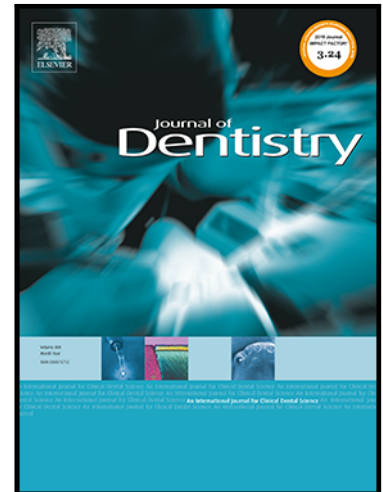


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In-vivo colour stability of enamel after ICON[®] treatment at 6 years of follow-up: a prospective single center study.

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Title: In-vivo colour stability of enamel after ICON® treatment at 6 years of follow-up: a prospective single center study.

Short title: In vivo long-term colour stability of resin infiltration

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Abstract (250)

Objectives: This *in-vivo* clinical study provides subjective and objective documentation on colour stability of enamel after resin infiltration at a mean observation time of six years after treatment.

Methods: 76 teeth previously treated with ICON® due to hypomineralized lesions of enamel were recalled for a follow-up at(.....). Colour stability was assessed: i) subjectively by patients using FDI-colour matching criteria; ii) objectively by calculating CIEDE2000 colour differences between the affected/treated and sound enamel in each tooth at T₀ (baseline), T₁ (one year) and T₂ (six years) based on spectrophotometric data. Analysis of correlation between FDI and CIEDE2000 data was performed.

Results: Two teeth were lost to follow-up prior to 72 months. No unwanted effects were reported by patients. Number of FDI scores 1 and 2 were 13.5%, 90.6% and 93.2% at T₀, T₁ and T₂, respectively. ΔE_{00} was evaluated at 6.8 (SD3.8) at T₀. ΔE_{00} was 5.8 (SD3.1) between T₀ and T₁ and 1.3 (SD0.6) between T₁ and T₂. ΔE_{00} reduction (T₁-T₀) was significantly but only fairly correlated with FDI scores at any follow-up.

Conclusions: This study shows that caries infiltration satisfactorily masks aesthetically relevant lesions after longer follow-up. Subjective and objective outcomes showed a fair correlation mainly for the initial masking effect.

Clinical Significance: This prospective clinical trial demonstrates the excellent subjective and objective colorimetric stability of enamel treated with ICON® 6 years after treatment.

1.Introduction

In-vitro and *in-vivo* investigations and clinical data have demonstrated the immediate aesthetic outcomes that follow resin infiltration [1–3]. However, as pointed out in a systematic review [4] the follow-up period is usually too short (max 12 months) to allow definitive conclusions about the long-term outcomes of this treatment. Moreover, the clinical documentation has been predominantly based on photographic images. The main drawback of photographic images is colorimetric error caused by camera settings (i.e., white balance) and lighting conditions. Unless these sources of error are adequately addressed, photography cannot be used to objectively document tooth colour variability after resin infiltration. Standardized conditions are necessary to avoid errors in the photographic documentation of the tooth colour, which may be influenced not only by camera settings and ambient lighting, but also possible dehydration of the enamel. Therefore, all the tooth colour assessment procedures should be performed before the teeth are exposed to dehydration [5,6].

A very recent *in-vivo* study found a mean colour difference of 5.57 ± 2.6 between hypomineralized and sound enamel when followed up 2 years after treatment [7]. However, the study was limited in only reporting colour differences using the CIELAB colour-difference metric for the colour stability after ICON® treatment. Another *in-vivo* study assessed the colour stability

of mild to moderate fluorosis after resin infiltration 12 months after treatment, but the methodology was entirely based on photographic images [8].

In addition, an *in-vitro* study evaluated colorimetric changes after resin infiltration on artificially created hypomineralized enamel lesions using spectrophotometric measurements [9]. Although a significant colorimetric effect was observed, reverting white spot lesions back toward the adjacent sound enamel, no qualitative colour data were provided. Moreover, another *in-vitro* study investigated the staining behaviour of demineralized enamel treated with artificial saliva, with daily application of 0.05% NaF and resin infiltration (ICON®, DMG) [10]. The ICON® group showed significantly higher staining after exposure to coloured solutions (red wine or coffee) than all other tested groups.

Controversies exist about what represents colour stability after resin infiltration. Leland *et al.* investigated the colour changes produced by ICON® in a recent *in-vitro* study, after accelerated aging was simulated by eating and drinking and the polishing represented by brushing/cleaning of teeth [2]. Accelerated aging produced a ΔE of 6.1 which decreased to 2.1 after polishing, indicating that 66% of the initial staining was eliminated by polishing. A very recent *in-vitro* clinical study by Jansen *et al.* to assess the possibility of whitening infiltrated and artificially stained enamel carious lesions, showed that all the tested bleaching agents could recover the visual appearance of infiltrated and stained caries-like enamel lesions [11].

We previously performed a retrospective clinical study to assess objective and subjective aesthetic outcomes of ICON® for enamel hypomineralized lesions on a large cohort of young adolescents [1]. The aesthetic outcome was subjectively evaluated by dentists with skills in cosmetic dentistry using FDI-colour match criteria and compared with objective colorimetric changes measured by a spectrophotometer. Our *in-vivo* clinical study results showed that the aesthetic outcome of resin infiltration was highly effective, according to both visual qualitative and spectrophotometric objective assessment [1]. The methodology for measuring the color difference between two different measurement points (affected and sound enamel) before and after ICON® procedure, shown in our previous study [1], has been used by others, such as in a recent evaluation study aiming to assess the influence of colour changes during the re-wetting process as a possible predictor for the final aesthetic outcome of ICON® [5]. Colorimetry in dentistry is based on objective assessments, mainly through the use of a spectrophotometer, and on subjective assessments that collect the impressions of patients or doctors. Objective measurements allow calculations of colour differences using different metrics (CIEDE2000, CIELAB, etc...) while subjective assessments allow the determination of thresholds of perception and acceptance [1, 12, 13].

Aim

The aim of this *in-vivo* study was to prospectively assess the subjective and objective colour stability after resin infiltration in patients with enamel hypomineralization lesions treated with ICON® 6 years after treatment.

2. Materials and Methods

2.1 Patients

All consecutive patients with hypomineralized enamel lesions on labial surface due to Early Caries Lesions (ECLs) and/or Developmental Defects of Enamel (DDE) who, in 2015, were treated using ICON® (DMG, Hamburg, Germany) infiltration were recalled for a follow-up at....., The follow-up visits were scheduled in 2021. Photographic and spectrophotometric assessments of the previously treated teeth were collected. Signed informed consent was obtained by all participants and the study received Ethical Committee approval (n.4280). All procedures were in accordance with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

2.2 Qualitative visual evaluation

Visual assessments were made directly by patients and then supported by the availability of digital photographs (Nikon D7100, 105 mm Macro lens, R1C1 Macro flash) with standardized camera settings (shutter speed 1/80 and aperture settings f32) and lighting conditions.

The qualitative visual evaluation was expressed by patients at T₀ (before treatment), T₁ (one year after treatment) and T₂ (six years after treatment), by applying the Fédération Dentaire Internationale (FDI) approved clinical aesthetic colour match and translucency criteria Evaluation Scale:

1. Clinically excellent/very good (Good colour match. No difference in shade and translucency);
2. Clinically good (Minor deviations);
3. Clinically sufficient/satisfactory (Clear deviation but acceptable. Does not affect aesthetics);
4. Clinically unsatisfactory (Localized-clinically unsatisfactory, but can be corrected by repair);
5. Clinically poor (Unacceptable. Replacement necessary.)

According to this classification, a five-point scale is used to qualitatively assess colour stability [14].

2.3 Quantitative spectrophotometric evaluation and measurements

Quantitative evaluation was assessed using a calibrated reflectance spectrophotometer (SpectroShade, MICRO, Serial NHDL1407, MHT, Arbizzano di Negrar, Verona, Italy) as previously explained [1]. Spectral reflectance was measured every 8nm and the on-board software of the MHT instrument calculated CIE (1976) $L^*a^*b^*$ colour coordinates of the measurement points on the enamel surface. The L^* value measures the lightness ranging from 0 (black) to 100 (white), a^* measures redness ($a^* > 0$) or greenness ($a^* < 0$), and b^* measures yellowness ($b^* > 0$) or blueness ($b^* < 0$).

To define colour stability two measurement points (sound and affected enamel) were chosen on each tooth at: T_0 (before treatment); T_1 (1 year after treatment) and T_2 (six years after treatment) (Figure 1). The colour difference between the two measurement points for each tooth was defined by the CIEDE2000 colorimetric [15-17] known as ΔE_{00} , which is perceptually more uniform than the older CIELAB equation. Thus ΔE_{00} between T_1 and T_0 and ΔE_{00} between T_2 and T_1 was calculated and compared with perceptibility (PT) and acceptability (AT) thresholds. Based on prior experiences [18,19], thresholds of 1.1 for PT and 3.3 for AT were used. A value of ΔE_{00} greater than 3.3 indicates a clearly detectable visual colour difference; ΔE_{00} between 3.3 and 1.1 indicates a perceptible (but acceptable) colour difference; a ΔE_{00} smaller than 1.1 indicates no visible difference.

2.4 Comparison between visual and spectrophotometric evaluations

The subjective assessment was compared with the objective spectrophotometric measurements to verify the degree of correlation between the two methods. We considered whether there was a relationship between FDI scores at the three assessment points and the instrumental assessments (ΔE_{00} values). Spearman's rank correlation coefficient was used to evaluate the relationship between visual and spectrophotometric measurements because of the ordinal scale nature of the visual assessments.

Figure 1 about here

2.5 Outcome measurements

Primary outcome

Objective measurement of colour stability of the treated teeth was by spectrophotometric assessment. Two measurement points (one on sound and one on the adjacent affected enamel) were selected to calculate the colour difference ΔE_{00} at: T_0 (baseline condition), T_1 (1 year after treatment) and T_2 (six years after treatment). The expected colour difference between T_1 and T_0 is < colour difference between T_2 and T_1 ; >Acceptability Threshold (AT: 3.3) and < Perceptibility Threshold (PT: 1.1), respectively.

Secondary outcome

The effect of infiltrative resin treatment on hypomineralized enamel lesions by means of FDI score change at T_0 (before treatment), at T_1 (1 year after treatment) and at T_2 (six years after treatment); the correlation between the qualitative FDI-scores and the quantitative by calculating CIEDE2000 colour differences (ΔE_{00}).

2.6 Statistical analysis

The R software program (R Foundation for Statistical Computing, Vienna, Austria, ver. 4.0.5) was used for the statistical analyses. A Wilcoxon signed rank test with continuity correction was used to compare the distributions of FDI scores values, testing the null hypothesis that the distributions are identical. A paired t-test was used to compare the means ΔE_{00} values, testing the null hypothesis that the average of the differences between the series of paired observation is zero. The correlation between visual and spectrophotometric measurements was quantified using the Spearman's rank correlation coefficient as a measure of the monotonic correlation between the two variables. The correlation coefficient ranges from -1 to $+1$, where ± 1 indicates a perfect monotonic relationship and 0 indicates no monotonic relationship.

3.Results

3.1 Study population

Fifteen patients (mean age 21, range 19–24) with hypomineralized enamel lesions on the labial surface due to ECLs and/or DDE, who were treated using resin infiltration, were recalled for a visit 6 years after treatment. One patient, accounting for two teeth, was lost at this follow-up point. A total of 74 permanent teeth (45 ECL and 29 DDE) from the 14 patients (10 female – 57 teeth and 4 male – 17 teeth) were assessed, with a mean of 5 teeth treated per patient (range 2–10). Therefore, a total of 222 (74 teeth \times 3) FDI scores and 148 ΔE_{00} were evaluated, accounting for after treatment and at 6 years of follow-up assessments of the affected and sound measurement points of each tooth.

3.2 Qualitative visual evaluations

The distribution of FDI scores at pre-treatment, post-treatment and long-term follow-up is shown in Table 1. Clinically excellent and good evaluations (score 1 and 2) were 13.5%, 90.6% and 93.2% at T₀, T₁ and T₂, respectively. These results indicate that the aesthetic effect was positively assessed immediately after treatment, and it was further improved at long-term follow-up.

Table 1. Distribution FDI scores at T₀, T₁ and T₂

FDI score	FDI T ₀	FDI T ₁	FDI T ₂
Total sample			
1	0.0%	59.5%	71.6%
2	13.5%	31.1%	21.6%
3	23.0%	5.4%	4.1%
4	44.6%	2.7%	1.4%
5	18.9%	1.4%	1.4%
ECL			
1	0.0%	60.0%	80.0%
2	15.6%	35.6%	20.0%
3	37.8%	4.4%	0.0%
4	42.2%	0.0%	0.0%
5	4.4%	0.0%	0.0%
DDE			
1	0.0%	58.6%	58.6%
2	1.3%	24.1%	24.1%
3	0.0%	6.9%	10.3%
4	48.3%	6.9%	3.4%
5	41.4%	3.4%	3.4%

Wilcoxon signed rank test was used to determine if FDI scores distributions at different times of follow-up were identical. Continuity correction was applied taking into account that visual assessments were continuous in theoretical nature. The differences in FDI scores distribution were significant between T_0 and T_1 for the Total sample and in both ECL (Early Caries Lesions) and DDE (Developmental Defects of Enamel) groups ($p < 0.001$). The differences were significant between T_1 and T_2 for the Total sample and in the ECL group ($p < 0.001$) but insignificant in the DDE group.

3.3 Quantitative spectrophotometric evaluations

ΔE_{00} values were calculated based on CIELAB color space L^*a^*b coordinates at two points of time. Descriptive statistics of this coordinates is shown in Table 2.

Table 2. CIELAB color space coordinates and ΔE_{00} mean and standard deviations

	L^*	a^*	b^*	ΔE_{00}
Total sample				
T_0	75.23 (7.92)	3.99 (3.6)	16.41 (9.58)	6.75 (3.83)
T_1	72.1 (5.63)	4.43 (3.08)	19.78 (5.69)	2.12 (1.52)
T_2	72.3 (5.44)	4.57 (2.78)	19.17 (5.53)	2.49 (2.05)
ECL				
T_0	76.59 (4.81)	3.92 (2.99)	16.21 (7.87)	5.41 (2.47)
T_1	72.47 (3.17)	4.97 (2.32)	20.71 (4.26)	1.48 (1.06)
T_2	72.59 (3.01)	4.97 (2.19)	20.15 (4.23)	1.78 (1.19)
DDE				
T_0	73.11 (10.93)	4.09 (4.45)	16.72 (11.9)	8.83 (4.61)
T_1	71.53 (8.14)	3.59 (3.88)	18.32 (7.22)	3.07 (2.30)
T_2	71.87 (7.91)	3.93 (3.45)	17.66 (6.89)	3.23 (2.26)

ΔE_{00} was evaluated on 74 teeth and the mean value between T_1 and T_0 was of 5.81, while the ΔE_{00} between T_2 and T_1 mean value was of 1.26 (Table 3).

Table 3. ΔE_{00} values (mean, Std. Dev and 95% Confidence interval) between T_1T_0 and T_2T_1 .

ΔE_{00}	n	mean	sd	95% Conf. Interval
Total sample				
$\Delta E_{00}(T_1-T_0)$	74	5.811	3.132	(5.085, 6.536)
$\Delta E_{00}(T_2-T_1)$	74	1.261	0.6375	(1.114, 1.409)
ECL				
$\Delta E_{00}(T_1-T_0)$	45	5.757	3.26	(4.778, 6.737)
$\Delta E_{00}(T_2-T_1)$	45	1.109	0.4904	(0.962, 1.257)
DDE				
$\Delta E_{00}(T_1-T_0)$	29	5.893	2.978	(4.76, 7.026)
$\Delta E_{00}(T_2-T_1)$	29	1.497	0.7662	(1.206, 1.789)

3.4 Comparison between visual and spectrophotometric evaluations

ΔE_{00} between T_1 and T_0 was significantly correlated with all three FDI measurements (Table 4). Positive correlation indicates that larger colour differences (ΔE_{00}) were observed in patients with higher FDI scores (note that patients with Clinically unsatisfactory or clinically poor visual assessments have more possibilities to improve colour). Between T_1 and T_2 there was a negative correlation which indicates that larger colour differences at the T_1-T_0 stage are often associated with smaller FDI scores (clinically excellent or clinically good). In the ECL and DDE groups the signs of correlation coefficients were the same (see Table 4); lower significance may be the result of lower number of teeth. ΔE_{00} between T_2 and T_1 was not significantly correlated with FDI score at T_0 , T_1 and T_2 neither for all sample nor in ECL and DDE groups.

Table 4. Correlation between ΔE_{00} and FDI

	FDI_ T_0	FDI_ T_1	FDI_ T_2
Total sample			
$\Delta E_{00}(T_1-T_0)$	0.288 (p=0.013)*	-0.359 (p=0.002)*	-0.329 (p=0.004)*
$\Delta E_{00}(T_2-T_1)$	0.004 (p=0.972)	0.174 (p=0.138)	0.171 (p=0.144)
ECL			
$\Delta E_{00}(T_1-T_0)$	0.367 (p=0.013)*	-0.284 (p=0.059)	-0.239 (p=0.113)
$\Delta E_{00}(T_2-T_1)$	-0.045 (p=0.767)	0.093 (p=0.542)	-0.030 (p=0.845)
DDE			
$\Delta E_{00}(T_1-T_0)$	0.179 (p=0.351)	-0.474 (p=0.009)*	-0.485 (p=0.008)*
$\Delta E_{00}(T_2-T_1)$	-0.277 (p=0.146)	0.267 (p=0.160)	0.277 (p=0.145)

* p<0.05

4. Discussion

This clinical study has, for the first time, assessed long term *in-vivo* colour stability after ICON® treatment by means of spectrophotometric calculations and patient-based subjective evaluations. Colour stability was assessed six years after treatment on hypomineralized enamel lesions treated by ICON® on 74 permanent teeth in 14 young adults. The results showed that at six years of follow-up the mean colour difference ΔE_{00} (T2–T1) was $1.261+0.637$, which indicated an excellent long-term colour stability. Aesthetic outcome of the colour stability was assessed by patients and more than 92% of them gave an FDI score of 1 or 2, corresponding to a clinically very satisfactory result.

In-vivo and *in-vitro* studies in recent years have extensively documented the clinical aesthetic efficacy of ICON® treatment, with repair of the enamel translucence and rise of the refractive index of the hypomineralized enamel [1,9,20-23]. However, the aspect that remained to be verified was the *in-vivo* stability of the treatment's outcome with a long period of follow-up.

The assessment of colour stability can be achieved with the use of photographic documentation or with the use of spectrophotometers. In both cases, the standardization of the methods used is fundamental both for providing data that can be compared, even from different sources and studies, and for the rigor required in presenting aesthetic results. The available literature on colorimetric stability after resin infiltration shows different types of approaches. For example, for the use of digital images, the colour stability after resin infiltration was recently assessed on a sample of three clinical cases presenting with white fluorotic spots. Although durability of the improvement of the patients' aesthetic outcomes was documented at 12 months of follow-up, the documentation was based uniquely on photographic images, performed with different camera settings and lighting conditions [8]. The study by Kobbe *et al.* to assess the influence of colour changes during the re-wetting process as a possible predictor for the result after ICON®, was entirely based upon digital photographic images but with standardized camera conditions [5]. As reference for colour standardization, a piece of a neutral grey card (18 % grey; Mennon, Lake Forest, USA) was used and positioned close to the tooth of interest. In the case of studies using a spectrophotometer, a recent clinical study assessed the stability of the aesthetic outcome of resin infiltration on post-orthodontic white spot lesions after at least 24 months after treatment. The spectrophotometric colour stability calculations used the older CIELAB colour-difference metric, which is less reliable than the CIEDE2000 metric [24]. In the present study the colour change between the two measurement points before, after and at six years of follow-up for

each tooth was defined by CIEDE2000, known as ΔE_{00} , which is more perceptually uniform than the older CIELAB equation [15-17, 25].

In-vitro studies to assess ICON® colour stability showed great variability in the methodology used: i) differences in the material used as a sample (test material discs, human enamel, bovine enamel); ii) differences in the solutions used to test the colour stability (artificial saliva, colored solutions, coffee or wine); iii) differences in observation time and iv) methodology used for detecting the colour itself [10, 23, 26-28]. Overall, these *in-vitro* studies assessed the colorimetric stability of ICON® over a short or very short period (immediate evaluation, after 4-6 or 8 weeks), showing that ICON® was subject to greater colour variation or susceptibility to accelerated aging compared to the tested materials [27,28]. The study by Chen *et al.* demonstrated that after 96 h of accelerated aging ICON® showed a significant colour change (4.70 ± 0.69) [27]. The study by Zhao *et al.* showed a colour difference of 12.7 ± 4.7 after immersion into coffee staining solution for 180 h at 37°C [28].

Moreover, in 2014 Borges *et al.* evaluated the *in-vitro* color stability of ICON® after exposure to different staining solutions and showed that the resin-infiltrated group exhibited the highest staining values, but the repolishing procedures resulted in significantly decreased color change [10]. The clinical significance of these results was that absence of tooth brushing probably could increase the staining susceptibility. In addition to re-polishing, patients treated with the ICON® can undergo tooth whitening, as recently shown in the study by Jansen *et al.* [11]. This evidence represents an important advance in clinical research on ICON® as it shows that resin infiltration can be integrated with other dental procedures and therefore treating the patient with the ICON® does not rule out other therapeutic choices. [1,11,29].

The current study presents, for the very first-time, data on colour stability six years following treatment of ICON®. The study has shown that colour stability remains with an average ΔE_{00} of 1.261 ± 0.637 and CI :1.114, 1.409. The human eye perception thresholds used in this study were 1.1 for PT and 3.3 for AT. However, these are conventional and experimental values that deserve further research. Though, the colorimetric data, with an average ΔE_{00} of 1.261 ± 0.637 , indicates that colour stability is maintained over time [18, 19].

In the current study the subjective observations of patients according to the Fédération Dentaire Internationale (FDI) approved clinical aesthetic colour match and translucency criteria Evaluation scale were assessed, with more than 92% having score 1 and 2, indicating a clinically excellent/good results [14]. Clinically excellent and good evaluations were 13.5%, 90.6% and

93.2% at T₀, T₁ and T₂, respectively, indicating that the aesthetic effect was further improved at long-term follow-up. The analysis of FDI correlation with ΔE_{00} confirmed the objective evaluation of colour stability. The results of our previous study on subjective and objective evaluation of in-vivo aesthetic outcome of ICON® showed that a ΔE_{00} threshold of 1.73 can be used to determine whether the outcome is clinically excellent or good, after treatment. The results of the present study showed that the colour stability at 6 years of follow-up is correlated with a ΔE_{00} threshold of 1.261, which further improves the already excellent aforementioned results.

Finally, no patient reported side effects or complaints. In addition, no unwanted effects such as tooth or soft tissue hypersensitivity, loss of vitality, discoloration or gingival changes were observed after treatment. Furthermore, none of the patients required correction of treatment with additional aesthetic prosthetic procedures.

In conclusion, this study provides data based on the use of a rigorous, reproducible and updated methodology in the field of dental colorimetry, further supporting the results of previous studies based on shorter observation periods on the first-rate stability of enamel colour after resin infiltration procedure. The excellent colorimetric stability demonstrated in this study should further encourage the choice of resin infiltration as the first option for the minimally invasive treatment of hypomineralized enamel lesions in aesthetic sectors.

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Declaration of interest

The authors declare that they have no known competing financial interest or personal relationships that could have appeared to influence the work reported in this paper.

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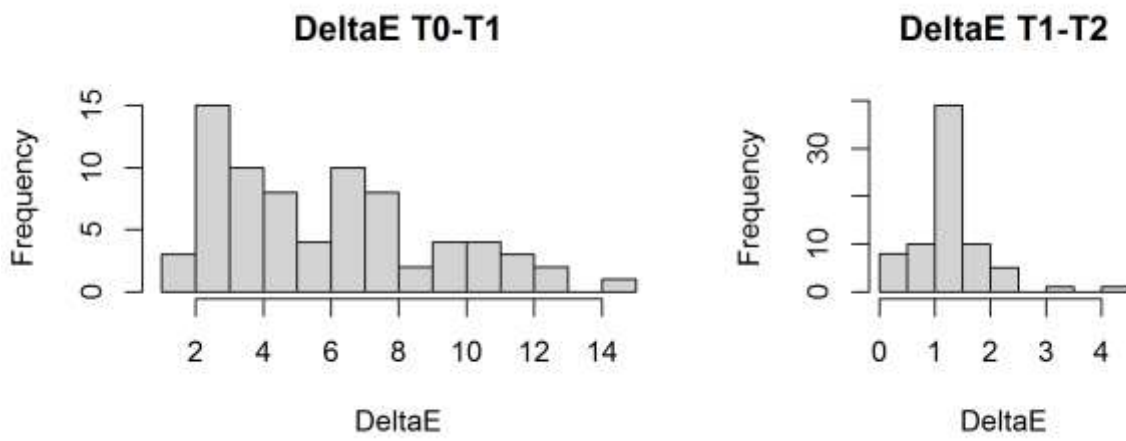
Caption to Figure 1

Example of case record clinical spectrophotometric (SP) documentation: (a–b–c) before, after and 6 years after treatment SP overall clinical crown polarized image; (d–e–f) before, after and 6 years after treatment colour distribution and overall detailed mapping (Vita 3D Master Scale); (g–h–i–l–m–n) affected and sound enamel two measurement points before, after and 6 years after treatment; (o–p–q) CIE L*a*b* colour coordinates of the measurement points.



Figure 1

Fig 2.



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