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Optic changes due to innovative experimental formulations for bleaching non-vital teeth. In vitro study

D. PASCALAU[a], T. F. MARINCA[b], S. BUDURU[a,*], A.-S. MESAROS[a]
[a] *"Iuliu Hatieganu" University of Medicine and Pharmacy, Faculty of Dental Medicine, Department of Prosthetic Dentistry, Cluj-Napoca, Romania*
[b] *Technical University of Cluj-Napoca, Department of Mechanical Engineering, Cluj-Napoca, Romania*

Objectives: This study aims to evaluate the efficiency of experimental materials for the internal bleaching of non-vital teeth, compared to commercially available agents based on peroxides.
Methods: Sixty extracted teeth divided in six groups underwent endodontic treatment followed by simulated discoloration. For each group, a different bleaching formulation was used. The dental colour was assessed by spectrophotometry.
Results: According to the spectrophotometric analysis, the most effective results were achieved for groups treated with TiO_2 /SiO_2 /Aa and TiO_2 /SiO_2 with small amount of sodium perborate addition.
Conclusions: Our study reveals that internal bleaching with oxidative systems is a plausible innovation.

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Keywords: Spectrophotometry, Internal bleaching, silicon dioxide, Titanium dioxide

1. Introduction

Optical properties of dental tissues are very important form two points of view: aesthetics of the smile and status of health of the tooth. The most important optical parametrs of tooth colour are shade, luminosity and saturation. However, in present days systems such as CIEL*a*b* or CIEDE2000 are using numeric values for luminosity and chromatic parameters in order to calculate colour differences. The monitoring of tooth color parameters and establishing if there are modifications or not is done in an objective manner by using spectrophotometers. Endodontically treated teeth suffer intrinsic changes due to the intrapulpal haemorrhage, the tissular decay products, sometimes the insufficient access to the pulp chamber, which creates a barrier that doesn't allow full removal of infected tissue, the presence of chromophore debris or due to incorrect filling techniques [1,2]. These changes determine a series of effects upon dental tissues, such as discoloration. The treatment of choice for discoloration is dental bleaching. Bleaching of non-vital teeth is achieved by oxidizing the conjugated double bonds of the chromophore organic compounds deposited in dental tissues following endodontic treatment. The bleaching process can be performed internally, externally or by combining these two techniques. Because the teeth are non-vital and the discoloration starts from inside the tooth, internal bleaching techniques are preferred. These procedures often use peroxides (hydrogen and carbamide peroxide) and newly sodium perborate as effective bleaching agents. But they cause a series of important side effects [3-5]. Among the local effects there are hypersensitivity, gingival irritation, changes in enamel microhardness, micromorphological defects related to demineralization, and adverse effects on restorative materials [6]. This effect on the enamel texture, surface morphology and hardness can increase the susceptibility to fracture. Moreover, studies show that the great loss of dental tissue during the preparation of endodontic access cavity is a key factor in the aetiology of fracture [7]. There are also reported systemic effects such as irritation of gastro-intestinal mucosa, soreness and burns of digestive tract and minor abdominal discomfort [8].

Endodontically treated teeth with coronal discoloration can undergo internal bleaching procedures using the "walking bleach" technique [9,10].

One of the recent concerns in the aesthetic dentistry research field is to lower the side effects caused by dental bleaching with peroxides. The identification of some bleaching materials with mild chemical behaviour, with less or absent side effects and a higher benefit-risk ratio, is challenging. Some trials using plant extracts (Aloe Vera, Pomegranate Peel, Grape Seed Extract, Green Tea, and Sodium Ascorbate) as bleaching agents were performed [11].

The aim of this in-vitro study is to evaluate the bleaching effect and to assess the potential changes in texture and surface morphology of endodontically treated teeth, using experimental inorganic oxides TiO_2 and SiO_2, respectively. Both TiO_2 and SiO_2 are used in pharmaceutical and food industries [12], as carrier for catalysts in catalytic industrial processes [13,14], mostly

TiO_2 as inorganic nanoparticles inducing oxidative stress [15,16] and also as active or inert components in dental materials processing [17-19].

2. Experimental

Sixty extracted human teeth were used in this in-vitro study. The teeth were cleaned with distilled water, any trace of soft tissue was removed, and then the standard endodontic treatment was performed [20,21]. The treatment was conducted manually, respecting the clinical procedures. The antiseptic treatment was done with a 5.25% Sodium Hypochlorite solution, with abundant irrigation, using 5 ml of solution per canal. The root canal filling was performed with gutta-percha cones in the lateral condensation technique using Calcium hydroxide paste (Adseal, Meta Biomed, United States of America) as sealing material. The temporary crown filling was done with Citodur (DoriDent, Wien, Austria) cement.

The endodontically treated teeth have undergone artificial staining. They were immersed in a concentrated solution of black tea (2 g in 100 ml distilled water) [22] for 24 hours, then they were extracted from the concentrated solution and immersed in artificial saliva. The study began after 24 hours from the immersing in artificial saliva. A series of trials were conducted in order to achieve the best formulation in terms of bleaching agent concentration, texture and consistency. The consistency of each formulation was paste-like and similar among them. The teeth were divided in 6 groups of 10 teeth each. Groups 1-5 were treated with five different bleaching formulations (Table 1) while Group 6 is the control (endodontically treated, without bleaching).

The bleaching technique was started 24 hours after staining. The temporary filling material was removed together with a 2 mm-thick part from the cervical canalar obturation. A 2 mm layer of glass-ionomer cement was placed in the gap in order to prevent root resorption. A certain quantity of bleaching material has been placed in the pulp chamber in a layer of 2 mm and the temporary filling was performed with Citodur cement according to the Walking Bleach technique.

A number of three novel formulations were used in bleaching groups 1-3. For groups 4 and 5 there were used commercially known bleaching agents (P and OE, respectively).

Between examinations, the teeth were kept in artificial saliva. The final coronal filling was done with light-curing hybrid composite resin using the oblique layering technique (G-aenial, GC, Europe) on day 14, after the final spectrophotometric analysis.

Table 1. The groups' teeth specimens and the corresponding bleaching formulation. Composition, abbreviation and components weight ratio of bleaching formulations

Group teeth specimens	Bleaching formulation composition	Bleaching formulation abbreviation	Components weight ratio
Group 1	TiO_2 + Ascorbic acid + distilled water	TAa	T:Aa:W (0.3:0.1:0.1)
Group 2	TiO_2 + SiO_2 + Ascorbic acid + distilled water	TSAa	T:S:Aa:W (0.125:0.063:0.063:0.15)
Group 3	TiO_2 + SiO_2 + Tetrahydrate sodium perborate (P) + distilled water	TSP	T:S:P:W (0.125:0.063:0.063:0.25)
Group 4	Tetrahydrate Sodium Perborate + distilled water	P	P:W (0.8:0.3)
Group 5	Hydrogen peroxide 35% (Opalescence Endo 35%)	OE	OE 35%
Group 6	Control	C	-

In order to evaluate the evolution of the treated teeth colour it was used the spectrophotometry technique on Vita Easyshade Advance 4.0 spectrophotometer. The first analysis was performed at the beginning of the study (day 0). The evolution of dental colour was evaluated in a 14 days period. The bleaching paste was removed on day 3 and day 7, the pulp chamber was cleaned with distilled water and was renewed with a fresh layer from the same bleaching material and the temporary filling was done. After every stage (days 3, 7 and 14), the dental colour change was analysed by spectrophotometric images captured in a corrected light environment. The photographs were captured from a standard height and distance without using the flash. In order to obtain standardized results for the spectrophotometric analysis, the chosen dental surface to be examined (buccal surface, cervical region) was marked by using dental polyethylene capes with round holes, equal in diameter with the tip of the spectrophotometer. Three measurements were recorded for each tooth at days: 0, 3, 7 and 14. The data analysis was performed based on the trichromatic system CIE L*a*b*. The luminosity coordinate (L*), the chromatic coordinate in the red-green axis (a*) and the chromatic coordinate in the yellow-blue axis (b*) were measured according to VITA 3D Master Shade Guide and CIEDE2000 [23]. For the determination of the colour difference (ΔEab (1) and ΔE_{00} (2)) the following equations were used [24,25]:

$$\Delta Eab = \sqrt{\Delta \mathbf{L}*^2 + \Delta \mathbf{a}*^2 + \Delta \mathbf{b}*^2} \tag{1}$$

$$\Delta E_{00} = \left[\left(\frac{\Delta L'}{K_L S_L}\right)^2 + \left(\frac{\Delta C'}{K_C S_C}\right)^2 + \left(\frac{\Delta H'}{K_H S_H}\right)^2 + R_T\left(\frac{\Delta C'}{K_C S_C}\right)\left(\frac{\Delta H'}{K_H S_H}\right)\right]^{\frac{1}{2}} \tag{2}$$

The whiteness index was calculated in order to show the colour shift towards shades of white.

X-ray diffraction (XRD) analysis was performed for characterizing crystalline structure of the purchased oxides. X-ray diffraction patterns were recorded using Inel model Equinox 3000 diffractometer, which operates in reflection with CoKα radiation (λ = 1.7903 Å). The diffractometer equipped with CPS120 detector allows instantaneous diffraction signal detection at 90º with 0.08º resolution. Samples were investigated on a rotating Al support to reduce the preferential orientation effects. Acquisition time for diffractograms was 10 minutes. The performed statistical analysis was Chi squared test ($p < 0.005$).

3. Results

The results obtained by spectrophotometric analysis are expressed according both to VITA Easyshade Classical Shade Guide (Table 2) and VITA 3D Master Shade Guide. Colour difference (Fig. 1) Whiteness index and Luminosity variation (Fig. 2) were calculated based on trichromatic parameters values (L*a*b*) and plotted versus time.

The experimental groups that presented statistically significant results ($p<0.05$) are marked with * in Figs. 1 and 2.

XRD patterns of TiO_2 and SiO_2 samples are illustrated in Fig. 3. Indexing of XRD patterns was performed using JCPDS file (21-1272). X-ray pattern of TiO_2 indicates crystallization in tetragonal system from spatial group I41/amd characteristic for anatase. X-ray pattern of SiO_2 sample reveals an amorphous structure.

The colour shades according to VITA Classical Shade Guide show few modifications, most of them are present in the group 5 (A3.5 to B2; A3.5 to A2; C4 to B3; A4 to B3; A3 to A2; B3 to B1; A4 to A3 and B4 to B2). Minimal changes are displayed in group 1 (B4 to A3.5; C4 to B4; B4 to A3.5) and also in group 6 (A3 to A2; A4 to A3.5; A3.5 to A3 and B3 to B2). Groups 2, 3 and 4 presented a similar amount of colour shade modifications, most important ones are: in group 2 (A4 to A2), in group 3 (B3 to B1) and in group 4 (B3 to A1 and A4 to A2) as seen in Table 2. The values of colour parameters L*a*b* and calculated colour difference (ΔEab and ΔE_{00}) for groups 1-6 are presented in Table 3 [26-28].

The clinical classification of ΔEab and ΔE_{00} values are illustrated in Table 4 [29, 30]. The mean values of ΔEab and ΔE_{00} determined for groups 1-6 were clinically significant. According to the CIELAB trichromatic system, the first analysis (3rd day) shows a clinically acceptable value for group 1 and the maximal acceptable value for the control group. The other groups show clinically obvious modifications, similar for group 2 and 4, slightly higher for group 3 and very high for group 5. The next analysis was performed in the 7th day. The results were similar with the previous analysis for group 1 and 3. Groups 2 and 4 had a significant increase in values. Group 5 values are also increased but in a smaller amount.

Table 2. Colour shades indexes for Groups 1-6 teeth specimens, after bleaching treatment, according to VITA Classical Shade Guide

Group	Sample	Day 0	Day 3	Day 7	Day 14	Group	Sample	Day 0	Day 3	Day 7	Day 14
1	1	A4	A4	A4	A4	4	1	C4	C4	C4	C4
	2	B3	B3	B3	B3		2	B3	A2	B2	A1
	3	A1	A1	A1	A1		3	C4	C4	C4	C4
	4	B4	A4	A3.5	A3.5		4	C4	A4	A3.5	A3.5
	5	A4	A4	A4	A4		5	B3	B3	B2	A2
	6	C4	C4	B4	B4		6	A4	A4	A3.5	A3
	7	A4	B4	A4	A4		7	B3	B2	B2	B2
	8	A4	A4	A4	A4		8	A4	A3	A2	A2
	9	B4	A4	A4	A3.5		9	A4	A3.5	A3	A2
	10	A3	A3	A3	A3		10	B3	B3	B2	B2
2	1	C4	C4	C4	C4	5	1	A3.5	B3	B3	B2
	2	A4	A4	A4	A3.5		2	A3.5	B3	B3	B2
	3	B2	A1	A1	A1		3	A3.5	B3	B3	A2
	4	A2	A1	A1	A1		4	C4	A4	A4	B3
	5	A4	A4	A3	A2		5	A4	A3.5	A3	B3
	6	C4	B4	B3	B3		6	A3	A3	A3	A2
	7	A4	A3.5	A3.5	A3		7	B3	B2	B2	B1
	8	B1	B1	B1	B1		8	A4	A3.5	A3	A3
	9	A1	A1	A1	A1		9	C4	A4	A3.5	A3
	10	A3	A3	A3	A3		10	B4	B3	B3	B2
3	1	A3.5	A3.5	A3.5	A3.5	6	1	A4	C4	C4	A4
	2	A4	A3.5	A3.5	A3.5		2	A2	B2	A2	A2
	3	A3	A2	A2	A2		3	A3	A3	A2	A2
	4	A1	A1	A1	A1		4	A1	A1	A1	A1
	5	A3	B2	B2	B2		5	A4	C4	A4	A3.5
	6	A3	A3	A3	A3		6	A3.5	A3.5	A3.5	A3
	7	A3.5	A3.5	A3	A3		7	B3	B3	B2	B2
	8	A2	A2	A2	A2		8	A1	A1	A1	A1
	9	A1	A1	A1	A1		9	A4	A3.5	A3.5	A3.5
	10	B3	B2	B2	B1		10	C4	C4	C4	A4

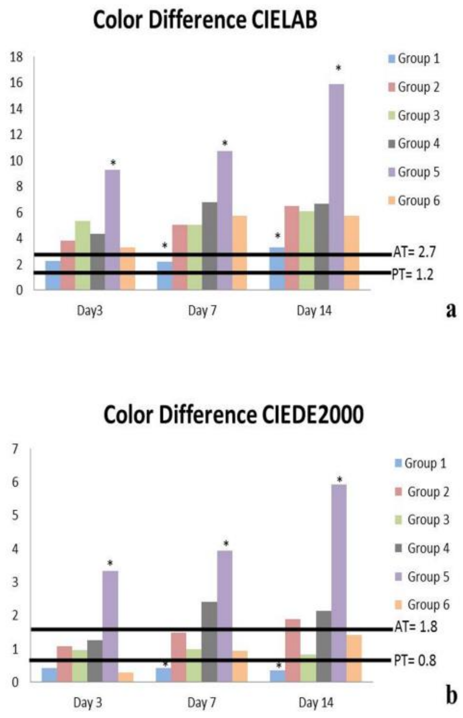

Fig. 1. Graphical representation of Colour Difference mean values calculated with CIELAB (a) and CIEDE2000 (b) trichromatic systems for Groups 1-6 teeth specimens versus time (days). Legend: AT = acceptability threshold; PT= perceptibility threshold

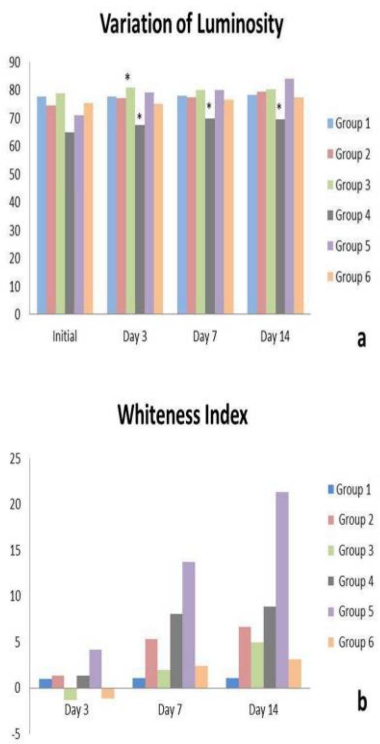

Fig. 2. Graphical representation of Variation of Luminosity (a) and Whiteness index (b) mean values for Groups 1-6 teeth specimensversus time (days)

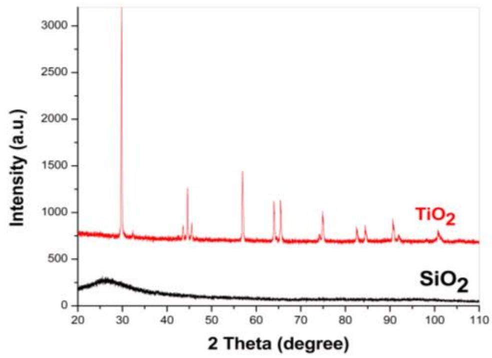

Fig. 3. XRD patterns of TiO_2 and SiO_2 samples

4. Discussions

The endodontically treated teeth following the bleaching protocol were assessed using the spectrophotometric analysis of colour difference and variation of luminosity at the 3rd, 7th and 14th day of bleaching process.

The final analysis was conducted in the 14th day. Group 1 changed a rank in the classification becoming clinically acceptable for 50% observers. Groups 2, 3 and 4 had slightly increased values against group 5 whose value increased significantly.

According to the newest classification system CIEDE2000, only groups 1 and 6 have values under the clinically perceptible range, whereas groups 2, 3 and 4 are both clinically perceptible and acceptable. Group 5 surpasses the clinically acceptable value by the 3rd day. In the 7th day, group 1 remains clinically imperceptible. The mean values for group 2 stand at the border of clinically acceptable threshold. Higher values surpassing the acceptable range are observed for groups 4 and 5. The last day of examination shows increased values, surpassing the clinically acceptable range for groups 2, 4 and 5, while group 3 and 6 remains under this range, being clinically perceptible only.

The most important colour parameter in the bleaching process is luminosity. It is defined as the quantity of reflected light by a certain surface. Using this parameter, bright colours (high level of luminosity) can be distinguished from dark colours (low level of luminosity). Therefore, in the bleaching process probably the most detectable parameter for the human eye is the variation of luminosity (the tooth appears brighter when the luminosity increases). In this study, the higher variation of luminosity is clearly observed for group 5. It has a linear increase from the beginning until the 14th day. The same behaviour has been observed for group 2 with a plateau between the 3rd and 7th days. Groups 3 and 4 present a similar behaviour, a slight increase between the initial day and the 3rd day. The values for the control group increased slightly because the teeth were kept in artificial saliva.

The whiteness index (Fig. 3) shows that groups 3 and 6 of teeth specimens shifted to shades of white by the 3rd day of the bleaching process. For group 1, the whiteness index remained unmodified, showing that the formulation had a poor capacity of bleaching.

*Table 3. Mean values of colour parameters L*a*b* determined by spectrophotometric analysis and calculated colour difference (ΔEab and ΔE_{00}) for groups 1-6*

Group	Color parameter	Day 0	Day 3	Day 7	Day 14	Color difference					
						ΔEab			ΔE_{00}		
						Day 3	Day 7	Day 14	Day 3	Day 7	Day 14
1	L*	77.69	77.77	77.94	78.38	2.23	2.18	3.27	0.41	0.42	0.36
	a*	3.34	3.54	3.66	3.60						
	b*	29.31	28.75	28.69	29.01						
2	L*	74.41	77.14	77.32	79.50	3.80	5.03	6.48	1.06	1.49	1.88
	a*	4.42	4.10	3.81	3.92						
	b*	27.21	26.43	25.04	26.44						
3	L*	78.96	80.92	80.00	80.34	5.32	5.03	6.06	0.96	0.98	0.81
	a*	1.71	1.60	1.60	1.42						
	b*	23.34	24.74	25.22	24.54						
4	L*	64.91	67.6	69.88	69.46	4.37	6.79	6.66	1.26	2.40	2.14
	a*	5.50	4.82	4.31	4.56						
	b*	28.92	27.89	26.40	26.74						
5	L*	71.00	79.15	80.12	84.04	9.29	10.71	15.91	3.32	3.95	5.92
	a*	3.85	2.08	1.53	1.02						
	b*	34.32	32.57	31.00	26.93						
6	L*	75.28	75.10	76.45	77.44	3.2	5.74	5.75	0.28	0.94	1.41
	a*	2.66	2.95	2.24	1.96						
	b*	24.70	24.62	23.07	22.55						

Table 4. Clinical classification of ΔEab and ΔE_{00}

ΔEab	Clinical significance
1.2	Only 50% perceptible by healthy observers (PT)
2	Clinically acceptable value
2.7	Clinically acceptable value for 50% observers (AT)
3.7	Maximum value that is clinically acceptable for some observers; any upper modification is obvious
ΔE_{00}	**Clinical significance**
0.8	Only 50% perceptible by healthy observers (PT)
1.8	Clinically acceptable value for 50% observers (AT)

A significant increase of whiteness index is observed for groups 2, 3, 4 and 5, demonstrating similarities in the bleaching behaviour between the novel hybrid formulations (groups 2 and 3) and the commercially used bleaching agents (groups 4 and 5). The colour of group 6 shifted to white shades in the 7th day of bleaching, and then the values of whiteness index remained constant showing an initial loss of pigment attributable to the fact that the specimens were kept in artificial saliva.

Further studies could involve the enhancement of TiO_2 internal bleaching effect by photosensitization. Prospective studies should be done on the protein content of human enamel influence from freshly extracted teeth on root resorption after treatments with hybrid bleaching agents.

5. Conclusions

Results regarding the utility of the developed bleaching system reveal good bleaching effect on discoloured non-vital teeth. The efficiency of the novel bleaching systems using TiO_2 as oxidizing agent instead of the commercially available products could take advantage of the limited oxidative stress in dental environment with less potentially deleterious side effects. The most effective bleaching results were achieved by formulations including TiO_2 /Aa; TiO_2 /SiO_2 /Aa and TiO_2 /SiO_2 /small amount of sodium perborate.

The obtained results in respect to the bleaching treatments performed in this study show the efficiency of using them concomitantly with the antioxidant, rather than applying the antioxidant consecutively. This opportunity is very important as it leads to the shortage of treatment procedures. A significant increase of whiteness index is observed for formulations including TiO_2 /Aa; TiO_2 /SiO_2 /Aa and TiO_2 /SiO_2 /small amount of sodium perborate, in the same range of values with the widely used bleaching agents.

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*Corresponding author: smarandabuduru@yahoo.com