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ORIGINAL ARTICLE

Hydrogen peroxide release kinetics into saliva from different whitening products: a double-blind, randomized clinical trial

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Abstract The objective of this study is to compare salivary hydrogen peroxide (HP) release kinetics and potential toxicity of systemic exposure of four different whitening products. A double-blind, randomized controlled trial was conducted in a Portuguese dental faculty clinic. Two hundred forty volunteers were randomized to eight intervention groups. Participants were randomly assigned to receive active or placebo applications of one of four different products: Opalescence 10% PF™ (OPL), Vivastyle® 10%™ (VS10%), Vivadent Paint On Plus™ (PO+), and Trés White Supreme™ (TWS). Saliva collection was obtained by established methods at different times. The HP salivary content was determined by a photometric method. Salivary HP variations, total amount of salivary HP, and counts of subjects above the safe daily HP dose were the main outcome measures. All whitening systems significantly released HP to the saliva when compared to placebo, and all showed different release kinetics. The adaptable tray system (TWS) presented a risk increase of 37% [20–54%, 95% confidence interval] when compared to the other systems. The use of an adaptable tray whitening system with higher concentration of HP increases the toxicity potential.

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Introduction

In recent years, tooth whitening has become one of the most rapidly growing oral care sectors, fuelled by the patients demand for both healthy and cosmetically attractive smiles. The challenge to enhance the cosmetic appearance of teeth has led to the launch of a multitude of improved toothpastes, in-office or home prescribed professional whitening kits, and mass market technologies for tooth whitening [1–4]. From the usual 10% carbamide peroxide in custom-made bleaching trays to the more recent strip and paint-on delivery systems, all is available to the clinician. However, this diversity has launched to the market products from which its efficacy, longevity, and safety have not been fully studied [5]. In fact, the peroxide released into the saliva from tooth bleaching is potentially available for ingestion [6–8], and with the proliferation of new formulations, it is necessary to ascertain their real efficacy and safety when compared to traditional standard of care systems like the nightguard vital bleaching with 10% carbamide peroxide. Therefore, the aim of this randomized controlled trial was to determine the safety profiles, mainly the hydrogen peroxide (HP) release into the saliva, and the patient total HP exposure of whitening products intended for home-use like Opalescence 10% PF™ (OPL; Ultradent®, USA) and Vivastyle® 10%™ (VS10%; Ivoclar-Vivadent®, Liechenstein) from a paint-on formulation like Vivadent Paint On Plus™ (PO+; Ivoclar-Vivadent®, Liechenstein) and a standard tray formulation like Trés White Supreme™ (TWS; Ultradent®, USA) in healthy individuals, comparing the different systems.

Objectives

The aims of this study were to compare the effects of four different whitening systems on the HP released into the saliva.

The study hypotheses were as follows:

1. There is a significant difference in the HP released into the saliva from the four whitening systems.
2. There is a significant difference in HP released into the saliva between active and placebo products of each whitening system.

Subjects and methods

Trial design

This study employed human subjects in a randomized, double-blind, single-center, eight-arm, placebo-controlled, parallel phase IV clinical trial with medical devices to evaluate the clinical safety during the whitening treatments.

Study participants

Patients were recruited between January and July 2007 from a population of patients of a Portuguese University Clinic through advertisement and were eligible if healthy and above 18. Recruitment was supervised by research assistants.

Exclusion criteria were the presence of systemic conditions that may cause oral dryness and the taking of current xerostomic medication, known allergies to ingredients, and patients who are pregnant or nursing; both records were obtained self-reportedly from volunteers.

In total, 240 participants gave their written informed consent, and saliva samples were collected at the oral biology research group (GIBO) laboratory. The study protocol was approved by the local ethics committee at the Instituto Superior de Ciências da Saúde Egas Moniz, Portugal. All steps of the study were planned and according to the principals outlined in the Declaration of Helsinki [9] (Table 1).

Interventions

Visit 1

Prior to visit 1, the whitening products used in this study were firstly titrated for its HP content by a described method using cerium as a titrating agent [10, 11].The initial concentration of each lot of the whitening products was analyzed until a minimum of three replicates within the interval of 0.5% was obtained. All products were refrigerated at 4°C until used in the study. At the screening visit, exclusion criteria were verified for each participant, and from there, they were randomly allocated to one of eight groups marked from A to H accordingly to computer-generated randomization software (GraphPad Quick-Calcs website: http://www.graphpad.com/quickcalcs/randomize1.cfm, accessed on December 2006). Both placebo and active products for each whitening system were transferred by external personnel into identical opaque containers and named according to the randomization software from A to H. A code for randomization was kept in an opaque envelope and kept in a safe and opened only at the end of the study. Data were analyzed by a third party blinded to the allocation results, which were at that point referred to as treatment A to H in the SPSS worksheet (SPSS, Inc., Chicago, IL, USA).

Since the four whitening systems present different formulations, blinding was only effective between active and placebo groups for each system. Nevertheless, the measurements of interest were objective and not susceptible to interpretation, thus removing the potential bias for lack of blinding.

Table 1 Whitening products used in the clinical study with manufacturers, application type, lot numbers of products, labeled carbamide peroxide (CP), and corresponding hydrogen peroxide (HP)

Product	Active agent	Application type	Instructions to use	Manufacturer	Lot number	Label CP%	Label HP%
Vivadent Paint On Plus™	HP	Self-application paint on varnish	Two times a day, 10 min applications at home (14 days)	Ivoclar-Vivadent®	G27174 (A) NLL2020 (P)	–	6
Vivastyle® 10%™	CP	Customized nightguard gel	One hour application per day at home (14 days)	Ivoclar-Vivadent®	HL1018 (A) NRG3133 (P)	10	3.62
Opalescence 10% PF™	CP	Customized nightguard with reservoirs gel	Two hours application per day at home (14 days)	Ultradent®	B1KGY (A)	10	3.62
Trés White Supreme™	HP	Standardized professional supervised self-applied nightguard	One hour application per day (10 applications)	Ultradent®	B2KHW (A) B2K2D (A) B2JRM (A)	–	10

The Ultradent placebos products do not possess lot numbers

A active product, *P* placebo product

Participants were instructed to present themselves between 8 and 11 a.m. at the laboratory the following week. The participants were told to refrain from eating and drinking (except water) for 2 h prior to the investigation to minimize effects of diurnal variability in salivary composition [12, 13].

Visit 2

Upon arrival at the laboratory, participants were instructed to perform a supervised tooth brushing with modified Bass technique [14] since the manufacturer recommends brushing immediately before applying their product. All subjects were provided with a non-whitening dentifrice (Aquafresh Extreme clean, GlaxoSmithKline, Brentford, UK) and a medium soft-bristled manual toothbrush (Akzenta, Lugano, Switzerland) and waited for 1 h. Before whitening protocol, a baseline unstimulated saliva sample (about 2 min) was taken for determination of the reagent blank value. After the start of the bleaching protocol, saliva samples were taken at 1, 2, 3, 5, 8, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 75, 90, 105, and 120 min accordingly with the whitening protocol advised by the manufacturers. The amount of whitening product that each participant applied was weighted to the nearest milligram on an analytical balance (Denver Instrument Company, Denver, CO, USA) to determine the initial amount of HP to which the subject was exposed (initial content).

Previous to saliva collection in the VS10% system, patients were instructed to wipe off the excess gel with a clean finger, and the patients for OPL system were instructed that after removing the excess gel, they should rinse with water twice and disgorge to a clean vial (rinse sample). During the saliva collection, accordingly to described methods [15, 16], subjects were advised to keep their mouths closed and only at the pre-determined times to expectorate into previously weighted 15-ml falcon tubes. After this procedure, the saliva contained in the falcon tube was weighed, and salivary volume was determined in milliliters. The saliva was analyzed to evaluate the amount of HP each subject would probably have swallowed if spitting did not occur (saliva sample).

After the bleaching protocol, the remaining product on the teeth was removed, and the participants were asked to rinse their mouths with distilled water to wash out remnants of the whitening material in order to determine the remaining HP (remaining). All participants were weighted with an analytical balance (Body line, Ufesa, Vitoria, Spain).

Enzymatic determination of peroxide

A photometric method based on the reaction of 4-aminoantipyrin and phenol with H_2O_2 catalyzed by horseradish peroxidase was used for determination of peroxide in salivary samples [6–8] since the 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) method presented interaction with saliva constituents [17]. For determination of peroxide, a calibration of peroxide was carried out. An enzyme reagent with aminophenazone (4-aminoantipyrin) (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. The reagent was stored at 4°C; 30 μl of saliva was added to 1,470 μl enzyme reagent in a 1.5-ml cuvette (Nuova Aptaca, Italy). The cuvette was hand stirred to allow for the components to mix properly. Directly afterwards, the absorbance was measured at λ=510 nm (Heλios, Unicam, Germany). Results were expressed as milligrams HP per milliliter or milligrams HP present.

At least nine solutions of different HP concentrations were made out of a HP standard solution in order to get an absorption in the range of 0.1–1.5. Only confidence coefficients >0.99 were accepted as valid.

The complete mean peroxide release into saliva from whitening products was divided through the subject's body weight in order to calculate mean daily dose per kilogram. This value was then compared with the safe daily dose derived from the study by Weiner et al. [18]. The safe daily intake is indicated by the application of the traditional 100-fold factors to the NOEL (IGHRC), which gives a safe daily intake level of 0.26 mg/kg/day [7].

Outcomes

The HP released into the saliva was expressed as mean± standard error of the mean of milligrams of HP obtained at different time points.

Overall HP obtained in the different samples (initial content, rinse sample, content in saliva, and remaining content) was expressed as mean±standard error of the mean of the total HP obtained divided by the participant body weight (milligrams HP per kilograms).

To better quantify risk differences of the HP exposure above safe daily intake of the different whitening systems, a contingency table compiling the counts of subjects with salivary HP above 0.26 mg kg^{-1} was obtained. Additional analyses were performed to calculate association measures like the absolute risk increase and number needed to harm.

Statistics

All data analysis was carried out according to a pre-established plan. Data and analyses were computed using a computer statistical package (SPSS v.15, SPSS Inc., Chicago, IL, USA).

The results were expressed as milligrams of HP, milligrams of HP per kilogram of body weight, and number of patients above the safe daily intake.

Comparison between the study groups was performed with *t* tests or χ^2 tests as appropriate. The mean values were reported, followed by standard error of the means. A 5% significance level was used for all statistical comparisons.

Results

Participant baseline demographic characteristics

A total of 240 persons were selected for participating in the study (Fig. 1). They were randomly assigned to one of the eight study groups, and there were no dropouts. Baseline characteristics of the eight groups are depicted in Table 2. Chi-square test and Student's *t* test were employed for testing differences between categorical and continuous variables, respectively. There were no statistically significant differences ($P>0.05$) between baseline characteristics for the placebo and active subgroups of each whitening system.

Hydrogen peroxide release kinetics into saliva from different whitening products

In this series of experiments, the HP release kinetics into the saliva of different whitening products was determined and compared to respective placebo and also between each product. Before the whitening protocol, a baseline non-stimulated saliva sample was taken for determination of the reagent blank value.

Figure 2 shows original time course chart recordings of the HP release kinetics of 10% carbamide peroxide whitening gel (OPL) intended for a minimum 2-h use when compared to placebo. The results show a sustained HP release during the 120-min protocol; however, the magnitude of the HP release was higher during the initial 30 min release ($P<0.05$).

Figure 3 shows original time course chart recordings of the HP release kinetics of 10% carbamide peroxide whitening gel (VS10%) intended for a 1-h use when compared to placebo. The results show a high release kinetics in the first ten minutes which decreases during the following 45 min of the protocol when compared to the placebo ($P<0.01$).

Figure 4 shows original time course chart recordings of the HP release kinetics of 10% HP in a pre-loaded adaptable whitening tray (TWS) intended for a 1-h use when compared to placebo. The results show a high and sustained release of HP during the 60-min protocol when compared to the placebo product ($P<0.01$).

Figure 5 shows original time course chart recordings of the HP release kinetics of 6% HP in a "paint-on" formulation (PO+) intended for 10 min use (twice a day) when compared to placebo. The results show that the highest peroxide release occurs in the first 2 min, which is significantly different when compared to placebo ($P<0.01$).

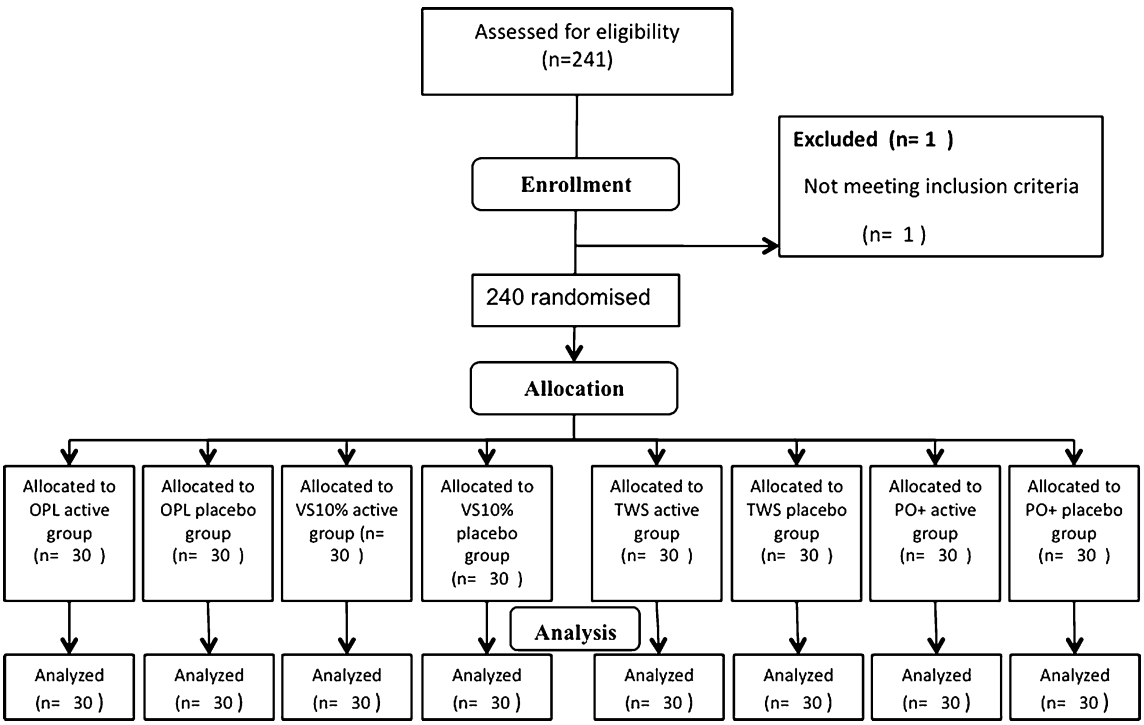

Fig. 1 Study design diagram

Table 2 Mean±SEM for baseline characteristics

	OPL		VS10%		TWS		PO+	
	Active	Placebo	Active	Placebo	Active	Placebo	Active	Placebo
Gender								
Male	21	23	15	14	10	10	11	13
Female	9	7	16	14	20	20	19	17
Age (years)	22.65±1.23	23.23±2.09	20.93±0.42	21.00±0.50	21.48±0.53	19.67±0.30	22.12±2.79	21.77±2.58
Body weight (kg)	61.08±1.65	60.38±2.72	64.14±2.35	63.07±2.00	59.18±2.07	59.96±2.99	64.35±11.96	65.19±2.58

Hydrogen peroxide toxicity from different whitening products

Figure 6 shows the mean±SEM of the initial content, initial rinses, saliva, and the remaining of HP content per kilogram of weight of the different whitening systems used in this study.

(a) OPL. The results show that the initial amount of HP applied is above of the safe level of intake, while initial content, rinse, and remaining gel retrieved were significantly different when compared to the placebo group. Moreover, OPL presented a very low release into the saliva during the protocol when compared to the placebo results ($P>0.05$). Since OPL can be used overnight, we estimate that the maximum exposure level would equate to 0.04 mg H_2O_2/kg (saliva content+remaining), also well below the safe daily dose of 0.26 mg H_2O_2/kg.

(b) VS10%. The results show that this product presented a similar initial amount of HP applied when compared to OPL ($P>0.05$), since both gels present a concentration of 10% carbamide peroxide. The amount of HP retrieved from the saliva and from the remaining was significantly different when compared to the placebo group ($P<0.01$). Both of these values were below the safe daily intake of HP.

(c) TWS. The results show that the initial content, the saliva content, and the remaining content were significantly different when compared to the placebo group ($P<0.01$).

(d) PO+. The results show that the initial amount of HP is below the safe daily intake of HP (0.26 mg/kg) and that the initial content of HP and the amount retrieved are significantly different when compared to the placebo group ($P<0.01$). Since PO+ is recommended for use once/twice a day, it is conceivable that the adoption of the twice-a-day regimen will result in an enhancement of the daily peroxide release into saliva than that reported here. The daily exposure to HP from PO+ (releasing 0.005±0.001 mg H_2O_2/kg) used two times daily equates to 0.01 mg H_2O_2/kg, also below the safe daily dose and therefore not of toxicological concern.

Figure 7 shows the mean±SEM of maximum possible exposure (saliva content+remaining content) per kilogram of weight of the four whitening products used in this study. The results present different toxicological concerns, while TWS presented results of maximum exposure well above the safe daily dose (0.26 mg H_2O_2/kg) and was significantly different when compared to OPL, VS10%, and PO+ ($P<0.01$), which presented results of no toxicological concern.

Table 3 shows a contingency table for determination of absolute risk increase of different whitening products regarding the safe daily intake level. The results show that the number needed to harm is 3 for TWS, which means that

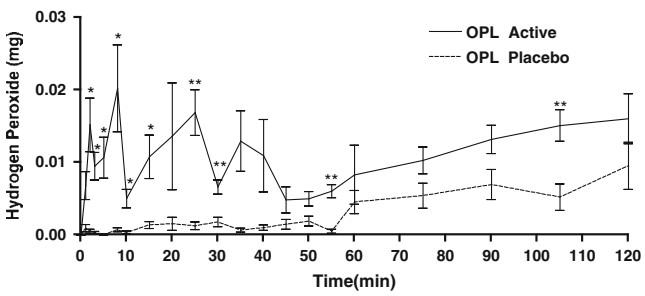

Fig. 2 Original chart recording of mean±SEM of hydrogen peroxide release into saliva during application of OPL in upper and lower teeth for 120 min ($n=30$; *$P<0.05$; **$P<0.01$ when compared with placebo)

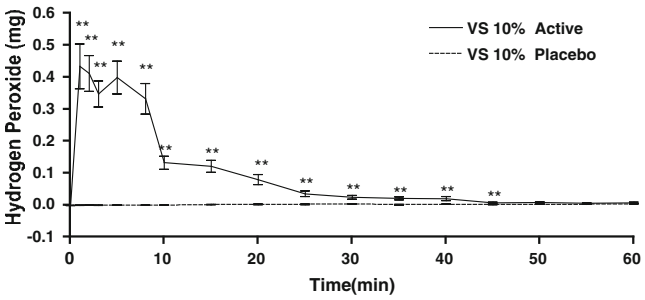

Fig. 3 Original chart recording of mean±SEM of peroxide release into saliva during application of VS10% in upper and lower teeth for 60 min ($n=30$; **$P<0.01$ when compared with placebo)

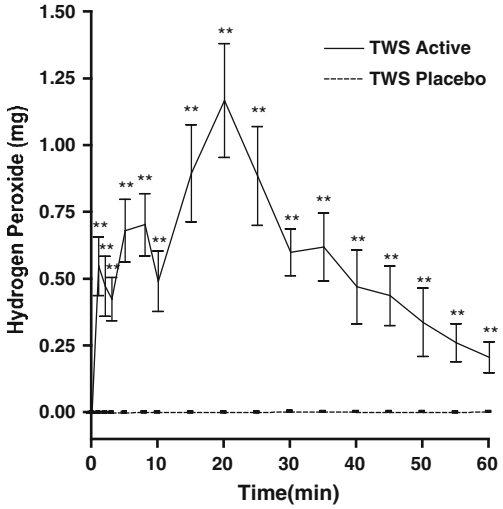

Fig. 4 Original chart recording of mean±SEM of peroxide release into saliva during application of TWS in upper and lower teeth for 60 min (n=30; **P<0.01 when compared with placebo). Note that there were significant differences between active and placebo groups during the 60-min duration of the whitening protocol

for every three whitening treatments, patients on TWS will experience a potential systematic overexposure to HP.

Absolute risk increase

$$\mathrm{TWS} = 37 \pm 17\%[20 - 54\%]95\%\mathrm{CI}$$

Number needed to harm

$$\mathrm{TWS} = 3[2 - 5]95\%\mathrm{CI}$$

Discussion

The results of this study suggest that based on the peroxide release kinetics data of this study, the product with higher HP content (manufacturer's claim) used in this study was the only one that presented mean exposures of HP in the oral cavity which exceeded the safe daily intake adopted for this study of 0.26 mg H_2O_2/kg and therefore should be used with caution in patients. However, the results also suggest that an initial mouth rinsing with water after tray application reduces significantly the initial HP peak release, thus reducing the exposure to HP content.

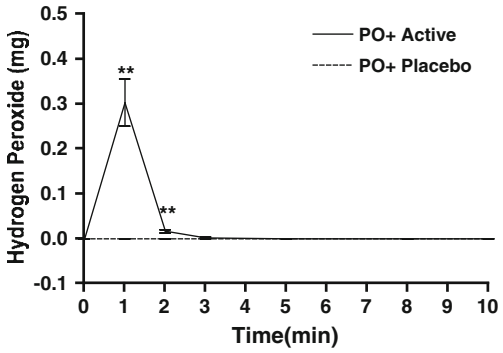

Fig. 5 Original chart recording of mean±SEM of peroxide release into saliva during application of PO+ in upper and lower teeth for 10 min (n=30; **P<0.01 when compared with placebo)

Every product presented different peroxide release kinetics, which is consistent with our in vitro results [19] and suggests that besides the type tray used, the formulation of the product (viscosity, HP concentration, matrix composition, and type of application) will interfere with the peroxide release into the oral cavity. Thus, the results of this study indicate that the use of a standard tray with higher HP content possesses an unfavorable risk benefit ratio when compared to the other products and that increasing the HP concentration in whitening products should be done with caution to prevent the risk of increased toxicity.

For the preparation of this study, we conducted a search on Cochrane database, which retrieved a review published in 2006 [20] referring to dental whitening studies in the last 40 years, with a total of 416 articles identified. However, these studies enrolled small number of patients; employed varied designs, preparations, and doses; and included diverse study populations. Of the initial 416 articles, only 25 met the inclusion criteria (randomized controlled trials and quasi-randomized controlled trials of dentist-dispensed or over-the-counter tooth whitening products with a chemical action, for home use) and presented data that could be used in the Cochrane review analysis. This review concluded the need for pragmatic long-term and independent clinical studies where it is possible to access the efficacy and safety of such products. Also regarding HP toxicity, very few data exists with only three previously published studies [6–8] with a small number of enrolled patients. For this purpose, a large-scale randomized, double-blind, single-center, eight-arm, placebo-controlled, parallel phase IV clinical trial was designed specifically to determine the safety profile of different whitening products in healthy individuals, comparing different systems.

Sixty (60) patients were randomly allocated to each whitening system used in the safety study. Baseline characteristics were determined (age, weight, and gender) and compared between groups (active vs. placebo).

In this study, we have evaluated the peroxide release and toxicity from classical trays intended for home use (OPL and VS10%), from a paint-on formulation (PO+) and a standard tray formulation (TWS). Although some methods for determination of peroxides can be influenced by oxidizable salivary components, the photometric method utilizing peroxidase and 4-aminoantipyrin is shown to be accurate and that oxidizing components do not interfere

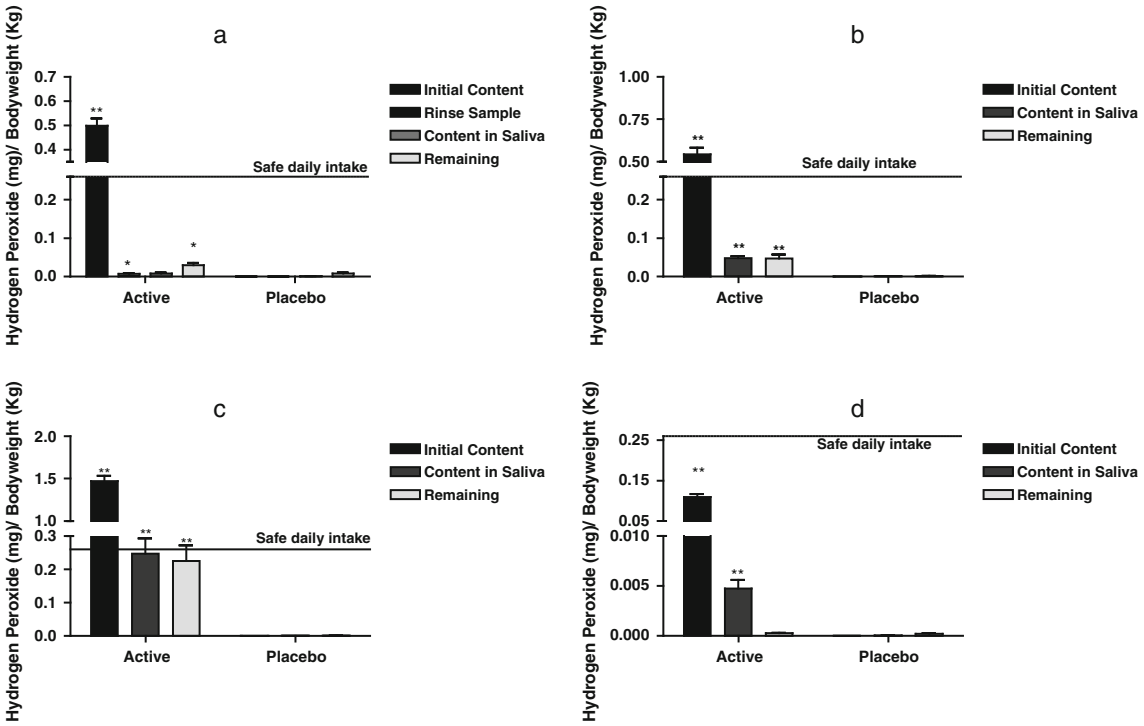

Fig. 6 Bar charts showing mean±SEM of individual hydrogen peroxide exposition in initial content, initial rinses, saliva, and the remaining of hydrogen peroxide content per kilogram of weight of the different whitening systems used in this study. *$P<0.05$ and **$P<0.01$ when compared with placebo. **a** OPL application (120 min; $n=30$). **b** VS10% application (60 min; $n=30$). **c** TWS application (60 min; $n=30$). **d** PO+ application (10 min; $n=30$)

significantly with the color complex [6–8, 21] in contrast and comparison with the ABTS method. The highest concentration in the saliva was observed in the initial minutes after application of the whitening systems (Figs. 2, 3, 4, and 5) but rapidly decreased, except for the pre-loaded adaptable whitening tray which presented a sustained release during the whole whitening protocol, probably due to whitening systems like Opalescence containing carboxymethylene polymer (Carbopol) to prolong the release of peroxides [22, 23]. Despite the fact that OPL contains the same amount of peroxides than VS10%, it showed a significantly lower kinetics profile probably due to its viscosity and the initial mouth rinsing with water. The significantly lowest exposure and fastest kinetic peroxide profile were presented by the paint-on formulation (PO+), releasing its HP content in the initial 2 min of the whitening protocol. The significantly higher initial HP content was observed in the TWS system and presented mean exposures of HP in the oral cavity higher than the safe daily intake established for this study of 0.26 mg H_2O_2/kg (Fig. 7). Thus, it is suggested that besides the type tray used, the formulation of the product (viscosity, HP concentration, matrix composition, and type of application) interferes with the peroxides released into the oral cavity.

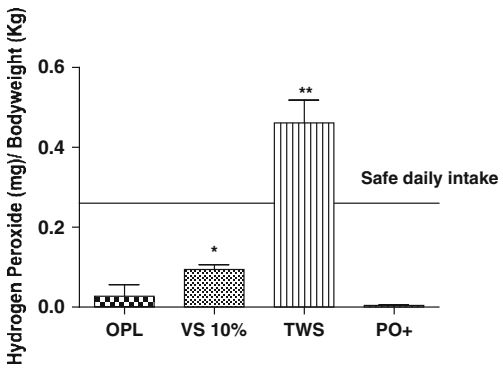

Fig. 7 Bar chart showing mean±SEM of maximum individual hydrogen peroxide exposition (released into saliva and measured in remaining; $n=30$; *$P<0.05$ when compared to PO+ system; **$P<0.01$ when compared to the other systems)

Table 3 Contingency table for determination of absolute risk increase of different whitening products regarding the safe daily intake level

Product	Above safe daily intake		
	Yes	No	Total
OPL	0	30	30
VS10%	0	30	30
TWS	11	19	30
PO+	0	30	30

The amount of peroxides retrieved at the end treatment was significantly lower in the paint-on formulation when compared with the tray formulations, suggesting that the existence of some sort of an external barrier may prevent the release of HP into the saliva with increased effectiveness when compared with paint-on formulations. This finding is supported by other studies with similar conclusions [6, 7]. In all the whitening systems used in this study, more than 75% of the peroxide was not recovered in the saliva or in the remaining gel/varnish at end treatment. Accordingly, to a diversity of studies, a number of different reasons could be accounted for, namely, the degradation of peroxide by salivary and cellular enzymes [7], reaction with external dental stains [24], diffusion through dental hard tissues [25], antimicrobial activity [26], and swallowing of bleaching gel despite the fact that the subjects were advised to expectorate saliva completely. OPL presented a scarce user's instructions, advising from a 2- to 8-h application. In our study, the application time was limited to 2 h of saliva collection since it is uncomfortable to swallow dry for longer times by the volunteers; however, since the remaining peroxide after the 2-h protocol was determined, the worst-case assumptions were made regarding 100% ingestion of saliva collection and remaining gel for all the products used. Moreover, it was possible to ascertain that the prolonged use of this system (more than 2 h) is only due to the patient's comfort since only 7% of the initial content was recovered from the tray after the 2-h use.

The amount of peroxide charged in the custom trays was diverse between subjects since the patients placed the gel at their own will, accordingly to the user's instructions. The average application for the custom trays was of 400–450 mg per tray, while the standardized tray presented an average value of 1,000–1,200 mg per tray. Considering these initial values, it thus could be helpful to disgorge or rinse with water the initially released maximum of HP immediately after setting the bleaching tray. In fact, if the request to disgorge/rinse with water is not added to the manufacturer's instructions, the peroxide released into the saliva is substantially increased.

Nonetheless, based on the peroxide release kinetics data of the present study, the daily exposure to HP resulting from the application of the tooth whitening agents tested only presented a toxicological concern to TWS (standard tray) group. This product presented an absolute risk increase of 37±17%, and the number needed to harm was of three patients. All the custom-tray and paint-on formulations intended for home use presented much lower values than the safe daily intake level for peroxides, which was determined to be 0.26 mg/kg/day regarding the traditional 100-fold safety factor [7, 18]. This sufficiently large margin of safety between the dose that was used with no effect in animal studies and the human exposure to HP during tooth whitening provides confirmation that there is no increased systemic risk for the human when using some of these products.

Some conclusions can be drawn on the relationship between the amount of peroxide released into the oral cavity and the intensity of intraoral effects, namely, the presence of ulcerations since the only product with values above the safe daily intake was the only one where patients presented oral ulcerations following treatment. These side effects of peroxides on oral structures are commonly described in the literature, namely, transient inflammatory reactions and irritation of the gingiva and/or tooth sensitivity, and in a recent review published by the *Cochrane Database of Systematic Reviews* [20], the authors report the existence of these side effects in several studies and that the patients should be informed of them. While such effects are usually not severe and resolve within days [27], excessive and unintentional exposure of oral structures to peroxides should be reduced to the minimum possible [28].

In conclusion, the results of this study demonstrate that the use of an adaptable tray whitening system containing 10% HP induces a mean exposure higher than the safe daily intake, which can equate with an increased risk of HP toxicity. Moreover, it is strongly suggested that adding to the manufacturer's instructions, the request to disgorge/rinse with water immediately after setting the bleaching tray maintains the benefits (HP inside the tray) while diminishing in an important way the HP salivary exposure, and therefore, its use could be less detrimental and recommended.

Conflicts of interest The authors declare that they have no conflict of interest and that the whitening systems were provided by Ultradent®, USA and Ivoclar-Vivadent®, Liechenstein.

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