OBJECTIVE HUMAN TOOTH COLOUR MEASUREMENTS AS A MEANS OF DETERMINING CHRONOLOGIC AGE *IN VIVO* AND EX *VIVO*

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ABSTRACT

Colour is a subjective sensation and as such is difficult to use in a quantitative study. However a number of clinical studies on extracted teeth have shown a good correlation between tooth colour and age.

The purpose of this study was to examine the usefulness of a specific spectrophotometer in determining tooth colour on extracted and non-extracted teeth and to look for a possible age relationship.

There were two parts in this study. An *ex vivo* study concentrated on collected tooth material. Single rooted teeth were selected out of each of the 5-year-age groups (ages ranged from 15-89 years). Colour measurements were performed on the mesial and vestibular aspects of the roots as well on the mid-vestibular aspects of the enamel crown. An *in vivo* study concentrated on the use of this specific shade taking system in living patients (n=70). Statistical analysis of the results revealed regression formulas for both *ex vivo* and *in vivo* situations displaying adjusted R-squares between 0.48 and 0.56. It may be concluded that age related trends were found. Having its shortcomings, the shade taking system was found to perform well as a convenient adjunct to dental age estimation in both the living and the deceased.

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Keywords: age calculation, tooth colour, spectrophotometer, *in vivo*, *ex vivo*

INTRODUCTION

Identification of unknown corpses or human skeletal remains is often problematic because of lack of medical or dental ante mortem identifiers. In addition, the impact of trauma makes comparative means of identification impossible. In these cases reconstructive types of identification are then entertained As part of this biological profiling it is important to have an indication of the chronological age of the skeletal remains as well as information on biological group, gender and circumstantial evidence. In general, age estimation is important in the process of identification since it is helpful in concentrating or limiting the search within the target group of missing people and is an adjunct for court magistrates to help out in establishing verdicts.

Information on the age of a subject is also requested in other totally different forensic cases such as adoption cases, immigration issues or criminal cases. In the latter it is important to know whether a suspect has reached the age of majority.

Especially in the field of forensic odontology a large volume of research has been published on this subject. Dental age estimation in a forensic context has been performed on radiological as well as morphological parameters and the authors recently published a review of most commonly used dental age estimation techniques.[1] Amongst the latter, are techniques based on age related parameters such as attrition, periodontal ligament attachment, secondary dentin formation, translucency of root dentin, root resorption, and cementum deposition.[2-5] These techniques often require the forensic odontologist to prepare the tooth in thin sections or to produce a mid-tooth section for further analysis. These time consuming age-estimation techniques could be abandoned if a valid and time-effective alternative were available.

A method based on the evaluation of tooth colour could be such a technique, although the infrequent use of tooth colour for age estimation may already point out the difficulty in objective assessment. However, the general observation that teeth tend to darken with age has already been noted by some authors,

trying to confirm the correlation between tooth colour and age. Ten Cate *et al*[6] compared teeth of unknown age with teeth of known age based on the colour of root surfaces as the only indicator of age. They showed that the age of the individuals to whom the teeth belonged was estimated within an age range of +/- 10 years. Solheim[7] reported a significant correlation between age and colour of both tooth crowns and roots. However, most results were obtained from visual grading, which is prone to subjective bias.

When the colour of an object, e.g. a tooth, is to be measured, two other parameters that influence colour perception must be standardized. These are light source and observer. Sunlight does not always have the same intensity of different wavelengths and can vary during the day, depending on the place or the environment. In addition, the sensitivity of the human eye is highly observer dependent.[8-10] A spectrophotometer and a colourimeter are instruments that use a constant light source, and transfer the measurements into tri-stimulus values according to the CIE-system[11] and thus exclude observer subjectivity. The CIELAB system, a modified CIE system specifies any colour into tri-stimulus values L, a, and b. L stands for lightness (the black-white factor), a for the red-green factor and b for the yellow-blue factor.

Lackovic and Wood[12] investigated the correlation between colour of tooth roots and chronological age. Their results strongly support the suspicion that chronological age is directly related to increased root colouration: they even found a significant difference between the colouration of the four root surfaces, especially for the mesial surface, as well as between non-molar and molar tooth roots.

In 2001 Odioso[13] investigated the impact of demographic variables (such as age, gender and ethnicity) on tooth colour. The measurements were taken from the crown surface of anterior teeth. They came to the conclusion that as a tooth ages, its colour becomes darker (-L) and more yellow (+b). Age is shown to be the most significant predictor of tooth colour. In a more recent study[14] models were created to predict the age of a human being based on the root colour of freshly extracted anterior teeth among others. The models show a linear correlation between chronological age and tooth colour enabling an age estimation based on tooth colour.

However, such an estimate was only accurate up to 11,5 years.

The purpose of the present study was to evaluate the influence of colour on dental age estimation using a computer-aided shade taking system avoiding the bias inherent to observer subjectivity and to develop new mathematical regression models for age estimation based on objective colour assessment on extracted and non-extracted teeth

MATERIALS AND METHODS
Ex vivo study.

The tooth collection of Ten Cate *et al*[6] was used for this project. They obtained a sample of extracted teeth of known age and gender from Ontario (Canada) dental practitioners. These teeth were cleaned with pumice and stored in corked glass jars. The teeth were originally pooled by gender into five-year age groups: 15-19, 20-24, 25-29 and so on until 85-90. For the present *ex vivo* study, the single rooted intact teeth were selected out of each of the five-year-age groups. A total of 1332 colour measurements were performed: 560 on male and 772 on female teeth (Table 1). Colour measurements were performed on the vestibular aspect of the tooth crown (CV) as well as on the mesial (MR) and vestibular (VR) surface of the root. Each specific tooth surface was measured 5 times and the results were averaged. Colour evaluation was carried out with the ShadeEye-NCC™ Dental Chroma Meter (Shofu, San Marcos, CA, USA) [15-16]. The ShadeEye-NCC™is a technologically advanced shade taking system especially designed for its application in dentistry. The cordless measuring unit digitally analyses the shades and immediately transmits the data to the main unit via an infrared interface. It records L(brightness), a(red-green), b(blue-yellow) values according to the CIE-LAB system without being affected by lighting conditions. All colour measurements were done with this shade taking system while the tooth was held against a black background. The tip of the measuring unit made contact with the enamel or dentine surface at a vertical level of at least 4 mm a way from the vestibular cementum-enamel junction. Each single measurement consisted of an automatic run of three colour measurements that are automatically averaged. This procedure was repeated 5 times for each specific tooth surface evaluated. Apart from the results of the measurements, gender, age group and specific location on the tooth surface were recorded.

Table 1: *Representation of the amount of colour measurements performed for the ex vivo study, categorised according to gender and location of the measurement on the tooth. Each colour measurement represents a total of five consecutive measurements of which the data were averaged to obtain a single value for a specific location on a specific tooth. (Different locations are CV: crown vestibular; RM: root mesial; RV: root vestibular).*

| Age group | Male | | | | Female | | | | |
	CV	RM	RV	Subtotal	CV	RM	RV	Subtotal	Total
15-19	2	2	2	6	3	5	5	13	19
20-24	4	17	14	35	7	15	11	33	68
25-29	11	23	25	59	13	23	23	59	118
30-34	7	26	18	51	13	30	24	67	118
35-39	6	16	18	40	9	9	8	26	66
40-44	11	16	18	45	23	38	39	100	145
45-49	14	37	37	88	9	18	16	43	131
50-54	13	23	22	58	26	43	29	98	156
55-59	13	24	21	58	22	39	42	103	161
60-64	15	30	27	72	20	28	28	76	148
65-69	2	6	6	14	16	31	30	77	91
70-74	-	2	2	4	12	20	20	52	56
75-79	-	2	2	4	2	4	4	10	14
80-84	3	5	5	13	4	6	5	15	28
85-89	3	6	4	13	-	-	-	-	13
Total	104	235	221	560	179	309	284	772	1332

In vivo study

The *in vivo* part of this project consisted of the colour measurement of teeth in individuals seeking dental treatment at the University Hospitals of the Katholieke Universiteit Leuven. The same shade taking system as earlier reported was used to determine the L-, a- and b-values of three upper front teeth: left or right upper central and lateral incisor and upper canine. Measurements were performed on the vestibular enamel of the specific air dried tooth crown after cleaning them with a mixture of pumice and water, at least 2 mm coronal to the gingival border and after cleaning and rinsing of these surfaces with pumice and slow rotating devices. 70 subjects were randomly selected (Table 2). Selection criteria were: the evaluated teeth should be intact, unrestored, unbleached and free of calculus and specific discolorations. Subjects were non-smoking western European Caucasians. For each specific surface, three repeated measurements were performed while the patient was asked to sit completely still. A total of 210 measurements from 70 subjects were obtained and divided into 5 year-age groups. Again with each colour measurement, age, tooth, sex and ethnical group were recorded.

Table 2: *Representation of the amount of colour measurements performed for the in vivo study, categorised according to gender. Three colour measurements (upper central and lateral incisors and canine) represent 1 patient.*

Age group	Male	Female
15-19	3	6
20-24	24	21
25-29	9	15
30-34	9	9
35-39	3	12
40-44	15	3
45-49	6	9
50-54	3	6
55-59	3	3
60-64	3	12
65-69	9	9
70-74	0	6
75-79	3	3
80-84	6	0
85-89	0	0
Total	**96**	**114**

Table 3: *Regression models for age calculation based on the colour values obtained from both ex vivo and in vivo studies. For a specific age calculation, include the obtained L-, a- and b-values in the model if necessary. Different locations exist CV: crown vestibular; RM: root mesial; RV: root vestibular. For each location, a separate regression model is obtained: include either 1 or 0 into the model depending on whether the colour measurement was performed, respectively, on that specific location or not. Gender G=0 for male and G=1 for female. For the parameter Tooth substitute the numerical value (11, 12, 13, 21, 22 or 23) of the specific tooth that was evaluated into the specific regression parameter for that tooth. The other tooth parameters become zero. In case of tooth 13, all tooth parameters become zero.*

EX VIVO STUDY RESULTS

MALE r^2

    Including location

        Age = 165.79 – 2.06 L – 4.95 a + 1.04 b + 38.90 CV + 2.89 RM   0.49

    Including location and gender

        Age = 162.08 –2.15 L – 5.05 a + 1.43 b + 43.60 CV + 6.07 G   0.49

FEMALE

    Including location

        Age = 166.01 – 2.23 L – 5.31 a + 1.67 b + 48.20 CV   0.48

    Including location and gender

        Age = 162.08 –2.15 L – 5.05 a + 1.43 b + 43.60 CV + 6.07 G   0.49

IN VIVO STUDY RESULTS

    Including interaction between tooth and parameter L

        Age = 247.58 – 3.34 L – 3.70 a + 1.07 b + 0.20 T11 +0.11 T12 + 0.35 T21 + 0.29 T22   0.56
        + 0.12 T23

Statistical Analysis

Pearson correlation coefficients were calculated between the different mean L-, a- and b-values measured and age of the individual and multiple regression analysis was performed on the data obtained with and without integrating parameters like location of measurement, gender and type of tooth evaluated. In order to be able to perform multiple regression analysis the parameter age was converted into a continuous variable by averaging each age group (e.g. the class of 15-19 years was replaced by the continuous variable 17 years). This method is acceptable because there are 14 age-classes and they are all ordinal. A specific parameter was included into the model if it generated a significant difference of $p<0.01$. The final model thus included only the most relevant variables. Also an analysis of variance was performed to highlight whether there was an influence between the type of tooth evaluated and the colour measurement obtained and thus whether the type of tooth had an effect on the age estimation. All statistical procedures were run with the SAS statistical software package (SAS Institute, Cary, NC, USA).

RESULTS

Ex vivo study

Statistical analysis of the relationship between the L-, a-, b- values obtained through the shade taking system and the chronological age revealed a poor Pearson correlation coefficient (r) of approximately 0.44 and 0.49 for female and male teeth respectively. Multiple regression analysis delivered comparable results: $r^2 = 0.20$ and 0.26 respectively. When the specific location (CV, RM and RV) of the colour measurement was entered into the model as well as gender (G), then a significant influence was found. Pearson correlation coefficients rise up to 0.63 and multiple regression analysis comes up with r^2 equal to 0.49 (Table 3). That a significant influence of the parameter location on the colour measurement exists was confirmed after analysis of variance with $p<0.0001$.

A general conclusion of the statistical analysis was that whatever model was used, there was always a slight tendency present to underestimate the real age for the higher age categories and to overestimate the chronological age in the lower age categories based on the colour parameters L, a and b. These tendencies remained when integrating parameters location and gender into the regression model.

In vivo study

Table 3 represents the results of the *in vivo* study. Gender and parameter b had no effect on the age calculation and were left out of the regression model. On the other hand, a significant effect was noticed for the type of tooth (T). However, since all three teeth were measured for each individual subject, the parameter tooth would not have a large effect on the age prediction of an individual. But the interaction between tooth and a colour

parameter (L, a or b) obtained might have a significant effect. Figs. 1a-c show a visual effect of tooth type on the colour measurements. Therefore, the possible interaction effect of both parameters was investigated with analysis of variance and a strong r² of 0.56 was found with a significant contribution of the interaction between the type of tooth evaluated and the parameter L (Table 3).

DISCUSSION

Since the goal of the study was to enable objective colour measurements of teeth, the object, light source and perceiver had to be standardized. Therefore a *colourimeter* device marketed as a shade taking system was used.

In this study a modified CIE system was used, namely the CIELAB system. There are two advantages in using this CIELAB system over the CIE system designed in 1971. Firstly, the specification is easier to interpret in terms of the psychophysical dimensions of colour perception in the CIE system namely brightness (L), hue (H) and colour (C). Secondly it is possible to estimate the magnitude of the differences between two colour stimuli using the chromaticity diagram. The L axis is known as the lightness and extends from 0 (black) to 100 (white). The other two coordinates (a and b) represent the red/green and yellow/blue fractions respectively.

The major drawback of the Shade-Eye NCC Dental Chroma Meter is that the cordless appliance has a measuring tip that analyses only a small area of the tooth surface. The tip needs to be in full contact and perpendicular to the investigated tooth surface. This is problematic when evaluating the curved surface of a small root. In that case full contact between tip and surface is impossible and this might have had an influence on measurements. This differs from the technique of Lackovic and Wood[12] who used colour measurements of tooth root surfaces that had been scanned.

The present system is relatively easy to handle as far as data interpretation are concerned. The compact nature of the device should allow its inclusion as a complimentary dental age estimating procedure which may confirm the findings of other techniques but based on an additional parameter which is objectively obtained.

The results obtained from the *ex vivo* study gave similar results to those from earlier studies,[12,14] although not as high correlation coefficients could be achieved.

In Ten Cate's study,[6] a human observer was used as the measuring tool for colour determination whereas in the work of Lackovic and Wood[12] a flat bed scanner and six selected points for each tooth surface were used for colour determination and ultimately correlation with chronological age. While flat bed scanning devices are common-place there are many variables that need to be accounted for including scanner-bulb properties, position on the scanner and use of a colour standard, amongst others. The human eye-brain "tool" possesses a remarkable ability to integrate information. Perhaps using human vision as a measuring tool should be evaluated in further studies in this aspect of age determination. A study by Solheim[17] using visual analysis for age estimation performed relatively well when compared to more cumbersome methods. Finally it is conceivable that collections of other teeth from other ethnic groups and other geographic locales could result in higher or lower correlations. The teeth in the present study and the studies of both Ten Cate *et al*[6] and Lackovic and Wood[12] all used teeth that were grouped in five-year ranges of known gender and were stored dry. Further studies should investigate teeth of similar anatomic sites on precisely known chronological age and explore possible subtle changes of colour over time. Finally the present investigative system allows measurement *in-vivo* which is a new development. It would be unethical to extract teeth from a living person in order to determine age and it is interesting to note that the same trends towards increased coloration of the teeth is seen in both extracted teeth and teeth in live individuals.
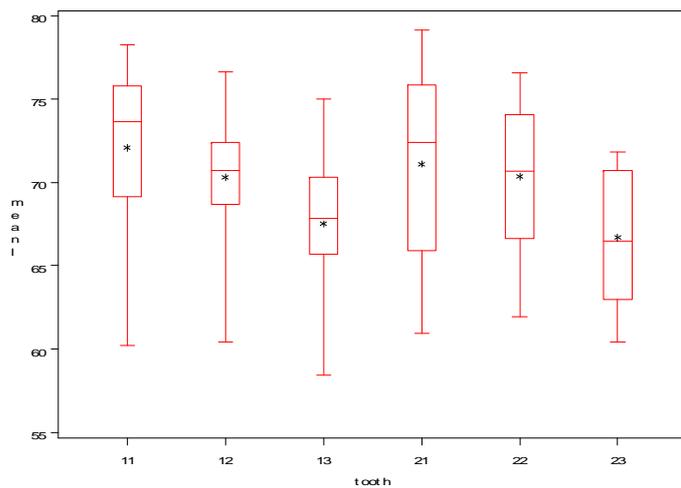
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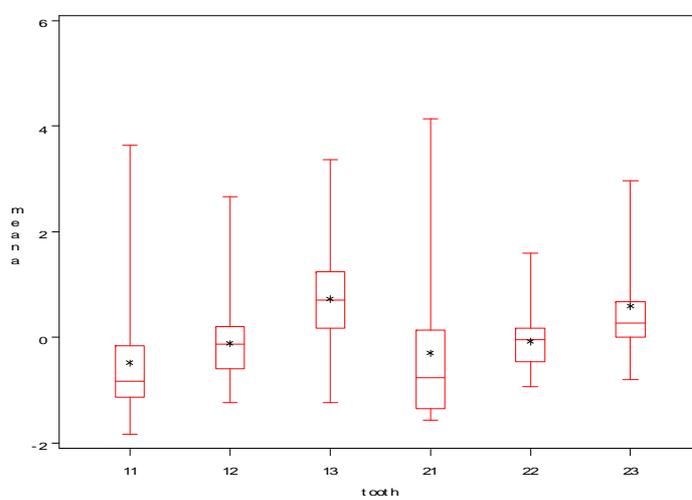
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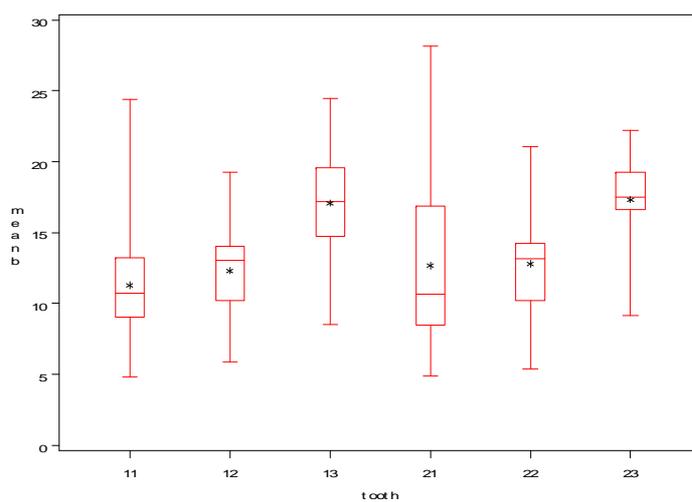
1c.

Figs 1a-c: *L-, a-, b- values obtained from colour measurements in in vivo study represented in the form of box plots and categorised according to the type of tooth. Visual observation reveals differences in colour values between teeth (11/21: upper central incisor; 12/22: upper lateral incisor; 13/23: upper canine). The same trend is noted between contralateral teeth.*

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