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Assessing the bleaching effect of an experimental stabilized chlorine dioxide agent used for internal bleaching purposes

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Aim: Assessing the intracoronal bleaching effectiveness of an experimental chlorine dioxide product, based on the walking bleach technique. **Methods:** Extracted bovine incisors were artificially stained with bovine blood and filled with zinc phosphate cement at cementoenamel junction level. Teeth were divided into 3 groups (n=10): (SP) sodium perborate added with distilled water, (CD) chlorine dioxide and (C) control – dry cotton inserted into the pulp chamber. Bleaching agents were used at 0, 7 and 14 days. VITA Easyshade^{tm} (ΔEab) was used to analyze tooth color at the 7th, 14th and 21st days, based on the CIE2000 system. Data were analyzed through ANOVA and Tukey's test. **Results:** There were no statistically significant differences in Δb, ΔE, ΔE00 and ΔWI_D between CD and the control group. These parameters have shown significant differences between CD and SP, which differed from the control. However, they did not show significant differences either in the control group or in CD at the 7th, 14th and 21st days. Values recorded for SP at the 7th day differed from those recorded at the 14th and 21st days. Δa has shown differences within the same group at the 7th, 14th and 21st days. There was no difference between groups, when they were compared at the same day (7th and 14th). The control group differed from SP at the 21st day. CD did not differ from the other two groups. ΔL did not show differences between groups and times. **Conclusion:** Stabilized chlorine dioxide (0.07%, at pH 3.5) should not be used as intracoronal bleaching agent along with the walking bleach technique.

Keywords: Tooth bleaching. Borates. Chlorine compounds. Dental materials.

1. Introduction

Tooth discoloration can result from intrinsic or extrinsic factors. Procedures such as polishing tooth surfaces, microabrasion, direct or indirect restoration and dental bleaching techniques have been developed to treat this cosmetic impairment. Dental bleaching treatments provide fast and expressive results without wearing the dental structure away[1]. Consequently, they have become remarkably popular[2]. Internal bleaching is the technique most widely used in non-vital teeth due to its quite reasonable cost, safety and pleasing aesthetic results[3].

Root-filled teeth whitening was firstly performed with hydrogen peroxide or sodium perborate, since the late nineteenth century[4]. Spasser[5] (1961) has introduced a technique, according to which, sodium perborate and distillated water solution is injected into the pulp chamber for a few days; subsequently, this procedure is repeated until the enamel reaches the desired color. This technique is known as Walking Bleach. A simultaneous internal and external bleaching technique based on peroxide carbamide was described in the literature in 1997[6]. The adverse effect known as external cervical root resorption can result from certain internal bleaching techniques[7]. This issue appears to be directly linked to hydrogen peroxide diffusion in the periradicular area. Thus, hydrogen peroxide should not be used as treatment agent, either in association with sodium perborate, or not[8,9].

Reports on external bleaching carried out with chlorine dioxide precursors were published, mainly in the UK, in the first decade of the current century. This technique applies sodium chloride, in association with anhydrous citric acid, to whiten tooth surface; its effects result from chlorine dioxide release on tooth surface[10]. Several studies have shown that this technique can harm the dental tissue due to the solution's low pH; thus, its application should be discouraged[10-14]. However, chlorine dioxide has oxidative antimicrobial activity and it may remain used as alternative to other internal bleaching agents[15]. Nevertheless, the literature lacks studies focused on investigating its use in a lesser acidic stabilized formula. Therefore, the aim of the current study was to assess the effects of stabilized chlorine dioxide used as internal bleaching agent in comparison to those of sodium perborate and the control group.

2. Materials and Methods

Sixty (60) permanent bovine mandibular incisors with intact crowns were used in the current study. All organic and inorganic debris were removed from extracted teeth with the aid of scalers; dental elements were stored in chloramine-T solution, at 37°C/pH 7, for one week, for disinfection purposes. Teeth were selected and only the ones presenting A2 color, in compliance with the Vittapan Classical scale, were used. Root canals were sectioned right below the amelocemental junction, with the aid of diamond disk attached to a handpiece, whereas crowns were opened with spherical diamond burs (KG Sorensen, Barueri, SP, Brazil, model 1018HL). Tooth pulp was removed with the aid of scalers and the first third of the root canal was expanded with spherical diamond burs (KG Sorensen model 1016HL).

All teeth were stained by using a variation of the technique described by Freccia and Peters[16] (1982). Crowns were centrifuged together with fresh bovine blood, at high speed (5,000 rpm), three times a day. The aforementioned blood was replaced on a daily basis; the pigmentation procedure was repeated for 15 days. Then, dental elements were rinsed with running water for 2 minutes, and stored in saline solution.

Thirty (30) stained crowns were selected and covered with 1-mm thick protective base (ZnPO4, SS White, Juiz de Fora, Brazil), which was fixed 1 mm below the buccal cementoenamel junction. Each crown was randomly assigned to one of the three groups.

The SP group was treated with sodium perborate (Odontofarma, Londrina, Brazil) and distilled water solution at 2:1 mg/ml. The bleaching agent was applied to the tooth until it filled the buccal surface inside the pulp chamber; then, a provisional restoration (Coltosol, Coltrane, France) was used to seal the cavity. The bleaching agent was applied at treatment days 0, 7 and 14. Specimens were rinsed with distilled water and gently dried with triple syringe, before each bleaching exchange.

The CD group was treated with 0.07% chlorine dioxide (from the TescaClor product, which has 5% chlorine dioxide), in association with carbopol, and it generated a product with pH 3.5. The gel was applied to the tooth until it filled the buccal surface inside the pulp cavity; then, a provisional restoration (Coltosol, Coltrane, France) was used to seal the cavity. The bleaching agent was applied at treatment days 0, 7 and 14. Specimens were rinsed with distilled water and gently dried with triple syringe, before each bleaching exchange. Dry cotton was inserted into the pulp chamber of teeth in the control group at 0, 7 and 14 days after the procedures described above. All dental elements were stored in gauze (soaked in water), at 37°C and relative humidity of 100%.

Easyshade intraoral spectrophotometer (Vita, Zahnfabrik H. Rauter GmbH & Co. KG, Bad Sackingen, Germany) was used for tooth color assessment under controlled conditions (artificial light and temperature of 16°C). A mold, whose opening had the same diameter as the tip of the spectrophotometer, was prepared with hot glue to ensure that the same point was assessed on each specimen. Color change (T1 and T2) was herein obtained through CIEDE2000 formula, which uses coordinates h (hue) and C (chroma): ΔE00 = [(ΔL 0 /KLSL)2 + (ΔC 0 / KCSC)2 + (ΔH 0 /KHSH)2 + RT * (ΔC 0 /KCSC) * (ΔH 0 /KHSH)]1/2. (23) In addition, the perceptibility threshold was set at ΔE00 ≤ 0.8, whereas the clinical acceptability threshold was set at ΔE00 ≤ 1.8.[17] Data were also obtained through a formula recommended by CIELAB, according to which, coordinates L (indicating brightness), a and b (red-green and yellow-blue, respectively) interact as follows: ΔEab = $[(\Delta L)^{2} + (\Delta A)^{2} + (\Delta B)^{2}]^{0.5}$. Color variations Δa, Δb and ΔL, as well as the Whiteness index ΔWI_D, were calculated[18].

Data were subjected to Kolmogorov-Smirnov test, which has shown that they were within the normality range; then, they were subjected to analysis of variance (ANOVA) and to Tukey's test, at 5% significance level.

3. Results

There were no statistically significant differences in color measurement (Δb, ΔE and ΔE00) and whiteness index (ΔWI_D) between CD and the control group. There were sig-

nificant differences in these parameters between the CD and SP groups, which also differed from the control group ($p<0.05$). Moreover, no significant differences were observed either in the control group or in the CD group at the 7th, 14th and 21st days. However, values recorded for the SP group at the 7th day differed from those recorded at the 14th and 21st days. Data are shown in Table 1.

Table 1. Effect of bleaching agents on the color change of materials subjected to bleaching treatment

Groups	Period (days)	Δa*	Δb*	ΔL*	ΔE	Δ00	ΔWI_D
Control	7	-0.15±1.14 a	0.72±5.82 a	1.22±3.02 a	5.6±3.4 c	2.87±1.84 c	0.18±8.16 c
	14	-0.33±1.43 ab	0.62±5.96 a	1.72±3.20 a	5.6±3.8 c	3.14±1.93 c	0.96±8.14 c
	21	-0.30±1.46 ab	1.12±6.37 a	2.32±2.80 a	6.4±3.6 c	3.40±1.77 c	0.65±8.48 c
Sodium Perborate	7	-1.66±1.32 abc	-12.34±8.92 b	3.41±2.77 a	14.5±6.3 b	7.98±3.36 b	19.17±10.36 b
	14	-2.02±0.77 bc	-25.44±2.82 c	4.01±2.33 a	26.1±2.8 a	14.12±1.30 a	34.73±3.90 a
	21	-2.22±1.09 c	-28.84±3.40 c	6.01±2.99 a	29.7±3.8 a	16.04±1.71 a	39.95±5.37 a
Chlorine Dioxide	7	-0.03±1.55 a	-0.09±3.28 a	1.38±4.52 a	5.3±2.3 c	3.4±1.28 c	0.87±8.22 c
	14	-0.42±1.31 abc	1.21±3.75 a	2.28±5.11 a	6.2±2.3 c	3.92±1.44 c	0.81±7.45 c
	21	-0.56±1.43 abc	-0.59±5.17 a	3.28±4.24 a	6.9±2.6 c	4.02±1.41 c	3.63±8.17 c

Different letters indicate statistically significant difference based on Tukey's test, at 5% significance level.

Δa did not show differences inside the same group at the 7th, 14th and 21st days (Table 1). There was also no difference between groups, when they were compared at the same day (7th and 14th). The control group differed from SP in the analysis conducted at the 21st day. CD did not differ from any of the two other groups.

The analysis applied to ΔL (Table 1) did not show differences between groups and times.

4. Discussion

Sodium perborate efficiency, either in association with distilled water or with hydrogen peroxide, has been known for years[4,5,19-23]. In addition, it is widely used for internal bleaching due to its reasonable cost and reliability. Sodium perborate was herein associated with distilled water at 2:1 g/ml, rather than with hydrogen peroxide, which may be linked to external root resorption[3,8]. However, sodium perborate, either in its mono-, tri- or tetrahydrate form, releases hydrogen peroxide. H_2O_2 release can generate different radicals and reactions in the body. Besides being related to external root resorption, free radicals can act on lipids, proteins and nucleic acids[8]. Thus, the discovery of a bleaching agent that does not release hydrogen peroxide would be a remarkable breakthrough.

Chlorine dioxide has been randomly used in beauty salons in the UK[10,11]. However, precursor elements such as sodium chlorite and citric acid are used to obtain chlorine dioxide, since they cause a chemical reaction that releases it right away[10-12]. However,

this reaction has severe impact on hard dental tissue, since it leads to increased dentinal sensitivity and enables faster future staining[11]. Studies have shown that topographic changes take place when dental tissues get in contact with solutions at pH 2, or lower[10]. This factor leads to enamel tissue deterioration, tooth surface roughening, minerals' erosion and to changes at atomic level, including calcium leaching[12].

Several studies have shown that chlorine dioxide works as bleaching agent in discolored teeth[10-13] and composites[24]. Chlorine dioxide attacks stains, be them organic or inorganic, at subatomic level, such as ClO2 + e- = ClO2[12]. However, the chlorine dioxide used in these studies derived from anhydrous citric acid and sodium chloride. Because of the acidic nature of this association, apart from its other deleterious effects, the aforementioned studies have emphasized that sodium chloride should be recommended to be used in tooth bleaching procedures.

Chlorine dioxide pH level was selected due to its proximity to the agents[10,12], as well as to its comparability to that of hydrogen peroxide bleaching solutions, which range from 2.1[13] to 3.7[20]. The adopted chlorine dioxide concentration was based on Ablal et al.[13] (2013), although the aforementioned authors used an agent produced by mixing sodium chloride with anhydrous citric acid, rather than a stabilized agent. The bleaching observed in their study was associated with teeth dehydration caused by the temperature resulting from light activation; this factor could explain the immediate bleaching effect observed in their study[13]. Thus, prolonged exposures did not improve bleaching effect[14].

If one takes into consideration the non-difference in luminosity (ΔL) among all assessed groups, the significant difference in color measurement (Δb, ΔE and ΔE00) and whiteness index (ΔWI_D) recorded for SP, in comparison to the CD and Control groups, can be associated with the potential of SP bleaching to remove stains and, consequently, to decrease Δa and Δb. This factor provided the analyzed specimens with stronger greenish and bluish color, and it has changed the perceptibility threshold.

Although the perceptibility threshold observed for the control and CD groups was slightly higher than 1.8 (Δ00), which clinically indicated discoloration, it happened due to elimination of easily-removed extrinsic pigments from bovine teeth. It is possible saying that immersing the specimens into water, similar to the control group, did not have effective bleaching effect like the one observed for Chlorine dioxide. The citric acid and sodium chlorite combination enabled fast chlorine dioxide release, in its gaseous form, which acted almost immediately on nearby surfaces[11]. However, the chlorine dioxide-based agent was not capable of bleaching teeth discolored with bovine blood in the current study. The negative result observed for the chlorine dioxide-based experiment was likely associated with the use of stabilized chlorine dioxide liquid, rather than with the active destabilized form deriving from the association between citric acid and sodium chloride, which, however, is unsafe for such a use[10,11].

The use of bovine dental elements in the current study was justified by common human teeth standardization difficulties associated with both dentin thickness and the incidence of reactionary dentin. Bovine incisors are similar to human teeth; thus, they have been used as substitutes in assays focused on assess the effectiveness of dental products[23]. Other studies have used cattle incisors in mandibular tooth whiten-

ing tests, which included both external[25] and internal bleaching[26,27]. According to these tests, specimens were pigmented with bovine blood in order to simulate discoloration types often found in clinical practice[16]. The herein used teeth were standardized in A2 and randomly distributed among the analyzed groups, after staining protocol application. Although teeth with slight difference in color were used in the current study, the visually different ones were removed from it. This factor can be considered a limitation of the present study, since spectrophotometry-based analysis was not performed at that time.

Although shade evaluation could have been performed by calibrated observers, based on the Vita scale, in controlled environment[20], the use of the spectrophotometer provides lower margin of error, enables more accurate results and presents higher reproducibility[28].

Chlorine dioxide is categorized as primary oxidizing agent. However, its stabilized form was not capable of bleaching bovine incisors in the current study, unlike sodium perborate, which is also a well-known oxidizing product. Using chlorine dioxide at other concentrations or pH levels, or adding certain adjuvant agents to it, may help achieving the desired effect.

In conclusion, keeping in mind the limitations of the current study, it was possible concluding that stabilized chlorine dioxide (0.07% at pH 3.5) cannot perform as internal bleaching agent.

Data availability

Datasets linked to the current article will be available upon request to the corresponding author.

Disclosure statement

The authors declare no conflict of interest.

Author contribution

Conceptualization: Lopes MB, Berger SB. Data curation: Felizardo KR.

Formal analysis: Lopes MB, Berger SB Funding acquisition: Lopes MB Investigation: Hirata BS, Felizardo KR

Methodology: Hirata BS, Guiraldo RD Project administration: Lopes MB Resources: Ramos NBP, Guiraldo RD.

Supervision: Lopes MB

Validation: Guiraldo RD Visualization: Berger SB

Writing - original draft: Lopes MB, Guiraldo RD, Ramos NBP, Hirata BS

Writing - review &editing: Felizardo KR, Berger SB, Lopes MB

All authors actively participated in the discussion of the manuscript's findings and have revised and approved the final version of the manuscript.

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