

The use of tea might not directly simulate the type of intrinsic staining usually observed in patients on the clinic, where stains may be induced by blood, tetracycline stains etc as absolute colour value might be different. The tea technique used for intrinsic discoloration in our study however would still give a valid trend of colour change. This has been previously documented in literature (Sulieman et al. 2003). The main crux of this study was based on the new method's ability to measure whole teeth surfaces. In a sense, the staining method used in this study did not matter as stained teeth needed to have a surrounding area of unstained enamel with the standard QLF analysis system.

With stain removal, both methods longitudinally monitored the process. However it was noted that while the ΔE values from the spectrophotometer method returned to a range of readings similar to the baseline values pre staining, while the ΔF values from QLF did not. This could be explained by the white light scattering effect. The whiter a tooth becomes the more the light scattering intensity and, as a consequence, the lower the fluorescence intensity as captured by the QLF system. As the bleaching progresses teeth thus lose some their fluorescing properties. The spectrophotometer detects full reflected light spectrum and give L*, a* and b* colour values. Bleaching changes the reflected light spectrum and as such changes the L*, a* and b * values. Thus changes

in tooth fluorescence properties as they are bleached will not be detectable to the spectrophotometer. For this reason, it is concluded that QLF is more sensitive in detecting slight changes in fluorescence due to bleaching than the spectrophotometry method.

Both techniques require a subjective input in the placement of the '*inner patch'* and the spectrophotometer unit respectively during the analysis stage. The intra examiner reliability for both methods was high although the spectrophotometer had a higher ICC value than QLF. Although no inter examiner reliability assessment was carried out in this study, the results of the intra reliability test seem to be in support of an *in vitro* study looking at the intra examiner and inter examiner repeatability of QLF which also reported the high reproducibility and reliability of QLF [19].

The Easy shade spectrophotometer used in this study does not allow the user to visualise the tooth image to the patient at chair side as it provides only a numerical output of the data which needs to be recorded manually. QLF however has the advantage that it allows immediate visual and quantitative comparison of live images with the previously recorded baseline images. These can be shown to the patient and can be archived. Some spectrophotometers are able to visualise and store images [8].

Within the limits of the study, the new QLF method appears to monitor staining and whitening effectively and reliably. The ability of this method to be able to measure whole tooth surfaces now removes the limitation of previous QLF analysis requiring surrounding sound enamel around lesions. This improves its application to intrinsic and extrinsic stain measurements in *in vitro* and *in vivo* situations. This has the

potential to measure tooth fluorescence changes in patients and also to test the efficacy of various whitening and bleaching products and procedures in clinical practice. At present there is no published data available demonstrating this *in vivo* application. The effect of variables such as the placement of the analysis patch on unsound or stained reference area, moisture and camera angulations on the final outcome needs further investigation; however the new method looks promising to quantify staining and bleaching (stain removal). The method needs to be refined by manufacturing a device connected to the intraoral QLF handpiece containing an external reference material for routine use in the *in situ* and *in vivo* environment.

The null hypothesis in this study has been rejected. There is a correlation between QLF and spectrophotometry in measuring stain progression and whitening.

Conclusion

It was demonstrated *in vitro* that the use of an external reference material in combination with the inner patch QLF analysis technique had the ability to detect and measure the progression of staining and its removal longitudinally on whole tooth surfaces. QLF has shown a high correlation with the spectrophotometry method in this regard. The method is reproducible, reliable and can be used to study aspects of intrinsic staining and its removal on whole teeth surfaces.

Conflict of Interest

The authors declare that they have no conflict of interest

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