©Operative Dentistry, 2020, **45-4,** 368-376

Assessment of Peroxide in Saliva During and After At-home Bleaching With 10% Carbamide and Hydrogen Peroxide Gels: A Clinical Crossover Trial

MC Mailart • PA Sakassegawa • CRG Torres • RM Palo • AB Borges

Clinical Relevance

This study suggests that at-home bleaching systems are safe in relation to toxicity based on peroxide levels in saliva since the amount of peroxide potentially ingested is much lower than the estimated toxic dose.

SUMMARY

Objectives: This study evaluated the presence of peroxide in saliva using at-home bleaching systems containing hydrogen peroxide (HP) and carbamide peroxide (CP) with a prefilled tray (PT) or conventional tray (CT).

Methods and Materials: Participants received bleaching treatments after the sequence randomization (n=10): PT-HP/Opalescence-Go10%; CT-HP/WhiteClass10%; and CT-CP/OpalescencePF10%. Saliva was collected at the following times: baseline; at 1, 5, 15, and 30 minutes after administration; and at 3, 5, and 8 minutes after the tray was removed. Colorimetric analysis using analytic spectrophotometry was performed. The salivary flow (SF) was monitored during use of trays. Data about peroxide concentration (PC) were submitted to repeated-measures analysis of variance and Tukey tests (5%), and toxic dose was calculated based on body weight. The relation between SF and PC was verified with the Pearson correlation test.

Results: There was a significant difference for bleaching (p=0.0001) and time (p=0.0003) but

Mariane C Mailart, PhD student, Restorative Dentistry, Institute of Science and Technology, São Paulo State University – UNESP, São José dos Campos, Brazil

Paula A Sakassegawa, undergraduate student, Restorative Dentistry, Institute of Science and Technology, São Paulo State University – UNESP, São José dos Campos, Brazil

Carlos RG Torres, associate professor, Restorative Dentistry, Institute of Science and Technology, São Paulo State University – UNESP, São José dos Campos, Brazil

Renato Miotto Palo, PhD, Restorative Dentistry, Institute of Science and Technology, São Paulo State University – UNESP, São Jose dos Campos, Brazil

*Alessandra Buhler Borges, associate professor, Restorative Dentistry, Institute of Science and Technology, São Paulo State University – UNESP, São José dos Campos, Brazil

*Corresponding author: Av Eng Francisco José Longo, 777, Jd São Dimas, São José dos Campos, São Paulo 12245-000, Brazil; e-mail: alessandra.buhler@unesp.br

https://doi.org/10.2341/19-127-C

not for interaction (p=0.3121). PC was lower for CT-CP in relation to PT-HP and CT-HP. After tray removal, expectoration, of the remaining gel, and mouth rinsing, no peroxide was detected in saliva. Correlation between SF and PC was considered weak (r=0.3379). The overall mean SF was 50.44% during tray use. In general, PC in saliva was 68.72% lower than the estimated toxic dose (0.26 mg/kg/day) considering all the bleaching systems.

Conclusions: Higher peroxide levels were detected in saliva with 10% HP gels. Nevertheless, they were below estimated toxic dose and were considered safe in relation to toxicity.

INTRODUCTION

Tooth bleaching is an esthetic treatment frequently performed in dental offices as it is minimally invasive and has proven clinical efficacy.[1,2] The most common active ingredient in bleaching gels is hydrogen peroxide, used in its original compound or produced by chemical reaction of its precursor carbamide peroxide. Due to its low molecular weight and high instability, hydrogen peroxide is able to diffuse into dental tissues, decomposing in free radicals that act on chromophores.[3,4] As a result of the oxidative reaction, the chromophores are converted into less complex structures, resulting in alteration of the optical properties of tooth substrate, increasing its reflectance and consequently resulting in a visual impression of a whiter tooth.[1,3,5]

Bleaching treatment can be used for teeth with intrinsic or extrinsic discoloration, as well as vital and nonvital teeth.[3,6,7] For vital teeth, three techniques can be performed: in office; at home; and using over-the-counter products.[8,9] The at-home bleaching technique was first described by Haywood and Heymann[10] in 1989 using 10% carbamide peroxide overnight. Several bleaching systems are available in the market with different peroxide concentrations, presentations, and suggested protocols of use. The more concentrated gels (up to 10% hydrogen peroxide) are marketed as at-home bleaching treatment alternatives to reduce the time of tray use during the day compared with the conventional at-home bleaching protocol.[2,6,10]

Although bleaching treatment is considered safe and effective,[1,2,11] potential adverse effects caused by peroxide have been described in literature. The most relevant negative consequences related to tooth bleaching are tooth sensitivity during and after bleaching procedures and gingival irritation. Additionally, peroxide release in saliva and potential gel ingestion have also been described.[3,6,12,13]

The potential toxicologic effect of hydrogen peroxide in contact with soft tissues or swallowed is a subject of concern by researchers and clinicians.[14] A safe hydrogen peroxide exposure level (no observed effect level) was determined in a previous study with catalase-deficient mice, reporting a maximum dose of 26 mg/kg/d.[15] This corresponds to the dose level in humans, considering the conventional uncertainty factor of 100-fold, of 0.26 mg/kg/d.[16,17] Peroxide release in the oral environment can be related to bleaching gel composition, viscosity, hydrogen peroxide concentration, amount of gel dispensed in the tray, type of tray, and adaptation in the dental arches.[16,18,19] Although peroxide levels in saliva are usually considered safe with lower-concentrated products, the more concentrated products may reach levels above the estimated toxic dose.[18] Even though many products are already available in the dental market with higher peroxide concentrations and different gel viscosity and tray designs, their safety regarding peroxide released in saliva has not been fully established.

This randomized crossover clinical trial aimed to evaluate the amount of peroxide released in saliva during tray use with different bleaching systems and compare it with the estimated toxic dose. Additionally, salivary flow during tray use was measured. The following null hypotheses were tested: 1) there is no difference among bleaching systems in relation to presence of peroxide in saliva, 2) there is no increase in salivary flow of participants during tray use, and 3) there is no difference in salivary flow during the use of the different bleaching systems.

METHODS AND MATERIALS

Ethical Aspects

This study was approved by the committee for the protection of human participants of the local university, and it was registered at the ReBEC website (Brazilian Clinical Trials Registry virtual platform). The structure of this article followed the protocol established by the Consolidated Standards of Reporting Trials Statement (CONSORT).[20]

Study Design

This study followed a factorial 3×8 design, considering the following as experimental factors: bleaching systems at three levels: prefilled trays with 10% hydrogen peroxide (PT-HP) (Opalescence Go 10%, Ultradent Products, Inc. South Jordan, UT, USA),

Table 1: *Eligibility Criteria*

Inclusion Criteria	Exclusion Criteria
Good general health	Smoking or alcoholic-dependent patients
> 18 years old	Pregnancy
Absence of noncarious cervical lesions, active caries, gingival recession, or periodontal disease	Tooth sensitivity
Do not use orthodontics appliance or removable prosthesis	Bruxism habits
Presence of all teeth from first molar to first molar (upper and lower) without restorations	Periapical alterations
Availability of recurring returns	Use of medicaments that alter salivary flow

customized trays with 10% hydrogen peroxide (CT-HP) (White Class 10%, FGM Produtos Odontológicos, Joinville, SC, Brazil), and customized trays with 10% carbamide peroxide (CT-CP) (Opalescence PF 10%, Ultradent Products) and time at eight levels (baseline; 1, 5, 15, and 30 minutes; and 3, 5, and 8 minutes after the tray was removed). These factors were tested in a prospective, randomized, and crossover clinical trial with three phases involving participants selected according to preestablished criteria (Table 1). To exclude a reduced salivary flow (SF) condition (lower than 2 mL/min), the participants were submitted to an SF analysis based on collection of unstimulated saliva during 5 minutes. The SF was calculated considering volume of saliva (mL) divided by time (minutes).

Participants were submitted to tooth bleaching treatment using prefilled trays or customized trays. Peroxide concentration in saliva measured at different times was the main dependent variable. The treatment sequence was randomized. The PICO (problem/patient/population, intervention/indicator, comparison, outcome) question was stated, and the parameters were defined: P = adult patients with discolored teeth; I = 10% hydrogen peroxide in prefilled or customized trays; C = 10% peroxide carbamide in customized trays; and O = peroxide concentration in saliva. The main research question was: Does 10% hydrogen peroxide in prefilled or customized trays present similar peroxide concentration in saliva compared with 10% carbamide peroxide in customized trays? Peroxide detected in saliva was compared with the estimated toxic dose ingestion. Additionally, as secondary outcomes, salivary flow during tray use and comparison among treatments were determined.

Sample-size Calculation

The sample size was determined using a power of 80% and alpha of 5%, considering means and standard deviations from a previous study.[21] The calculated effect size was 0.9, which resulted in a sample size of eight participants. Then, 10 participants were selected considering possible dropouts. The data were obtained using the G-Power 3.1 software.[22]

Group Division and Randomization Process

The participants underwent the bleaching treatments: 10% hydrogen peroxide in prefilled tray (PT-HP/Opalescence Go), 10% hydrogen peroxide in customized tray (CT-HP/White Class), and 10% carbamide peroxide in customized tray (CT-CP/Opalescence PF). The composition and manufacturers of each bleaching gel are presented in Table 2. The sequence of treatments that each participant received was generated using the software RANDOM.ORG True Random Number Service (www.random.org).

Table 2: *Composition of the Bleaching Gels Tested*

Bleaching Gel	Composition	Peroxide Concentration	Manufacturer
Opalescence Go	Glycerin, 20% water; carbomer; 10% hydrogen peroxide; sodium hydroxide; PVP (polyvinylpyrrolidone); silica; xylitol; disodium phosphate; 3% potassium nitrate; sucralose; flavor; 0.25% sodium fluoride; EDTA (ethylenediamine tetraacetic acid); sodium lauryl sulfate.	10% Hydrogen peroxide	Ultradent Products, Inc. South Jordan, UT,/USA
White Class	10% hydrogen peroxide; neutralized carbopol; 5% potassium nitrate; sodium fluoride; aloe vera; calcium gluconate; stabilizer; humectant; deionized water.	10% Hydrogen peroxide	FGM Produtos Odontológicos, Joinville, SC/ Brazil
Opalescence PF	Glycerin; 20% water; xylitol; 10% carbamide peroxide; carbomer; propylene glycol-300; polyacrylic acid; sodium hydroxide; EDTA (ethylenediamine tetraacetic acid); 0.5% potassium nitrate; 0.25% sodium fluoride.	10% Carbamide peroxide	Ultradent Products, Inc. South Jordan, UT,/USA

Blinding

The participants and operator were not blinded for the procedures since the type of tray used cannot be masked. Nevertheless, the examiner was blinded for chemical analysis.

Laboratory Procedures

Alginate impressions of maxillary and mandibular dental arches of patients were taken to obtain casts to produce the customized trays. Reservoirs 1 mm (±0.1 mm) thick were created on the facial surfaces of anterior teeth, including the first premolars in both arches, applying a light-cured resin (Ultradent LC Block-Out Resin) on the casts. The resin layer thickness was standardized using a thickness gauge with blunt tips that was positioned on the thirds of the buccal surface of each tooth before and after the resin application. Then, customized trays were fabricated with 0.9-mm-thick vinyl acetate sheets (Sof-Tray Regular, Ultradent Products) using the thermoforming process. Trays were precisely trimmed/scalloped completely involving tooth surface (1 mm incisal or occlusal to gingival margin), and its adaptation was verified on the casts. A small and controlled flame was gently applied to the edges to ensure maximum fit on cervical area, according to manufacturer's instructions. The trays were placed over teeth to verify the adaptation in the participants' mouths.

Study Intervention

The bleaching procedures were performed in three phases, according to the treatment randomization sequence. The participant underwent each of the three bleaching systems on a different day, during the morning, with a washout period of 1 week between treatments. The operator dispensed a standardized quantity (0.350 g for maxillary arches and 0.250 g for mandibular arches) of the bleaching gel inside the tray. This amount was calculated based on the average of three participants in a pilot study because the amount of gel present in the prefilled trays (0.799g±0.023g for maxillary and 0.766g±0.023g for mandibular) was too high and clinically impracticable to be applied in the customized trays. Any excess overflow of gel was carefully removed with gauze swabs. The prefilled trays were removed from the package and placed on the dental arches following manufacturer's instructions. Participants used the bleaching systems for 30 minutes.

Detecting Peroxide in Saliva

A spectrophotometry method based on the reaction of 4-aminoantipyrine and phenol with hydrogen peroxide was adopted. This method allows the oxidation of peroxide by peroxidase enzyme, resulting in solution color change from transparent to pink.[21] To detect hydrogen peroxide, an enzymatic reagent was prepared. The amount of hydrogen peroxide in saliva was quantified using an analytic spectrophotometer (Bioespectro SP-22, Curitiba, PR, Brazil), which related light absorbance with peroxide concentration of each sample, according to the Beer-Lambert law.

An aqueous solution containing aminophenazone (4-aminoantipyrine; 4 mmol/l), phenol (24 mmol/l), and peroxidase (0.4 U/mL) dissolved in 0.1 M phosphate buffer at pH 7.0 was used as the enzyme reagent. The reagents were stored at 4°C.[23] Before the analysis, a calibration curve with a standard hydrogen peroxide solution of known concentration was determined to obtain accurate measurement. The concentration of hydrogen peroxide solution was verified with potassium permanganate titration using a potentiometric titrator (HI 902, Hanna Instruments, Woonsocket, RI, USA). This curve is defined by absorbance values (optical density) of solution in relation to hydrogen peroxide concentration.

Participants were advised not to eat or drink for 2 hours before the analysis. During the analysis, they were instructed to swallow dry in order to collect whole saliva.[16] Thus, they should avoid swallowing consciously even when they desired to do it, since they needed to expectorate their saliva in graduated tubes during the study. At the beginning of the experiment, before insertion of the trays, participants expectorated saliva in the graduated tubes for 5 minutes. Then, the bleaching trays were inserted in the mouth, and saliva samples were collected again at 1, 5, 15, and 30 minutes.

After the tray was removed, the remaining gel was removed from tooth surfaces with a toothbrush without toothpaste for 30 seconds followed by expectoration; and the mouth was rinsed abundantly for 1 minute. Two minutes after the tray was removed, saliva samples were again collected. Subsequently, saliva was collected after 5 and 15 minutes, that is, 33, 38, and 48 minutes after the beginning of the procedure. For each analysis, 1000 μL of the collected saliva was added inside acryl-cuvettes (Sarstedt, Nümbrecht, Germany) with 1000 μL of enzymatic reagent, resulting in different

pinkish solutions, depending on the amount of peroxide present. After 48 minutes, total salivary flow was measured.

Comparison With Estimated Toxic Dose

This measurement was performed based on body weight (kg). Each participant was weighed to determine individual safe dose. The values were compared to safe daily dose, determined in previous studies (0.26 mg/kg/day).[15,16] To calculate the daily dose of peroxide, the values were expressed in milligrams of hydrogen peroxide per kilogram of body weight.

Statistical Analysis

To analyze data distribution, the Kolmogorov-Smirnov normality test was applied (α=0.05), and for homoscedasticity calculation, the Levene test was used (α=0.05). The relation between salivary flow and peroxide released in saliva was determined.

For comparison of peroxide presence in saliva and salivary flow, according to the bleaching systems, repeated measures analysis of variance and post hoc Tukey test were applied (5%); the bleaching treatments were the fixed factor, and the participants were the repeated factor. The relation between salivary flow and presence of peroxide in saliva was verified with the Pearson correlation test. The tests were performed using Statistica for Windows (StatSoft, Tulsa, Oklahoma, USA) and Graphpad Prism (Graphpad Prism Software, La Jolla, CA, USA).

RESULTS

There was no loss of participants or missed appointments during the study. The flow chart shows the distribution of participants among the groups (Figure 1). Eight participants were women and two were men, with average age of 27.2 ± 2.75 years. The average weight of participants was 57.0 ± 11.81 kg.

Peroxide Concentration in Saliva

Two-way repeated measures analysis of variance showed significant differences for bleaching system ($p<0.0001$) and time factors ($p=0.0003$) but not for interaction ($p=0.3121$). Hydrogen peroxide concentration in saliva was lower for CT-CP/OpalescencePF than for PT-HP/OpalescenceGo and CT-HP/White Class. When compared to the daily estimated toxic dose (0.26 mg/kg/d), the peroxide concentration in saliva was 64.83% lower for PT-HP/OpalescenceGo, 68.38% for CT-HP/White Class, and 72.97% for CT-CP/OpalescencePF (Figure 2). After tray removal, no peroxide was detected in saliva. Hydrogen peroxide concentration in saliva for each bleaching system compared with estimated toxic dose is shown in Figure 3.

Salivary Flow

Comparison of salivary flow before and after bleaching procedures showed significant differences for all groups ($p=0.0001$). Nevertheless, no differences were observed among bleaching systems at the same time ($p=0.0342$). The correlation between salivary flow and peroxide concentration was weak for all bleaching systems ($r=0.3775$ for PT-HP/OpalescenceGo; $r=0.3322$ for CT-HP/White Class; and $r=0.0162$ for CT-CP/OpalescencePF). Salivary flow increased by 42.38% for PT-HP/OpalescenceGo, 54.60% for CT-HP/White Class, and 54.34% for CT-CP/OpalescencePF from baseline to during treatment (Figure 4).

DISCUSSION

Considering the results, the first and second null hypotheses were rejected as there was a significant difference in presence of hydrogen peroxide in saliva among the bleaching systems, and salivary flow increased during tray use. The third null hypothesis was accepted because there was no significant difference in salivary flow during the study among the groups. The 10 participants received three different types of treatment as appropriate for a crossover study. The participants were 20% men and 80% women Although there is a discrepancy in participants' gender, the quality of saliva samples were not supposed to be affected as salivary flow and proteome profile are not influenced by sex.[24,25] This model allows for spectrophotometric analysis of peroxide in the same person, thereby decreasing the influence of variables related to oral environment such as pH, flow, and salivary content. The 1-week washout period was chosen to ensure that peroxide was eliminated from saliva and teeth, since it has been previously shown that residual oxygen may remain in the tooth structure after bleaching procedures.[26,27]

Considering possible toxicity caused by ingestion of peroxide,[2,12,18,28] this study evaluated the presence of hydrogen peroxide in saliva with a spectrophotometric method, which is considered accurate for this investigation.[21] This analysis was based on measuring light absorption by the dye generated by the reaction between hydrogen peroxide and the

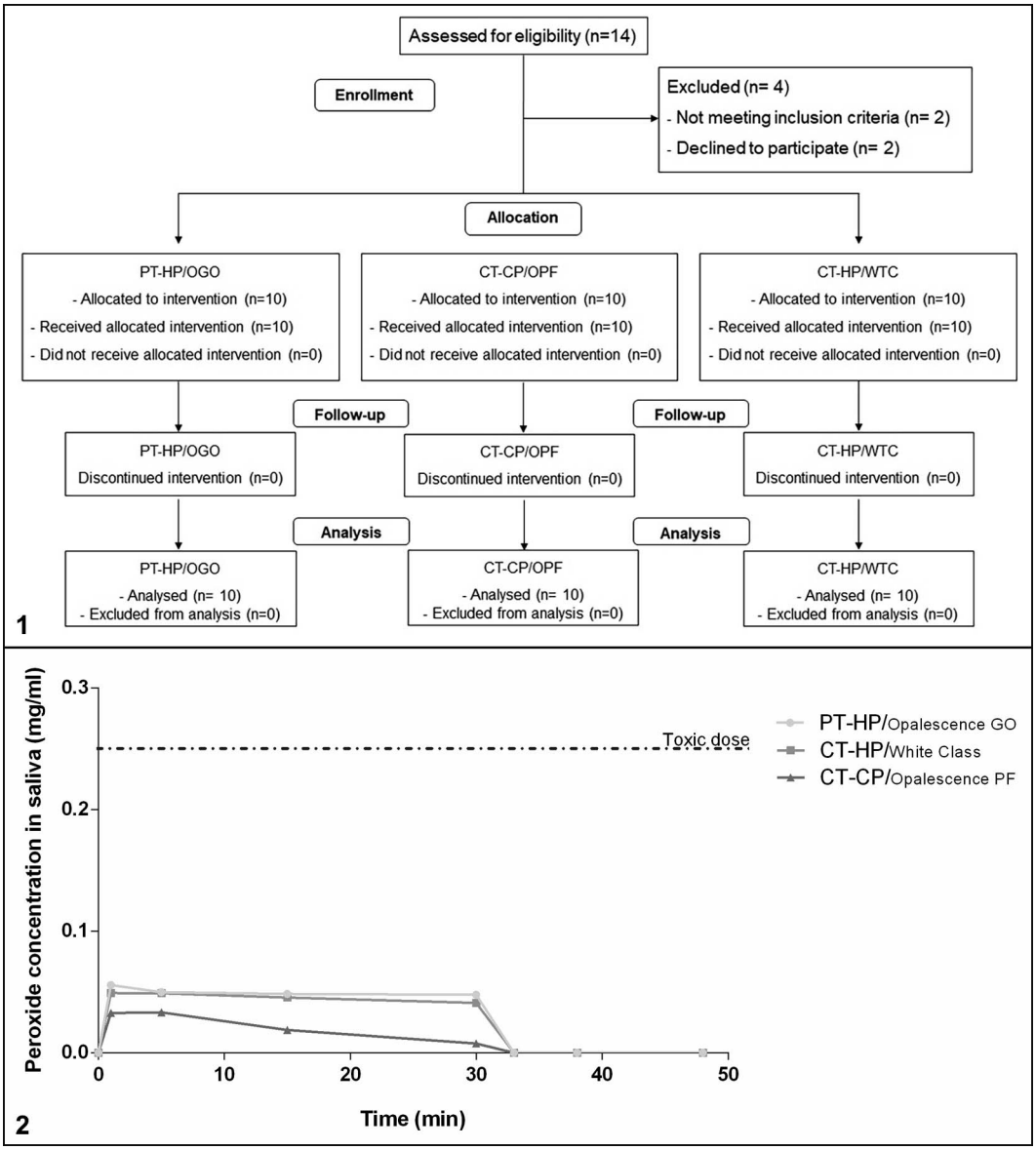

Figure 1. *Flow chart of the study (CONSORT).*

Figure 2. *Peroxide concentration in saliva in relation to time in comparison to toxic dose.*

chemical components of the solution, using an analytical spectrophotometer and considering the Beer-Lambert law. Thus, the light absorption by the molecules in a solution is directly proportional to their absorbance, path length (how long the light needs to overcome the solution) and concentration.[29,30]

The higher concentrations of peroxide in saliva were detected in bleaching systems with 10% hydrogen peroxide, despite type of tray used. Although in a lower concentration, hydrogen peroxide released by carbamide peroxide gel was also detected in the first minutes of the study, but it decreased during the analyzed period. It is important to highlight that after the tray removal, gel removal with toothbrush, and rinse, no peroxide was detected in saliva with all bleaching systems. This result was also observed in a previous study with 10% carbamide peroxide gels dispensed in customized trays.[16] The presence of peroxide in saliva was measured at 30 minutes because this is the time of use usually recommended by the manufacturers of 10% hydrogen peroxide gels.

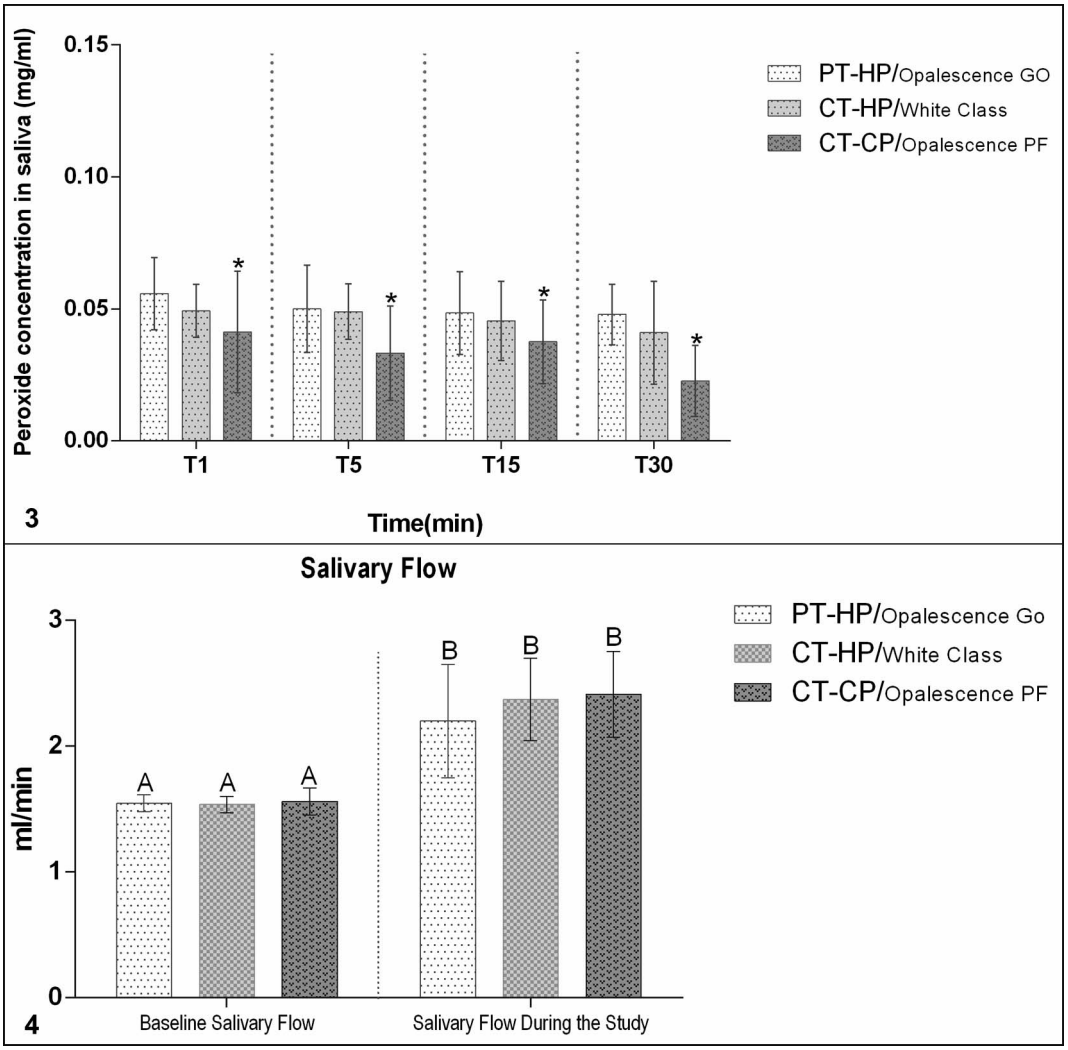

Figure 3. *Hydrogen peroxide concentration in saliva for each bleaching system according to time (asterisk shows significantly lower peroxide concentration in saliva inn each time,* $p<0.05$*).*

Figure 4. *Mean baseline salivary flow and salivary flow during the study (mL/min) for different bleaching systems and results of the Tukey test (different letters mean significant differences, 5%).*

Carbamide peroxide decomposes, generating approximately 3.5 parts of hydrogen peroxide and 6.5 parts of urea,[14] resulting in a real concentration of 3.5% of hydrogen peroxide versus the 10% hydrogen peroxide of the other two products tested. In this way, it was in fact expected that hydrogen peroxide detected in saliva would be lower than the 10% carbamide peroxide gel.

Human saliva is considered an effective defense against possible toxic effects due to the presence of enzymes such as peroxidase and catalase, which degrade peroxide.[14,16] Additionally, salivary flow can be influenced by several factors, including drugs, taste sensations, olfactory stimulus, circadian cycle, as well as movements and tactile stimulation of mucosa.[31] The trays are considered a tactile stimulation inside the mouth, and this is related to the overall 50% increase in salivary flow of participants in this study compared with initial salivary flow (before the use of trays). The relation between bleaching system and salivary flow was considered weak. Although prefilled trays are adaptable, they cannot accurately copy a patient's dental arch compared with customized trays; however, this did not influence salivary flow change in participants.

Even though peroxide was detected in participants' saliva, the peroxide concentration was considered much lower than the estimated toxic dose. Different protective mechanisms are related to peroxide degradation in organisms, such as oxida-

tion reaction by salivary peroxidases, catalase reaction from bacteria, consumption of peroxide due to its antimicrobial action, and the diffusion of peroxide through the tooth.[21] Bleaching gels also have different viscosities, which can favor or not the retention of gels inside the trays.[18] Nevertheless, bleaching systems containing the same hydrogen peroxide concentration did not show differences regarding the presence of peroxide in saliva, even with prefilled trays containing a higher amount of bleaching agent compared with customized trays. Although the prefilled tray is universally designed and presents a higher amount of bleaching gel, the gel is more viscous and is concentrated in the incisal/occlusal region of the tooth, which does not favor its overflow to saliva. Moreover, the tray extends beyond marginal gingiva and therefore acts as an external barrier, reducing the eventual contact between saliva and bleaching gel.

In this study, peroxide concentration in saliva was considered low and safe in relation to estimated toxic dose, even using at-home gels with higher concentrations of peroxide. Thus, at-home bleaching procedures are not expected to produce significant risk to human health under present conditions tested. Care should be taken to ensure these favorable results. When customized trays are used, they should be properly manufactured, and trimmed, and the adaptation to dental arches should be checked. Also, the patient must be oriented regarding the correct gel application, avoiding excessive amounts that may promote overflow. It is important to highlight that even though reservoirs may not interfere with bleaching efficacy and gel degradation,[19,32] when they are not present, more gel could be lost to the oral environment, thereby increasing the risk of peroxide contact with soft tissues, release to saliva, and, consequently, ingestion.[32] Additionally, patients must be instructed to rinse the mouth abundantly and expectorate after tray removal as our findings indicate no detectable presence of peroxide in saliva after these procedures.

Considering that the peroxide concentration in saliva within the tested parameters was extremely low, it can be inferred that the bleaching agent is retained within the tray, suggesting that the amount of gel does not decrease significantly during the time of use, maintaining the bioavailability of the bleaching agent to interact with the tooth surface without compromising bleaching efficacy. Indeed, in additional studies conducted by our research group, whitening efficacy was also not influenced by the type of tray used or the gel degradation pattern.[33,34]

CONCLUSIONS

Higher peroxide levels were detected in saliva with 10% hydrogen peroxide at-home bleaching gel compared with 10% carbamide peroxide gel, despite the type of tray used. Nevertheless, a sufficiently large margin of safety was observed with all bleaching systems tested compared with the estimated toxic dose. Salivary flow increased around 50% during tray use, independent of the bleaching system tested.

Acknowledgements

The authors wish to thank the following companies for the materials donation: Ultradent Products, South Jordan, UT, USA and FGM, Joinville, SC, Brazil. This study was supported by FAPESP (São Paulo Research Foundation: 2016/13044-4, first author's scholarship; 2016/25644-6, second author's scholarship); and CNPq/PQ (310320/2017-8, corresponding author's research productivity scholarship).

Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of the Institute of Science and Technology committee for the protection of human participants. The approval code issued for this study is No. RBR-34F49C.

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 24 September 2019)

REFERENCES

1. Joiner A (2006) The bleaching of teeth: A review of the literature *Journal of Dentistry* **34(7)** 412-419.
2. Kose C, Calixto AL, Bauer JR, Reis A, & Loguercio AD (2016) Comparison of the effects of in-office bleaching times on whitening and tooth sensitivity: A single blind, randomized clinical trial *Operative Dentistry* **41(2)** 138-145.
3. Dahl JE & Pallesen U (2003) Tooth bleaching—A critical review of the biological aspects *Critical Reviews in Oral Biology and Medicine* **14(4)** 292-304.
4. Torres CR, Crastechini E, Feitosa FA, Pucci CR, & Borges AB (2014) Influence of pH on the effectiveness of hydrogen peroxide whitening *Operative Dentistry* **39(6)** E261-E268.
5. Kwon SR & Wertz PW (2015) Review of the mechanism of tooth whitening *Journal of Esthetic and Restorative Dentistry* **27(5)** 240-257.
6. Bernardon JK, Sartori N, Ballarin A, Perdigão J, Lopes GC, & Baratieri LN (2010) Clinical performance of vital bleaching techniques *Operative Dentistry* **25(1)** 3-10.

7. Sulieman MA (2008) An overview of tooth-bleaching techniques: Chemistry, safety and efficacy *Periodontology 2000* **48** 148-169.
8. Dietschi D, Benbachir N, & Krejci I (2010) In vitro colorimetric evaluation of the efficacy of home bleaching and over-the-counter bleaching products *Quintessence International* **41(6)** 505-516.
9. Heymann HO (2005) Tooth whitening: Facts and fallacies *British Dental Journal* **198(8)** 514.
10. Haywood VB & Heymann HO (1989) Nightguard vital bleaching *Quintessence International* **20(3)** 173-176.
11. Haywood VB & Heymann HO (1991) Nightguard vital bleaching: How safe is it? *Quintessence International* **22(7)** 515-523.
12. Goldberg M, Grootveld M, & Lynch E (2010) Undesirable and adverse effects of tooth-whitening products: A review *Clinical Oral Investigations* **14(1)** 1-10.
13. Watt BE, Proudfoot AT, & Vale JA (2004) Hydrogen peroxide poisoning *Toxicological Reviews* **23(1)** 51-57.
14. Li Y (2011) Safety controversies in tooth bleaching *Dental Clinics of North America* **55(2)** 255-263.
15. Weiner ML, Freeman C, Trochimowicz H, de Gerlache J, Jacobi S, Malinverno G, Mayr W, & Regnier JF (2000) 13-week drinking water toxicity study of hydrogen peroxide with 6-week recovery period in catalase-deficient mice *Food and Chemical Toxicology* **38(7)** 607-615.
16. Hannig C, Zech R, Henze E, Dreier S, & Attin T (2005) Peroxide release into saliva from five different home bleaching systems in vivo *American Journal of Dentistry* **18(1)** 13-18.
17. Interdepartmental Group on Oral Health Risk from Chemicals (IGHRC) (2003) Uncertainty factors: their use in human health risk assessment by UK government; Retrieved March 9th, 2019. http://www.iehconsulting.co.uk/IEH_Consulting/IEHCPubs/IGHRC/cr9.pdf
18. Marques DN, da Mata AD, Silveira JM, Marques JR, Amaral JP, & Guilherme NF (2012) Hydrogen peroxide release kinetics into saliva from different whitening products: A double-blind, randomized clinical trial *Clinical Oral Investigations* **16(1)** 155-163.
19. Matis BA, Yousef M, Cochran MA, & Eckert GJ (2002) Degradation of bleaching gels in vivo as a function of tray design and carbamide peroxide concentration *Operative Dentistry* **27(1)** 12-18.
20. Schulz KF, Altman DG, Moher D, CONSORT group (2010) CONSORT 2010 statement: updated guidelines for reporting parallel group randomized trials *International Journal of Surgery* **9(8)** 672-677.
21. Hannig C, Zech R, Henze E, Dorr-Tolui R, & Attin T (2003) Determination of peroxides in saliva-kinetics of peroxide release into saliva during home-bleaching with Whitestrips and Vivastyle *Archives of Oral Biology* **48(8)** 559-566.
22. Faul F, Erdfelder E, Lang AG, & Buchner A (2007) G*Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences *Behavior Research Methods* **39(2)** 175-191.
23. Torres CR, Wiegand A, Sener B, & Attin T (2010) Influence of chemical activation of 35% hydrogen peroxide bleaching gel on its penetration and efficacy—in vitro study *Journal of Dentistry* **38(10)** 836-843.
24. Heft MW & Baum BJ (1984) Unstimulated and stimulated parotid salivary flow rate in individuals with different ages *Journal of Dental Research* **63(10)** 1182-1185.
25. Quintana M, Palicki O, Lucchi G, Ducoroy P, Chambon C, Salles C, & Morzel M (2009) Inter-individual variability of protein patterns in saliva of healthy adults *Journal of Proteomics* **72(5)** 822-830.
26. Torres CGR, Koga AF, & Borges AB (2006) The effects of antioxidant agents as neutralizers of bleaching agents on enamel bond strength *Brazilian Journal of Oral Sciences* **5 16** 971-976.
27. Sasaki RT, Flório FM, & Basting RT (2009) Effect of 10% sodium ascorbate and 10% α-tocopherol in different formulations on the shear bond strength of enamel and dentin submitted to a home-use bleaching treatment *Operative Dentistry* **34(6)** 746-752.
28. Naik S, Tredwin CJ, & Scully C (2006) Hydrogen peroxide tooth-whitening (bleaching): Review of safety in relation to possible carcinogenesis *Oral Oncology* **42(7)** 668-674.
29. Fuwa K & Vallee BL (1963) The physical basis of analytical atomic absorption spectrometry: The pertinence of the Beer-Lambert law *Analytical Chemistry* **35(8)** 942-946.
30. Ricci RW, Ditzler M, & Nestor LP (1994) Discovering the Beer-Lambert Law *Journal of Chemical Education* **71(11)** 983.
31. Humphrey SP & Williamson RT (2001) A review of saliva: Normal composition, flow, and function *Journal of Prosthetic Dentistry* **85(2)** 162-169.
32. Matis BA, Hamdan YS, Cochran MA, & Eckert GJ (2002) A clinical evaluation of a bleaching agent used with and without reservoirs *Operative Dentistry* **27(1)** 5-11.
33. Mailart MC, Ferracioli CS, Torres CRG, Palo RM, & Borges AB (2017) Evaluation of degradation of hydrogen peroxide in customized or prefilled trays: Randomized clinical trial [in Portuguese] *Brazilian Oral Research* **31(Supplement 2)** 302
34. Mailart MC, Palo RM, Torres CRG, & Borges AB (2018) Evaluation of at-home bleaching efficacy with different systems: Randomized clinical trial [in Portuguese] *Brazilian Oral Research* **32(Supplement 2)** 309.