Visual and digital comparative tooth colour assessment methods and atomic force microscopy surface roughness

SADJ October 2013, Vol 68 no 9 p412 - p421

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ABSTRACT

This study compared digital and visual colour tooth colour assessment methods in a sample of 99 teeth consisting of incisors, canines and pre-molars. The teeth were equally divided between Control, Ozicure Oxygen Activator bleach and Opalescence Quick bleach and subjected to three treatments. Colour readings were recorded at nine intervals by two assessment methods, VITA Easyshade and VITAPAN 3D MASTER TOOTH GUIDE, giving a total of 1782 colour readings. Descriptive and statistical analysis was undertaken using a GLM test for Analysis of Variance for a Fractional Design set at a significance of P<0.05. Atomic force microscopy was used to examine treated enamel surfaces and establish surface roughness. Visual tooth colour assessment showed significance for the independent variables of treatment, number of treatments, tooth type and the combination tooth type and treatment. Digital colour assessment indicated treatment and tooth type to be of significance in tooth colour change. Poor agreement was found between visual and digital colour assessment methods for Control and Ozicure Oxygen Activator treatments. Surface roughness values increased two-fold for Opalescence Quick specimens over the two other treatments, implying that increased light scattering improved digital colour reading. Both digital and visual colour matching methods should be used in tooth bleaching studies to complement each other and to compensate for deficiencies.

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ACRONYMS

AFM:	atomic force microscopy
CIE:	Commission Internationale de l'Éclairage
GLM Test:	General Linear Models

INTRODUCTION

The field of aesthetic dentistry has blossomed in response to consumer demand for a "perfect smile". Technologies associated with developing life-like tooth- coloured restorative materials, veneers, crowns and acrylics, as well as the expanding tooth bleaching industry, have escalated to meet this demand. However, concomitant progress in the creation of an accurate, simple, cost effective and universal tooth colour assessment method, is less apparent. Traditionally, tooth colour assessment is by visual colour comparison with tooth shade guides and charts. While such guides are both quick and cost effective, they are hardly commensurate with new technologies, despite continual improvements. Dental shade guides still do not cover the colour distribution of natural teeth,[1] are hampered by a lack of system in the colour space and are prejudiced by the variety of materials used in production[2] resulting in inconsistency between commercially available shade guides. Furthermore, human evaluation of tooth shade has been judged as unreliable for reasons such as subjectivity, experience, fatigue, colour blindness and bias, among many others.[3] In addition, a tooth will vary in colour when viewed under different lighting conditions (metamerism). Thus ambient light has to be standardised before tooth colour is assessed, to exclude variables such as the light source, time of day, the surrounding environment eg. wall colour and the angle at which the tooth is viewed, among other confounders.

In pursuit of accuracy, dental researchers have looked towards the paint, plastics, printing and textile industries for guidance on tooth colour determination. Colour perception and space in these industries are based on the Commission Internationale de l'Éclairage (CIE) LAB system standards, which uses units to express colour differences.[4] The units are generated by colourimeters, spectrophotometry and computer calculations which express colours numerically.[2] In the search for an accurate and precise tooth shade measuring system, these technologies have been incorporated within the new generation of portable digital tooth colour assessment devices. Such instruments utilise an intense light source to radiate the tooth and sensors to measure the ensuing spectral colours. Since these devices do not rely on personal judgment and are unaffected by surrounding light,[5] incorrect tooth colour readings are minimised. Unfortunately the instruments have not met expectations. Poor repeatability, reproducibility and efficiency, complexity, expense and cumbersomeness are reasons hindering the widespread clinical use of digitally-based tooth colour readers. Given the complexities of tooth colour and the limitations, advantages and disadvantages of the two main tooth colour assessment methods, it is not surprising literature is divided on which is best. Digital shade assessment has been reported to be superior to human shade assessment[6] while evidence for the exact opposite is also available.[7] Such inconsistency occurs in both *in vivo* and *in vitro* situations;[2,3] affected by levels of experience of observer populations;[8] by variations between studies of natural teeth and prostheses; and by instances when different shade guide tabs are compared with each other. Further confusion results by including photographic images, computerised matching methods and other tooth colour matching techniques.[4,9] Why is tooth colour so hard to measure accurately? The dentine is the primary source of colour and is modified by the translucency and thickness of overlying enamel.[6] Consequently teeth are not homogeneous in colour, being darker at the gingival margin, due to the thinner enamel, varying through the tooth body to the incisal tip which appears lighter due to its narrow bucco-lingual edge and a tapering off of the intervening dentine. The tooth body colour lies somewhere in between these extremes and is therefore taken to represent basic tooth colour.[10] Therefore, the facial middle third of the tooth has been recommended for tooth colour assessment.[11,12] This pragmatic recommendation is seemingly by default and disregards other factors which play a role in the interaction of light with the tooth surface, such as the pellicle and tooth stains, both extrinsic or intrinsic. All of these influence the light scattering, reflection, transmission and absorption properties of enamel and dentine, thereby affecting the colour reading.[13,14] In addition, natural topographic tooth contour, with its surface anomalies, grooves, ridges and bands, diverts light in different ways[2] and hence alter the perceived colour. Finally the hydroxyapatite crystals themselves contribute significantly towards light scattering and the propagation of light through teeth.[15] Indeed, studies by Darling, Huynh and Fried[16] and Joiner[2] found that natural and artificial demineralisation of the enamel increased the light scattering co-efficient by two to three orders of magnitude.

Little consensus is apparent regarding the influence of bleaching on tooth enamel and the effect of light on the tooth. *In vitro* studies have mainly focussed on tooth surface changes following peroxide bleaching with concentrations ranging from 5.3% to 38%. Joiner[17] has reviewed 91 studies investigating changes in surface morphology and chemistry of enamel and dentine, as well as surface micro-hardness following bleaching. The majority (64) of these studies showed no substantive changes in enamel surface morphology following bleaching, for example those by Sulieman *et al*.[18] and Lopes *et al*.[19] In contrast, Jiang *et al*.[20] advised that high concentration bleaching agents should be used with caution, because they could have an adverse effect on the tooth enamel. Unfortunately, no detail has been given of these "adverse effects". Further studies report enamel loss;[21] increased surface roughness or surface porosity;[22,23] increased pitting, exposure and loss of the underlying prism structure of enamel[24,25,26] among other effects. Surface changes shown by the above *in vitro* studies did not replicate the *in vivo* setting. This may be due to the low pH of the products used,[17] time of bleaching[27] or the method of surface roughness analysis utilised. The above studies emphasise the uncertainties of bleaching agents possibly effecting microscopic alteration to tooth structure and thereby altering tooth colour readings, over and beyond the tooth lightening effect of the bleach itself.

In addition, it highlights the crucial role of methodology when investigating microscopic enamel surface changes. For this reason, the surface roughness of treated teeth was assessed in this study using atomic force microscopy (AFM), to clarify possible increased light scattering within the sample. One main advantage of AFM over enamel surface roughness techniques such as profilometry, is that the resolution of the AFM is orders of magnitude greater than that offered by the profilometer and therefore its imaging capabilities are so much more sensitive. Secondly, scanning electron microscopy (SEM), demands damaging sample preparation techniques, which can affect the surface and create artefacts.[28]

This study is part of a larger investigation into tooth bleaching products and their effects on tooth surfaces. The first publication compared the bleaching capacity of a new ozone-based product with a "gold standard" and a distilled water control.[29] The current report addresses the results of AFM examination of enamel surface after bleaching and compares two methods of tooth colour assessment. The latter aspect of the investigation received particular attention when designing the study: it was planned that there would be a large data base for comparison – colour readings taken at nine intervals for digital and visual colour readings giving a total of 1 782 comparative recordings. Secondly, the influence of four variables could be used to tease out significance within the study, namely: three treatments, three tooth types, nine recording intervals and two colour assessment methods. Thirdly, subtle colour changes occurring in bleaching would stretch the tooth colour assessment capabilities of both methodologies. Finally, tooth surface examination would show the effect of the treatment regime on enamel surface roughness and provide clues which could link surface roughness to comparative colour reading anomalies.

The research questions which arose from the synopsis of literature are as follows:

1. Do the tooth colour assessment methods reflect a similar trend of treatment outcomes, ease of use, reproducibility, repeatability and cost?
2. Can the experimental variables be teased out to pinpoint possible confounders, affecting the performance of digital and visual tooth colour determination?
3. Does enamel surface roughness, as measured by AFM, differ between the experimental treatments?

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, ethics clearance certificate (M050760). The sample consisted of 99 teeth utilised for a tooth bleaching study which has previously been published.[29] For the purposes of this study, the 99 teeth were equally allocated to three treatment regimes, one Control and two tooth-bleaching products (Ozicure Oxygen Activator (O_3, RSA) and Opalescence

Quick with 35% carbamide peroxide (Ultradent, USA). The thirty three teeth in each group comprised 12 incisors, six canines and 15 pre-molars and were sub-divided into three replicates of 11 teeth in each. The teeth were subjected to bleaching three times according to each manufacturer's instructions (Controls were treated with distilled water) and tooth colour readings taken on nine occasions during the study. Two methods were used for tooth colour assessment on each occasion: visually with the Vitapan 3D Master tooth guide (VITA, Germany) and digitally with the VITA Easyshade spectrophotometer (VITA, Germany). This gave a total number of 1 782 colour readings. Colour assessment took place in the same position in a dental surgery. The walls of the surgery were painted a light grey matt finish with day light globes as the only light source. Blinds closed the windows, eliminating external light sources and the surgery door was shut. A black matt background was used during tooth colour assessment for both methods.

VISUAL TOOTH COLOUR ASSESSMENT

Prior to the study and following a period of colour assessment training, the investigator (AAG) completed an intra-examiner test to ensure consistent colour assessment. The data was subjected to a Cochran-Mantel-Haenszel test with P= 0.1841, indicating that tooth colour was consistently read. The Vitapan 3D Master Tooth Guide (VITA, Germany) has 29 tabs arranged in a three step system which first assesses tooth lightness, then tooth chroma and finally tooth hue. Colour was assessed by comparing the middle third facial tooth surface with the tab (Figure 1). For statistical evaluation, 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 to 29. These ranked numbers were also used for the descriptive analysis of the data.

DIGITAL TOOTH COLOUR ASSESSMENT

The VITA Easyshade spectrophotometer (VITA, Germany) has a maximum colour selection of 81 shades. The manufacturer recommends automatic self-recalibration of the instrument prior to each colour assessment. Consequently recalibration was done prior to each individual tooth colour assessment. For each tooth colour reading, the probe tip was placed flush on to the tooth and perpendicular to the facial, mid third of the tooth surface and an electronic colour assessment completed (Figure 2). The readings were repeated to adhere to the manufacturer recommendation that three corresponding tooth colour assessments are needed for a positive tooth colour match.

Figure 1: Visual tooth colour assessment using the Vitapan 3D Master Tooth Guide. The shade tab is held against the tooth to determine an accurate tooth colour.

Figure 2: Digital tooth colour assessment using the VITA Easyshade (VITA, Germany). The probe is placed flush on the mid-third of the tooth body and perpendicular to the tooth surface. Three corresponding and consecutive tooth colour readings are needed for a positive tooth colour match.

As was the case with visual colour assessment, each of the alpha-numeric tooth shades within the digital library was rank numbered from the lightest (0M3) to the darkest (5M3). These numbers, ranking from one to 81, were used in the statistical and descriptive evaluation.

The researcher was blinded during the entire study to the previous tooth colour readings, for both visual and digital methods. Data recording tooth colour shades were captured on a Microsoft Office Excel spreadsheet and converted to the corresponding numerical value. The numerical values were descriptively and statistically analysed using SAS (SAS for Windows Version 9.1, SAS Institute Inc., Cary, NC: USA). Statistical analysis was by the General Linear Models (GLM) test for Analysis of Variance for a Fractional Design set at a significance of $P<0.05$. Direct statistical testing between the tooth colour reading methods was not possible, because each has its own systematic tooth shade series, unique to the operating system with different numbers of colour options, 29 for visual, 81 for digital.

SURFACE ROUGHNESS - ATOMIC FORCE MICROSCOPE PROCEDURE

After bleaching, one tooth showing the least colour change and one tooth with the greatest colour change in each experimental group, were selected and allowed to dry. Each tooth was carefully cleaned with a cotton bud soaked in ethanol (CAS 64175) and dried with an Easy Duster (SPI Supplies), West Chester, USA (*+H6Ø6Ø76Ø5ABLO*). Tooth specimens were individually mounted on a round steel disc using Pratley Quickset Clear (Pratley®), so that the surface of interest was uppermost, parallel to the disc surface. Scanning was restricted to ten micrometres in the X and Y- axes due to the curvature of the teeth. The images were analysed using Fast Fourier Transform analysis to derive the 2D power spectrum of the image and the notch filter was used to remove peaks due to noise. A height histogram was plotted to record the distribution of heights along the selected scan profile.

A research notebook was kept throughout to reflect personal observations and record findings during the study.

RESULTS

General

For the purpose of this study, visual tooth colour assessment will refer to tooth colour assessment captured with the Vitapan 3D Master Tooth Guide (VITA, Germany) and digital tooth colour assessment for the VITA Easyshade (VITA, Germany) method. There were 16 "no readings" within the digital group: nine in the Ozicure Oxygen Activator group where notably the colour of one canine could not be assessed on four occasions. One Opalescence Quick treated incisor was responsible for six of the seven "no reads" for the group. All Control tooth specimens were successfully colour- assessed digitally.

Statistical tooth bleaching results

The statistical analysis utilised four of the nine colour readings for investigation of independent variables, resulting in 396 colour readings for visual assessment and 391 for digital assessment, to determine the influence of treatment, number of treatments, tooth type and variable combinations. These were the baseline and the 24 hour post-bleach readings, following each of the three bleach treatments. The 24 hour readings are regarded as the most stable following bleaching.[29]

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

Independent variables	Dependent variable – tooth colour					
	Visual tooth colour assessment method			Digital tooth colour assessment method		
	F value	P value	No. of observations	F value	P value	No. of observations
Treatments: n=3 (Control, Opalescence, Ozicure)	9.72	<0.0001	396	4.34	0.0137	391
Number of tooth bleaches: n=3	3.41	0.0176	396	1.35	0.2589	391
Tooth type: n=3 (canine, incisor, premolar)	3.21	0.0416	396	7.88	0.0004	391
Tooth type and treatment	2.75	0.0280	396	0.81	0.5209	391

Table 1 shows the F and P values for the experimental variables by tooth colour assessment method. Visual tooth colour assessment 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). Digital colour assessment indicated treatment (F=4.34; P=0.0137) and tooth type (F=7.88; P=0.0004) to be of significance in tooth colour change. All other variable combinations proved insignificant.

DESCRIPTIVE TOOTH BLEACHING RESULTS

Comparative graphs of tooth type versus colour assessment method are given to illustrate the trends in colour change and performance of both colour assessment methods. The graphs were constructed from 891 colour readings for the visual method and 875 readings for the digital method, slightly reduced because of "no reads". Figures 3-5 show bleaching trends for Control (Figure 3); Ozicure Oxygen Activator (Figure 4) and Opalescence Quick (Figure 5) with (a) representing incisors, (b) the canines and (c) premolars. The graphs are all laid out in the same way. The X-axis gives the sequence of the nine tooth colour assessments in the study. The left hand Y1-axis gives the mean tooth colour reading for visual assessment, the right Y2-axis for digital assessment. Visual colour assessment of bleached specimens showed progressive tooth lightening. It can be seen that there is little congruence between the two colour assessment measures

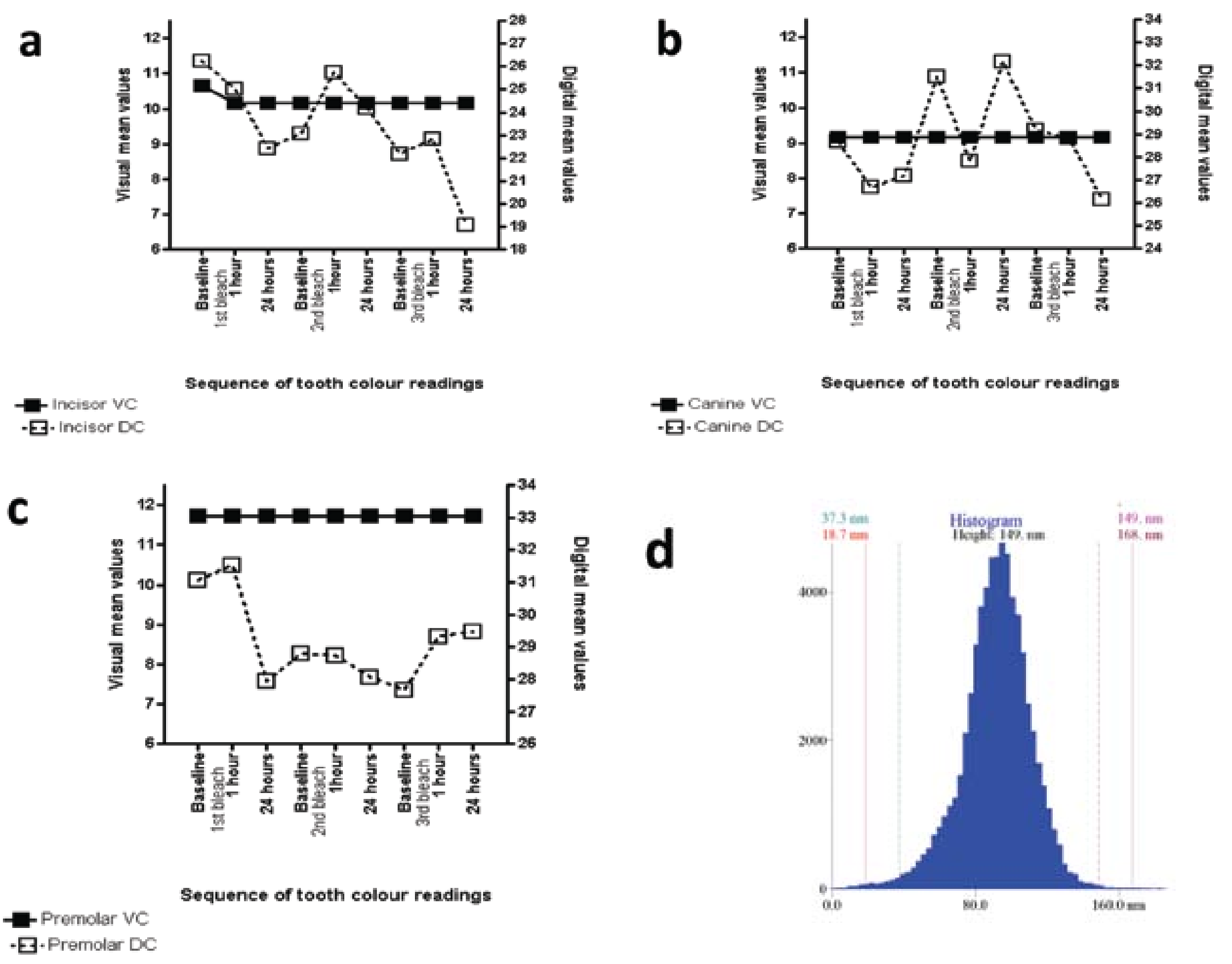

Figure 3: Visual (V) and digital (D) bleaching colour assessment trends for Control (C) **(a)** incisors **(b)** canines and **(c)** premolars. Height histogram of the premolar tooth surface roughness, showing a left skewed Gaussian distribution is given in **(d)** where the y-axis represents frequency and the x-axis represents height (nm).

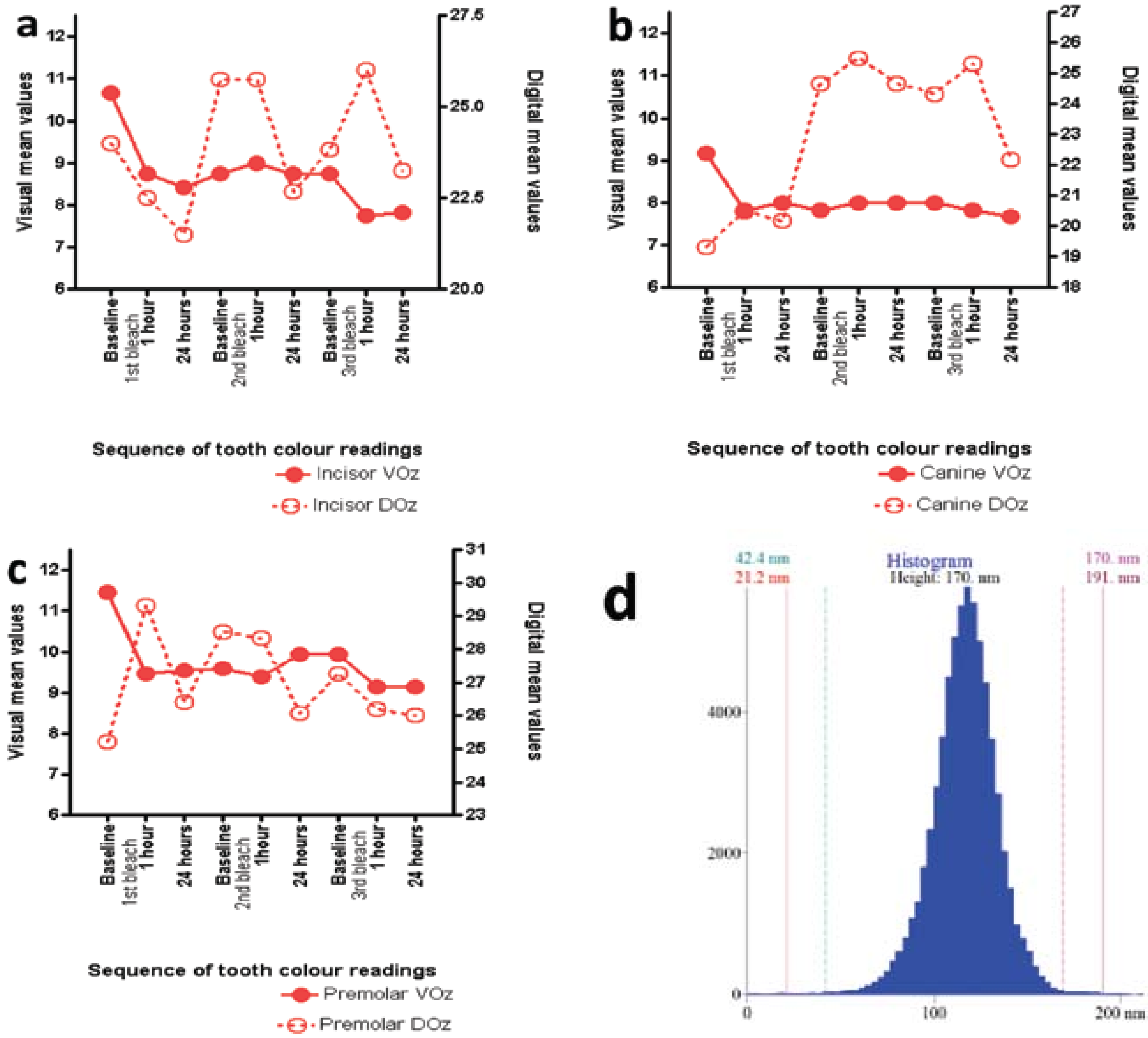

Figure 4: Visual (V) and digital (D) bleaching colour assessment trends for Ozicure Oxygen Activator (Oz) **(a)** incisors **(b)** canines and **(c)** premolars. Height histogram of canine tooth surface roughness showing a bell-shape or symmetrical Gaussian distribution is given in **(d)** where the y-axis represents frequency and the x-axis represents height (nm).

for all three Control tooth types (Figure 3 a, b, c) and Ozicure Oxygen Activator incisors and canines (Figure 4 a, b) with digital colour readings marked by peaks and troughs. The trend for Ozicure Oxygen Activator premolars (Figure 4c) and all Opalescence Quick treated tooth types (Figure 5a, b, c) show better agreement between the two colour assessment methods following sequential bleaching. In both cases mean tooth colours were consistently confined to just over 20% of the spectrum range of each colour assessment technique: visually assessed teeth fell between shades 6-12 (Y1 axes in Figures 3-5) and digital colour assessment were between15-34 (Y2 axes in Figures 3-5).

SURFACE ROUGHNESS

Problems emerged when the experimental teeth were viewed in the AFM. These related to viewing constraints caused by the curvature of the teeth themselves, adhesion of the mounting medium and the stability of the tooth during scanning. Thus only one tooth was scanned in each group with a variable number of scans possible on the flattest portion of the tooth. As it happened, the suitable teeth were all those showing the least amount of colour change. The Control premolar enamel tooth surface (Figure 6), shows many irregularities with long, dark, scratch-like marks typical of masticatory wear and tear. The Ozicure Oxygen Activator bleached canine tooth surface (Figure 7) and Opalescence Quick bleached incisor tooth surface (Figure 8), were more regular than that of the Control and showed no deep scratch marks. However, Ozicure Oxygen Activator had a narrower depth margin between the valleys and the peaks when compared with Opalescence Quick, as indicated by the colour bar scale to the left of the micrographs.

Surface roughness data were obtained from 10 scans from the Opalescence Quick treated incisor and 10 scans from a Control premolar. Unfortunately, only seven usable scans were achieved from the Ozicure Oxygen Activator canine, due to the curvature of the crown surface. The histograms in Figures 3d, 4d, and 5d represent the surface roughness of a single scan typical for each treatment regime. Important features on each histogram are the maximum peak height, given at the top of the peak and the horisontal distance between the peak and the left and the right tails respectively, which is an indication of surface regularity. The Control premolar histogram (Figure 3d) represents a skew Gaussian distribution where peak to tail length on the left side of the histogram is longer than the corresponding length on the

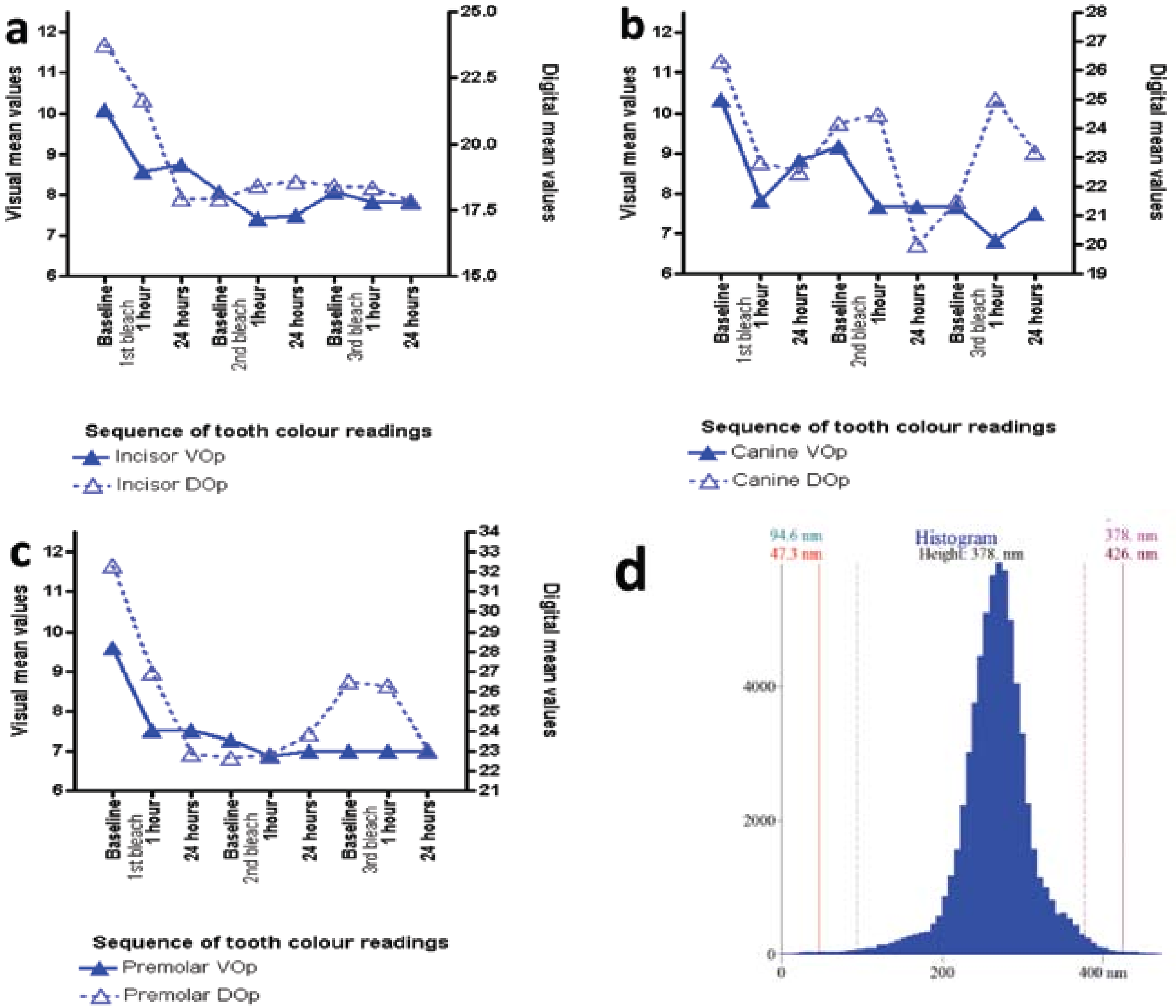

Figure 5: Visual (V) and digital (D) bleaching colour assessment trends for Opalescence Quick (Op) **(a)** incisors **(b)** canines and **(c)** premolars. Height histogram of incisor tooth surface roughness showing a bell-shape or symmetrical Gaussian distribution is given in **(d)** where the y-axis represents frequency and the x-axis represents height (nm).

right side of the histogram. This indicates a preponderance of small peaks and valleys dominated by fewer, but greater, peaks and valleys, interpreted as being the scratches seen in the micrograph (Figure 6). An average surface roughness of 14.7nm was recorded for the Control. Although there is a slight skewing to the left of the Ozicure Oxygen Activator treated canine surface histogram (Figure 4d), the left and right peak to tail distances are nearly equal giving a more bell-shaped or symmetrical Gaussian distribution. This indicates that the roughness is symmetrical around the peak value. Average surface roughness for Ozicure Oxygen Activator was 13.3nm. While the histogram of the Opalescence Quick incisor enamel surface bleaching is also an almost symmetrical Gaussian distribution (Figure 5d), the right side shows slight predominance, implying a preponderance of larger peaks and a greater surface roughness than the Control and Ozicure Oxygen Activator specimens. This was confirmed by the data in that Opalescence Quick average enamel surface roughness was 32.6nm. Hence, Opalescence Quick treatment produced the greatest enamel surface roughness. On the other hand, both bleaching systems yielded a more regular surface than the Control samples, as revealed by the Gaussian symmetry of the bleached surfaces, compared to the skewed histogram of the Control.

EXPERIMENTAL OBSERVATION

Methodologically, visual colour assessment was fast and easy to use, whereas the digital colour assessment method was time consuming and problematic. The instrument proved capricious and it was often impossible to obtain a consistent tooth colour after three consecutive assessments. Instead, the displayed tooth colour readings bounced across the entire available spectrum, bearing no relation to the actual tooth colour being assessed. When this occurred, the instrument had to be switched off, re-started and re-calibrated before continuing. Consequently, it took approximately 25 minutes for digital tooth colour assessment of one replicate of 11 teeth. By contrast, visual tooth colour assessment for the same number of teeth took approximately 10 minutes to complete. At the time of the study, the price tag for the Vita Easyshade was just under R 40 000 versus slightly over R 1 100 for the Vitapan 3d Master Tooth Guide (VITA, Germany).

DISCUSSION

Our results show that visual colour assessment seemed statistically more discerning when tooth colour change was tracked, following sequential bleaching compared with digital tooth colour assessment. Visual colour change showed significance for the four variables, treatment, number of

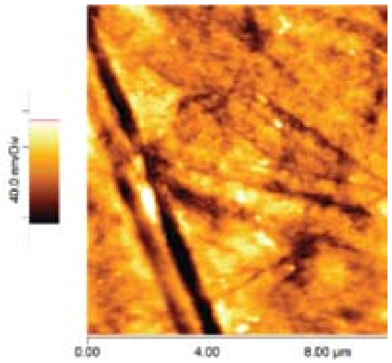

Figure 6: AFM micrograph of a Control premolar tooth surface. The dark lines on the micrograph represent typical scratch-like markings found on human teeth as a result of masticatory wear and tear. The average surface roughness was 14.7nm.

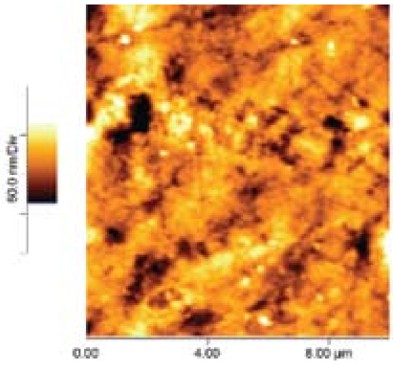

Figure 7: AFM micrograph of the Ozicure Oxygen Activator bleached canine tooth surface. The average surface roughness was 13.3nm.

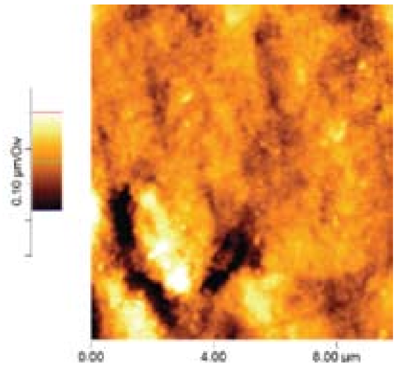

Figure 8: AFM micrograph of the Opalescence Quick bleached incisor tooth surface. The average surface roughness for the area was 32.6nm..

bleaches, tooth type and the linked combination tooth type* treatment. (Statistical significance following digital colour assessment was confined to treatment and tooth type. Descriptive data analysis showed a steady tooth lightening for visual assessment, whereas digitally assessing specimens resulted in colour inconsistencies throughout. Particularly perplexing were the digital colour readings for the Control teeth, which showed marked colour lightening over the nine colour assessment intervals and Ozicure Oxygen Activator incisors and canine teeth which seemingly went darker with bleaching. Coupled to the vagaries and time consuming nature of the digital colour assessment method, one could conclude that visual tooth colour assessment is superior to digital colour assessment as has been shown in other studies.[7,30] However, this is a simplistic conclusion and must be further explored within the context of our methodology, the atomic force microscopy surface roughness data and current literature.

Paul *et al.*[31] maintain that current technologies, related to tooth shade determination, offer a 33% improvement in accuracy and a more objective match in 93.3% of cases, compared with observations by the human eye or conventional techniques. Olms and Setz[14] highlighted procedures which they felt affected repeatability, reproducibility and precision of their VITA Easyshade measurements when examining metal ceramic crowns. Firstly, they stress the use of a customised jig to ensure the same spot on the crown is measured, a recommendation endorsed by others.[2,32] Much of our colour variation could be ascribed to the absence of such a jig resulting in inconsistent site colour readings ,although all colour was assessed within the facial middle third of the tooth body as recommended.[11,12] Producing individual jigs for 99 teeth is clearly beyond the scope of our study and we further question the logic of using a probe spot, 3-5mm diameter, as being representative of tooth colour for an entire tooth. We suggest that one of the limitations of digital tooth colour assessment is its reliance on precise, repeatable, and positioning of the probe tip for colour reading.

Secondly, Olms and Setz[14] concede that the homogenous surface of their crowns ensures shade stability, although they point out that even minor surface variations, due to the presence of abrasions, impurities and individual layering of the veneered crowns, can lead to slight shade differences between readings. Our exhaustive literature review has emphasised the problems associated with colour determination of teeth. We submit that current digital tooth shade technologies are, as yet, better suited to uniform dental material colour determination rather than natural teeth. This view is echoed by others.[3,14,32]

Olms and Setz[14] observe that calibration information is rarely included in studies on digital colour assessment. They used a calibration interval of five measurements for VITA Easyshade whereas Celik *et al.*[33] re-calibrated the VITA Easyshade after 10 measurements. Olms and Setz[14] feel that short re-calibration intervals have a positive impact on the repeatability of measurement data. Re-calibration was required between each tooth and unfortunately became one of the frustrations of our study.

The human eye can discern very small differences in shade.[3,14] When this is coupled to the reduced spectrum of colours of the 29 tab Vitapan 3D Master Tooth Guide and a single, trained investigator, error rates are minimised and our visual colour results appear reliable. The wider colour library of 81 shades coupled with the on going capriciousness of digital assessment created a tension within this aspect of the study. The research protocol required recording the first uniform series of three colour data readings, displayed by the instrument as representing the correct colour for a specific tooth. This was done, provided the investigator felt the colour recorded fitted reasonably within the true tooth colour range: if not, a "no read" was recorded for the specimen. Investigator frustration plus increased shade choices could have compounded errors of the digital colour reading and hence the erratic and absent data. Our experience was not unique; instrument errors were identified by Al Saleh *et al.*[6] when they examined light scattering properties in teeth ceramic crowns and shade tabs. We therefore do not know to what extent methodological limitations affected the recording of subtle colour change, as encountered in bleaching and this should be acknowledged as one of the failures of this study. We are also the first to report "no reads," using digital tooth colour assessment and are unable to explain why two of the experimental teeth garnered 10 of the total of 16 "no reads".

The most fascinating part of the study lay in the surface roughness data which implies that bleaching alters, or does not alter tooth surfaces, depending on which question is asked. Firstly, both bleaching systems created a more regular surface compared to the Control, where irregular masticatory surface scratches were evident, causing the skewed Gaussian surface roughness curve. No such scratches were observed in the bleach- treated teeth, supporting those authors who found that bleaching does alter surface enamel.[21-26] In contrast, the surface roughness of the Ozicure Oxygen Activator specimen is no greater than that of the Control, corroborating the conclusions of the majority of studies, reviewed by Joiner,[17] which indicated that no substantive changes in enamel surface morphology occur following bleaching. Although bleach-induced surface roughening was approximately symmetrical around the mean, tooth enamel bleached with Opalescence Quick, showed a surface roughness which increased two-fold when compared with that of normal tooth enamel and Ozicure Oxygen Activator surfaces. This confirms that bleaching products are not uniform when interacting with enamel.[23] Further work is called for on AFM measured enamel surface roughness following tooth bleaching and its effects on colour determination.

The findings on surface roughness have relevance within the first two purposes of this study. Increased roughness indicates greater light scattering.[2,15,16] We speculate that greater scattering could result in more spectra reaching the detector and thereby an improved colour reading, which would give rise to the marked agreement between the visual and digital colour assessment slopes of teeth treated with Opalescence Quick. This speculation is supported by the findings of Pedriera de Freitas *et al*,[27] who showed that gloss and increased enamel surface roughness as measured by AFM are inter-related.

It can be argued that our enamel surface roughness findings are based on a very small sample, (which we endeavoured to extend, but were hampered by specimen and technique constraints). We seem to be the first to examine entire teeth in the AFM – more usually enamel fragments are prepared for AFM examination[2,3] although others have trimmed teeth as a minimum measure to improve overall specimen handling.[27] We embarked on examination of whole teeth to avoid any artefacts caused by specimen preparation. However, problems with specimen adhesion, stability within the microscope and finding a suitable flat area of tooth surface to optimally record surface topography were encountered, preventing examination of the full number of planned specimens. Our study proves it is possible to examine entire teeth within the AFM. However, based on our experience, we advise that prior selection of tooth specimen surfaces is imperative, as those with minimal curvature are best for optimal viewing and measurement. We recommend Pratley® as an adhesive to ensure specimen stability within the microscope.

CONCLUSION

This paper contributes towards the debate surrounding visual and digital tooth colour assessment methods and tooth bleaching. With regard to the three research questions investigated: (a) only in part did the tooth colour assessment methods reflect a similar trend of treatment outcomes for both bleaching agents and the Control. Within the context and limitations of our study, visual colour assessment proved more satisfactory than the digital method for tooth colour determination when it came to ease of use, agreement, reproducibility, repeatability and cost. (b) of all experimental variables examined, only surface roughness findings could be linked to improved digital tooth colour determination given the limitations of the AFM data (c) bleaching effects on the enamel were not uniform.

Several recent publications concur in the view that, while digital tooth colour shade determination has the potential for accuracy, precision, repeatability and reproducibility, the technology still needs improvement. "Therefore, modified and optimised software or hardware could improve [tooth colour] matching between human and machine-driven outcomes in the future."[3] "Chances are that new, affordable, high-quality colour matching instruments and technology will contribute to successful work with colour and aesthetic dentistry"[32] and "methods and techniques for determining ... tooth colour will continue to evolve with time."[2] Our findings endorse these sentiments. We further suggest, as have others, that "wherever possible both instrumental and visual colour matching method should be used, as they complement each other."[32]

Declaration: No conflict of interest declared.

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