©Operative Dentistry, 2016, **41-6,** 578-586

Clinical Evaluation of Genotoxicity of In-office Bleaching

M Rezende • JL De Geus • AD Loguercio • A Reis • D Kossatz

Clinical Relevance

In-office bleaching with 35% hydrogen peroxide gel was not genotoxic for gingival and lip tissues during a one month evaluation period.

SUMMARY

Objective: The aim of this study was to evaluate the genotoxicity of in-office bleaching with 35% hydrogen peroxide in epithelial cells from the gingival and lip tissues.

Methods and Materials: Thirty volunteers with central incisors shade A1 or darker were selected for this study. The gingival tissue of the teeth to be bleached was isolated with a light-polymerized resin dam, and the 35% hydrogen peroxide gel was administered during three 15-minute applications over the course of the 45-minute application period. Two bleaching sessions with a one-week interval in between were performed. Exfoliated oral mucosa gingival epithelial cells and upper lip lining were collected at baseline and one month after the in-office dental bleaching. The scraped cells were placed on clean glass slides and smears were prepared. After staining with Giemsa solution, two blinded examiners performed cell and micronuclei counts under a 100× optical microscope. Tooth sensitivity was evaluated using the Visual Analogue Scale (VAS). Shade evaluation was recorded before and one month after the bleaching treatment with the value-oriented shade guide Vita Bleachedguide 3D-MASTER and the spectrophotometer Vita Easyshade. Data from the shade guide units and the micronuclei (MN) frequency were subjected to a Mann-Whitney test (α=0.05). The overall difference between before and one month after the bleaching treatment (ΔE and ΔSGU), absolute risk, and intensity of tooth sensitivity (TS) were calculated, as was the 95% confidence interval (CI).

Results: The frequency of MN was not increased after bleaching with 35% hydrogen peroxide in both study groups ($p>0.05$). The absolute risk of TS of the participants was 93% (95% CI, 79%-98%), with a mean VAS intensity of 5.7 ± 2.9 (95% CI, 4.6-6.8). Meaningful whitening was observed after bleaching. The change in shade guide units in the Bleached-

Márcia Rezende, DDS, MS, postdoctoral student, Department of Restorative Dentistry, University Estadual de Ponta Grossa, Ponta Grossa, Brazil

Juliana Larocca De Geus, DDS, MS, doctoral student, Department of Restorative Dentistry, University Estadual de Ponta Grossa, Ponta Grossa, Brazil

*Alessandro Dourado Loguercio, DDS, MS, PhD, professor, School of Dentistry, State University of Ponta Grossa, Ponta Grossa, Brazil

Alessandra Reis, DDS, PhD, professor, School of Dentistry, Department of Restorative Dentistry, University Estadual de Ponta Grossa, Ponta Grossa, Brazil

Stella Kossatz, DDS, MS, PhD, professor, School of Dentistry, Department of Restorative Dentistry, University Estadual de Ponta Grossa, Ponta Grossa, Brazil

*Corresponding author: Rua Carlos Cavalcanti, 4748 Bloco M, Sala 64-A, Uvaranas, Ponta Grossa, Paraná, Brazil 84030-900; e-mail: aloguercio@hotmail.com

DOI: 10.2341/15-207-C

guide 3D-MASTER was 2.3 ± 1.4. In terms of ΔE, the change in color was 7.7 ± 3.5.

Conclusions: The in-office bleaching did not induce DNA damage to the gingival and lip tissue during the bleaching period. Although effective whitening was observed, most of the participants experienced TS.

INTRODUCTION

In a survey[1] from a population of 407 adults, about one-third of participants were not satisfied with their dental appearance, and tooth color was the primary reason for this dissatisfaction. In a more recent study,[2] the same research group reported that more than 80% of the group of participants reported that they wished to have their teeth bleached. This desire has been responsible for the increase in the demand for bleaching procedures in dental offices.

Despite the effective results in whitening produced by bleaching procedures, the profession and the public have been aware of certain risks related to dental bleaching, such as tooth sensitivity (TS) and gingival irritation.[3] While the release of free radicals is capable of converting the complex pigment molecules from the dental structure into smaller and less saturated chains[3,4] to attain effective whitening, these free radicals may also be able to react with the soft tissue and cause injuries, such as burns and ulceration.[4-8]

It is known that the DNA of cells exposed to chemical or physical agents may become damaged. In this situation, chromosomal fragments called micronuclei (MN) are observed as a result of atypical mitoses. Depending on the extent of cellular damage, the consequences may include impairment of the cell cycle, cell death, and even the formation of a neoplasm.[4,9,10] An increased frequency of chromosome breaks has been recently demonstrated to be an initial event in carcinogenesis, suggesting that these alterations may play a significant role in assessing oncogenic risk.[11,12] An increased frequency of MN in exfoliated cells from oral mucosa has traditionally served as an index for evaluating the genotoxicity of exposure to various carcinogens, mainly because this technique is simple, painless, and cheap and has been used as an adjunct in molecular epidemiology.[13]

Although there are several studies in the literature that have evaluated the genotoxicity of bleaching agents, most of these studies are experimental in animals, and only a few of them have been conducted on humans.[10,14-17] Klaric and others[16] analyzed the genotoxic effect of two hydrogen peroxide (HP)–containing bleaching products, HP 28% and 35%, on oral mucosal cells. The authors concluded that both preparations demonstrated potential genotoxic effects. However, the study of De Geus and others[17] found that at-home bleaching with 10% carbamide peroxide did not induce DNA damage to the gingival tissue. The results of the study of Almeida and others[10] supported the findings of De Geus and others[17] because after the application of two concentrations of carbamide peroxide (10% and 16%), no difference between the two groups of carbamide peroxide gels was observed in terms of mutagenic stress on gingival epithelial cells. This controversy among studies highlights the need for further investigations into this issue. Therefore, the aim of this study was to evaluate an in-office bleaching process with 35% hydrogen peroxide in terms of its efficacy, TS, and genotoxicity in epithelial cells from the gingival and lip tissues.

The null hypothesis tested was that in-office dental bleaching with 35% hydrogen peroxide did not induce DNA damage to the gingival and lip tissue during the bleaching period.

METHODS AND MATERIALS

This clinical investigation was approved (protocol 172.988) by the Scientific Review Committee and by the committee for the protection of human subjects of the local university. This report follows the protocol established by the CONSORT statement.[18] Based on pre-established criteria, 30 volunteers who searched for dental bleaching were selected for this study. This study was performed between November 2013 and March 2014. Two weeks before the bleaching procedures, all of the volunteers received dental prophylaxis with pumice and water in a rubber cup and signed an informed consent form.

Eligibility Criteria

The participants who were included in this clinical trial were between 18 and 33 years of age and had good general and oral health. The participants were required to have six caries-free maxillary anterior teeth and no periodontal disease. The central incisors were shade A1 or darker, as judged by comparison with a value-oriented shade guide (Vita Classical, Vita Zahnfabrik, Bad Säckingen, Germany). Although patients with baseline tooth color A1 or darker are not often included in clinical trials, as such baseline color does not leave much ability to measure color differences using shade guide units, there is great demand from these patients, who are requesting bleaching treatments. As the primary outcome of this

study was not centered on color change, we included such patients (A1 or darker) in this clinical trial.

Participants with anterior restorations or dental prosthesis, orthodontics apparatus, or severe internal tooth discoloration (tetracycline stains, fluorosis, and pulpless teeth) were not included in the study. Additionally, pregnant/lactating women, participants with any other pathology that could cause sensitivity (such as recession, dentin exposure, or the presence of visible cracks in teeth), smokers, bruxers, or participants who had previously undergone tooth-whitening procedures were also excluded.

Sample Size Calculation

The sample size calculation was based on the frequency of MN per 1000 cells in adults. In the pilot study it was observed that the normal frequency of MN was about 1 ± 1.1.[17,19-22] In order for the bleaching procedure to be considered safe, it was expected that we would find a mean difference of not more than 1.0. Thus, we needed a minimum sample size of 30 participants with a power of 80% and an alpha of 5%.

Bleaching Procedure

A lip and cheek retractor (ArcFlex, FGM, Joinville, Santa Catarina, Brazil) was placed in the participant's mouth to avoid the contact of the bleaching gel with the cheek, lips, and tongue. Then the gingival tissue of the teeth to be bleached was isolated from the bleaching agent using a light-polymerized resin dam (Top Dam, FGM). In every two teeth, the light-cured gingival barrier was activated for 20 seconds using a LED light-curing unit (Gnatus, Ribeirão Preto, São Paulo, Brazil) set at 1200 mW/cm^2. The 35% hydrogen peroxide gel (Whiteness HP Maxx, FGM) was used during three 15-minute applications over the course of the 45-minute application period. Two bleaching sessions within a one-week interval were performed on each patient.

TS Evaluation

The TS was evaluated up to 24 hours using the Visual Analogue Scale (VAS).[6-8,17,23] The participants were asked to place a line perpendicular to a 10-mm-long line with zero at one end indicating "no TS" and a 10-mm end indicating "unbearable TS." Then the distance (in millimeters) from the zero end was measured with the aid of a millimeter ruler. The higher sensitivity score, as reported by the patient in the first 24 hours after the first and second tooth-whitening sessions, was used for estimation purposes.

Patients who had high levels of TS were instructed to get in touch with the researchers to be examined and treated with painkillers, anti-inflammatories, and/or desensitizing topicals.

Color Evaluation

Two calibrated evaluators with a previous agreement of at least 85%, as determined by weighted kappa statistics, recorded the shade of the maxillary right central incisor during different time assessments. Shade evaluation was recorded before the procedure and one month after the bleaching treatment. The color evaluation was performed with the value-oriented shade guide Vita Bleachedguide 3D-MASTER (Vita Zahnfabrik). The Vita Bleachedguide 3D-MASTER (Vita Zahnfabrik) contains 15 shade tabs with lighter shades and it is already organized from the highest (0M1) to the lowest (5M3) value.[24] Additionally, an objective color evaluation was performed with the spectrophotometer Vita Easyshade (Vita Zahnfabrik).

The measurement area of interest for shade matching was the middle one-third of the facial surface of the maxillary central incisor, according to the American Dental Association guidelines. Color changes were calculated from the beginning of the active phase through the individual recall times by calculating the change in the number of shade guide units (ΔSGU), which occurred toward the lighter end of the value-oriented list of shade tabs. In the event of disagreements between the examiners during the shade evaluation, a consensus was reached.

The spectrophotometer measurement with a silicone guide was determined using the parameters of the Easyshade device, which indicated the following values: L*, (a*), and (b*), in which L* represents the value from 0 (black) to 100 (white) and a* and b* represent the shade, where a* is the measurement along the red-green axis and b* is the measurement along the yellow-blue axis. The color comparison before and after treatment was given by differences between the two colors (ΔE), which were calculated using the formula $\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$.[25]

Sample Collection for MN

Epithelial cells that were exfoliated from the oral mucosa gingival and upper lip lining were collected at baseline and one month after the in-office dental bleaching. Before the cell collection, the participants

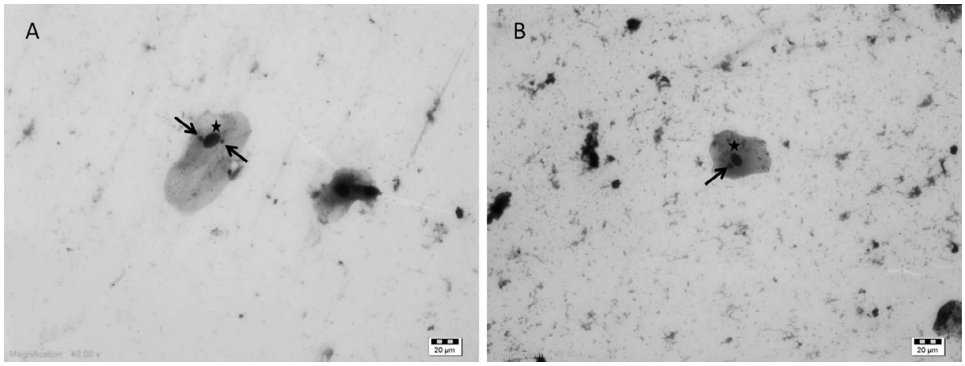

Figure 1. *Cells with micronuclei. In both (A) and (B) one can see the presence of micronucleus (indicated by an arrows) and the central nucleus of the cell (indicated with a star).*

rinsed their mouths with tap water for one minute. Subsequently, the cells were scraped with wooden spatulas from the marginal gingival and upper lip lining.[17,20,21,26,27] The scraped cells were placed on clean glass slides and smears were prepared. The smear was dried with a jet of air from a triple syringe for one minute at a distance of approximately 30 cm, thus avoiding excessive dehydration of the cells.[17,27]

Staining Procedures

The staining protocol was prepared immediately after the smear collection. Five to six drops of Giemsa stock solution (Cinética Química, Jandira, São Paulo, Brazil) were applied directly over the slide for two minutes and then the slides were washed in a container with tap water (container 1=three to four washes; container 2=two to three washes). The differentiation of the cells was performed in a third container (1200 mL of tap water and one drop of glacial acetic acid; Vetec Química Fina Ltda, Duque de Caxias, Rio de Janeiro, Brazil). After this process, the slide was dried for one minute in the same manner as described in the sample collection item. Then three drops of the adhesive Entellan (Merck KGaA, Darmstadt, Germany) were applied on the visibly dry slide for cover-glass positioning.[17,27]

Evaluation of the Slides

A single researcher performed the microscopic examination of the cells to avoid inter-examiner variation. An experienced oral pathologist trained this examiner. Calibration was made by reading five slides, randomly selected, from this study sample. An agreement of at least 80% was required (between the oral pathologist and the examiner) before beginning this phase of the study.

At least 1000 cells from each participant were evaluated during each period with the staining procedure. Cell counting was performed under an optical microscope with 100× magnification and, when MN were found, the magnification was increased to 400× (Nikon E800, Tokyo, Japan). The criteria for inclusion in the total cell count were the following: 1) the cytoplasm was intact and lying relatively flat; 2) there was little or no overlap with adjacent cells; 3) there was little or no debris; and 4) the nucleus was normal and intact, with the nuclear perimeter smooth and distinct.[28]

The parameters for identifying the micronucleus were as follows: 1) a rounded, smooth perimeter suggestive of a membrane; 2) less than one-third of the diameter of the associated nucleus but large enough to discern shape and color; 3) staining intensity similar to nucleus; 4) texture similar to nucleus; 5) same focal plane as nucleus; and 6) an absence of overlap with or bridge to nucleus (Figure 1).[28] Dead or degenerating cells (karyolysis, karyorrhexis, nuclear fragmentation) were excluded from evaluation. Nuclear blebbings (a micronucleus-like structure connected to the main nucleus by a bridge) were also not considered.

Statistical Analysis

The data were tabulated using the software SigmaPlot 5.0 for Windows (Systat Software Inc, San Jose, CA, USA). The Shapiro-Wilk test and the Bartlett test were used to test the normality and equal variance of the data. As at least one of these assumptions were not met, MN data were subjected to the Wilcoxon Signed Rank test with a level of 95% of confidence. The overall ΔE and ΔSGU, absolute risk, and intensity of TS were calculated, as was the 95% confidence interval (CI). Data from TS intensity between bleaching sessions were compared with the Wilcoxon Signed Rank test with a level of 95% of confidence.

RESULTS

A total of 104 participants were screened to select 30 participants who met the inclusion criteria. The distribution of patients according to the baseline

Table 1: *Distribution of the Participants According to the Baseline Tooth Color of the Vita Classical Scale*

	Baseline Tooth Color					
	A1	B2	A2	C1	C2	A3
No. of patients	8	7	5	3	1	6

tooth color can be seen in Table 1. The mean age of the participants was 23.5 ± 4.8 years, with a range of 18 to 33 years. Most of the participants were women (63%). All of the participants attended the recall visits during the bleaching protocol.

No significant difference was observed in the TS intensity between the first and second bleaching sessions ($p>0.05$) (Table 2). The overall absolute risk of TS of the participants was 93% (95% CI, 79%-98%), with a mean VAS intensity of 5.7 ± 2.9 (95% CI, 4.6-6.8). Five patients took an analgesic to alleviate the bleaching-induced TS (Tylenol, Janssen-Cilag Farmacêutica), and one patient self-administered an anti-inflammatory drug (Nimesulida, Medley, Campinas, Brazil).

Table 3 reports the means and standard deviations as well as medians and interquartile ranges of SGU, L*, a* and b* values. Meaningful changes towards whitening were observed after bleaching (Table 3; $p<0.05$). The change in the shade guide units within the Bleachedguide 3D-MASTER was 2.3 ± 1.4. In terms of ΔE, the change in color was 7.7 ± 3.5. The frequency of MN was not increased after bleaching with 35% hydrogen peroxide in both tissues (Table 4; $p>0.50$).

DISCUSSION

The authors of this study consider whitening at-home to be the first option for the treatment of discolored teeth.[29-31] The use of low concentrations of hydrogen peroxide gel causes less pulp irritation[32,33] and can minimize the risk and intensity of bleaching-induced TS, being a safer alternative for bleaching purposes. However, although at-home bleaching has some advantages over in-office bleaching, there are still some patients who do not adapt well to the at-home protocol, as it requires the daily use of a bleaching tray. Others desire to have a faster outcome and therefore ask for quicker ways to achieve the same whitening result. Under these circumstances, in-office bleaching is usually performed, and, thus, researchers should conduct further studies about the safety and efficacy of this protocol.

The present study showed an effective bleaching of approximately two SGU when evaluated with the Vita Bleachedguide. This is lower than the change detected by other authors[34-37] who also used 35% hydrogen peroxide. The small SGU changes detected in this study can be attributed to the fact that this study included patients with lighter teeth (shade A1 or darker), while the previous ones selected patients with darker teeth (shade C2 or darker). In a multivariable regression analysis, from pooled data of 11 clinical trials of dental bleaching performed by the same research group, Rezende and others[38] identified a significant relationship between baseline color in relation to color change estimates, meaning that the darker the baseline tooth color, the higher the degree of whitening. Using low-concentrated whitening strips, Gerlach and Zhou[39] observed the same. They reported that the lighter the baseline tooth color, the lower the degree of whitening. These studies corroborate our findings and suggest that patients with lighter teeth do not respond to the bleaching regimen as well as patients with darker teeth, perhaps because of the lower amount of available substrate for hydrogen peroxide oxidation.

Table 2: *Means and Standard Deviations of Tooth Sensitivity (TS) Intensity Experienced by Patients from the First and Second Session to Different Periods Using Visual Analog Scale (VAS) Pain Scales*[a]

Periods	First Session	Second Session
Immediately after	2.1 ± 2.5 A	2.4 ± 2.8 A
Up to 1 h	3.0 ± 2.7 B	2.9 ± 3.1 B
Up to 24 h	1.1 ± 2.0 C	1.9 ± 2.9 C

[a] *Means identified with the same letter are statistically similar.*

In the present study, we observed a high absolute risk of bleaching-induced TS that affected 93% of the patients who reported pain at least once during the onset of the treatment. This high risk is in accordance with the findings of previous studies.[8,40-42] Although the etiology of TS is not yet well understood, it is likely the result of the activation of nociceptors,[43,44] which is caused by the inflammatory reaction in the pulp tissue.[45,46] In the face of the high risk of bleaching TS after in-office bleaching, clinical alternatives to minimize this undesirable side effect were the focus of several investigations. The use of nonsteroidal anti-inflammatory drugs,[40,41,47] antioxidants,[48] and steroidal anti-inflammatory drugs[49] was not effective in minimizing this side effect. On the other hand, topical approaches, such as the application of potassium nitrate[34] or Gluma desensitizer PowerGel[8] (GLU; Heraeus Kulzer, Hanau, Germany), before in-office bleaching offer good alternatives to

Table 3: *Means, Standard Deviations (SDs), Medians, and Interquartile Ranges of the Shade Guide Units (SGUs) and L*, a*, and b* Parameters Before and After Bleaching*

Color Parameters	Mean ± SD		Medians and Interquartile Range	
	Baseline	1 Month	Baseline	1 Month
SGU (Bleachedguide)	6.5 ± 1.7	4.2 ± 1.1	6 (5/8)	4 (4/4)
L*	84.4 ± 3.9	86.8 ± 3.8	84.8 (80.9/86.7)	87.3 (85.6/88.5)
a*	−2.3 ± 1.2	−1.9 ± 1.2	−2.3 (−3.4/−1.6)	−1.9 (−2.7/−1.3)
b*	18.8 ± 4.3	13.6 ± 4.9	18.6 (16.3/21.6)	14.1(10.2/17.4)

significantly reduce the bleaching-induced TS produced by in-office bleaching.

With regard to the soft gingival tissues, clinicians can avoid the contact of in-office bleaching gels with the gingival tissue by applying a light-cured gingival barrier; however, the hydrogen peroxide often comes into contact with the oral tissue. This contact causes burns and ulcerations due to the oxidative stress induced by the hydrogen peroxide that may ultimately lead to genomic damage,[16] which highlights concerns about the safety of in-office bleaching protocols.

The frequency of MN in normal oral mucosa is between 0.5 and 2.0/1000 cells,[20-22] which is within the range we detected in the present study at baseline and one month post-bleaching. These findings suggest that the high-concentration hydrogen peroxide gel under controlled conditions did not seem to induce DNA damage to the gingival and lip tissues when applied in two bleaching sessions within a one-week interval. Although a higher concentration of hydrogen peroxide is used in the in-office bleaching protocol, clinicians can demonstrate good control of the product application. In general, the use of light-cured gingival barrier and the short exposure duration of the product may compensate for the increased hydrogen peroxide concentration used in in-office bleaching protocols.

Altogether, this explains why the results of this study are similar to those involving at-home bleaching, in which bleaching was not associated with an increased MN frequency.[17] On the other hand, the results of the present study are not in agreement with the findings of an in-office bleaching study.[16]

Table 4: *Means and Standard Deviations of the Micronuclei Frequency (MN) per 1000 Exfoliated Gingival and Lip Tissues*

	Before	After	*p*-Value[a]
MN gingival tissue	0.4 ± 0.6 A	0.5 ± 0.5 A	0.52
MN lip	0.3 ± 0.5 A	0.4 ± 0.6 A	0.50

[a] *Wilcoxon Signed Rank test, Capital letters indicate statistically similar groups.*

Klaric and others[16] speculated that the high number of MN after in-office bleaching could be attributed to the inadequate polymerization of the gingival light-curing barrier or even to the failure to promote an adequate protection to mitigate/eliminate the problem. Perhaps rubber dam isolation, with protection of the gingival tissue with petroleum jelly, might provide a better isolation of the operative field without the need to use a lip and cheek retractor.

An inadvertent contact of the highly concentrated bleaching material with the soft tissues of the oral cavity could induce increased levels of MN in the oral epithelial cells, as oxygen free radicals released from peroxides are important etiologic agents in the development of many pathological conditions.[50-52] This reinforces the need for caution during in-office bleaching,[50] as under controlled in-office conditions, such as those of the present study, this is not expected to occur.

Additionally, the study of Klaric and others[16] involved samples that were collected only 72 hours after bleaching. Considering that the oral epithelial cells turn over every 14 days,[53] it is theoretically not impossible to observe the genotoxic effects of acute exposure in shorter periods of time. In light of this, the present study collected samples one month after bleaching, as this period of time is within the regeneration cycle of the cells from the gingival tissue.[54] However, in light of these conflicting results, further clinical trials should focus on this topic and involve collection of samples at different time periods in order to investigate this controversy.

One should also mention the limitations of the current study. Most of the participants in this study were young adults with light baseline tooth color, which affects the generalizability of the findings of the present investigation to the overall population. Bonassi and others[13] compiled data from 5424 subjects with epithelial MN values obtained from 30 laboratories worldwide and concluded that several conditions may affect the MN frequency; in particular, age was shown to be highly significant. Further studies that include young and elderly

participants should be conducted in this field in order to prove this hypothesis.

CONCLUSIONS

In-office bleaching with 35% hydrogen peroxide did not induce DNA damage to the gingival and lip tissues during the bleaching period. Although effective whitening was observed, most of the participants experienced TS.

Acknowledgements

The authors would like to thank FGM Dental Products for the donation of the bleaching gel used in this investigation. The authors also declare that they have no conflict of interest.

Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of the Research Ethics Committee of the University Estadual de Ponta Grossa. The approval code for this study is 172.988.

Conflict of Interest

The authors of this manuscript certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

(Accepted 9 May 2016)

REFERENCES

1. Samorodnitzki-Naveh GR, Geiger SB, & Levin L (2007) Patients' satisfaction with dental esthetics *Journal of the American Dental Association* **138(6)** 805-808.
2. Samorodnitzky-Naveh GR, Grossman Y, Bachner YG, & Levin L (2010) Patients' self-perception of tooth shade in relation to professionally objective evaluation *Quintessence International* **41(5)** e80-e83.
3. Carey CM (2014) Tooth whitening: What we now know *Journal of Evidence-based Dental Practice* **14(Supplement)** 70-76.
4. Goldberg M, Grootveld M, & Lynch E (2010) Undesirable and adverse effects of tooth-whitening products: A review *Clinical Oral Investigation* **14(1)** 1-10.
5. Dahl JE, & Pallesen U (2003) Tooth bleaching—A critical review of the biological aspects *Critical Reviews in Oral Biology and Medicine: An Official Publication of the American Association of Oral Biologists* **14(4)** 292-304.
6. Gurgan S, Cakir FY, & Yazici E (2010) Different light-activated in-office bleaching systems: A clinical evaluation *Lasers in Medical Science* **25(6)** 817-822.
7. He LB, Shao MY, Tan K, Xu X, & Li JY (2012) The effects of light on bleaching and tooth sensitivity during in-office vital bleaching: A systematic review and meta-analysis *Journal of Dentistry* **40(8)** 644-653.
8. Mehta D, Venkata S, Naganath M, LingaReddy U, Ishihata H, & Finger WJ (2013) Clinical trial of tooth desensitization prior to in-office bleaching *European Journal of Oral Science* **121(5)** 477-481.
9. Igarashi M, Nagata M, Itoh S, Yamoto T, & Tsuda S (2010) Relationship between DNA damage and micronucleus in mouse liver *Journal of Toxicological Science* **35(6)** 881-889.
10. Almeida AF, Torre Edo N, Selayaran M dos S, Leite FR, Demarco FF, Loguercio AD, & Etges A (2015) Genotoxic potential of 10% and 16% carbamide peroxide in dental bleaching *Brazilian Oral Research* **29(1)** 1-7.
11. Hagmar L, Bonassi S, Strömberg U, Brogger A, Knudsen LE, Norppa H, & Reuterwall C (1998) Chromosomal aberrations in lymphocytes predict human cancer: A report from the European Study Group on cytogenetic biomarkers and health (ESCH) *Cancer Research* **58(18)** 4117-4121.
12. Bonassi S, Hagmar L, Strömberg U, Montagud AH, Tinnerberg H, Forni A, Heikkilä P, Wanders S, Wilhardt P, Hansteen IL, Knudsen LE, & Norppa H (2000) Chromosomal aberrations in lymphocytes predict human cancer independently of exposure to carcinogens *Cancer Research* **60(6)** 1619-1625.
13. Bonassi S, Coskun E, Ceppi M, Lando C, Bolognesi C, Burgaz S, Holland N, Kirsh-Volders M, Knasmueller S, Zeiger E, Carnesoltas D, Cavallo D, da Silva J, de Andrade VM, Demircigil GC, Domínguez Odio A, Donmez-Altuntas H, Gattas G, Giri A, Giri S, Gómez-Meda B, Gómez-Arroyo S, Hadjidekova V, Haveric A, Kamboj M, Kurteshi K, Martino-Roth MG, Montero Montoya R, Nersesyan A, Pastor-Benito S, Favero Salvadori DM, Shaposhnikova A, Stopper H, Thomas P, Torres-Bugarín O, Yadav AS, Zúñiga González G, & Fenech M (2011) The Human Micro Nucleus project on exfoliated buccal cells (HUMN(XL)): The role of life-style, host factors, occupational exposures, health status, and assay protocol *Mutation Research* **728(3)** 88-97.
14. De Castro Albuquerque R, Gomez RS, Dutra RA, Vasconcellos WA, Gomez RS, & Gomez MV (2002) Effects of a 10% carbamide peroxide bleaching agent on rat oral epithelium proliferation *Brazilian Dental Journal* **13(3)** 162-165.
15. Da Costa Filho LC, da Costa CC, Sória ML, & Taga R (2002) Effect of home bleaching and smoking on marginal gingival epithelium proliferation: A histologic study in women *Journal of Oral Pathology and Medicine: Official Publication of the International Association of Oral Pathologists and the American Academy of Oral Pathology* **31(8)** 473-480.
16. Klaric E, Par M, Profeta I, Kopjar N, Rozgaj R, Kasuba V, Zeljezic D, & Tarle Z (2013) Genotoxic effect of two bleaching agents on oral mucosa *Cancer Genomics & Proteomics* **10(5)** 209-215.
17. De Geus JL, Rezende M, Margraf LS, Bortoluzzi MC, Fernández E, Loguercio AD, Reis A, & Kossatz S (2015) Evaluation of genotoxicity and efficacy of at-home bleaching in smokers: A single-blind controlled clinical trial *Operative Dentistry* **40(2)** E47-E55.
18. Schulz KF, Altman DG, Moher D, & CONSORT Group (2011) CONSORT 2010 Statement: Updated guidelines

for reporting parallel group randomized trials *International Journal of Surgery* **9(8)** 672-677.

19. Holland N, Bolognesi C, Kirsch-Volders M, Bonassi S, Zeiger E, Knasmueller S, & Fenech M (2008) The micronucleus assay in human buccal cells as a tool for biomonitoring DNA damage: The HUMN project perspective on current status and knowledge gaps *Mutation Research* **659(1-2)** 93-108.
20. Angelieri F, Moleirinho TCG, Carlin V, Oshima CTF, & Ribeiro DA (2010) Biomonitoring of oral epithelial cells in smokers and nonsmokers submitted to panoramic Xray: Comparison between buccal mucosa and lateral border of the tongue *Clinical Oral Investigation* **14(6)** 669-674.
21. Nerseyan A, Muradyan R, Kundi M, & Schulte-Hermann R (2011) Impact of smoking on the frequencies of micronuclei and other nuclear abnormalities in exfoliated oral cells: A comparative study with different cigarette types *Mutagenesis* **26(2)** 295-301.
22. Naderi NJ, Farhadi S, & Sarshar S (2012) Micronucleus assay of buccal mucosa cells in smokers with the history of smoking less and more than 10 years *Indian Journal of Pathology and Microbiology* **55(4)** 433-438.
23. Rezende M, Loguercio AD, Reis A, & Kossatz S (2013) Clinical effects of exposure to coffee during at-home vital bleaching *Operative Dentistry* **38(6)** E229-E236.
24. Paravina RD, Johnston WM, & Powers JM (2010) New shade guide for evaluation of tooth whitening—Colorimetric study *Journal of Esthetic and Restorative Dentistry* **19(5)** 276-278.
25. Commission Internationale de l'Eclairage (1978) Recommendations on Uniform Color Spaces, Color Difference Equations, Psychometric Color Terms, 1978; Supplement 2 to CIE Publication 15 (E2-31.1) 1971 (TC-1.3) 1978 Paris *Paris, France: Bureau Central de la CIE*.
26. Bansal H, Sandhu VS, Bhandari R, & Sharma D (2012) Evaluation of micronuclei in tobacco users: A study in Punjabi population *Contemporary Clinical Dentistry* **3(2)** 184-187.
27. Bortoluzzi MC, Campagnoli EB, Milan JR, Reinheimer A, Masson M, & Capella DL (2014) Frequency of micronucleus in oral epithelial cells after exposure to mate-tea in healthy humans *Medicina Oral Patologia Oral y Cirugia Bucal* **19(4)** e345-e349.
28. Tolbert PE, Shy CM, & Allen JW (1992) Micronuclei and other nuclear anomalies in buccal smears: Methods development *Mutation Research* **271(1)** 69-77.
29. Zekonis R, Matis BA, Cochran MA, Al Shetri SE, Eckert GJ, & Carlson TJ (2003) Clinical evaluation of in-office and at-home bleaching treatments *Operative Dentistry* **28(2)** 114-121.
30. Haywood VB (2003) Frequently asked questions about bleaching *Compendium Continuing Education in Dentistry* **24(4A)** 324-338.
31. Auschill TM, Hellwig E, Schmidale S, Sculean A, & Arweiler NB (2005) Efficacy, side-effects and patients' acceptance of different bleaching techniques (OTC, in-office, at-home) *Operative Dentistry* **30(2)** 156-163.
32. Bernardon JK, Sartori N, Ballarin A, Perdigão J, Lopes GC, & Baratieri LN (2010) Clinical performance of vital bleaching techniques *Operative Dentistry* **35(1)** 3-10.
33. Mondelli RF, Azevedo JF, Francisconi AC, Almeida CM, & Ishikiriama SK (2012) Comparative clinical study of the effectiveness of different dental bleaching methods—Two year follow-up *Journal of Applied Oral Science* **20(4)** 435-443.
34. Tay LY, Kose C, Loguercio AD, & Reis A (2009) Assessing the effect of a desensitizing agent used before in office-tooth bleaching *Journal of the American Dental Association* **140(10)** 1245-1251.
35. Reis A, Dalanhol AP, Cunha TS, Kossatz S, & Loguercio AD (2011) Assessment of tooth sensitivity using a desensitizer before light-activated bleaching *Operative Dentistry* **36(1)** 12-17.
36. Papathanasiou A, Kastali S, Perry RD, & Kugel G (2002) Clinical evaluation of a 35% hydrogen peroxide in-office whitening system *Compendium of Continuing Education in Dentistry* **23(4)** 335-338.
37. Onwudiwe UV, Umesi DC, Orenuga OO, & Shaba OP (2013) Clinical evaluation of 16% and 35% carbamide peroxide as in-office vital tooth whitening agents *Nigerian Quarterly Journal of Hospital Medicine* **23(2)** 80-84.
38. Rezende M, Loguercio AD, Kossatz S, & Reis A (2016) Predictive factors on the efficacy and risk/intensity of tooth sensitivity of dental bleaching: A multi regression and logistic analysis *Journal of Dentistry* **45** 1-6.
39. Gerlach RW, & Zhou X (2001) Vital bleaching with whitening strips: Summary of clinical research on effectiveness and tolerability *Journal of Contemporary Dental Practice* **2(3)** 1-16.
40. Paula E, Kossatz S, Fernandes D, Loguercio A, & Reis A (2013) The effect of perioperative ibuprofen use on tooth sensitivity caused by in-office bleaching *Operative Dentistry* **38(6)** 601-608.
41. De Paula EA, Loguercio AD, Fernandes D, Kossatz S, & Reis A (2013) Perioperative use of an anti-inflammatory drug on tooth sensitivity caused by in-office bleaching: A randomized, triple-blind clinical trial *Clinical Oral Investigations* **17(9)** 2091-2097.
42. Bonafé E, Bacovis CL, Iensen S, Loguercio AD, Reis A, & Kossatz S (2013) Tooth sensitivity and efficacy of in-office bleaching in restored teeth *Journal of Dentistry* **41(4)** 363-369.
43. Fried K, Sessle BJ, & Devor M (2011) The paradox of pain from tooth pulp: Low-threshold "algoneurons"? *Pain* **152(12)** 2685-2689.
44. Gibbs JL, Melnyk JL, & Basbaum AI (2011) Differential TRPV1 and TRPV2 channel expression in dental pulp *Journal of Dental Research* **90(6)** 765-770.
45. Caviedes-Bucheli J, Ariza-García G, Restrepo-Méndez S, Ríos-Osorio N, Lombana N, & Muñoz HR (2008) The effect of tooth bleaching on substance P expression in human dental pulp *Journal of Endodontics* **34(12)** 1462-1465.
46. Costa CA, Riehl H, Kina JF, Sacono NT, & Hebling J (2010) Human pulp responses to in-office tooth bleaching

Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontics **109(4)** e59-e64.

47. Charakorn P, Cabanilla LL, Wagner WC, Foong WC, Shaheen J, Pregitzer R, & Schneider D (2009) The effect of preoperative ibuprofen on tooth sensitivity caused by in-office bleaching *Operative Dentistry* **34(2)** 131-135.
48. De Paula EA, Kossatz S, Fernandes D, Loguercio AD, & Reis A (2014) Administration of ascorbic acid to prevent bleaching-induced tooth sensitivity: A randomized triple-blind clinical trial *Operative Dentistry* **39(2)** 128-135.
49. Rezende M, Bonafe E, Vochikovski L, Farago PV, Loguercio A, Reis A, & Kossatz S (2016) Pre- and postoperative dexamethasone does not reduce bleaching-induced tooth sensitivity: A randomized, triple-masked clinical trial *Journal of the American Dental Association* **147(1)** 41-49.
50. Floyd RA (1997) The effect of peroxides and free radicals on body tissues *Journal of the American Dental Association* **128(Supplement)** 37S-40S.
51. Fenech M, Crott J, Turner J, & Brown S (1999) Necrosis, apoptosis, cytostasis and DNA damage in human lymphocytes measured simultaneously within the cytokinesis-block micronucleus assay: Description of the method and results for hydrogen peroxide *Mutagenesis* **14(6)** 605-612.
52. Ribeiro DA, Marques ME, & Salvadori DM (2005) Assessment of genetic damage induced by dental bleaching agents on mouse lymphoma cells by single cell gel (comet) assay *Journal of Oral Rehabilitation* **32(10)** 766-771.
53. Thomson PJ, Potten CS, & Appleton DR (1999) Mapping dynamic epithelial cell proliferative activity within the oral cavity of man: A new insight into carcinogenesis? *British Journal of Oral & Maxillofacial Surgery* **37(5)** 377-383.
54. Newman MG, Takei HH, Klokkevold PR, & Carranza FA (2012) *Carranza's Clinical Periodontology* **11th edition** Elsevier, St Louis, MO.