Systematic review of in vitro studies evaluating tooth bleaching efficacy

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Abstract: Purpose: To review and assess the literature on in vitro studies evaluating tooth bleaching efficacy considering the use of a negative control, type of tooth substrate, storage medium, color evaluation methods, and evaluation time points. **Methods:** The following databases were searched: PubMed (MEDLINE), Web of Science. Search used Medical Subject Headings (MeSH) in PubMed in addition to free text. The following limits were applied: English, articles published between January 1989 and October 2017. Additional free text key terms included: in vitro, tooth bleaching, placebo, negative control, overall CIELAB color change (ΔE*ab), change in shade guide units (ΔSGU), tooth color stabilization, evaluation time points, bovine teeth, and staining. Search was repeated in Web of Science but no additional articles were identified. A total of 11 studies were included for qualitative and quantitative analysis. **Results:** The meta-analysis of nine included studies that reported ΔE*ab values, revealed that the NC statistically exceeded the perceptibility threshold (PT) of 1.2 ($P< 0.05$). The estimate was 2.872 with lower and upper bounds of 1.955 and 3.790, respectively. (*Am J Dent* 2020;33:17-24).

Clinical significance: Randomized controlled trials are gold standards to evaluate bleaching efficacy of different materials. However, in vitro studies offer a way to screen for potential bleaching efficacy. It is vital to determine an appropriate cut-off value for determining bleaching efficacy in vitro and further apply for clinical relevance.

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Introduction

The ever-increasing demand for beautiful teeth has driven the growth of the global tooth whitening market. The United States tooth whitening market was valued at $1.7 billion in 2019, and is expected to grow substantially and reach over $2 billion in 2024.[1] Improvements in product efficacy achieved through advancements in technology are enabling competitive brands to effectively compete by offering superior product features and therapeutic benefits.[1]

The International Organization for Standardization (ISO) creates documents that provide requirements and guidelines to ensure that materials and products are fit for their purpose.[2] Thus, ISO offers a platform for all stakeholders to join together and create collaborative solutions that yield decisions to improve and support healthcare. The ISO 28399 standard is one of the standards obtained through international consensus for 'Products for External Tooth Bleaching', that are used for changing the color of natural teeth towards a lighter or whiter shade. The standard includes test methods for laboratory assessment of tooth bleaching efficacy.[3] Despite the fact that it has been used widely since its publication in 2011, there still remain issues to be addressed to identify the most reliable ways to measure efficacy, determine the best substrate for specimen preparation, and define how to properly interpret bleaching efficacy results.

Tooth bleaching efficacy has been evaluated visually with shade guides and instrumentally with electronic color measuring devices. Frequently used devices are spectrophotometers, colorimeters, and imaging systems for traditional digital imaging and spectral imaging.[4] The 'ISO/TR 28642' outlines the interpretation of color compatibility results under controlled conditions and methods. Based on the report, color compatibility between dental materials and human tissues present a very good match if the color difference is at or below ΔE*ab =1.2, while a difference above ΔE*ab=2.7 is considered to be an unacceptable match.[4] Thus, the use of perceptibility (PT) and acceptability thresholds (AT) are clearly defined for dental materials and human tissues related to color compatibility. A few studies[5,6] have proposed the use of perceptibility and acceptability threshold as a reference number to determine bleaching efficacy. The use of a negative control group is a common practice for in vitro studies[7,8] to confirm the validity of the experimental design and procedure. However, the application of thresholds for the interpretation of bleaching efficacy with regards to values obtained from the negative control groups has not been fully investigated.

Another important aspect for bleaching efficacy interpretation is the time point of post-bleaching tooth color measurement. Laboratory studies that evaluated the efficacy of bleaching materials on extracted human or bovine teeth either did not report when the post bleaching measurement was made or made measurements ranging from immediately after bleaching treatment to up to 6 months post treatment. A study[9] that measured post-bleaching tooth color at different time points showed an increase in overall color change (ΔE*ab) and lightness (ΔL*) up to 1 week followed by a gradual stabilization at 6 weeks post bleaching. This is in accordance with clinical recommendations to wait for 2-6 weeks for the post bleaching color to stabilize.[10]

Therefore, this study reviewed the literature on in vitro studies evaluating bleaching efficacy considering the use of a negative control, type of tooth substrate, storage medium, color evaluation methods, and evaluation time points. The first null hypothesis tested was that the overall color change (ΔE*ab) of the negative control groups measured instrumentally would not exceed the PT of 1.2. Additionally, the change in shade guide units (ΔSGU) of the negative control groups measured visually would not be equal to zero.

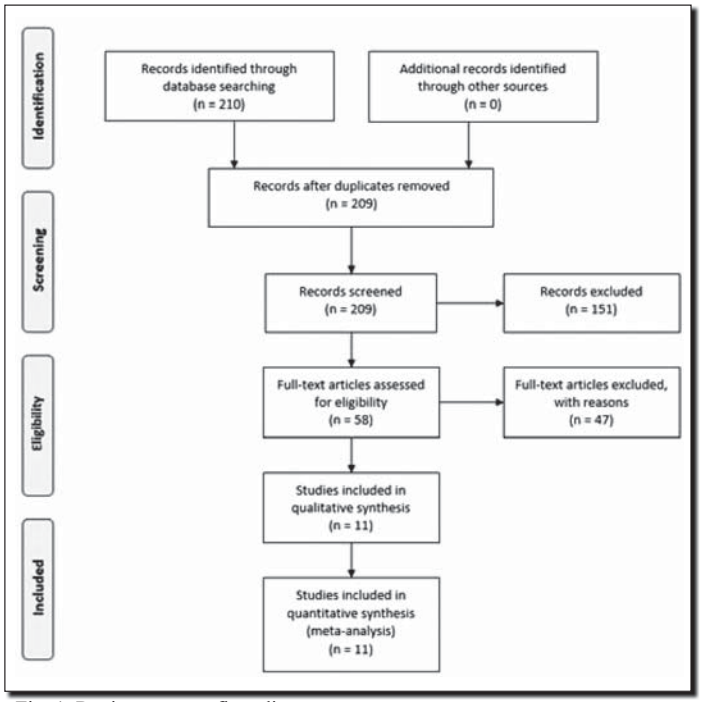

Fig. 1. Review process flow diagram.

Materials and Methods

Selection criteria of studies - Types of studies - All in vitro studies comparing bleaching methods based on the classification into four categories were evaluated:[11] professionally applied in-office (OW), professionally dispensed at-home (HW), over-the-counter (OTC) tooth bleaching products or do-it-yourself (DIY) bleaching material (via chemical bleaching action) with a negative control group were included.

Types of test substrates - Extracted human or bovine teeth that were unstained or artificially stained were considered.

Types of interventions - OW, HW, OTC, or DIY bleaching materials that have a bleaching action rather than an abrasive action to remove superficial stains were considered. Various forms and delivery systems such as gels, paste, liquids, trays, paint-on films, bleaching strips, with or without light-activation systems were included.

Types of outcome measures - The data assessing bleaching efficacy are determined using one of the two methods:

1. Visual measurements by examiners using acceptable shade guides (e.g. VITA Classical[a] or VITA Bleachedguide 3D-Master[b]) or equivalent guides. The shade guide tabs are ordered according to value where lower numbers indicate higher value or lightness. The change in shade guide units (ΔSGU) was calculated by subtracting the number of post-treatment (post-tx) tabs to the baseline tabs.

2. Instrumental measurements obtained using digital imaging/software, spectrophotometers, and colorimeters. These instruments provide the value of three color-coordinates: Lightness (L*) ranging from 0 to 100 black representing black and white, respectively; chroma in red-green (a*); and chroma in yellow-blue (b*). ΔE*ab was computed by the square root of the sum of squared values of the differences in the L*a*b* scales between the baseline and follow-up assessments as described by the Commission International de L'Eclairage.[12] The overall color change does not indicate the direction of change in the composite assessment of tooth color. In this review, however, given that all materials produce whiter teeth, the direction of change is positive towards an increase in lightness and negative towards a reduction in yellow-blue chroma.

Studies that measured tooth color visually or instrumentally and reported ΔE*ab or ΔSGU were included.

Quality assessment of studies - Included studies were assessed on three criteria: randomization of specimens (yes, no, not reported); blinding of operators and examiners (yes, no, not reported); and information on the training of operators and exa-

Table 1. Characteristics of excluded studies (N=47).

Name	Year	Reason for exclusion
White[25]	2000	No data on ΔE*ab available
Pretty[26]	2001	Evaluation of stain removal efficacy
Clelland[27]	2002	ΔE*ab not reported for the negative control group
White[28]	2002	No data on ΔE*ab available
Dostalova[29]	2004	ΔSGU not reported for the negative control group
Wetter[30]	2004	No negative control
Wetter[31]	2004	No data on ΔE*ab available
Sulieman[32]	2005	No negative control: negative control used was light activated with Xe/Halogen, Plasma arc, Optilux, laser diode
Wiegand[33]	2005	No negative control
Adeyemi[34]	2006	Evaluation of stain removal efficacy
Dietschi[35]	2006	No negative control
Duschner[36]	2006	No data on ΔE*ab available
Sulieman[37]	2006	No negative control
Joiner[38]	2008	Evaluation of stain removal efficacy
Lee[39]	2008	Evaluation of stain removal efficacy
Lima[40]	2008	Evaluation of stain removal efficacy
Manton[41]	2008	No negative control
Patel[42]	2008	No data on ΔE*ab available
Polydorou[43]	2008	No negative control
Wriedt[44]	2008	No negative control
Bruzell[45]	2009	No negative control
Goharkhay[46]	2009	No negative control
Lima[47]	2009	No negative control
Al Machot[48]	2010	ΔE*ab and ΔSGU not reported for the negative control group
Dietschi[49]	2010	No negative control
Markovic[50]	2010	No negative control
Scaminaci Russo[51]	2010	Measured the color stability of bleached and non-bleached teeth to staining cycles
Travassos[52]	2010	No negative control
Borges[53]	2011	No negative control
Caneppele[54]	2011	ΔE*ab not reported for the negative control group
Llambes[55]	2011	No negative control
Cunha[56]	2012	No negative control
D'Arce[57]	2012	No negative control
Grundlingh[58]	2012	ΔSGU not reported for the negative control group
Liang[59]	2012	No negative control
Lima[60]	2012	No negative control
Fornaini[61]	2013	No negative control
Hahn[62]	2013	No negative control
Jin[63]	2013	No negative control
Kwon, Wang[64]	2013	Bleaching efficacy was measured on enamel and dentin specimens separately
Kwon, Wertz[65]	2013	No negative control
Liang[66]	2013	No negative control
Tam[67]	2013	In situ study
Bennett[68]	2015	No data on ΔE*ab available
Kwon, Kurti[69]	2015	No negative control
Bortolatto[70]	2016	No negative control
Kwon, Dawson[71]	2016	No negative control

Table 2. Characteristics of included studies with instrumental measurements.

Author	Year	Substrate	Storage medium	Groups	N	Instrument	Summary of data		Measurements
Jones[14]	1999	Human teeth Maxillary central incisors	Distilled water			Minolta CR-221	Mean ΔE* at 2 wks of Tx	SE	Baseline at 1 wk and at 2 wks Not clear how much time elapsed after post tx to measurement
				NC: Distilled water	10		1.1	0.47	
				HW: 10% CP (2 hrs × 14)	10		5.7	0.74	
				OW: 35% HP with argon (3 min appl with 30 sec laser × 5)	10		2.7	0.44	
Kishta-Derani[15]	2007	Human teeth Maxillary anterior teeth	Artificial saliva			Minolta CR321	Mean ΔE* at 2 wks of Tx	SD	Baseline, at 1 wk and at 2 wks Not clear how much time elapsed after post tx to measurement
				NC: Artificial saliva	12		2.9	1.55	
				OTC: 10 % CP (one paint application ×14)	12		3.8	1.81	
				OTC: 19% sodium per-carbonate (one paint application ×14)	12		5.6	1.84	
				OTC: Urea peroxide (one paint application × 14)	12		4.4	1.89	
				OTC: 8.7% HP (one paint application ×14)	12		5.5	2.15	
Knösel[16]	2011	Human teeth Incisors and Canines	Artificial saliva			Photoshop digital images	Mean ΔE* at 2 wks of Tx	CI 95%	Baseline, 2, 4, 12, 26 wks post bleaching
				NC: No treatment	77		3.9	3.37:4.35	
				HW: 15% CP (8 hrs × 5)	77		6.9	5.55:7.12	
				OW: 38% HP (3 ×15 min)	77		6.3	5.45:7.17	
Meireles[17]*	2012	Bovine teeth Incisors 6x6x3 Mm blocks	Distilled water			Vita Easyshade	Mean ΔE* at 1 wk Post-Tx	SD	Baseline, 1 wk at tx and 1 wk post tx
				NC: Distilled water	15		5.5	2.9	
				HW: 10% CP (4 hrs ×14)	15		15.1	5	
				OW: 37% CP with light activation (60 min × 3)	15		13.6	3.5	
Kwon, Oyoyo[18]	2013	Human teeth canines	Artificial saliva			Vita Easyshade	Mean ΔE* at 4 wks Post-Tx	SD	Baseline, 1-hr, 1 day, 1-, 4-, 8-, 12-, 16-, 20-, 24 wks post tx
				NC: Opalescence Boost Bases	10		1.2	0.6	
				OW: 40% HP (60 min × 3)	10		7.9	3.1	
				OW: 40% HP with light activation (60 min × 3)	10		14.6	3.1	
Dantas[19]	2015	Bovine teeth 6×6×2 mm blocks	Artificial saliva			Vita Easyshade	Mean ΔE* at 2 wks Post-Tx	CI 95%	Baseline 7-, 14-, 21-days post tx
				NC: No treatment	15		4.3	1.8	
				HW:10% CP (4 hrs ×14)	15		10.5	1.8	
				OW:35% HP (45 min ×2)	15		10.7	1.8	
Kwon, Meharry[20]	2015	Human teeth Molars	Artificial saliva			Vita Easyshade	Mean ΔE* at 1 wk Post-Tx	SD	Baseline 1-week, 1-month, and 3-month post tx
				NC: Water of Grade 3	20		3.2	2	
				DIY: Strawberry mix (5 min × 3)	20		4	1.9	
				OTC: 9.5 % HP Strips (2 hrs × 7)	20		10	2.6	
				HW: 10% CP (6 hrs × 14)	20		13.8	2.4	
				OW: 25% HP Zoom light (45 min × 3)	20		17.3	3.3	
Park[21]	2016	Human teeth Molars	Artificial saliva			Vita Easyshade	Mean ΔE* at 1 mt Post-Tx	SD	Baseline, 1-day and 1-month post tx
				NC: Glycerin Gel	15		1.4	0.7	
				OW: 20% HP (30 min × 3)	15		11.7	2.8	
				OW: 25% HP (30 min × 3)	15		10	2.8	
				OW: 25% HP with light activation (30 min × 3)	15		15.1	2.2	
Santana[22]*	2016	Human teeth Third molars 3×3×3 mm blocks	Humid environment			Photoshop digital images	Mean ΔE* at 12 hrs Post-Tx	SD	Baseline, 12 hrs post tx
				C: Distilled water	10		3	2	
				OW: 37.5 % HP (8 min × 4)	10		4	3	

* Stained teeth.

miners (yes, no, not reported). The overall rating of bias was modified to fit the study and based on the scale reported in the Risk of Bias tool 2.0 of Cochrane Collaboration and applied to included studies.[13] Studies that met all the criteria for evaluating quality, were rated with 'low' bias. Studies that met partly, one or more of the criteria, were rated with 'moderate' bias. Studies that did not meet one or more of the criteria were rated with 'high' potential for bias.

Search methods for identification of studies - The following databases were searched: PubMed (MEDLINE), Web of Sci-

ence. Search used Medical Subject Headings (MeSH) in PubMed in addition to free text. The following limits were applied: English, articles published between January 1989 and October 2017. Additional free text key terms included: in vitro, tooth bleaching, placebo, negative control, overall color change (ΔE*ab), change in shade guide units (ΔSGU), tooth color stabilization, evaluation time points, bovine teeth, and staining. The search was repeated in Web of Science but no additional articles were identified.

Search strategy - Search ("Tooth Bleaching/methods" [Mesh] OR "Tooth Bleaching/statistics and numerical data"[Mesh]) in vitro Sort by: Relevance, Filters: Publication date from 1989/01/01 to 2017/10/01; English.

Data collection and analysis - A total of 209 articles were retrieved from the initial search after removing all duplicates. Two authors (SR and MW) independently reviewed the titles and abstracts of all articles. Disagreements between the two review authors were resolved by consensus. Upon abstract review, 151 articles did not meet the inclusion criteria and were excluded. Full copies were obtained of all remaining articles, which also included those where there was uncertainty about the inclusion criteria. Full text articles of 58 titles[14-71] were further reviewed to extract data and confirm uncertainties of inclusion criteria based on the abstract review (Fig 1). Details of studies excluded following this stage were entered into the characteristics of excluded studies (Table 1).

Heterogeneity was verified using the I2 test with a significance level P< 0.10. Data were meta-analyzed with the inverse variance method and random effects model. To meta-analyze data generated using the overall color change (ΔE*ab) and the change in Vita Classical shade guide units (ΔSGU), the confidence intervals or standard errors were converted to standard deviation data. One study[19] did not specify whether the interval provided for variability was a confidence interval. We assumed the interval was a confidence interval, and transformed the interval into a standard deviation to continue with additional analyses. Another study[24] provided values of '0' for measures of variability. Those values were transformed to 0.001 to continue with additional analyses. Meta-analyses were undertaken to analyze the null hypotheses that ΔE*ab of negative controls would not exceed the perceptibility threshold of 1.2 and that ΔSGU would not be zero. Sub meta-analyses were further carried out to test the hypothesis that the OW, HW, and OTC groups would exceed the upper bound of the negative controls for instrumental measurements. Additionally, random effects meta-regression was performed to evaluate the effect of "concentration" on the overall estimate for OW, HW, and OTC. Analysis was conducted with R version 3.6.0 (meta package) and SAS[b] version 9.4.

Results

Characteristics and quality of included studies - This review included 11 selected in vitro studies.[14-24] Of the 11 studies, nine used instrumental measurements: spectrophotometer (N=5),[17-21] chromameter (N=2),[14,15] and digital images with software (N=2)[16,22] while four studies[15,20,23,24] used visual measurements with the VITA Classical shade guide. Of the nine studies using instrumental measurements, two also performed visual measurements.[15,20]

There were a total of 621 human and bovine teeth in the nine studies that used instrumental measurements. Out of 621 teeth, there were 184, 212, 137, 68, and 20 teeth for the NC, OW, HW, OTC, and DIY groups respectively. There were a total of 254 teeth in the four studies that used visual measurements with the Vita Classical shade guide. Out of the 254 teeth, there were 53, 50, 63, 68, and 20 for the NC, OW, HW, OTC, and DIY groups respectively. Tables 2 and 3 present information on the tooth substrate, storage medium, shade assessment instrument, measurement time points used by study.

The time point of instrumental or visual assessment after bleaching varied. The majority of studies had a measurement at 12-24 hours post-treatment[18,21-24] and at 1-2 weeks post-treatment.[16-20] Three studies[16,18,20] included post-treatment measurements of up to 3-6 months. In two studies[14,15] measurements were made post-treatment but it was not clear how much time elapsed from post-treatment to the actual measurement.

Artificial saliva (54%) was the most common storage medium, followed by distilled water (27%), saline solution (9%), and humid environment (9%). The treatment agent for the negative control included saline, distilled water, artificial saliva, glycerin, bleaching agent base, or no treatment at all.

Of the 11 included studies nine (82%) reported the randomization of specimens into treatment groups, four (36%) reported blinding of examiners/operators, and six (55%) reported training and/or calibration of examiners/operators. Table 4 shows the risk bias of all included studies.

Characteristics of excluded studies – Table 1 displays the characteristics of excluded studies and lists the exclusion reasons of each of 47 out of the 58 studies. The most common reason for exclusion was the lack of a negative control in the study design (N=28) followed by lack of reporting of ΔE*ab values (N=6). Some studies did use a negative control group but did not report ΔE*ab or ΔSGU* associated with it in their results (N=5). Other exclusion reasons included evaluation of stain removal rather than bleaching efficacy, in situ study design, and measurement of enamel and dentin substrate separately.

Meta-analysis results - The meta-analysis of nine included studies that reported ΔE*ab values revealed that the NC statistically exceeded the PT of 1.2 (P< 0.05) (Fig. 2). The estimate was 2.872 with lower and upper bounds of 1.955 and 3.790, respectively. Thus, even the negative controls demonstrated perceivable tooth color change, when measured instrumentally. The heterogeneity (I2= 94.48) of the studies reporting ΔE*ab values of negative controls was statistically significant (P< 0.001). Six studies were either below[14,18,21] or above[16,17,19] the estimate while only three studies[15,20,22] were close to the estimate. When further using the upper bound of 3.790 as a reference point to determine bleaching efficacy, we found that OW and HW were statistically higher (P< 0.05) than the cut-off value (Figs. 3, 4). There was high heterogeneity among different OW (I2= 98.33), HW (I2= 97.73) and OTC (I2=94.59) products evaluated (Figs. 3-5). The analysis of four included studies that reported ΔSGU included zero in the confidence interval (P> 0.05), indicating no color change of negative controls when measured visually with shade guides

Table 3. Characteristics of included studies with visual measurements.

Author	Year	Substrate	Storage medium	Instrument	Groups	N	Summary of data		Measurements
Leonard[23]	1998	Human teeth Incisors, canines	0.9% saline	Vita Classic			Mean ΔSGU at 16 hrs post-tx	SD	Baseline and and premolars 16 hrs post tx
					NC: Saline	11	-0.1		
					HW: 10% CP`	33	5.1		
Kishta-Derani[15]	2007	Human teeth Maxillary anterior teeth	Artificial saliva	Vita Classic			Mean ΔSGU at 2 wks of tx	SD	Baseline, at 1 wk and at 2 wks
					NC: Artificial saliva	12	0.6	1	Not clear how time elapsed after post tx to measurement
					OTC: 10 % CP (one paint application × 14)	12	2.6	2.3	
					OTC: 19% sodium percarbonate (one paint application × 14)	12	4.2	2.7	
					OTC: Urea peroxide (one paint application × 14)	12	3.1	2.6	
					OTC: 8.7% HP (one paint application × 14)	12	4.6	2.8	
Rees[24]*	2009	Human teeth Third molars	Distilled water	Vita Classic			Mean ΔSGU at 24 hrs post-tx	SD	Baseline and 24 hrs post tx
					NC: Water	10	0	0	
					HW: 10% CP (2 hours ×7)	10	13.2	1.3	
					OW: 15% HP (1 hour)	10	13.5	1.0	
					OW: 16% HP (15 min × 3)	10	10.9	2.9	
					OW: 16% HP (15 min × 3)	10	11.5	2.1	
Kwon, Meharry[20]	2015	Human teeth Molars	Artificial saliva	Vita Classic			Mean ΔSGU at 4 wks post-tx	SD	Baseline 1-week, 1-month, and 3-month post tx
					NC: Water of Grade 3	20	1.3	1.4	
					DIY: Strawberry mix (5 min × 3)	20	-0.1	1.8	
					OTC: 9.5 % HP Strips (2 hrs × 7)	20	8.1	2	
					HW: 10% CP (6 hrs × 14)	20	7.7	3	
					OW: 25% HP Zoom Light (45 min ×rese 3)	20	8	2	

* Stained teeth

Table 4. Quality assessment of included studies.

Author	Randomization	Blinding	Training	Risk of bias	Funding
Leonard[23]	Yes	NR	Yes	Moderate	Bleaching materials were provided by manufacturer(s)
Jones[14]	No	NR	NR	High	Bleaching materials were provided by manufacturer(s)
Kishta-Derani[15]	Yes	Yes	Yes	Low	Bleaching materials were provided by manufacturer(s)
Rees[24]	Yes	NR	NR	Moderate	NR
Knösel[16]	Yes	Yes	Yes	Low	NR
Meireles[17]	NR	Yes	Yes	Moderate	NR
Kwon, Oyoyo[18]	Yes	NR	NR	Moderate	Bleaching materials were provided by manufacturer(s)
Dantas[19]	Yes	Yes	Yes	Low	Supported by FGM Produtos Odontologicos
Kwon, Meharry[20]	Yes	NR	Yes	Moderate	NR
Park[21]	Yes	NR	NR	Moderate	Bleaching materials were provided by manufacturer(s)
Santana[22]	Yes	NR	NR	Moderate	NR

NR: not reported.

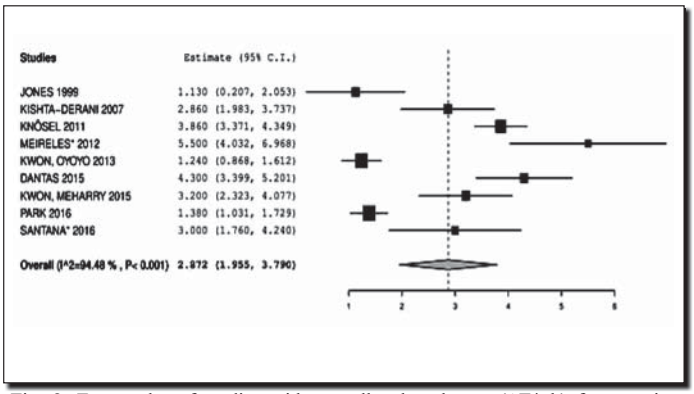

Fig. 2. Forest plot of studies with overall color change (ΔE*ab) for negative controls.

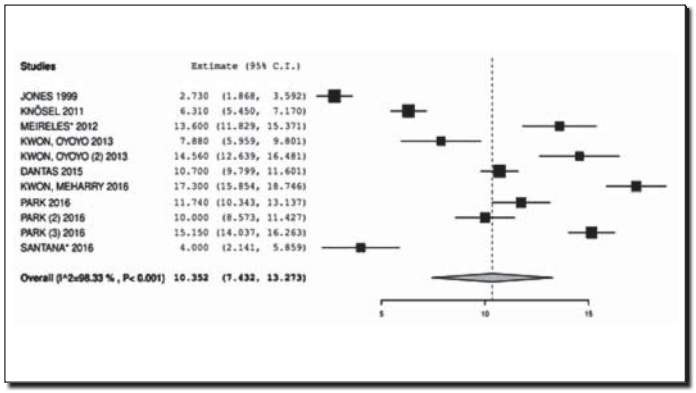

Fig. 3. Forest plot of studies with overall color change (ΔE*ab) for office whitening groups.

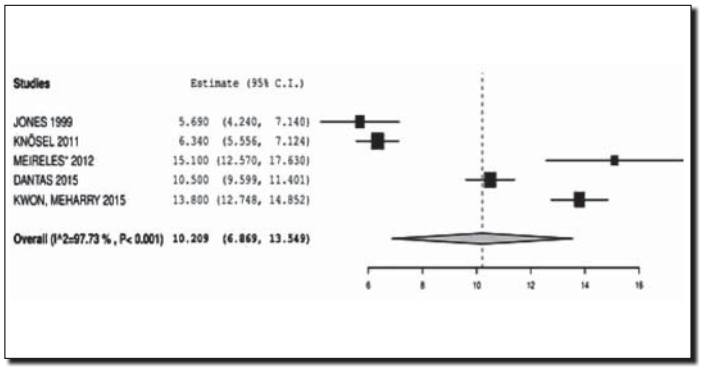

Fig. 4. Forest plot of studies with overall color change (ΔE*ab) for home whitening groups.

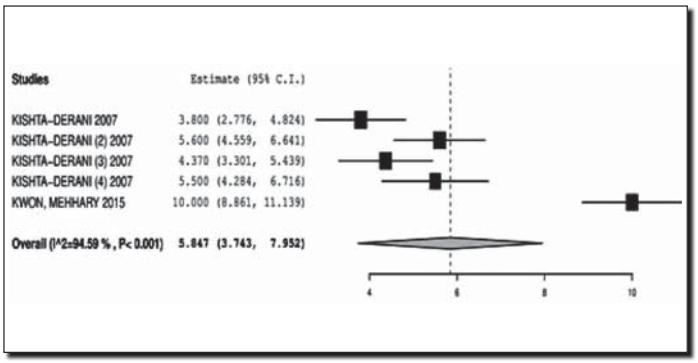

Fig. 5. Forest plot of studies with overall color change (ΔE*ab) for OTC groups.

(Fig. 6). A sub-meta-analysis was performed on different bleaching regimens on studies with instrumental measurements only since there were not enough studies for the visual assessments.

Random effects meta-regression showed that concentration had a very weak, or no statistically significant effect on the overall estimate for OW (P= 0.116), HW (P= 0.166), and OTC (P= 0.729).

Discussion

As product development advances and the consumer market increases in the field of tooth bleaching, it is important for oral health professionals to develop well-designed in vitro studies that provide evidence to support efficacy of bleaching products and regimens. Despite the fact that our literature search yielded over 200 articles, out of the 58 selected for full review, the most common reason for exclusion for the meta-analysis was the lack of a negative control or not reporting the results of the control group. The control group consists of elements that present exactly the same characteristics of the experimental group, except for the variable applied to the latter.[7,8] Thus, the control group enables the experimental study of one variable at a time and should be included in in vitro study designs.

So far there has been no study that evaluated the color stability of teeth from the time of extraction to disinfection and storage over time. It is assumed that extracted teeth stored in saline or artificial saliva will be stable, with no perceivable tooth color change. This is the first review that analyzed the overall tooth color change of negative controls in studies that used instrumental and visual color assessments. The reviewed studies included tooth color measurement as early as immediately after bleaching treatment to up to 6 months post-treatment. In the current study, a meaningful homogenous range of times was defined as to include at 2 weeks of treatment to 4 weeks post-treatment in the meta-analysis. Based on the results, the first null hypothesis was rejected. ΔE*ab of the negative controls did exceed the PT of 1.2. However, the second null hypothesis was not rejected, supporting the assumption that extracted teeth do not change color over time when evaluated visually with a shade guide. It is also important to note the high heterogeneity of the studies reporting ΔE*ab values of the negative controls. On exploring potential factors that may contribute to higher standard errors, it was noted that studies using digital images with Adobe Photoshop[16,22] and artificially stained teeth[18,22] had a wider range in confidence intervals. In contrast, studies[18,21] that used nail varnish to limit the bleaching area and enable repeated measurements showed the least variation.

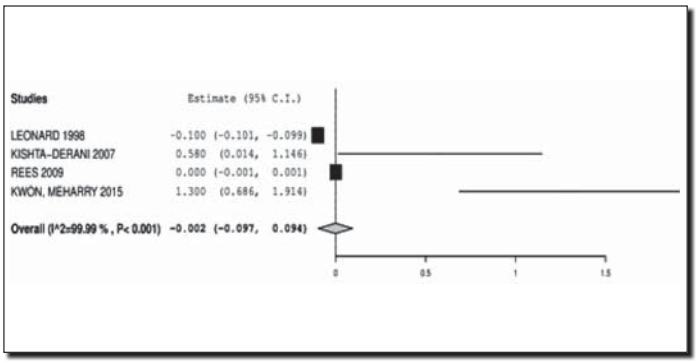

Fig. 6. Forest plot of studies with change in shade guide units (ΔSGU) for negative controls.

The findings of this review prompt re-evaluation of the current interpretation of bleaching efficacy as described in ISO 28399, which considers the bleaching efficacy of a product acceptable if ΔE*ab, after the treatment, resulting from increased ΔL* and decreased Δb* is two or greater compared to that before the treatment. With a lower bound of 1.955 and an upper bound of 3.790, many of the negative controls may be considered erroneously as an efficacious bleaching material. A recent study[5] summarized the interpretation of bleaching efficacy through PT and AT. Based on the study, a bleaching material is deemed not effective when ΔE*ab is equal or less than 1.2; moderately effective when between 1.2 and 2.7; good when between 2.7 and 5.4; very good when between 5.4 and 8.1; and excellent when exceeding 8.1. Application of this interpretation again would deem negative controls moderately effective.

The question remains as to how to determine when a bleaching material should be considered effective when evaluated in vitro. In this review the upper bound of "ΔE*ab =3.790" as a potential cut-off value was further tested to determine bleaching efficacy. Based on the meta-analysis, all HW and OW products tested exceeded this value while the OTC products were close to the border with a lower bound of 3.743. Noteworthy was also the high heterogeneity of the studies included. The concentration of the home bleaching groups varied from 10 to 15% carbamide peroxide; over-the-counter bleaching groups had a range from 10% carbamide peroxide to 9.5% hydrogen peroxide; and the office bleaching group varied from 37% carbamide peroxide to 40% hydrogen peroxide. Al-

though high heterogeneity is expected for testing bleaching materials with varying concentrations and treatment regimens specific for their purpose it is not desired nor anticipated in the negative controls. Therefore, it would be desirable to include step-by-step directions from specimen preparation to storage and measurement methods to standardize the methods for bleaching efficacy evaluation with the aims to obtain more reliable results among the negative controls, which would then also support the validity of the study design. Additionally, the inclusion of a positive control could be suggested to further validate the in vitro model.

One study[5] used the CIELAB color difference formula. In the future, it is also recommended to evaluate results obtained from the CIEDE2000 formula, since it has been shown to better represent the human perception of color differences (95% agreement with visual findings) when compared to the CIELAB formula (75% agreement).

Within the limitations of this review, it is concluded that with an estimate of $\Delta E^*ab = 2.872$, the overall color change of negative controls used in in vitro studies exceeds the perceptibility and acceptability threshold. Therefore, it is vital to further investigate the appropriate cut-off value for determining bleaching efficacy in vitro.

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Disclosure statement: The authors declared no conflict of interest. All authors of this manuscript certified that they had no proprietary, financial or other interest in any products presented in this article.

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References

1. Global teeth whitening market research report, Segment by major players, types, applications and regions, 2014-2024. Maia Research Analysis, 2019.
2. International Organization for Standardization. We're ISO: We develop and publish International Standards. https://www.iso.org/standards.html. Last accessed on May 9, 2019.
3. International Organization for Standardization ISO 28399 Dentistry - Products for external tooth bleaching. Geneva, Switzerland: ISO; 2011.
4. International Organization for Standardization. ISO TR 28642 Dentistry-Guidance on colour measurement. Geneva, Switzerland: ISO; 2016.
5. Paravina RD, Perez MM, Ghinea R. Acceptability and perceptibility thresholds in dentistry: A comprehensive review of clinical and research applications. *J Esthet Restor Dent* 2019;31:103-112.
6. Perez MM, Herrera LJ, Carrillo F, Pecho OE, Dudea D, Gasparik C, Ghinea R, Bona AD. Whiteness difference thresholds in dentistry. *Dent Mater* 2019;35:292-297.
7. Kinser PA, Robins JL. Control group design: enhancing rigor in research of mind-body therapies for depression. *Evidence-based complementary and alternative medicine: eCAM* 2013;2013:140467.
8. Horwitz W. Good laboratory practices in analytical chemistry. *Anal Chem* 1978;50:521-524.
9. Kwon SR, Li Y, Oyoyo U, Aprecio RM. Dynamic model of hydrogen peroxide diffusion kinetics into the pulp cavity. *J Contemp Dent Pract* 2012;13:440-445.
10. ADA Council on Scientific Affairs. Tooth whitening/bleaching: Treatment considerations for dentists and their patients. ADA, 2009.
11. Perdigão J. *Tooth whitening: An evidence-based perspective*. Springer International Publishing Switzerland, 2016;3-10.
12. Commission Internationale de l'Eclairage. Colorimetry: Technical Report. CIE Pub. Bureau Central de la CIE 1986.
13. Higgins JP, Altman DG, Gotzsche PC, Juni P, Moher D, Oxman AD, Savovic J, Schulz KF, Weeks L, Sterne JAC. The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. *BMJ* 2011;343:d5928.
14. Jones AH, Diaz-Arnold AM, Vargas MA, Cobb DS. Colorimetric assessment of laser and home bleaching techniques. *J Esthet Dent* 1999;11:87-94.
15. Kishta-Derani M, Neiva G, Yaman P, Dennison J. In vitro evaluation of tooth-color change using four paint-on tooth whiteners. *Oper Dent* 2007;32:394-398.
16. Knosel M, Reus M, Rosenberger A, Attin T, Ziebolz D. Durability of bleaching results achieved with 15% carbamide peroxide and 38% hydrogen peroxide in vitro. *Eur J Esthet Dent* 2011;6:342-356.
17. Meireles SS, Fontes ST, Coimbra LA, Della Bona A, Demarco FF. Effectiveness of different carbamide peroxide concentrations used for tooth bleaching: An in vitro study. *J Appl Oral Sci* 2012;20:186-191.
18. Kwon SR, Oyoyo U, Li Y. Effect of light activation on tooth whitening efficacy and hydrogen peroxide penetration: An in vitro study. *J Dent* 2013;41(Suppl 3):e39-e45.
19. Dantas AA, Bortolatto JF, Roncolato A, Merchan H, Floros MC, Kuga MC, Oliveira Junior OB. Can a bleaching toothpaste containing Blue Covarine demonstrate the same bleaching as conventional techniques? An in vitro, randomized and blinded study. *J Appl Oral Sci* 2015;23:609-613.
20. Kwon SR, Meharry M, Oyoyo U, Li Y. Efficacy of do-it-yourself whitening as compared to conventional tooth whitening modalities: An in vitro study. *Oper Dent* 2015;40:E21-E7.
21. Park S, Kwon SR, Qian F, Wertz PW. The effect of delivery system and light activation on tooth whitening efficacy and hydrogen peroxide penetration. *J Esthet Restor Dent* 2016;28:313-320.
22. Santana MS, Bridi EC, Navarro RS, de Lima CJ, Fernandes AB, do Amaral FL, Franca FM, Turssi CP, Basting RT. Dental bleaching with ozone: Effects on color and enamel microhardness. *Acta Odontol Latinoamer* 2016;29:68-75.
23. Leonard RH, Sharma A, Haywood VB. Use of different concentrations of carbamide peroxide for bleaching teeth: An in vitro study. *Quintessence Int* 1998;29:503-507.
24. Rees JS, Gilmour AS, Patel B. A study in vitro to compare home and surgery vital bleaching techniques. *Eur J Prosthodont Restor Dent* 2009;17:166-169.
25. White DJ, Kozak KM, Zoladz JR, Duschner HJ, Gotz H. Effects of tooth-whitening gels on enamel and dentin ultrastructure - A confocal laser scanning microscopy pilot study. *Compend Contin Educ Dent* 2000;29(Suppl):S29-S34.
26. Pretty IA, Edgar WM, Higham SM. The use of QLF to quantify in vitro whitening in a product testing model. *Br Dent J* 2001;191:566-569.
27. Clelland NL, Dorosti Y, Seghi RR. Effects of carbamide peroxides on wear resistance and color change of enamel opposing porcelain. *J Prosthodont* 2002;11:81-85.
28. White DJ, Kozak KM, Zoladz JR, Duschner H, Gotz H. Peroxide interactions with hard tissues: Effects on surface hardness and surface/subsurface ultrastructural properties. *Compend Contin Educ Dent* 2002; 23(1a):42-48.
29. Dostalova T, Jelinkova H, Housova D, Sulc J, Nemec M, Miyagi M, Brugnera Junior A, Zanin F. Diode laser-activated bleaching. *Braz Dent J* 2004;15(Sp No):SI3-SI8.
30. Wetter NU, Barroso MC, Pelino JE. Dental bleaching efficacy with diode laser and LED irradiation: An in vitro study. *Lasers Surg Med* 2004;35:254-258.
31. Wetter NU, Walverde D, Kato IT, Eduardo C de P. Bleaching efficacy of whitening agents activated by xenon lamp and 960-nm diode radiation. *Photomed Laser Surg* 2004;22(6):489-93.
32. Sulieman M, MacDonald E, Rees JS, Addy M. Comparison of three in-office bleaching systems based on 35% hydrogen peroxide with different light activators. *Am J Dent* 2005;18:194-197.
33. Wiegand A, Vollmer D, Foitzik M, Attin R, Attin T. Efficacy of different whitening modalities on bovine enamel and dentin. *Clin Oral Investig* 2005;9:91-97.
34. Adeyemi AA, Jarad FD, Pender N, Higham SM. Comparison of quantitative light-induced fluorescence (QLF) and digital imaging applied for the detection and quantification of staining and stain removal on teeth. *J Dent* 2006;34:460-466.
35. Dietschi D, Rossier S, Krejci I. In vitro colorimetric evaluation of the effi-

cacy of various bleaching methods and products. *Quintessence Int* 2006;37:515-526.
36. Duschner H, Gotz H, White DJ, Kozak KM, Zoladz JR. Effects of hydrogen peroxide bleaching strips on tooth surface color, surface microhardness, surface and subsurface ultrastructure, and microchemical (Raman spectroscopic) composition. *J Clin Dent* 2006;17:72-78.
37. Sulieman M, MacDonald E, Rees JS, Newcombe RG, Addy M. Tooth bleaching by different concentrations of carbamide peroxide and hydrogen peroxide whitening strips: An in vitro study. *J Esthet Restor Dent* 2006;18:93-100.
38. Joiner A, Philpotts CJ, Ashcroft AT, Laucello M, Salvaderi A. In vitro cleaning, abrasion and fluoride efficacy of a new silica based whitening toothpaste containing blue covarine. *J Dent* 2008;36 Suppl 1:S32-S37.
39. Lee BS, Huang SH, Chiang YC, Chien YS, Mou CY, Lin CP. Development of in vitro tooth staining model and usage of catalysts to elevate the effectiveness of tooth bleaching. *Dent Mater* 2008;24:57-66.
40. Lima DA, Silva AL, Aguiar FH, Liporoni PC, Munin E, Ambrosano GM, Lovadino JR. In vitro assessment of the effectiveness of whitening dentifrices for the removal of extrinsic tooth stains. *Braz Oral Res* 2008;22:106-111.
41. Manton DJ, Bhide R, Hopcraft MS, Reynolds EC. Effect of ozone and Tooth Mousse on the efficacy of peroxide bleaching. *Aust Dent J* 2008;53:128-132.
42. Patel A, Louca C, Millar BJ. An in vitro comparison of tooth whitening techniques on natural tooth colour. *Br Dent J* 2008;204:E15.
43. Polydorou O, Hellwig E, Hahn P. The efficacy of three different in-office bleaching systems and their effect on enamel microhardness. *Oper Dent* 2008;33:579-586.
44. Wriedt S, Keller S, Wehrbein H. The effect of debonding and/or bleaching on enamel color - An in-vitro study. *J Orofacial Orthoped* 2008;69:169-176.
45. Bruzell EM, Johnsen B, Aalerud TN, Dahl JE, Christensen T. In vitro efficacy and risk for adverse effects of light-assisted tooth bleaching. *Photochem Photobiol* 2009;8:377-385.
46. Goharkhay K, Schoop U, Wernisch J, Hartl S, De Moor R, Moritz A. Frequency doubled neodymium:yttrium-aluminum-garnet and diode laser-activated power bleaching-pH, environmental scanning electron microscopy, and colorimetric in vitro evaluations. *Lasers Med Sci* 2009;24:339-346.
47. Lima DA, Aguiar FH, Liporoni PC, Munin E, Ambrosano GM, Lovadino JR. In vitro evaluation of the effectiveness of bleaching agents activated by different light sources. *J Prosthodont* 2009;18:249-254.
48. Al Machot E, Noack B, Hoffmann T. In vitro evaluation of two whitening regimens using color-analyzing methods. *Quintessence Int* 2010;41:145-156.
49. Dietschi D, Benbachir N, Krejci I. In vitro colorimetric evaluation of the efficacy of home bleaching and over-the-counter bleaching products. *Quintessence Int* 2010;41:505-516.
50. Markovic L, Fotouhi K, Lorenz H, Jordan RA, Gaengler P, Zimmer S. Effects of bleaching agents on human enamel light reflectance. *Oper Dent* 2010;35:405-411.
51. Scaminaci Russo D, Viano M, Bambi C, Nieri M, Giachetti L. Color stability of bleached teeth over time: An in vitro study. *Eur J Esthet Dent* 2010;5:300-310.
52. Travassos AC, Rocha Gomes Torres C, Borges AB, Barcellos DC. In vitro assessment of chemical activation efficiency during in-office dental bleaching. *Oper Dent* 2010;35:287-294.
53. Borges BC, Borges JS, de Melo CD, Pinheiro IV, Santos AJ, Braz R, Montes MA. Efficacy of a novel at-home bleaching technique with carbamide peroxides modified by CPP-ACP and its effect on the microhardness of bleached enamel. *Oper Dent* 2011;36:521-528.
54. Caneppele TM, Torres CR. Influence of surfactants on the effectiveness of bleaching gels. *Clin Oral Investig* 2011;15:57-64.
55. Llambes G, Llena C, Amengual J, Forner L. In vitro evaluation of the efficacy of two bleaching procedures. *Med Oral Patol Oral Cir Bucal* 2011;16:e845-e851.
56. Cunha AG, De Vasconcelos AA, Borges BC, Vitoriano J de O, Alves-Junior C, Machado CT, Dos Santos AJ. Efficacy of in-office bleaching techniques combined with the application of a casein phosphopeptide-amorphous calcium phosphate paste at different moments and its influence on enamel surface properties. *Microsc Res Tech* 2012;75:1019-1025.
57. D'Arce MB, Lima DA, Aguiar FH, Ambrosano GM, Munin E, Lovadino JR. Evaluation of ultrasound and light sources as bleaching catalysts - An in vitro study. *Eur J Esthet Dent* 2012;7:176-184.
58. Grundlingh AA, Grossman ES, Witcomb MJ. Tooth colour change with Ozicure Oxygen Activator: A comparative in vitro tooth bleaching study. *SADJ* 2012;67:332-337.
59. Liang S, Sa Y, Sun L, Ma X, Wang Z, Xing W, Jiang T, Wang Y. Effect of halogen light irradiation on hydrogen peroxide bleaching: An in vitro study. *Aust Dent J* 2012;57:277-283.
60. Lima FG, Rotta TA, Penso S, Meireles SS, Demarco FF. In vitro evaluation of the whitening effect of mouth rinses containing hydrogen peroxide. *Braz Oral Res* 2012;26:269-274.
61. Fornaini C, Lagori G, Merigo E, Meleti M, Manfredi M, Guidotti R, Serrai A, Vescovi P. Analysis of shade, temperature and hydrogen peroxide concentration during dental bleaching: In vitro study with the KTP and diode lasers. *Lasers Med Sci* 2013;28:1-6.
62. Hahn P, Schondelmaier N, Wolkewitz M, Altenburger MJ, Polydorou O. Efficacy of tooth bleaching with and without light activation and its effect on the pulp temperature: An in vitro study. *Odontol* 2013;101:67-74.
63. Jin J, Xu X, Lai G, Kunzelmann KH. Efficacy of tooth whitening with different calcium phosphate-based formulations. *Eur J Oral Sci* 2013;121:382-388.
64. Kwon SR, Wang J, Oyoyo U, Li Y. Evaluation of bleaching efficacy and erosion potential of four different over-the-counter bleaching products. *Am J Dent* 2013;26:356-360.
65. Kwon SR, Wertz PW, Dawson DV, Cobb DS, Denehy G. The relationship of hydrogen peroxide exposure protocol to bleaching efficacy. *Oper Dent* 2013;38:177-185.
66. Liang S, Sa Y, Jiang T, Ma X, Xing W, Wang Z, Wang Y. In vitro evaluation of halogen light-activated vs chemically activated in-office bleaching systems. *Acta Odontol Scand* 2013;71:1149-1155.
67. Tam LE, Bahrami P, Oguienko O, Limeback H. Effect of 10% and 15% carbamide peroxide on fracture toughness of human dentin in situ. *Oper Dent* 2013;38:142-150.
68. Bennett ZY, Walsh LJ. Effect of photo-fenton bleaching on tetracycline-stained dentin in vitro. *J Contemp Dent Pract* 2015;16:126-129.
69. Kwon SR, Kurti SR, Jr, Oyoyo U, Li Y. Effect of light-activated tooth whitening on color change relative to color of artificially stained teeth. *J Esthet Restor Dent* 2015;27(Suppl 1):S10-S17.
70. Bortolatto JF, Trevisan TC, Bernardi PS, Fernandez E, Dovigo LN, Loguercio AD, Batista de Oliveira Junior, Pretel H. A novel approach for in-office tooth bleaching with 6% H2O2/TiO_N and LED/laser system - A controlled, triple-blinded, randomized clinical trial. *Lasers Med Sci* 2016;31:437-444.
71. Kwon SR, Dawson DV, Wertz PW. Time course of potassium nitrate penetration into the pulp cavity and the effect of penetration levels on tooth whitening efficacy. *J Esthet Restor Dent* 2016;28(Suppl 1):S14-S22.