



Effect of Three Bleaching Protocols on Tooth Discoloration Caused by Hemoglobin

Narges Panahandeh¹, Shervin Sedighi², Shervin Mohammadkhani², Sogol Nejadkarimi³, Amir Ghasemi^{1*}

1. Dental Research Center, Research Institute for Dental Sciences, School of Dentistry, Shahid Beheshti University of Medical Sciences, Tehran, Iran
2. Private Practice, Tehran, Iran
3. Department of Operative Dentistry, School of Dentistry, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Article Info

Article type:

Original Article

Article History:

Received: 31 Jul 2023

Accepted: 05 Feb 2024

Published: 01 Aug 2024

* Corresponding author:

Dental Research Center, Research Institute of Dental Sciences, School of Dentistry, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Email: amir_gh_th@yahoo.com

ABSTRACT

Objectives: This study assessed the efficacy of different combinations of oxalic acid, 35% hydrogen peroxide, and 5.25% sodium hypochlorite (NaOCl) for bleaching of tooth discolorations caused by hemoglobin.

Materials and Methods: In this in vitro study, 40 sound extracted human premolars were disinfected and decoronated. Their primary color parameters were measured (T1). The teeth were then centrifuged with human blood for 3 days, rinsed, polished, and their color parameters were measured again (T2). They were randomly divided into 4 groups (N=10) and treated as follows: Group A: in-office bleaching with Pola-Office Plus followed by 30 seconds of light-curing, group B: 0.24 M oxalic acid for 5 minutes followed by in-office bleaching, group C: 5.25% NaOCl for 5 minutes followed by in-office bleaching, and group D: 0.24 M oxalic acid (5 minutes) followed by 5.25% NaOCl (5 minutes) and subsequent application of in-office bleaching gel. The color parameters of the teeth were measured again (T3). Data were analyzed by one-way ANOVA and paired sample t-test ($\alpha=0.05$).

Results: T2-T3 ΔE in groups B and D was significantly higher than that in group A ($P<0.05$); the difference between groups B and D was not significant. The mean ΔE and ΔL in group C were not significantly different from those in group A ($P>0.05$). ΔL significantly and equally increased in groups B and D after bleaching.

Conclusion: Application of oxalic acid followed by in-office bleaching gel is more effective than the bleaching gel alone for correction of tooth discolorations caused by hemoglobin.

Keywords: Tooth Bleaching; Hemoglobins; Hydrogen Peroxide; Oxalic Acid; Sodium Hypochlorite

➤ **Cite this article as:** Panahandeh N, Sedighi S, Mohammadkhani S, Nejadkarimi S, Ghasemi A. Effect of Three Bleaching Protocols on Tooth Discoloration Caused by Hemoglobin. *Front Dent.* 2024;21:29.

INTRODUCTION

Tooth bleaching is commonly performed for patients with tooth discolorations and those demanding whiter teeth [1]. However, this treatment is time-consuming, and has a risk of relapse, which has been acknowledged by dental clinicians [2]. In order to predict the speed and efficacy of bleaching treatment, the cause of tooth discoloration should be first identified since some discolorations better respond to bleaching treatments. For instance,

yellow discolorations caused by aging often quickly respond to treatment while blue-grayish discolorations caused by tetracycline are highly resistant to bleaching [3,4]. Also, tooth discolorations caused by iron hardly respond to bleaching treatments. Discolorations caused by hemoglobin are pretty common. Hemoglobin contains iron [5]. In traumatized teeth, hemoglobin may penetrate into the tooth structure and accumulate in the internal or external layers, causing tooth discoloration [6].

Several techniques have been suggested for correlation of hemoglobin-induced tooth discolorations. The chemical composition of the factor responsible for tooth discoloration is an important parameter to consider in selection of type of bleaching agent. Oxalic acid is the first product used for correction of hemoglobin-induced tooth discolorations [1]. It is widely used for elimination of iron oxide impurities in ceramic and paper industries, and its optimal efficacy for the abovementioned purposes has been well proven [7-9].

Sodium hypochlorite (NaOCl) has long been used as a bleaching agent for household and industrial purposes [10]. It is extensively used in dentistry for root canal irrigation in 0.5% to 5.25% concentrations [11], for dentin collagen removal in 10% concentration [12], and for tooth bleaching especially in fluorosed teeth as a safe, non-invasive and effective technique to remove yellow-brown hypo-mineralized enamel spots [13,14]. On the other hand, 35% hydrogen peroxide is commonly used as the most effective material for in-office bleaching of discolored teeth. It is applied on the tooth surface, and light or heat may be used to activate or reinforce it [15].

Comprehensive studies comparing the efficacy of the abovementioned bleaching agents for correction of hemoglobin-induced discolorations are lacking. Thus, this study aimed to assess the efficacy of different combinations of oxalic acid, 35% hydrogen peroxide, and 5.25% NaOCl for bleaching of tooth discolorations caused by hemoglobin.

MATERIALS AND METHODS

This in vitro, experimental study evaluated 40 sound human premolar teeth extracted for orthodontic treatment. The study protocol was approved by the ethics committee of Shahid Beheshti University of Medical Sciences (IR.SBMU.DRC.REC.1398.030).

The inclusion criteria were recently extracted sound teeth with no caries, restorations, developmental defects, enamel cracks, or tooth discolorations. The teeth were debrided, and the soft and hard tissue residues attached to the teeth were removed by a curette. The teeth were then cleaned with a prophylaxis brush

and pumice paste, and were then disinfected by immersion in 0.5% chloramine T solution for 1 week. They were then rinsed with water and stored in 0.9% saline at room temperature. The teeth were then randomly assigned to four groups (N=10). Digital photographs were obtained from the teeth and their color parameters were measured by a previously calibrated portable spectrophotometer (X-rite Ci6x; Grand Rapids, MI, USA) according to the CIE L*a*b* color space (T1). To ensure a standard reproducible position, the teeth were mounted in red dental wax. The labial surface of the teeth was photographed against a gray background.

Next, the teeth were decoronated at 1 mm below their cemento-enamel junction using a thin sectioning machine (Hamco Machines Inc., Rochester, NY, USA). The coronal part was retained, and the contents of the pulp chamber were removed. Next, the teeth were stained with hemoglobin according to the technique described by Freccia and Peters [16], which was later modified by Marin et al [17]. For this purpose, the teeth were placed in test tubes containing human blood (packed erythrocytes obtained from the Iranian Organization of Blood Transfusion) mixed with distilled water (to induce hemolysis) twice a day, each time for 25 minutes for 3 consecutive days, and centrifuged (Boeco C28-A, Boeckel & Co., Hamburg, Germany) at 3500 rpm. For the rest of the time, the teeth were stored in an incubator at 37°C and 100% humidity. Next, the teeth were rinsed with water for 20 minutes, and polished with a rubber cup and pumice paste to eliminate external stains. The color parameters of the teeth were measured by a spectrophotometer as explained earlier for the second time (T2). Next, the teeth in each group underwent bleaching as follows:

Group A: Pola-Office Plus bleaching gel (SDI, Australia) containing 37.5% hydrogen peroxide was applied in the pulp chamber and on the external surface of the teeth 4 times, each time for 8 minutes according to the manufacturer's instructions. The teeth were monitored during this time period, and the gel was agitated after 4 minutes. Next, the teeth were irradiated with a halogen light curing unit (Kerr Demetron LC;

Brea, CA, USA) from 1-2 mm distance for 30 seconds with a constant intensity to accelerate the bleaching reaction with hydrogen peroxide. Group B: First, 3% solution of 0.24 M oxalic acid [7] in water was prepared. To prepare 100 cc of oxalic acid solution, 3.026 g of oxalic acid dihydrate powder (CAS 6153-56-6; Merck Group, Darmstadt, Germany) with a molar weight of 126.07 g/mol was added to adequate amount of water to prepare 100 cc of acid solution. To adjust the pH at 3, ammonium hydroxide was gently added to the solution, and the pH was continuously measured until the desired pH was achieved [7]. This solution was poured into the pulp chamber and applied on the external tooth surface for 5 minutes [18]. The teeth were then rinsed with distilled water for 1 minute. Next, 37.5% Pola-Office Plus bleaching gel was applied in the pulp chamber and on the external tooth surface as explained for group A.

Group C: A solution of 5.25% sodium hypochlorite was prepared and poured into the pulp chamber and applied on the external tooth surface for 5 minutes. The teeth were then rinsed with distilled water for 1 minute. Next, 37.5% Pola-Office Plus bleaching gel was applied in the pulp chamber and on the external tooth surface as explained for group A.

Group D: First, 3% solution of 0.24 M oxalic acid [7] in water was prepared. This solution was poured into the pulp chamber and applied on the external tooth surface for 5 minutes [18]. The teeth were then rinsed with distilled water for 1 minute. A solution of 5.25% NaOCl was prepared and poured into the pulp chamber and applied on the external tooth surface for

5 minutes. The teeth were then rinsed with distilled water for 1 minute. Next, 37.5% Pola-Office Plus bleaching gel was applied in the pulp chamber and on the external tooth surface as explained for group A.

Afterwards, all teeth in all groups were rinsed with distilled water for 1 minute. The teeth were then immersed in saline for 1 week to eliminate the effect of dehydration caused by bleaching on tooth color. Next, the color parameters of the teeth were measured again (T3) as explained earlier. The color change (ΔE) was calculated for each tooth at different time points using the formula below:

$$\Delta E = \sqrt{\Delta a^2 + \Delta b^2 + \Delta L^2}$$

Data were analyzed using SPSS version 26 (SPSS Inc., IL, USA). The Kolmogorov-Smirnov test confirmed normal distribution of data. The changes in L^* , a^* and b^* color parameters between T1 and T2 were analyzed using paired sample t-test to confirm the discolorations caused by hemoglobin. The changes in color parameters (ΔL , Δa , Δb) and ΔE between groups before and after bleaching (between T2 and T3) were analyzed using one-way ANOVA. The Tukey HSD post-hoc test was used for pairwise comparisons of the groups after bleaching. Level of significance was set at 0.05.

RESULTS

Table 1 presents the mean L^* , a^* and b^* color parameters in the four groups at different time points. The mean ΔE was 7.49 ± 1.76 in group A, 10.56 ± 1.46 in group B, 8.97 ± 2.09 in group C, and 11.07 ± 1.58 in group D. The mean ΔE of

Table 1. Mean L^* , a^* and b^* color parameters in the four groups at different time points

Time point	Color parameters	Group A		Group B		Group C		Group D	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
Before staining	L^*	77.92	3.10	72.22	3.97	73.81	3.54	75.92	2.87
	a^*	2.01	0.76	0.91	0.89	0.88	0.76	0.80	0.45
	b^*	14.24	3.64	12.21	3.87	12.58	3.52	10.90	1.64
After staining	L^*	70.80	2.65	65.29	3.53	68.18	3.15	68.54	2.38
	a^*	1.43	0.70	2.14	0.98	1.07	0.63	1.50	0.83
	b^*	10.94	2.82	11.56	4.69	10.83	2.37	12.35	2.25
After bleaching	L^*	77.48	2.72	74.85	2.74	76.23	3.67	78.42	1.84
	a^*	0.15	0.43	0.12	0.39	-0.13	0.62	0.23	0.60
	b^*	8.27	1.31	7.87	3.67	7.32	1.25	7.84	1.69

SD: standard deviation

Table 2. Δa^* , Δb^* , ΔL^* and ΔE between T2 and T3 in groups A, B, C and D

Group	Color parameter	Mean	SD	Maximum	Minimum
A	ΔL^*	6.68	1.39	9.63	4.56
	Δa^*	-1.29	0.87	-0.09	-2.84
	Δb^*	-2.67	1.83	-0.20	-5.39
	ΔE^*	7.49	1.76	10.68	5.77
B	ΔL^*	9.56	1.33	11.31	7.23
	Δa^*	-2.02	0.93	-1.06	-4.19
	Δb^*	-3.69	1.43	-1.50	-5.82
	ΔE^*	10.56	1.46	13.12	8.36
C	ΔL^*	8.05	1.53	11.19	6.21
	Δa^*	-1.20	0.67	-0.20	-2.03
	Δb^*	-3.52	1.94	-1.33	-6.30
	ΔE^*	8.97	2.09	12.68	6.60
D	ΔL^*	9.87	1.71	12.61	7.18
	Δa^*	-1.27	0.72	-0.63	-2.80
	Δb^*	-4.51	1.58	-2.12	-6.92
	ΔE^*	11.07	1.58	13.68	8.79

SD: standard deviation

all specimens between T1 and T2 was 9.52. Considering the normal distribution of data ($P=0.2$), paired sample t-test was applied to compare the L^* , a^* and b^* color parameters before and after staining with hemoglobin, which showed that all color parameters experienced a significant change after staining in L^* ($P<0.001$), a^* ($P=0.012$) and b^* ($P=0.016$). The ΔE between T2 and T3 was analyzed and compared among the four groups using one-way ANOVA. Table 2 shows the mean change in color parameters (Δa^* , Δb^* , and ΔL^*) and ΔE between T2 and T3 in the four groups.

Comparison of groups regarding ΔE between T2 and T3:

One-way ANOVA revealed a significant difference in ΔE among the four groups ($P<0.001$). According to the Tukey HSD test, the mean ΔE was not significantly different in groups A and C ($P=0.243$). The mean ΔE was significantly higher in groups B ($P=0.002$) and D ($P<0.001$) than group A. However, the difference between groups B and D was not significant in this respect ($P=0.913$).

Comparison of groups regarding ΔL between T2 and T3:

One-way ANOVA revealed a significant difference in ΔL^* among the four groups ($P<0.001$). According to Tukey HSD test, ΔL^* was significantly greater in groups B ($P=0.001$) and D ($P<0.001$), compared with group A.

However, the difference between groups B and D was not significant regarding ΔL^* ($P=0.966$). In other words, the gradient of increase in L^* parameter was the same in groups B and D. Groups A and C had no significant difference in ΔL^* ($P=0.194$).

Comparison of groups regarding Δa^* between T2 and T3:

According to one-way ANOVA, the four groups were not significantly different regarding Δa^* ($P=0.095$).

Comparison of groups regarding Δb^* between T2 and T3:

According to one-way ANOVA, the four groups were not significantly different regarding Δb^* ($P=0.139$).

DISCUSSION

Considering the scarce information available regarding tooth discolorations caused by hemoglobin and effective treatments for their correction, this study was designed to assess the efficacy of different combinations of oxalic acid, 35% hydrogen peroxide, and 5.25% sodium hypochlorite for bleaching of tooth discolorations caused by hemoglobin. Color change was evaluated by spectrophotometry in this study considering its high precision and reliability [19]. In use of a spectrophotometer, unlike a digital camera, the environmental lighting does not affect the test results [20, 21].

The present results showed that the mean ΔE in group C (sodium hypochlorite alone) was not significantly different from that in group A (in-office bleaching gel alone). Gupta et al. [22] used sodium hypochlorite alone for treatment of tooth discolorations caused by fluorosis and reported that it was only effective for correction of mild discolorations, which was in line with the present findings. However, Penumatsa et al, [13], Flores et al, [14] and Belkhira and Douki [23] etched the enamel first, applied NaOCl, and then coated the enamel with resin materials and reported more favorable results. Similarly, Ghousia et al. [24] suggested demineralization and deproteinization of teeth prior to bleaching to increase the efficacy of the bleaching process and enable better penetration of the bleaching agent. Difference between the results of the abovementioned studies and the present findings may be due to conduction of enamel etching prior to bleaching in their studies.

In the present study, the L^* , a^* and b^* parameters experienced a significant change after hemoglobin staining ($P < 0.001$), and the teeth became darker, and redder (in the red-green axis). A reduction in b^* parameter was also noted, which means a reduction in yellowness in the yellow-blue axis, although it was not significant. The obtained ΔE was 9.52 compared with baseline, which indicated a clinically detectable color change. In a study by Kaneko et al, [25] the teeth were stored in a mixture of blood and iron sulfide at 37°C for 1 month, instead of centrifugation. They achieved more favorable color change in their study; however, their methodology was time-consuming. Thus, we applied the technique described by Freccia and Peters [16], which was later modified by Marin et al [17].

The porphyrin ring connected to iron in the heme molecule is probably responsible for hemoglobin-induced tooth discoloration [26, 27]. Hemoglobin is dark red in color, and can be responsible for the reduction in L^* and the increase in a^* parameter. Darkening of tooth color due to hemoglobin exposure has been documented in many previous studies [16, 17, 24, 25, 27]. According to Marin et al, [17] following hemolysis of red blood cells in dentinal tubules, intact hemoglobin remains

in dentin. Hemoglobin is bipolar [5], and this property conforms with the properties of the bleaching agents used in the present study. Water present in the composition of bleaching agents and hydroxide ions present in hydrogen peroxide bleaching gel, sodium hypochlorite and oxalic acid are all polar [28-31]. Thus, the selected bleaching agents probably have optimal efficacy for correction of hemoglobin staining. However, it should be noted that the bleaching results obtained in the present study may be attributed not only to correction of hemoglobin stains, but also to whitening of other molecules in the tooth structure by dissolving the organic matter and deproteinization of the dentin collagen [32]. Thus, interpretation of results should be done with caution.

In group A, following the application of Pola-Office Plus, which contains hydrogen peroxide, the L^* parameter significantly increased, which was in agreement with the results of previous studies that used this product for correction of conventional tooth discolorations [33-35]. The a^* parameter significantly decreased in group A, which was also reported by studies using bleaching products containing hydrogen peroxide such as the studies by Martin-Biedma et al, [35] and Lima et al [36]. However, according to the guideline of the American Dental Association [37] and a study by Luo et al, [33] change in a^* parameter occurs slower and in smaller magnitude in the tooth bleaching process and may not reach statistical significance. Difference between their statements and the current findings is probably due to the fact that the present study had an in vitro design, and such changes often occur faster and can be more accurately detected in vitro. The b^* parameter significantly decreased in group A. According to Gerlach et al, [34] the increase in L^* parameter and the reduction in b^* parameter indicate improvement of color and whitening of teeth.

In group B, application of oxalic acid followed by Pola-Office Plus significantly increased the L^* parameter and decreased the a^* and b^* parameters. The change in L^* parameter was significantly greater in group B than group A, which indicates achieving a lighter shade in group B. This result is probably due to removal

of iron oxide products by oxalic acid subsequent to degradation of the heme structure in hemoglobin [7,38,39]. The ΔE in group B was significantly different from that in group A, indicating higher efficacy of oxalic acid plus Pola-Office Plus compared with the application of Pola-Office Plus alone for color correction of discolored teeth. It has been reported that the combination of peroxide and oxalic acid has been the most effective for removal of natural colors [40].

In group C, application of sodium hypochlorite followed by Pola-Office Plus significantly increased the L^* and decreased the a^* and b^* color parameters. The change in L^* parameter and the mean ΔE in this group were not significantly different from the corresponding values in group A.

In group D, application of oxalic acid followed by sodium hypochlorite and then Pola-Office Plus significantly increased the L^* and decreased the a^* and b^* color parameters. The ΔL in this group had a significant difference with that in group A, indicating that a lighter color was achieved in group D, probably due to the presence of oxalic acid, since its effect was also significant on group B. The ΔE in this group was significantly different from that in group A. However, the mean ΔE was the same in groups B and D. The results of this study did not support the synergistic effect of sodium hypochlorite and Pola-Office Plus bleaching gel or oxalic acid for color correction of tooth discolorations caused by hemoglobin. Also, Δa^* was almost the same in all four groups, indicating that application of oxalic acid and sodium hypochlorite prior to the use of bleaching gel had no significant efficacy for reduction of yellowness.

CONCLUSION

Application of oxalic acid followed by in-office bleaching with Pola-Office Plus gel is more effective for correction of hemoglobin-induced discolorations compared with the application of Pola-Office Plus bleaching gel alone.

Conflict of interest: None

CONFLICT OF INTEREST STATEMENT

None declared.

REFERENCES

1. Plotino G, Buono L, Grande NM, Pameijer CH, Somma F. Nonvital tooth bleaching: a review of the literature and clinical procedures. *J Endod*. 2008 Apr;34(4):394-407.
2. Alqahtani MQ. Tooth-bleaching procedures and their controversial effects: A literature review. *Saudi Dent J*. 2014 Apr;26(2):33-46.
3. Haywood VB. Overview and status of mouthguard bleaching. *J Esthet Restor Dent*. 1991 Sep;3(5):157-61.
4. Hørsted-Bindslev P, Løvschall H. Treatment outcome of vital pulp treatment. *Endodontic Topics*. 2002 Jul;2(1):24-34.
5. Dickson CF, Kumar KK, Jacques DA, Malmirchegini GR, Spirig T, Mackay JP, et al. Structure of the Hemoglobin-IsdH Complex Reveals the Molecular Basis of Iron Capture by *Staphylococcus aureus*. *J Biol Chem*. 2014 Mar;289(10):6728-38.
6. Zollinger A, Attin T, Mohn D, Zehnder M. Effects of endodontic irrigants on blood and blood-stained dentin. *Heliyon*. 2019 May;5(5):e01794.
7. Lee SO, Tran T, Jung BH, Kim SJ, Kim MJ. Dissolution of iron oxide using oxalic acid. *Hydrometallurgy*. 2007 Jul;87(3-4):91-9.
8. Sultana U, Kurny A. Dissolution kinetics of iron oxides in clay in oxalic acid solutions. *Int J Miner Metall Mater*. 2012 Dec;19(12):1083-7.
9. Sjode A, Jonsson LJ, Nilvebrant N-O, editors. *Oxalic Acid in Bleaching Processes-Formation and Management*. 59th Appita Annual Conference and Exhibition: Incorporating the 13th ISWFPC (International Symposium on Wood, Fibre and Pulping Chemistry), Auckland, New Zealand, 16-19 May 2005: Proceedings; 2005: Appita Inc.
10. Bretherick L. *Bretherick's handbook of reactive chemical hazards*: Elsevier; 2016.
11. Torabinejad M. Root canal irrigants and disinfectants. *Endodontics: Colleagues for Excellence*. 2011:2-7.
12. Nakatani H, Mine A, Matsumoto M, Tajiri Y, Hagino R, Yumitate M, et al. Effectiveness of pretreatment with phosphoric acid, sodium hypochlorite and sulfinic acid sodium salt on root canal dentin resin bonding. *J Prosthodont Res*. 2020;64(3):272-80.
13. Penumatsa NV, Sharaneshia RB. Bleaching of fluorosis stains using sodium hypochlorite. *J Pharm Bioallied Sci*. 2015 Aug;7(Suppl 2):S766-8.
14. Flores AC, Reyes HF, Moscoso AG, Castanedo Cázares JP, Pozos Guillén Ad. Clinical efficacy of 5% sodium hypochlorite for removal of stains caused by dental fluorosis. *J Clin Pediatr Dent*. 2009 Apr;33(3):187-92.

15. Joshi SB. An overview of vital teeth bleaching. *J Interdiscip Dentistry*. 2016 Jan;6(1):3-13.
16. Freccia WF, Peters DD. A technique for staining extracted teeth: a research and teaching aid for bleaching. *J Endod*. 1982 Feb 1;8(2):67-9.
17. Marin P, Bartold P, Heithersay G. Tooth discoloration by blood: an in vitro histochemical study. *Dent Traumatol*. 1997 Jun;13(3):132-8.
18. Chapple J. Restoring discolored teeth to normal. *Dent Cosmos*. 1877 Sep;19:449.
19. Liberato WF, Barreto IC, Costa PP, de Almeida CC, Pimentel W, Tiozzi R. A comparison between visual, intraoral scanner, and spectrophotometer shade matching: A clinical study. *J Prosthet Dent*. 2019 Feb;121(2):271-5.
20. Joiner A, Luo W. Tooth colour and whiteness: A review. *J Dent*. 2017 Dec;67:S3-S10.
21. Zhu W, Liu C, Pan J. [Assessment of tooth bleaching efficacy with spectrophotometer]. *Hua Xi Kou Qiang Yi Xue Za Zhi*. 2014 Jun;32(3):259-62.
22. Gupta A, Dhingra R, Chaudhuri P, Gupta A. A comparison of various minimally invasive techniques for the removal of dental fluorosis stains in children. *J Indian Soc Pedod Prev Dent*. 2017 Jul;35(3):260.
23. Belkhir MS, Douki N. A new concept for removal of dental fluorosis stains. *J Endod*. 1991 Jun;17(6):288-92.
24. Ghousia S, Naik NS, Reddy VS. Spectrophotometric analysis on bleaching efficacy of blood stained demineralized and deproteinized dentin—An in vitro study. *J Clin Pediatr Dent*. 2010 Jul;34(4):303-8.
25. Kaneko J, Inoue S, Kawakami S, Sano H. Bleaching effect of sodium percarbonate on discolored pulpless teeth in vitro. *J Endod*. 2000 Jan;26(1):25-8.
26. Marin P, Heithersay G, Bridges T. A quantitative comparison of traditional and non-peroxide bleaching agents. *Dent Traumatol*. 1998 Apr;14(2):64-7.
27. Shokouhinejad N, Razmi H, Farbod M, Alikhasi M, Camilleri J. Coronal tooth discoloration induced by regenerative endodontic treatment using different scaffolds and intracanal coronal barriers: a 6-month ex vivo study. *Restor Dent Endod*. 2019 Aug;44(3):e25.
28. PubChem Compound Summary for CID 784, Hydrogen peroxide: National Library of Medicine (US), National Center for Biotechnology Information; 2020 [Available from: <https://pubchem.ncbi.nlm.nih.gov/compound/Hydrogen-peroxide>].
29. PubChem Compound Summary for CID 23665760, Sodium hypochlorite: National Library of Medicine (US), National Center for Biotechnology Information; 2004; 2020 [Available from: <https://pubchem.ncbi.nlm.nih.gov/compound/Sodium-hypochlorite>].
30. PubChem Compound Summary for CID 61373, Oxalic acid dihydrate: National Library of Medicine (US), National Center for Biotechnology Information; 2004; 2020 [Available from: <https://pubchem.ncbi.nlm.nih.gov/compound/Oxalic-acid-dihydrate>].
31. Sharp KA, Vanderkooi JM. Water in the half shell: structure of water, focusing on angular structure and solvation. *Acc Chem Res*. 2010 Feb;43(2):231-9.
32. Tartari T, Bachmann L, Maliza AGA, Andrade FB, Duarte MAH, Bramante CM. Tissue dissolution and modifications in dentin composition by different sodium hypochlorite concentrations. *J Appl Oral Sci*. 2016 May;24(3):291-8.
33. Luo W, Westland S, Brunton P, Ellwood R, Pretty IA, Mohan N. Comparison of the ability of different colour indices to assess changes in tooth whiteness. *J Dent*. 2007 Feb;35(2):109-16.
34. Gerlach RW, Barker ML, Sagel PA. Objective and subjective whitening response of two self-directed bleaching systems. *Am J Dent*. 2002 Sep;15:7-12A.
35. Martin-Biedma B, Gonzalez-Gonzalez T, Lopes M, Lopes L, Vilar R, Bahillo J, et al. Colorimeter and scanning electron microscopy analysis of teeth submitted to internal bleaching. *J Endod*. 2010 Feb;36(2):334-7.
36. Lima FG, Rotta TA, Penso S, Meireles SS, Demarco FF. In vitro evaluation of the whitening effect of mouth rinses containing hydrogen peroxide. *Braz Oral Res* 2012;26(3):269-74.
37. Siew C; American Dental Association. ADA guidelines for the acceptance of tooth-whitening products. *Compend Contin Educ Dent Suppl*. 2000;(28):S44-7.
38. Al-Mobarak NA. Effect of oxalic acid on the dissolution of magnetite coupled with iron of various surface area. *Int J Electrochem Sci*. 2008 Jun;3(6):666-75.
39. Santawaja P, Kudo S, Tahara A, Asano S, Hayashi JI. Dissolution of iron oxides highly loaded in oxalic acid aqueous solution for a potential application in iron-making. *ISIJ Int*. 2022 Dec 15;62(12):2466-75.
40. Ashaari Z, Hanim R, Tahir PM, Nizam N. Effects of Peroxide and Oxalic Acid Bleaching on the Colour and Gluing Properties of Some Tropical Bamboos. *J Biol Sci*. 2004;4(2):90-4.