



Research article

Assessment of the temporal variation of electrical potential and pH of different bleaching agents

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ABSTRACT

Introduction: Tooth whitening procedures are under continuous investigation to improve esthetic outcomes and reduce bleaching sensitivity (BS) precipitating from treatments. During the dental bleaching process it is known that the release of free radicals degrades the organic pigment molecules of the tooth and with this an amount of energy is released. Nonetheless, previous studies have never investigated the temporal correlation between of pH and electric potential (EP) generated in this treatment.

Objectives: Therefore, the objective of the present study was to investigate temporal variations of pH and EP associated with three different commercially available bleaching gels and the correlation levels between parameters of interest to provide relevant information regarding the kinetics of oxidation reactions in dental bleaching procedures.

Methods: The study was divided into 3 groups (n = 9) in function of hydrogen peroxide concentration (either 6%, 15% and 35%). The temporal evolution of pH and EP values were determined using a highly-accurate and previously calibrated pH meter at specific time-points (5, 10, 15 and 30 min).

Results: Data obtained were submitted to one-way ANOVA of repeated measures with Bonferroni post-test ($\alpha = 0.05$). The results of the study showed difference in the factor gel concentration ($p < 0,0001$), time ($p < 0,0001$) and interaction (gel/time) ($p = 0.002$) while throughout the intervals evaluated the groups remained relatively stable and without significant difference in the intragroup variation of pH ($p < 0.05$) and in EDP only with significant difference in the 5 min interval of the 35% concentration. A 2nd order polynomial relationship test showed high correlation levels.

Conclusion: It can thus be concluded that there is a negative relationship between EP and pH variation in the different gel concentrations.

Clinical significance: The findings of the present study suggest that bleaching gels of higher concentration may provoke BS that are more intense and durable due to significant electric depolarization of neuronal extensions of pulpal tissues.

1. Introduction

Tooth whitening procedures (at home or in-office) are considered ultraconservative and minimally invasive techniques because they do not require the removal of sound tissues, can be safely utilized on vital teeth, and are typically used to resolve visually perceivable dental discolorations from extrinsic and intrinsic origins. However, despite their widespread utilization and acceptance amongst patients and clinicians

worldwide, numerous studies have reported on the occurrence of adverse effects including changes onto enamel surface properties and bleaching sensitivity (BS; trans- or post-operative). This trans- or post-operative sensitivity is usually perceived by patients as small and recurrent "electric shocks" and this sensation was then later defined to as the "Zinger effect" [1].

Even though theories (i.e., transducer and hydrodynamic) have been proposed to explain the strong and positive correlation between dental

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bleaching procedures and the occurrence of BS, the majority of studies previously published were not focused on elucidating the factors, mechanisms or neuronal pathways involved. The hydrodynamic theory, which is the most accepted in the literature about sensitivity in general, describes that external stimuli (thermal, electrical, chemical, tactile or evaporative) are capable of promoting the movement of fluids within dentinal tubules. Such movement stimulates neuronal mechanoreceptors (odontoblastic extensions) that are sensitive to fluid pressure and are translated as a painful sensation by pulpal tissues [2].

According to previous studies, BS is one of the most common adverse effects precipitating from dental bleaching procedures. Its intensity (low, mild and severe) and duration (short-term or long-term) are directly correlated to exposure time and peroxide concentrations used [3, 4]. Modern techniques are typically conducted using low hydrogen peroxide concentrations (6%–15%), include desensitizing components in their formulations, and are mediated by violet LEDs to decrease the incidence of BS, lower free radical concentrations [4] and the cytotoxicity potential associated with the procedure [5, 6, 7].

Dental bleaching techniques are based on the utilization of peroxide-containing gels (e.g., hydrogen or carbamide) that typically generate large amounts of reactive oxygen species (ROS) [8, 9, 10]. Free radicals generated penetrate through enamel and reach deep regions of teeth (enamel-dentin junction), where they degrade conjugated C=C double bonds present in long-chained organic molecules (chromophores), thereby altering chromophores' optical behaviors (absorption and scattering) [11, 12, 13]. However, due to their low molecular weight and non-specific mechanisms of action, ROS can be found in locations as deep as the pulp chamber, and in concentrations that are considered concerning from the biocompatibility and cytotoxicity standpoints [14].

According to a recent review article published by Gentil de Moor et al. [10], oxidizing agents such as oxygen, hydroperoxyl, sodium hypochlorite, hydrogen peroxide, ozone and hydroxyl, are associated with different redox potential (E° , in Volts; +1.229 V, +1.510 V, +1.630 V, +1.780 V, +2.075 V and +2.800 V, respectively), wherein the higher the redox potential, the more effective the oxidizing agent will be in degrading conjugated C=C bonds [15]. Therefore, based on the context presented herein, and on the principle of nerve depolarization [16], it is possible to hypothesize that patients treated with bleaching agents of higher electrical potential (EP) will display stronger higher incidences of BS for greater release of free radicals mentioned and consequently greater local electrical variation. With this gap in knowledge in our minds, the present study aimed to investigate temporal variations of pH and EP associated with three different commercially available bleaching gels and the correlation levels between parameters of interest to provide relevant information regarding dental bleaching procedures and indirectly correlate our findings with their potential to induce the occurrence of BS. The rationale for the experimental design proposed in the present in vitro laboratorial study, which does not use any type of dental substrate, is justified by the fact that our objective was to characterize the temporal evolution of pH and EP without the presence of unmanageable external confounding factors such as molecular composition, mineralization levels, buffering capacity, porosity and tissue impedance that are typically present when mineralized tissues from either human or bovine origins are used. The null hypothesis tested was that bleaching gels of different HP concentrations would not display different kinetics.

2. Materials and methods

2.1. Study design

Three commercially available bleaching gels of varying peroxide concentrations (6%, 15% and 35%) were used in the present study (Table 1) to demonstrate the temporal evolution of pH and EP and the correlation levels between them.

Table 1. Description of commercially-available bleaching gels and their respective compositions.

Group	Product name	Composition
G1	Nano White Flex 6% (DMC, São Paulo, Brazil)	Phase 1–6% hydrogen peroxide (0.975 g); Phase 2 – thickening agent (0.325 g), titanium dioxide nanoparticles, organic dyes and chelator agents
G2	Lase Peroxide Flex 15% (DMC, São Paulo, Brazil)	Phase 1–15% hydrogen peroxide (10 g); Phase 2 – thickening agent (5 g), titanium dioxide nanoparticles, organic dyes and chelator agents; Phase 3 - Thinner (5 g)
G3	Lase Peroxide Flex 35% (DMC, São Paulo, Brazil)	Phase 1–35 % hydrogen peroxide (10 g); Phase 2 – thickening agent (5.0 g), titanium dioxide nanoparticles, organic dyes and chelator agents

2.2. Sample preparation

Samples ($n = 9$) of each bleaching gel investigated (containing either 6%, 15% or 35% H_2O_2) were individually mixed following the instructions provided by the manufacturer (available at www.dmcgroup.com.br). In brief, samples pertaining to groups 1 and 3 were prepared by individually dispensing drops (~50 μ L each) of phases 1 and 2 (24 drops of phase 1 to 8 drops of phase 2; 3:1 ratio) onto clean concave plastic mixing pads. Immediately after dispensing the necessary droplets of each phase, the material was then manually mixed using circular movements until a homogeneous color was achieved (~10 seconds/sample). Samples in group 2 (15% H_2O_2) were prepared in a manner similar to those in groups 1 and 3, but droplets (~50 μ L each) from phases 1, 2 and 3 were dispensed in a 1:1:1 ratio (10 drops of each phase). All samples in the present in vitro laboratorial study were prepared by one trained and calibrated operator to avoid operator-associated errors. Environmental conditions such as temperature and relative humidity were maintained constant throughout the entire study.

2.3. Measurements of pH and EP

Immediately after being prepared, individual samples of bleaching gels investigated (containing either 6%, 15% or 35% of H_2O_2) were subjected to longitudinal and real-time measurements of pH and EP using a high-resolution pH meter (Ph-2600; Instrutherm, São Paulo, Brazil) and highly-accurate dip-type glass-barrel electrodes (MI-401; Microelectrodes, NH, USA) that were previously calibrated using Ag–AgCl standardized internal references. The temporal evolution (0 min–30 min) of pH and EP were then manually recorded by a previously calibrated and trained operator to ensure consistency of data points acquired.

2.4. Statistical analysis

Significant predictors of response in the present study included pH, EP, time-points (either 0 min [T0], 15 min [T15] or 30 min [T30]) and hydrogen peroxide concentrations (either 6% [HP6], 15% [HP15] or 35% [HP35]). Experimental data acquired were tested for normality and homoscedasticity using the Shapiro-Wilks and Bartlett's tests, which indicated that our data was not normally distributed. After the assumptions of normality and homoscedasticity were met, data were then subjected to one-way repeated measures analysis of variance (ANOVA) and were followed by multiple comparisons with Bonferroni post-hoc corrections. A 2nd order polynomial relationship test was performed later. Summary statistical analyses in the present study were performed at a 5% significance level using a dedicated software (SPSS Version 22.0; IBM, Armonk, NY, USA).

3. Results

The results of the present study have been reported in terms of mean and standard deviation values of pH and EP and were plotted as line

graphs (Figures 1, 2, and 3) to illustrate the temporal evolution of parameters of interest and to facilitate the visualization of the correlation between them (Figure 4).

It is possible to observe from Figure 1, that bleaching gels investigated displayed distinct pH values at T0 that ranged from 7.70 ± 0.412 (HP35) to 8.18 ± 0.342 (HP6), wherein bleaching gels of lower H_2O_2 concentrations (HP6 and HP15) displayed initial pH values that were higher than those of HP35. Statistically significant differences were observed for the Time * HP interaction ($F(2,71) = 6.69$; $p = 0.002$; $n_2p = 0.35$ and $\pi = 0.94$), and also for the main factors time of analysis ($F(1,35) = 13.72$; $p < 0,0001$; $n_2p = 0.36$ and $\pi = 0.98$) and concentration of hydrogen peroxide ($F(2) = 25.36$; $p < 0,0001$; $n_2p = 0.68$ and $\pi = 1.00$). In Figure 1 it can be seen that the most significant effect with the greatest practical significance is conditioned by the concentration of HP. The statistical effect observed for the repeated measurement variable time, is clearly dependent on the gel used since there are no intra-group statistical differences.

Figure 2 illustrates that bleaching gels investigated also displayed electrical potentials that were different at T0 and varied from -112 ± 11.5 mV (HP6) to -55.6 ± 26.4 mV (HP35), wherein bleaching gels of lower concentrations (HP6 and HP15) were associated with EP values that were more negative. The analysis of the temporal evolution of pH has indicated that bleaching gels investigated either maintained their initial pH values (HP15) or became slightly more acidic (HP6 and HP35). In regards to the temporal evolution of EP, it is possible to observe that bleaching gels investigated displayed a similar behavior where they either maintained their initial electrical potential (HP15) or became less negative (HP6 and HP35). Statistically significant differences were observed for EP values for the Time * HP interaction ($F(6) = 4.95$; $p < 0,0001$; $n_2p = 0.29$ and $\pi = 0.99$), and for the main variable HP ($F(2) = 14.46$; $p < 0,0001$; $n_2p = 0.55$ and $\pi = 1.00$) and repeated-measure Time ($F(3) = 8.049$; $p < 0,0001$; $n_2p = 0.25$ and $\pi = 0.99$). These results can be seen in Figure 2.

Figure 3 - illustrate that parameters of interest (pH [primary axis, to the left] and EP [secondary axis, to the right]) are inversely correlated in all bleaching gels investigated (either HP6, HP15 or HP35). In combination, these results indicate that after being mixed, commercially available bleaching gels tend to experience a decrease in their redox potential over time (within 30 min). These findings were expected because H_2O_2 spontaneously dissociates into ROS when in contact with organic dyes present in their compositions (Phase 2; see Table 1 for compositions).

Figure 4 - have demonstrated that mean values of pH and EP varied on a 2nd order polynomial relationship where high correlation levels (R^2 ; HP35 = 0.9923, HP15 = 0.9904 and HP6 = 0.957) were observed for all bleaching gels investigated, thereby allowing bleaching gels to be rank ordered in terms of decreasing levels of correlation (HP35 > HP15 > HP6, respectively).

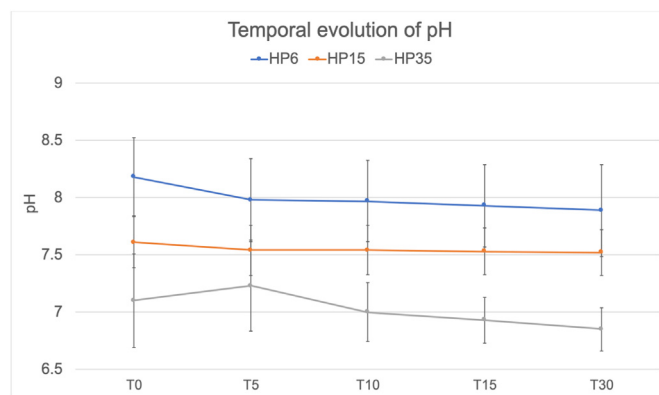


Figure 1. Complete data with mean and standard deviation of the temporal evolution of pH.

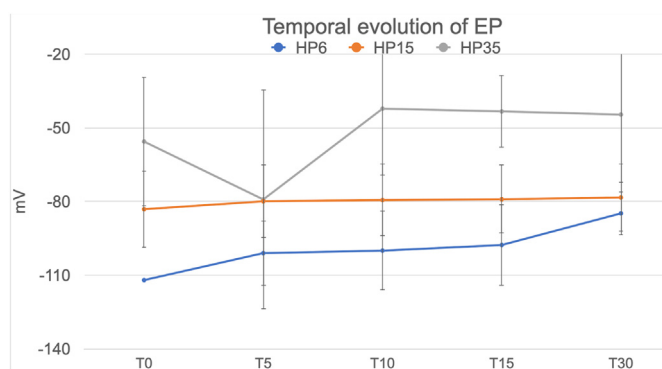


Figure 2. Complete data with mean and standard deviation of the Temporal evolution of EP.

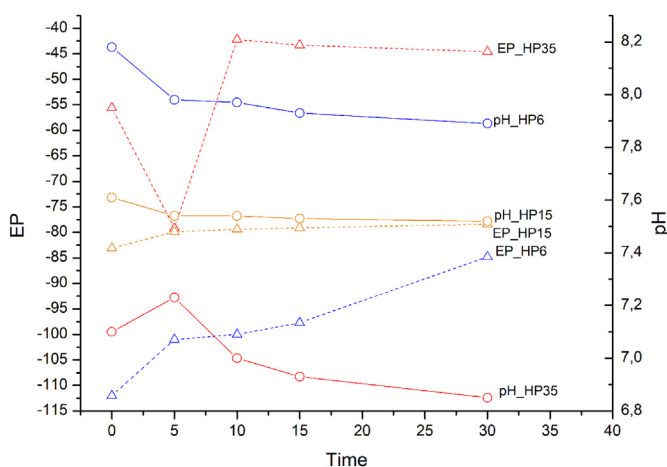


Figure 3. Mean Temporal correlation pH-EP (HP6) (HP15) (HP35).

4. Discussion

The objective of the present in vitro study was to investigate temporal variations in pH and EP from three commercially available bleaching gels, determine the correlation levels between parameters of interest and indirectly suggest bleaching gels' ability to induce the occurrence of BS. The null hypothesis tested was that bleaching gels of different HP concentrations would not display different kinetics. The results reported in the present study have shown that bleaching gels containing either 15% or 35% hydrogen peroxide displayed pH values that were slightly lower (7.61 and 7.10, respectively) when compared to that of 6%-containing gels (8.18). pH values reported were also shown to either be maintained or to slightly decrease over the course of 30 min (observation time). Therefore, the null hypothesis was rejected.

These results are critical from the efficacy standpoint because numerous studies have reported that alkaline gels typically generate large quantities of long-lived ROS when compared to acidic gels, which could justify why alkaline bleaching gels tend to yield esthetic outcomes that are more intense and durable. From the biocompatibility point of view, it is fundamentally important to understand how the evolution of pH may influence the penetration of ROS, the breakage of pigments and adversely impact the mechanical (decreased strength and hardness), biological (pulp viability) [14, 16, 17] and chemical properties of treated tissues (both hard and soft). The pH values reported in the present study have been corroborated by previous studies investigating the correlation between different pH conditioners and the chemical stability of bleaching gels (shelf life). According to results reported [18, 19], the large amounts of H^+ observed in highly concentrated bleaching gels helps to maintain the longitudinal chemical stability of materials investigated during

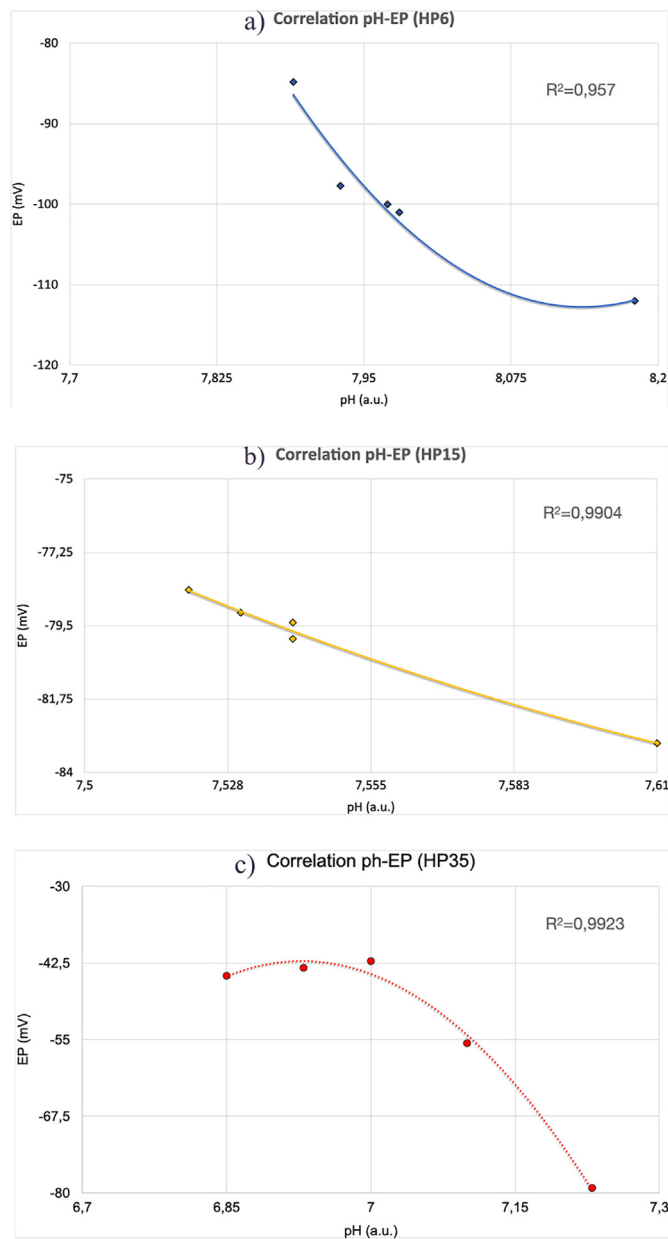


Figure 4. Presentation of correlation graphs between pH-EP of different concentrations of hydrogen peroxide a) (HP6); b) (HP15); and c) (HP35).

storage without etching the outermost surfaces of enamel [20, 21, 22]. These results are fundamentally important for clinicians and patients world-wide because the majority of in-office light-accelerated techniques are typically conducted with bleaching gels containing 35% H_2O_2 [4,23]. In addition, other studies demonstrated that acidic bleaching gels become alkaline immediately after being manipulated thereby promoting the efficient dissociation of H_2O_2 into ROS and further preventing acid etching of enamel [18, 24, 26].

Even though previous studies [20, 24, 25, 26] have investigated the evolution of pH values during dental bleaching procedures, the present study represents an effort to temporally correlate pH to EP, and provide with relevant information for the development of products and techniques that are capable of efficiently resolving dental discolorations without causing adverse effects on the viability and function of treated tissues. The rationale for the experimental design proposed in the present study was based on the need to remove unmanageable external confounding factors such as molecular composition, mineralization levels,

buffering capacity, porosity and tissue impedance that are typically present when mineralized tissues from either human or bovine origins are used.

The results reported in Figure 4, have clearly demonstrated the presence of a negative and strong correlation (HP6 [$R^2 = 0.957$], HP15 [$R^2 = 0.9904$] and HP35 [$R^2 = 0.9923$]) between the temporal variation of pH and EP, wherein parameters of interest were observed to inversely vary to one another (e.g., the higher the pH the lower the EP) as shown in Figure 3. According to studies that investigated the chemistry of light-activated dental bleaching procedures [10,27,28] this behavior can be explained by the process by which free radicals are generated during bleaching procedures, and by the release of ionic species that contribute to further increase the electric potential of bleaching gels investigated.

As previously mentioned in the present study, and as extensively reported in the literature, BS is one of the major adverse effects precipitating from the execution of at-home or in-office dental bleaching procedures. Even though theories have been proposed (i.e., transducer and hydrodynamic) to explain the mechanisms by which dental bleaching procedures cause BS (either trans- or post-operative), its etiology is still to be fully elucidated [29].

According to a recent systematic review and meta-analysis of the literature focused on in-office dental bleaching procedures mediated by bleaching gels of different H_2O_2 concentrations (either low, medium or highly concentrated) [24], higher levels of BS were observed on patients treated with bleaching gels of higher concentration. These findings could be used to partially support the central hypothesis of the present study that higher variation in EP values (as demonstrated in Figure 3) could lead to higher incidences of BS. The results of the present study regarding the temporal variation in pH values contradicts the findings of previous studies demonstrating that significant differences in pH were observed over time when irradiated with visible light and when in the presence of mineralized tissues [20, 23]. The results of the present study can be justified by the fact that the experimental design proposed did not use visible light or any type of mineralized tissues to avoid the presence of unmanageable confounding factors. In addition, the composition of bleaching agents investigated in the present study are slightly different than those previously reported and contain the presence of nitrogen-doped titanium dioxide nanoparticles. It is well-known from the literature, that H_2O_2 spontaneously dissociate in the presence of metal-oxides to favor the generation of ROS [6, 30]. Other studies [10, 13, 14, 31] have indicated that the breakage of conjugated C=C bonds, either by oxidative or reduction reactions, can release energy into the molecular environment where chromophores and ROS molecules are located and may further contribute to increase the variation in EP and pH values.

The results reported in the present study have clearly demonstrated the presence of a strong and positive correlation between pH and EP values wherein EP values inversely varied with the evolution of pH. It was also noticed that HP15 presented the lowest variation in pH and EP values of all bleaching gels investigated independently of the time-point considered (T0 – T30). From the clinical efficacy standpoint these results are critical because higher redox potential are typically correlated with immediate esthetic outcomes that are more intense and durable, as observed in HP35. Despite these results, it is anticipated that patients treated with highly concentrated bleaching gels will experience BS (either trans- or post-operative) that is more intense and durable due to this material's ability to generate a great ionic displacement flows that are capable of promoting strong depolarization of pulpal tissues [32].

The present study represents an effort to provide relevant information regarding how variations in pH and EP may influence the attainment of immediate esthetic results and the occurrence of BS. It is anticipated that the result of the present manuscript will positively impact subsequent research (either in vitro, in situ or clinical trials) and the development of novel dental bleaching materials. The present study offers to the field a new theory that strong variations in EP values, as observed in HP35, are capable to significantly depolarize neuronal extensions of pulpal tissues and cause sensations known as the Zinger effect. Subsequent studies

should investigate the effects of visible light and mineralized tissues on the evolution of pH and EP to confirm the findings reported in the present study and to substantiate the development of novel dental bleaching agents and the execution of robust clinical trials.

5. Conclusion

The present *in vitro* study has successfully characterized the temporal evolution of pH and EP values and their temporal correlations without the presence of unmanageable external confounding factors. Results reported have clearly demonstrated the establishment of strong and negative correlations for all bleaching gels investigated. In addition, the greater variation in pH and EP present in the higher concentration gel may be linked to the presence of SB in use, even reported in the literature, based on the principle of electrical depolarization of the neuronal extensions of the pulp tissues.

Declarations

Author contribution statement

Hermes Pretel: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Joatan Lucas de Sousa Gomes Costa: Performed the experiments; Wrote the paper.

Fernando Luis Esteban Florez: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Básia Rabelo Nogueira: Performed the experiments.

Osmir Batista de Oliveira Junior: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

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Data availability statement

Data included in article/supplementary material/referenced in article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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