Utilization of ozonated oil for internal dental whitening: a clinical, blind, and randomized study

Utilização do óleo ozonizado para clareamento dental interno: um estudo clínico, cego e randomizado

Utilización del aceite ozonizado para el blanqueamiento dental interno: un estudio clínico, ciego y aleatorio

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Poliana Maria Faveri Cardoso
Master's student in Dentistry
Institution: Universidade Estadual do Oeste do Paraná (UNIOESTE)
Address: Cascavel - Paraná, Brasil
E-mail: polif1704@gmail.com
Orcid: https://orcid.org/0000-0002-7073-278X

Fernanda Rafaela Ribeiro
Graduating in Dentistry
Institution: Universidade Estadual do Oeste do Paraná (UNIOESTE)
Address: Cascavel – Paraná, Brasil
E-mail: fernandarafaela1377@gmail.com
Orcid: https://orcid.org/0009-0001-4963-662X

Leonardo Dal Bosco
Master's student in Dentistry
Institution: Universidade Estadual do Oeste do Paraná (UNIOESTE)
Address: Cascavel – Paraná, Brasil
E-mail: leodalbosco08@gmail.com
Orcid: https://orcid.org/0009-0008-8299-0314

Rafael da Silva Vanolli
Graduating in Dentistry
Institution: Universidade Estadual do Oeste do Paraná (UNIOESTE)
Address: Cascavel – Paraná, Brasil
E-mail: rafahelvanolli@gmail.com
Orcid: https://orcid.org/0000-0002-4670-8878
ABSTRACT
To evaluate through a randomized, blind clinical study the effectiveness of two conventional bleaching agents modified by the incorporation of ozone gas on the degree of dental bleaching and color stability. Methodology: Forty-two teeth were selected with endodontic treatment and a degree of dental saturation (GS) equal to or greater than A2 and divided into 2 groups (n=21 teeth: PNaS: Sodium perborate + saline solution and PNaOZ: Sodium perborate + ozone sunflower oil with 16 ppm. Color GS was evaluated at the beginning of treatment and 7 days after the end of treatment, with the aid of Vita classical color scale. The statistical analysis of the color GS was performed by the Wilcoxon test, and the number of sessions by the Shapiro-Wilk test followed by the Mann-Whitney test, for correlation between GS and the number of clinical sessions, spearman correlation was performed. Resulted: There was a statistically significant difference between the initial (G1 = 15.0 ± 2.00 and G2 = 16.00 ± 1.00) and final (G1 = 5.00 ± 6.00 and G2 = 3 ± 3.00) GS values and PNaOZ had a greater color variation between the final and initial color. The number of clinical sessions was statistically lower for PNaOZ. No significant differences were found between the number of sessions and the GS. Conclusion: Adding ozone to sodium perborate accelerated dental bleaching, thus reducing the number of clinical sessions.

Keywords: dental bleaching, dental bleachers, ozone, gas.

RESUMO
Avaliar, por meio de um estudo clínico randomizado e cego, a eficácia de dois agentes clareadores convencionais modificados pela incorporação do gás ozônio no grau de clareamento dental e na estabilidade da cor. Metodologia: Quarenta e dois dentes foram selecionados com tratamento endodôntico e grau de saturação dentária (GS) igual ou superior a A2 e divididos em 2 grupos (n=21 dentes: PNaS: Perborato de sódio + solução salina e PNaOZ: Perborato de sódio...
+ óleo de girassol ozonizado com 16 ppm. A cor GS foi avaliada no início do tratamento e 7 dias após o término do tratamento, com o auxílio da escala de cores clássica Vita. A análise estatística da cor do SG foi realizada pelo teste de Wilcoxon, e do número de sessões pelo teste de Shapiro-Wilk seguindo do teste de Mann-Whitney, para correlação entre o SG e o número de sessões clínicas foi realizada a correlação de spearman. Resultados: Houve uma diferença estatisticamente significativa entre os valores de SG inicial (G1 = 15,0 ± 2,00 e G2 = 16,00 ± 1,00) e final (G1 = 5,00 ± 6,00 e G2 = 3 ± 3,00) e o PNaOZ apresentou maior variação de cor entre a cor final e a inicial. O número de sessões clínicas foi estatisticamente menor para o PNaOZ. Não foram encontradas diferenças significativas entre o número de sessões e o GS. Conclusão: A adição de ozônio ao perborato de sódio acelerou o clareamento dental, reduzindo assim o número de sessões clínicas.

Palavras-chave: clareamento dental, clareadores dentais, ozônio, gás.

RESUMEN
Evaluar mediante un estudio clínico aleatorizado y ciego la eficacia de dos agentes blanqueadores convencionales modificados por la incorporación de gas ozono sobre el grado de blanqueamiento dental y la estabilidad del color. Metodología: Se seleccionaron 42 dientes con tratamiento endodóntico y un grado de saturación dental (GS) igual o superior a A2 y se dividieron en 2 grupos (n=21 dientes: PNaS: Perborato sódico + solución salina y PNaOZ: Perborato sódico + ozono aceite de girasol con 16 ppm. El color GS se evaluó al inicio del tratamiento y 7 días después del final del tratamiento, con la ayuda de la escala de color clásica de Vita. El análisis estadístico de la GS color se realizó mediante el test de Wilcoxon, y el número de sesiones mediante el test de Shapiro-Wilk seguido del test de Mann-Whitney, para la correlación entre la GS y el número de sesiones clínicas se realizó la correlación de spearman. Resultados: Hubo una diferencia estadísticamente significativa entre los valores de GS inicial (G1 = 15,0 ± 2,00 y G2 = 16,00 ± 1,00) y final (G1 = 5,00 ± 6,00 y G2 = 3 ± 3,00) y PNaOZ tuvo una mayor variación de color entre el color final y el inicial. El número de sesiones clínicas fue estadísticamente inferior para PNaOZ. No se encontraron diferencias significativas entre el número de sesiones y la GS. Conclusiones: La adición de ozono al perborato sódico aceleró el blanqueamiento dental, reduciendo así el número de sesiones clínicas.

Palabras clave: blanqueamiento dental, blanqueadores dentales, ozono, gas.

1 INTRODUCTION

Ozone (O3), an allotropoform of oxygen, has strong oxidative properties and acts on bacteria, fungi, yeasts, viruses, and protozoa and eliminates bad odors. It is obtained from dissociating O2 molecules into O2 atoms, making it highly reactive (Mehlman; Borek, 1987).

Ozone can be administered in a variety of ways, including three: gas, water, and ozonized oil. Ozonated water is generally used as a mouthwash to kill bacteria, viruses, and fungi and is a...
safer alternative to gaseous ozone. In addition to being more stable, ozonized oil provides greater tissue permeability when compared to water and gas (German et al., 2013).

Ozone therapy has shown success when used in wound healing, dental caries, oral lichen planus, gum diseases such as gingivitis and periodontitis, halitosis, osteonecrosis of the jaw, postsurgical pain, dental biofilm, root canal cleaning, dentin hypersensitivity, and temporomandibular joint disorders (Suh et al., 2019).

Recently, studies have linked its use to tooth whitening, since ozone therapy produces an increase in the synthesis of ATP (adenosine triphosphate), increasing the supply of oxygen and potentially accelerating the whitening process (Al-Omiri et al., 2016; AL-Omiri et al., 2017).

However, the results found in the literature are contradictory. Some authors report that the use of ozone did not enhance whitening associated with carbamide peroxide and even reduced its effectiveness if applied in percentages lower than 8% carbamide (Manton et al., 2008). Others, involving hydrogen peroxide (H2O2), concluded that its use showed superior whitening capabilities when compared to ozone alone, and that when associated there was no improvement in whitening efficacy (Aykut-Yetkiner et al., 2017; Zanjani et al., 2015).

On the other hand, findings show that ozone has similar whitening capabilities to some commercially available whitening agents, such as highly concentrated carbamide peroxide at 45% or hydrogen peroxide at 37.5% (Al-Omiri et al., 2018). Its action in internal tooth whitening is still scarce in the literature, for this procedure the most commonly used material is sodium perborate (Barakah et al., 2019).

Sodium perborate is widely used and is stable when dry, but in the presence of acid, hot air, or water, it decomposes to form sodium metaborate, hydrogen peroxide, and oxygen. The gel interacts with the moist structure of the tooth and an oxidation-reduction reaction takes place. When associated with hydrogen peroxide, its action is accelerated (Ribeiro, 2018). Another factor to be noted is the scarcity of studies in the literature on the use of the association of O3 with sodium perborate and its application in internal tooth whitening of endodontically treated teeth.

In this context, this study aims to compare the efficacy of sodium perborate associated with ozone on the internal bleaching of darkened teeth subjected to chemical root canal preparation, in vivo. This study aimed to evaluate the efficacy of the bleaching agent sodium perborate associated with ozone therapy in bleaching darkened teeth after endodontic treatment using an in vivo clinical study.
2 MATERIALS AND METHODS

2.1 STUDY DESIGN

This prospective, randomized, blinded clinical study was carried out at the Dentistry Clinic of the State University of Western Paraná (UNIOESTE - Cascavel - PR) after the project was approved by UNIOESTE's Human Research Ethics Committee (15685419.7.0000.0107) and the Brazilian Registry of Clinical Trials (REBEC - opinion number: 9510).

2.2 ELIGIBILITY CRITERIA

Patients were selected based on a clinical and radiographic examination. Patients were also assessed for inclusion and exclusion criteria. The inclusion criteria were patients of both sexes, between 18 and 60 years old, absence of periapical lesions, absence of gingival inflammation, absence of external cervical root resorption, intact or minimally restored crowns, absence of orthodontic braces and the initial color of the teeth being A2 or more saturated compared to the Vita Classic color scale (Vita Lumin, Vita Zahnfabrik, Bad Säckingen, Germany). Exclusion criteria included patients who had already undergone a previous whitening procedure, pregnant and breastfeeding women, patients with a probing depth of more than 3 millimeters, and enamel malformations.

Initially, 42 patients were selected, 10 of whom were excluded because they were breastfeeding, pregnant, or had teeth with unsatisfactory or absent endodontic treatment. Therefore, 32 patients were included in the study, totaling 42 teeth, 21 teeth per group.

Patients who met the inclusion criteria signed the Free and Informed Consent Form (FICF) in two copies (Appendix 1).

2.3 SAMPLE CALCULATION

The sample calculation was based on probability distributions from the t-test family (Wilcoxon and Mann-Whitney tests for comparing two groups). The effect size used was 0.8, with type 1 error (α) of 0.05, a power of analysis (β error) of 0.8 resulting in a total of 32 individuals,
n=21 per group. The sample calculation was carried out using the GPower program, version 3.1.9.2.

University of Düsseldorf. The teeth were divided into two groups (n=21) according to the bleaching agent used: Sodium perborate + saline solution and PNaOZ: Sodium perborate + 16 ppm ozonized sunflower oil, as shown in Figure 1.

Figure 1 - Diagram of the clinical protocol and re-evaluations of the experimental groups.

Source: From the authors.

2.4 RANDOMIZATION

Randomization was carried out using an open-access program (GraphPad.com - https://www.graphpad.com/quickcalcs/randomize1.cfm).

Distribution of experimental groups
The selected patients were divided into two experimental groups according to Table 1.

Table 1 - Distribution of patients and type of application in each patient group.

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Application</th>
<th>Handling of whitening agent</th>
</tr>
</thead>
<tbody>
<tr>
<td>PNaS</td>
<td>Sodium perborate saline solution</td>
<td>Prophylaxis, absolute isolation, preparation of the cervical cap, application of the material using a number 1 spatula and provisional restoration.</td>
<td>Proportion 2:1, using two measures of whitening (sodium perborate) to one portion of vehicle (distilled water). Mix with flexible spatula until homogeneous.</td>
</tr>
<tr>
<td>PNaOZ</td>
<td>Sodium perborate + 16 ppm oozed sunflower</td>
<td>Prophylaxis, absolute isolation, preparation of the cervical cap, application of the material using a number 1 spatula and provisional restoration.</td>
<td>Proportion 2:1, using two measures of bleaching agent (sodium perborate) to one portion of vehicle (ozonized sunflower oil / Phlozon, Camborn, Santa Catarina, Brazil). Mix with a flexible spatula.</td>
</tr>
</tbody>
</table>

Source: from the authors.

2.5 OPERATOR CALIBRATION

A single operator was calibrated to carry out the clinical steps. A second operator carried out the randomization and distribution of the groups, and neither the patient nor the operator was aware of the product being tested. Three duly calibrated operators measured the degree of color saturation (GS).

2.6 STUDY INTERVENTIONS PREPARATION OF PARTICIPANTS

All patients underwent dental prophylaxis with a Robinson toothbrush (Microdont, São Paulo, SP, Brazil), attached to a contra-angle (Kavo, Joinville, SC, Brazil), with the aid of a pumice stone (Quimidrol, Joinville, SC, Brazil) and water. The color was assessed using a VITA scale (Vita Easyshade, Vita Zahnfabrik), and the data was recorded on a clinical form.

Subsequently, the height of the clinical crown was recorded with a millimeter instrument and rubber stop, before absolute isolation, for greater accuracy.
Absolute isolation was performed to protect the soft tissues and prevent contamination of the root canal. All the restorative material in the pulp chamber was. The gutta-percha was then removed with a spherical diamond tip at high speed (KG Sorensen, Barueri, SP, Brazil), under refrigeration, to access the pulp chamber. The gutta-percha was then partially removed, 2 millimeters below the gingival margin (Gupta; Saxena, 2014).

The cervical seal was created using Riva Light Cure, a composite resin-reinforced glass ionomer cement (SDI Limited, Bayswater, Victoria, Australia) in the area where the gutta-percha had been removed. The cervical barrier reproduced the position of the amelocementary junction and the level of the interproximal bone, protecting the dentinal tubules. On the proximal and lingual walls, the base was more extended towards the center of the pulp chamber (Dietrich et al., 2021).

2.7 WHITENING TREATMENT

At this point, the teeth were divided into two groups (table 3). The bleaching agents were inserted into the cavity using a number 1 spatula (Golgran, São Caetano do Sul, São Paulo, Brazil), filling the entire pulp chamber and leaving enough space for the restoration of the endodontic access (Gupta and Saxena, 2014). A cotton ball moistened in dentin adhesive, polymerized, was inserted and the cavity was provisionally sealed with Filtek Z250 XT composite resin (3M ESPE, St. Paul, MN, USA). Occlusal adjustment was carried out at each restoration change. The whitening agent was changed every seven days until the darkened tooth was slightly lighter than the adjacent tooth, limiting the number of internal whitening sessions to five (Fagogeni et al., 2021); the data obtained was recorded in the patient's clinical file. With each new application of the bleaching agent, the temporary restoration was removed and the pulp chamber was washed with a water spray to reapply the bleaching agent. After the end of the bleaching sessions, the pulp chamber was washed with water spray and a paste of calcium hydroxide (Biodinâmica, Ibiporã, Paraná, Brazil), and distilled water was applied. After 14 days, the temporary seal was removed and the shade was selected, the pulp chamber was rinsed with water, and acid etching was carried out for 30 seconds on enamel and 15 seconds on dentin, Single Bond adhesive (3M ESPE, St. Paul, USA) was applied and the final restoration was made with Filtek Z250 XT composite resin (3M ESPE, St. Paul, MN, USA).
2.8 ASSESSING THE DEGREE OF WHITENING

The shade was taken before the whitening treatment, using the upper central incisors as a reference. The subjective assessment was made by comparing the shade with the Vitapan Classical shade scale (Vita, Bad Säckingen, Germany). Fifteen days after the end of treatment, the shade recording procedure was repeated for a final assessment of tooth saturation. The color scale was set up in ascending order to brightness, from the brightest shade - B1 - to the least bright - C4 (Figure I). In this sequence, each hue received a score: B1 received a score of 1; A1 received a score of 2, and so on, making hue A2 score 5.

2.9 STATISTICAL ANALYSIS OF RESULTS

Wilcoxon's test was used to analyze the data related to color change, as it was non-parametric ordinal categorical data. To compare the number of sessions between the groups, the Shapiro-Wilk test was used to check for normality and homogeneity of variances, and, as the data had a non-normal distribution, the Mann-Whitney test was used. The Mann-Whitney test was also used for the difference between the initial and final values in the inter-group comparison, as this is a comparison between two independent samples. Finally, Spearman's correlation test was used to assess the correlation between the whitening achieved and the number of sessions performed. The statistical analyses were carried out using Bioestat® software 5.3 (Mamirauá Sustainable Development Institute, Tefé, Amazonas, Brazil).

3 RESULTS AND DISCUSSION

The results of the statistical analysis of shade change and number of clinical sessions for each experimental group are listed in Tables 2, 3, 4, and 5. In general, statistically significant differences were observed in the results of shade change and number of sessions in the intra-group and inter-group analyses.

The analysis in Table 2 shows that for both groups there was a statistically significant difference between the initial and final color in the intra-group analysis.
Table 2: Median values and interquartile deviation of the color score for the PNaS and PNaOZ groups.

<table>
<thead>
<tr>
<th></th>
<th>PNaS</th>
<th>PNaOZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>15.0 (± 2.00) a</td>
<td>16.0 (± 1.00) a</td>
</tr>
<tr>
<td>Final</td>
<td>5.00 (± 6.00) b</td>
<td>3.00 (± 3.00) b</td>
</tr>
</tbody>
</table>

*In the row, different capital letters mean that there is a statistically significant difference (p<0.05)
Source: from the authors.

The score adopted for the final colors was statistically lower than for the initial ones, according to the Wilcoxon test (p < 0.05).

The analysis in Table 3 reveals statistically significant differences in the number of sessions in the inter-group analysis between PNaS and PNaOZ, according to the Mann-Whitney test (p < 0.05). The analysis showed significantly lower values for PNaOZ.

Table 3: Median values and interquartile deviation of the number of sessions for the PNaS and PNaOZ groups.

<table>
<thead>
<tr>
<th></th>
<th>PNaS</th>
<th>PNaOZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median (± Deviation Interquartile)</td>
<td>3.00 (± 2.00) A</td>
<td>2.00 (± 3.00) B</td>
</tr>
</tbody>
</table>

*In the row, different capital letters mean that there is a statistically significant difference (p<0.05)
Source: from the authors.

Table 4 shows the values of the intergroup analysis of the initial and final color difference, according to the Mann-Whitney test (p < 0.05), where the PNaOZ values were statistically higher.

Table 4: Median values and interquartile deviation of the final and initial color difference for groups 1 and 2.

<table>
<thead>
<tr>
<th></th>
<th>PNaS</th>
<th>PNaOZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median (± Deviation Interquartile)</td>
<td>9.00 (± 5.00) A</td>
<td>12.00 (± 3.00) B</td>
</tr>
</tbody>
</table>

*In the row, different capital letters mean that there is a statistically significant difference (p<0.05)
Source: from the authors.

Finally, Table 5 shows the Spearman correlation values (p < 0.05) between the number of sessions and the difference between the initial and final color of the PNaS and PNaOZ groups, showing that there was no direct relationship between the number of sessions and color changes.

Table 5: Median values and interquartile deviation of the correlation between the number of sessions and the degree of whitening according to Groups PNaS and PNaOZ.

<table>
<thead>
<tr>
<th></th>
<th>PNaS</th>
<th>PNaOZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of sessions</td>
<td>3.00 (± 2.00) Aa</td>
<td>2.00 (± 3.00) Aa</td>
</tr>
<tr>
<td>Degree of whitening</td>
<td>9.00 (± 5.00) Aa</td>
<td>12.00 (± 3.00) Aa</td>
</tr>
<tr>
<td>(p)</td>
<td>0.0684</td>
<td>0.8599</td>
</tr>
</tbody>
</table>

*In the row, different capital letters mean that there is a statistically significant difference (p<0.05)
Source: from the authors.
Internal bleaching substances are used to promote the removal of endogenous and exogenous pigments after endodontic treatment, thus reducing the degree of tooth color saturation (GS). Thus, various substances have been proposed to whiten darkened teeth that have undergone endodontic treatment, such as sodium perborate, hydrogen peroxide, carbamide peroxide, and, more recently, substances containing ozone. The chemical reaction of all bleaching agents results in the formation of hydrogen peroxide, which decomposes into water and reactive oxygen radicals (Alkahtani et al. 2020).

The results found in this study concerning SG showed that both groups had statistically significant differences between the initial and final color, both were effective in whitening teeth. According to Frank (2022) and Schwendler et al. (2013) distilled water acts as the oxidizing agent for sodium perborate during the oxidation-reduction reaction that occurs when the bleaching agent is used in the pulp chamber, because distilled water acts as a vehicle for free oxygen radicals which, when penetrating the dentinal tubules, break down the long molecular chains that are responsible for tooth darkening by fractionating them into smaller molecules. In addition, the configuration of pigments are carbon rings and when they come into contact with oxidants, they donate electrons to the whitening agent and are converted into chain molecules with a lower molecular weight and light color, which are then diffused and eliminated through dental permeability (Ferreira et al., 2016).

The present study also evaluated the combination of ozone with sodium perborate in internal bleaching and it proved to be more effective than perborate with distilled water, both in terms of the number of sessions and GS, which may be due to the greater permanence of ozone in the tooth structure and its slow and gradual release by the oil. On the other hand, studies have shown that ozone used only in external tooth whitening, where the contact time is reduced, this association did not prove to be advantageous Manton et al. (2008) and Zanjani et al. (2015), differing from the study used.

In addition, the study design has a direct impact on the different results found. While this clinical study was carried out with a sample size of 21 teeth per group, the in vitro study by Manton et al. (2008) had a sample size of less than 10 teeth per group, which may have influenced the negative results found. From another perspective, in their research, which also consisted of clinical trials, Al-Omiri et al. (2016) and Al-Omiri et al. (2018) observed the synergistic effect
of ozone on whitening agents, enhancing the whitening results obtained compared to the pure whitening agent, in a sample of 16 and 35 patients per group, respectively.

Due to the scarcity of literature relating the number of clinical sessions in internal whitening with whitening efficacy, this study limited the methodology to assess whether there was a direct correlation between these factors, but even so, the group with ozone association statistically showed a lower number of whitening sessions, which can be evaluated as a positive factor, since studies such as Garrido et al. (2017) have shown that the number of sessions, although not correlated with color, negatively affects enamel microhardness. These changes in the physical properties and ultrastructure of dentin as the number of sessions increases were also identified by Castro et al. (2019).

Changes in the morphology of enamel and dentin occur during tooth whitening regardless of the whitening protocol used, these changes were observed by Ferreira et al. (2016) in which calcium levels showed a significant reduction and carbon levels (organic part) were altered in enamel, although these values were lower when sodium perborate and distilled water were used. In turn, Santana et al. (2016) observed that even the ozone group showed similar values of morphological changes, but they used the same number of clinical sessions for all the agents employed, and the benefit of using ozone is the lower number of clinical sessions, as found in this study. Ozone can also help in the tooth whitening process due to its oxidizing capacity, as well as being highly reactive, because when it comes into contact with the tooth structure it dissociates into water, hydroxyl radicals and peroxide radicals, helping to break down the oxidation process.

Of pigments (Santana et al., 2016). The whitening mechanism originated by ozone can be through bonding, substitution, or cleavage. During the bonding mechanism, a double bond originates from ozone forming ozonide with non-aqueous solvents, and when in the presence of water, the double bond formed is broken giving rise to the formation of by-products. Substitution occurs when one atom or functional group is replaced by another, while the cleavage mechanism occurs when ozone cleaves carbon-carbon bonds, producing organic compounds, these reactions occur (AL-Omiri et al., 2017; Perincek et al., 2007).

In this study, we opted to use resin-reinforced glass ionomer cement (RIVA/SDI) to make the cervical cap, which had its performance verified by the study by Newton et al. (2020), which observed that for all the materials tested, there was a certain permeability, and there was a better
performance of the resin-reinforced glass ionomer cement compared to the conventional one and it had worse results than coltosol. In this study, the agent used was sodium perborate, and Frank, (2022) reiterates the lower probability of this condition developing when using it, since among the internal bleaching agents it is the one with the most alkaline pH.

Cervical capping is essential because one of the main risks of internal tooth whitening is the possibility of developing OER in the whitened tooth. After all, the whitening agent can reach the periodontal tissues through the diffusion of the agent through the dentinal tubules and cause dentin denaturation, dissolution of the extracellular matrix covering the dentin and exposure of sequestered antigens, causing the dentin to be considered an immunologically different tissue, activating the foreign body inflammatory response and recruiting osteomodelling cells to the site (Schwendler et al., 2013).

Based on this, the creation of a cervical barrier becomes indispensable in internal bleaching due to the high rate of diffusion of the bleaching agent through the dentinal tubules. This barrier should be made over the obturator material, 3mm below the amelocementary junction, following the morphological conformation of the region. However, to inhibit the diffusion of bleaching agents and their by-products, in addition to having a good fit, the material used guarantees a safe and comfortable bleaching experience, while optimizing the bleaching process.

Whitening results (Santana et al., 2024). In this context, ozone can be used in conjunction with bleaching agents due to its high biocompatibility with dental tissues, not irritating the gingival tissues, in order to avoid CER (German et al., 2013).

Based on the results found, it can be concluded that the addition of ozone to sodium perborate, when used as an internal whitening agent, accelerates tooth whitening by removing dental pigments, thus reducing the number of clinical sessions.

5 CONCLUSION

This study led to the conclusion that sodium perborate as an internal bleaching agent showed satisfactory results both when used with distilled water and ozonated sunflower oil, with the latter requiring fewer clinical sessions to achieve the desired color.
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