©Operative Dentistry, 2010, **35-3**, 287-294

Laboratory Research

In Vitro Assessment of Chemical Activation Efficiency During In-office Dental Bleaching

AC Travassos • CRG Torres
A B Borges • DC Barcellos

Clinical Relevance

To accelerate the production of free radicals, chemical agents could act by reacting with H_2O_2, making bleaching faster, more efficient and safer, thus diminishing the possible damaging effects associated with the application of heat sources.

Alessandro Caldas Travassos, DDS, MS, Restorative Dentistry Department—São José dos Campos School of Dentistry, São Paulo State University—UNESP, SP, Brazil

*Carlos Rocha Gomes Torres, DDS, PhD, assistant professor, Restorative Dentistry Department—São José, dos Campos School of Dentistry, São Paulo State University—UNESP, SP, Brazil

Alessandra Buhler Borges, DDS, MS, PhD, assistant professor, Restorative Dentistry Department—São José dos, Campos School of Dentistry, São Paulo State University—UNESP, SP, Brazil

Daphne Câmara Barcellos, DDS, post-graduation student at the São José dos Campos School of Dentistry, São Paulo, State University—UNESP, SP, Brazil

*Reprint request: Avenida Engenheiro Francisco José Longo, 777, Jardim São Dimas, São José dos Campos, SP, Brazil, CEP: 12245-000; e-mail: carlosrgt@fosjc.unesp.br

DOI: 10.2341/09-256-L

SUMMARY

Purpose: This study compared five types of chemical catalyzing agents added to 35% hydrogen peroxide gel, with regard to their capacity of intensifying in-office dental bleaching results.

Methods: One-hundred and twenty bovine incisors were used, of which the crowns and roots were cut in the incisor-apical direction, to acquire the dimensions of a human central incisor. The specimens were sectioned in the mesiodistal direction by means of two longitudinal cuts, the lingual halves being discarded. The vestibular halves received prophylaxis with a bicarbonate jet, ultrasound cleaning and acid etching on the dentinal portion. Next, the specimens were stored in receptacles containing a 25% instant coffee solution for two weeks. After the

darkening period, initial measurement of the shade obtained was taken with the Easy Shade appliance, which allowed it to be quantified by the CIELab* method. The samples were divided into six groups, corresponding to the chemical activator used: a) none (CON); b) ferric chloride (CF); c) ferrous sulphate (SF); d) manganese gluconate (GM); e) manganese chloride (CM); f) mulberry root extract (RA). Each group received three 10-minute applications of the gels containing the respective activating agents. Next, a new shade measurement was made.

Results: The Analysis of Variance and Tukey tests (α=5%) showed statistically significant differences for the shade perception values (*p*=0.002). Groups GM, CM and RA showed significantly higher means than the control group.

Conclusion: The presence of some chemical activators is capable of resulting in a significant increase in tooth shade variation.

INTRODUCTION

Bleaching treatment uses certain types of oxidizing agents, such as hydrogen peroxide, carbamide peroxide and sodium perborate, which may be administered in various concentrations. In all the substances described, however, the bleaching agent will, directly or indirectly, be hydrogen peroxide, which, when it decomposes into free radicals through the influence of various mechanisms, starts the dental bleaching reaction. The free radicals released oxidize the pigments and coloring matters impregnated in the dental tissue, breaking the large aromatic chains of the darker coloring, transforming them into slightly lighter unsaturated linear chains. As the oxidizing process continues, these chains are converted into even lighter saturated linear chains, thus making the teeth whiter.[1-2]

The tooth bleaching procedure can be performed in-office, under the dentist's direct supervision, or at home, by means of individual mouth guards made after being molded or via strips obtained in drug stores and supermarkets, which keep the bleaching gel in close contact with the teeth for a period defined by the professional or the product manufacturer. Both types of treatments have advantages and disadvantages. The home bleaching treatment has the advantage of using gels with a lower hydrogen peroxide concentration, and being less expensive, when compared with in-office treatment. The disadvantage is the time required to achieve satisfactory results, generally ranging between one and two weeks;[3-4] whereas in-office bleaching is advantageous because of it being directly supervised by the professional, not requiring molds[5] and allowing for greater protection of the adjacent soft tissues. Also because it shows results in a shorter time than that taken by the home technique in spite of its high cost and greater H_2O_2 concentration.

In order for the free radicals resulting from H_2O_2 degradation to be released more rapidly, therefore making in-office bleaching more efficient, professionals have, over the years, used devices that transfer energy to the peroxide, generally in the form of heat, thus increasing its decomposition. In general, the most used heat sources are the heated tips of instruments and Halogen Lamps, Light Emitting Diodes (LED), Plasma Arc and LASER appliances.[6-7]

Although heat transfer possibly increases the efficiency of the bleaching process, this procedure has been subject to criticisms and questioning regarding the safety of its use and proof of its efficacy. Some articles have shown that inflammatory processes could occur due to heat transmission to the pulp tissue.[8-9]

Therefore, to increase the efficacy of the treatment with the desired safety, a possible solution could be to incorporate chemical agents derived from transmission metals, such as manganese and iron, or enzymes, such as catalase and peroxidase.[7,10-13] Zhao[14] observed that a natural solution of these enzymes could be obtained from a mulberry root extract, which could be used as a hydrogen peroxide catalyzer. The substances derived from these composites could act by reacting with H_2O_2 to accelerate the production of free radicals, making bleaching faster, more efficient and safer, diminishing the possible damaging effects association with the application of heat sources.

In view of the above exposition and awareness of the need for studies that prove the efficacy of chemical agents, in an endeavor to increase the efficiency of bleaching agents when using the in-office bleaching technique, the aim of this study was to compare five types of chemical catalyzing agents added to a hydrogen peroxide gel and compare them with the action of the bleaching gel without catalyzer. The null hypothesis evaluated was that the addition of chemical agents does not produce significantly greater bleaching than a standard gel without chemical activator.

METHODS AND MATERIALS

To prepare the specimens, 15 bovine mandibles were removed from recently slaughtered animals (Frigorífico Mantiqueira—São José dos Campos, SP, Brazil), from which 120 incisors were extracted with a straight 301 apical elevator (Quinelato, Rio Claro, SP, Brazil). After extraction, the teeth were cleaned and periodontal fibers were removed with a No 15 scalpel blade (Two Arrows RPC, Shangai Med SM, Shangai, China). To standardize the specimen size, two transverse cuts were made along the tooth axis, so that its final dimension in the incisor-apical direction would simulate that of a human maxillary central incisor tooth. For this

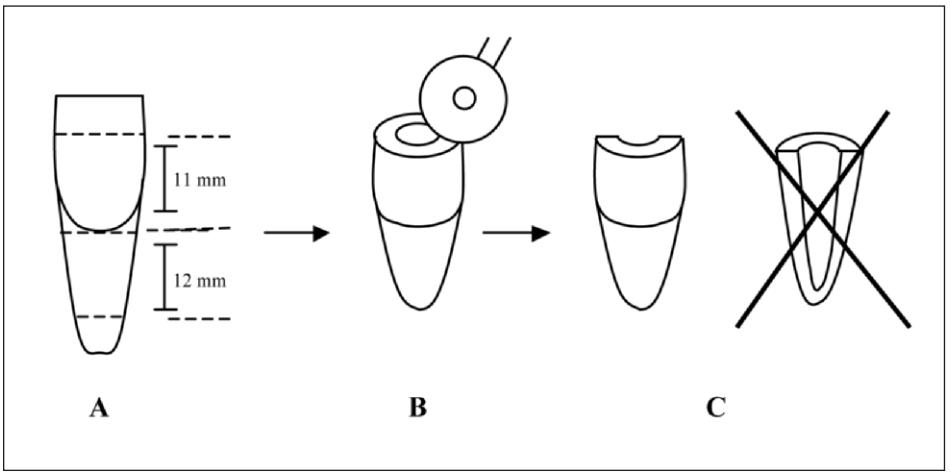

Figure 1. *Sectioning of teeth.*

purpose, the distance from the cervical limit to the incisal edge was demarcated at 11 millimeters and, in turn, the extent of the root was demarcated at 12 millimeters from the cervical limit in the apical direction. The cuts were made using a plaster cutting appliance (Kohlbach SA, Jaraguá do Sul, SC, Brazil), which cut the teeth until they were of the length demarcated on the vestibular face. (Figure 1B)

Next, two longitudinal sections were performed in the incisor-apical direction using Carborundum Disks (Dentaurum, Pforzheim, Germany) mounted in a straight handpiece (Dabi Atlante, Ribeirão Preto, SP, Brazil) in order to separate the tooth into the labial and lingual halves. When separated into halves, any type of pulp residue was removed from the labial halves. The lingual halves were discarded (Figure 1C).

After sectioning, the 120 teeth were submitted to prophylaxis with an abrasive sodium bicarbonate and water jet performed with the Profi II Ceramic appliance (Dabi Atlante, Ribeirão Preto, SP, Brazil) to remove extrinsic stains. To ensure that no type of residue was impregnated in the crowns of the teeth, they were also submitted to a distilled water bath in an UltraSonic Cleaner appliance (Odontobrás, Ribeirão Preto, SP, Brazil) for 10 minutes.

After all the cleaning procedures, the teeth were randomly divided into six groups with 20 teeth in each, with each group corresponding to one of the types of chemical activators studied. After this division, the teeth were separately stored in plastic receptacles, immersed in distilled water.

In order to have better visualization and obtain quantification of the bleaching gel efficiencies, the teeth were submitted to a darkening process by means of an adaptation of the method proposed by Sulieman and others.[15] This procedure was sufficient for the teeth to attain a shade close to C4, according to the Vita Classic scale (Vita Zahnfabrik, Bad Säckingen, Germany). For this purpose, the teeth had their internal portion etched with 37% phosphoric acid gel (DentalVille, Joinville, SC, Brazil) for 15 seconds, in order to open the dentinal tubules and make the darkening solution more efficient. Darkening was performed during two weeks, by immersing the teeth in a 25% instant coffee solution, which was prepared by dissolving 250g of instant coffee (Nescafé, Nestlé, São Paulo, SP, Brazil) in 750 grams of distilled water at 100°C. After the solution had cooled, the teeth were covered by it and kept in a bacteriological stove at 37°C for seven days. After the first week, the solution was changed, and a fresh one prepared, so that the teeth could be immersed for another week.

At the end of the second week, the specimens were removed from the coffee solution, washed and had their dentinal tubules sealed, which were opened by acid-etching before darkening. For this purpose, two layers of transparent nail varnish (Risque, Hiase, Taboão da Serra, SP, Brazil) were applied on the dentinal portion of the specimens. In addition to this procedure, the coronal vestibular portion of the specimens was also polished with diamond paste and a felt disk to remove extrinsic staining. After these procedures, the samples were again stored in the plastic receptacles and immersed in distilled water.

Next, the shades of the teeth were measured by a spectrophotometer Easy Shade (Vita Zahnfabrik, Bad Säckingen, Germany). At this time, each tooth was removed from the distilled water and wiped with a gauze compress. The vestibular faces were analyzed in thirds in the gingival-occlusal direction, corresponding to the gingival, middle and incisal thirds. The tip of the appliance was placed on each third so that it was perpendicular to the long axis of the tooth and the tooth shade could be recorded. For measurements in the cervical and incisal thirds, a distance of one millimeter above the cemento-enamel junction and below the incisal edge, respectively, was maintained. All the measurements were made by the same examiner. For each region analyzed, the values of the coordinates L*, a* and b* were recorded.

For dental bleaching, the bleaching gel Total Bleach (Clean Line, Taubaté, SP, Brazil) was used, modified by the manufacturer through the addition of various activating agents. This product was presented in two separate flasks of 10 g each, in which the first flask was composed of a solution of 50% hydrogen peroxide associated with a thickening agent. The second flask was composed of water, an alkalyzing agent, a coloring mat-

ter and a surfactant. When mixed in the proportion of three parts peroxide solution to one part alkaline solution, a 35% hydrogen peroxide gel was obtained. In this study, the peroxide solution was used exactly as it was supplied by the manufacturer, whereas the alkaline solution was modified by the addition of 0.02% by weight of the respective activating agents, resulting in the following experimental groups:

a) Control Group (CON) – No activating substance was added;

b) Group GM – activating solution containing 0.002g of manganese gluconate $(CH_2OH(CHOH)_4COO)_2 Mn{\cdot}2H_2O$—Gluconal—Purac, Campo dos Goytacazes, RJ, Brazil, CAS: 7544100);

c) Group CM—activating solution containing 0.002g of tetrahydrate manganese chloride ($MnCl_2{\cdot}4H_2O$—Vetec Química Fina Ltda, Rio de Janeiro, RJ, Brazil, CAS: 13446-34-9);

d) Group SF – activating solution containing 0.002g of heptahydrate ferrous sulphate ($FeSO_4{\cdot}7H_2O$—Lab Synth Ltda, Diadema, SP, Brazil, CAS: 77-82-63-0);

e) Group CF—activating solution containing 0.002g of ferric chloride ($FeCl_3{\cdot}6H_2O$—Vetec Química Fina Ltda, Rio de Janeiro, RJ, Brazil, CAS: 10025-77-1);

f) Group RA—In this group, when preparing the activating solution, the manufacturer replaced the deionized water by the mulberry root extract. To prepare this extract, a 10 cm piece of root was obtained and cut into little pieces that were then ground in a domestic food processor (Walitta Lique Faz, Philips, São Paulo, SP, Brazil) to reduce them into small grains. These little grains were weighed and a total of 0.02g was mixed with 88.9g of water. This first mixture was heated with a Bunsen burner until it started to boil. When it reached this stage, the mixture was filtered through filter paper. This solution was used to prepare 10g of the activator solution.

In all groups, at the moment of bleaching, the teeth were removed from the water, lightly dried with gauze and received the bleaching gel formed by the mixture of the solutions. Each group was placed in a receptacle suitable for the mixture. In order to form the gel, 60 drops of solution from the flask containing hydrogen peroxide were dispensed into the receptacle, then 20 drops of the solution from the flask containing the respective chemical activator were added to the H_2O_2.

The gel formed was applied to the entire labial extent of the crowns of the teeth in a uniform layer of around 2 mm for 10 minutes. Immediately after the gel application, the teeth were covered with an opaque steel to prevent any influence of ambient light on the bleach activation. After five minutes, the gel was agitated with a microbrush (Microbrush International, Orlando, FL, USA) to dissipate the bubbles formed and enhance bleach contact with the tooth surface. When the 10-minute period had elapsed, the specimens were washed with air-water spray, dried with gauze and then the procedure was repeated two more times, for a total of three applications, with the product remaining in contact with the teeth for a total of 30 minutes.

The teeth were then washed and returned to their respective flasks, wherein they were once again immersed in distilled water for around 20 minutes to rehydrate. When this time had elapsed, the groups were submitted to a new shade evaluation by the Easy Shade spectrophotomer in the same way as described for the initial assessment.

For each tooth, a mean of the values L*, a* and b*, obtained in the cervical, middle and incisal regions were calculated. Thus, for each specimen, the tooth shade was represented by only one value of L*, a* and b* (L^*_{means}, a^*_{means}, b^*_{means}). The shade values before and after treatment were compared for each tooth, arriving at the individual variation values of L_{means}^* (ΔL^*), a_{means}^* (Δa^*) and b_{means}^* (Δb^*). These values were obtained by means of the following equations:

$$\Delta L^* = L^*_{\text{final means}} - L^*_{\text{initial means}}$$

$$\Delta a^* = a^*_{\text{final means}} - a^*_{\text{initial means}}$$

$$\Delta b^* = b^*_{\text{final means}} - b^*_{\text{initial means}}$$

Next, the total shade variation or the variation in perception of shade of each tooth was calculated, as designated by the abbreviation ΔE^*, which was used for comparing the bleaching effect among the different groups.[5,7,15-17] This parameter, in turn, was calculated in accordance with the following formula:

$$\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$$

The data obtained were submitted to the analysis of variance for one factor (ANOVA), followed by the Tukey test. For all the analyses, a value of α=0.05%, was adopted, performed with the software Statistics for Windows (Statsoft South America, São Caetano do Sul, Brazil).

RESULTS

For all specimens in all groups, an increase was observed in the values of L* and a decrease was observed in the values of a* and b*.

Table 1 shows the results of the Analysis of Variance The value of $p<0.05$ allowed the null hypothesis to be rejected, indicating that there were significant differences among the groups.

In Table 2, the mean and standard deviation values for the different groups, as well as the results of the Tukey test, can be observed.

Analysis of Table 2 shows that the groups manganese gluconate, manganese chloride and mulberry root

Table 1: *Results of the One-factor Analysis of Variance*

Degree of Freedom	SQ	QM	F	p
5	27.980	7.176	3.898	0.0026*

**Significant difference.*

Table 2: *Mean and Standard-deviation Values*

Groups	Mean (sd)	Homogeneous Sets*		
Control Group	6.313 (0.950)	A		
Ferrous Sulphate	8.739 (2.571)	A	B	
Ferric Chloride	8.769 (3.482)	A	B	
Mulberry Root	8.971 (1.691)		B	
Manganese Chloride	8.977 (2.675)		B	
Manganese Gluconate	9.799 (3.660)		B	

**Sets accompanied by the same letters presented no significant differences.*

extract did not differ statistically, however, they presented significantly higher means than the control group.

DISCUSSION

Presenting the main advantage of being performed in a shorter time,[18] in-office bleaching has been the option preferred by persons who demand fast results and who feel disturbed by the nocturnal mouthguards used in home bleaching. On the other hand, this treatment modality may frequently present a risk to pulp vitality, due to the use of light sources and heated instruments on the teeth[2,6,10,15,18-19] for the purpose of providing energy to hasten the hydrogen peroxide degradation reaction. This may frequently lead to fractures and fissures in enamel and dentin,[20] tooth sensitivity and alterations to odontoblasts,[2,13,19] pulp inflammation[21] and inhibition of pulpal enzymes,[19] resulting from raising the intrapulpal temperature.

In view of the disadvantages related to in-office thermocatalytic light activation bleaching techniques presented by these authors and supported by the conclusions of authors, including Chen and others[10] and Frysh and others,[11] the current research used a methodology to study chemical hydrogen peroxide gel activation used in bleaching done directly by the dentist in an effort to establish an alternative method of increasing the speed of H_2O_2 decomposition in a more efficient and safer manner.

To conduct the study, the use of bovine teeth is justified, as they are easier to obtain and standardize, considering the large quantity of teeth required and because of ethical difficulties involved in obtaining healthy extracted human teeth.[22] As regards the structural similarity between bovine and human teeth, Wiegand and others[16] affirmed that the physical and chemical properties, such as composition, density, enamel diameter and Vickers hardness are very similar to that of human dentin. Schiavoni and others,[23] in 2006, also mentioned the similarity of bovine and human teeth in their study, demonstrating that bovine enamel could represent a feasible alternative to human enamel in permeability studies.

Darkening in a coloring solution was used so that a greater shade variation could be recorded after bleaching treatments, thereby facilitating the observation of significant differences, the concept being established in the literature.[15] The studies by Gaffar,[12] Sulieman and others[15] and Ley and others[17] used various types of beverages to darken the teeth used in their studies, obtaining good staining results. In the current study, the use of instant coffee is justified, because it allowed a more concentrated solution to be prepared than when using filtered coffee, thus hastening the darkening process.

Among the shade assessment methods, the option was for an objective method by means of using a spectrophotometer, thus eliminating the subjectivity of the shade scale method. The studies of Li[24] and Joiner[25] found the objective methods to be superior for assessing teeth. Moreover, according to Baltzer and Jinoian,[26] assessment by spectrophotometer is not influenced by the external medium and by the tone of the skin and tissues adjacent to the teeth.

When Table 2 was analyzed, it was found that a classification could be established in ascending order, related to the efficiency of the hydrogen peroxide gels containing chemical activators compared with pure hydrogen peroxide gel. Although the ferrous sulphate and ferric chloride groups resulted in slightly higher mean values than the control group, they showed no statistically significant differences. Whereas, the mulberry root extract, manganese chloride and manganese gluconate groups exhibited significantly higher shade perception variation means than the control group, representing an increase in efficiency of the process to the order of 42.10%, 42.19% and 55.21%, respectively, there was no statistical difference among them.

When in contact with tissues and organic fluids, hydrogen peroxide dissociates itself in different ways, and can form only water and oxygen, water and oxygen ion (O^{-2}), hydrogen ion (H^{+}) or free radicals, such as hydroxyl (HO•) or perhydroxyl (HOO•). The reaction speed and obtainment of these different products depend on the hydrogen peroxide concentration, the medium in which the reaction occurs, the temperature of the reagents, use of enzymes, such as catalases and peroxidases, and the interaction with transition metals (Fe, Cu, Ni, Cr, Pb, Mn) among others.[10,27]

The use of enzymes to accelerate H_2O_2 decomposition was investigated in the current study by the use of mulberry root extract, which has the enzyme peroxidase in its composition.[14] When mixed with hydrogen peroxide, peroxidase facilitates decomposition of the bleaching agent into free radicals. When reacting among them, the peroxidase molecules link and produce two hydrogen ions (H^+) that react with the hydrogen peroxide and result in the formation of water molecules and hydroxyl free radicals (HO•)[28] (Figure 2A). Mulberry root extract has been successfully used as a chemical activator for dental bleaching in the study conducted by Zhao[14] and has the advantage of being a material that comes from a natural medium, represents no risks and presents no contraindication. Considering that safety in dental treatments is greatly appreciated today, this activator has met these demands.

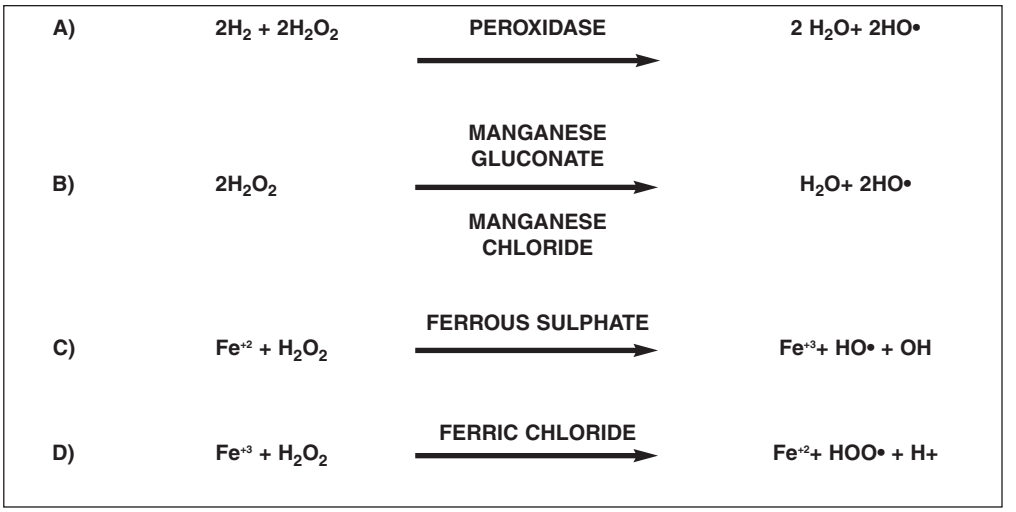

Figure 2. *Hydrogen peroxide decomposition reactions in the presence of the catalyzers.*

Interaction with transition metals, also mentioned by Chen and others[10] and Torres and others,[27] was assessed in the current study by the chemical activators linked to iron, such as ferrous sulphate and ferric chloride and to manganese, such as manganese gluconate and manganese chloride. According to the results obtained in this study, when being mixed with hydrogen peroxide at the time of application to teeth, all these composites brought about numerically superior results when compared with the effects of peroxide gel without activation. This numerical superiority of the means of the results is related to the interference of these chemical activators in the activation energy of the hydrogen peroxide dissociation reaction, acting as catalyzers, which will increase the speed of reactions and reduce the minimum quantity of kinetic energy the reagent molecules require for the reaction to start, thus providing another pathway of lower energy.[29]

There are differences, however, in the manner in which composites derived from manganese act in relation to the iron derivatives and also in the efficiency of these with regard to catalysis, which could explain the better results for the Mn composites (Table 2). As a result,when mixing manganese gluconate and chloride with 35% hydrogen peroxide, these chemical activators acted in a simple manner, accelerating peroxide degradation, forming water and free radicals without, however, dissociating themselves or participating in the reaction that does not occur with iron, which ends up participating in the reaction, making electron exchanges. The superiority in catalyzer efficiency probably occurred due to the higher oxidation number of manganese, which could reach up to a value of +5. The oxidation number refers to the reactivity of a substance, so that the higher the oxidation potential, the higher its reactivity would be. Therefore, compared with iron, which has a maximum oxidation number of +2, manganese has more affinity for hydrogen peroxide and is more easily incorporated into the degradation reaction (Figure 2B).

The role of a catalyzer of iron derivatives, in turn, results from a reaction between the Fe^{3+} ion coming from ferric chloride and the Fe^{2+} ion, coming from ferrous sulphate, with hydrogen peroxide, through a reaction known as the "Fenton Reaction." This reaction was initially used in the treatment of agricultural soil and industrial residues in an attempt to eliminate the toxic composites present. The addition of one of the mentioned iron ions causes the occurrence of an increase in the oxidative force of hydrogen peroxide, resulting in an increase in its degradation speed, causing free radicals to form more rapidly[30] (Figures 2C and 2D).

The greater efficiency of ferric chloride when compared with ferrous sulphate, although not statistically significant (Table 2), is probably due to the formation of free radical perhydroxyl as a product of the reaction with hydrogen peroxide. According to Torres and others, the perhydroxyl free radicals are more potent oxidants than hydroxyl radicals, so that their presence results in a better bleaching treatment outcome.

Toh[21] conducted a study to assess the effectiveness of bleaching treatment using a dual activation bleaching system (Hi Lite-Shofu) that uses a composite derived from manganese (manganese sulphate) and a composite derived from iron (ferrous sulphate) as chemical activation. After performing three bleaching sessions, using light activation in half the patients and chemical

activation in the other half, the authors analyzed the results obtained and concluded that there was no statistical difference between dual activation using light and only chemical activation of the gel. This similarity reinforces the option, confirmed in the current study, of using chemical activators without a reduction in bleaching treatment efficiency and, in addition, causing fewer side effects.

Gaffar[12] studied the efficiency of a bleaching gel containing 35% hydrogen peroxide, mixed with manganese gluconate, in order to accelerate peroxide decomposition and increase the speed of free radical release. The results obtained indicated that the presence of the chemical activator increased the efficacy of hydrogen peroxide to the order of around 1.5 to 8 times, which is in agreement with the results of the current study that showed a higher ΔE value for the group corresponding to manganese gluconate (Table 2).

From the results of this study, the authors could affirm that the use of chemical activation can significantly increase dental bleaching results as being an effective and safer substitution for activation by physical means, such as heated instruments or light. Complementary studies to compare bleaching using chemical activation with other means of activation should be conducted, both as regards bleaching effectiveness and pulp irritation, so that a safer treatment with the same efficiency can be offered. Another interesting aspect is that the increase in effectiveness observed with the use of chemical activators could allow lower concentrations of hydrogen peroxide to be used, thus guaranteeing the same process effectiveness and minimizing the irritating potential of the oxidant agent.

CONCLUSIONS

Based on the methodology used and in accordance with the statistical analysis of the results, it was concluded that:

a) The presence of the chemical activators manganese gluconate, manganese chloride and mulberry root extract resulted in significantly greater bleaching than the use of 35% hydrogen peroxide without activation.

b) The chemical activators ferric chloride and ferrous sulphate presented no significant differences in comparison with the control group.

c) Depending on the agent used, chemical activation can intensify the results of in-office dental bleaching.

(Received 25 August 2009)

References

1. Leonard RH Jr, Haywood VB & Phillips C (1997) Risk for developing tooth sensitivity and gingival irritation associated with nightguard vital bleaching *Quintessence International* **28(8)** 527-534.
2. Dahl JE & Pallesen U (2003) Tooth bleaching—A critical review of the biological aspects *Critical Reviews in Oral Biology & Medicine* **14(4)** 292-304.
3. Haywood VB & Heymann HO (1989) Nightguard vital bleaching *Quintessence International* **20(3)** 173-176.
4. Haywood VB (1992) History, safety and effectiveness of current bleaching techniques and applications of the nightguard vital bleaching technique *Quintessence International* **23(7)** 471-488.
5. Al Shethri S, Matis BA, Cochran MA, Zekonis R & Stropes M (2003) A clinical evaluation of two in-office bleaching products *Operative Dentistry* **28(5)** 488-495.
6. Walsh LJ (2000) Safety issues relating to the use of hydrogen peroxide in dentistry *Australian Dental Journal* **45(4)** 257-269; quiz 289.
7. Viscio D, Gaffar A, Fakhry-Smith S & Xu T (2000) Present and future technologies of tooth whitening *The Compendium of Continuing Education in Dentistry* **28** S36-S43.
8. Cohen SC (1979) Human pulpal response to bleaching procedures on vital teeth *Journal of Endodontics* **5(5)** 134-138.
9. Seale NS & Wilson CFG (1985) Pulpal response to bleaching of teeth in dogs *Pediatric Dentistry* **7(3)** 209-214.
10. Chen JH, Xu JW & Shing CX (1993) Decomposition rate of hydrogen peroxide bleaching agents under various chemical and physical conditions *Journal of Prosthetic Dentistry* **69(1)** 46-48.
11. Frysh H, Bowles WH, Baker F, Rivera-Hidalgo F & Guillermo G (1995) Effect of pH on hydrogen peroxide bleaching agents *Journal of Esthetic Dentistry* **7(3)** 130-133.
12. Gaffar A (1997) Oral compositions having accelerated tooth whitening effect United States Patent Application Publication US P 5648064.
13. Freedman B (2004) Booster and activator composition for tooth-whitening agents United States Patent Application Publication US P 0191188.
14. Zhao J (2005) Whitening Compound United States Patent Application Publication US P 0042185 Feb 24.
15. Sulieman M, Addy M, Macdonald E & Rees JS (2005) The bleaching depth of a 35% hydrogen peroxide based in-office product: A study *in vitro Journal of Dentistry* **33(1)** 33-40.
16. Wiegand A, Vollmer D, Foitzik M, Attin R & Attin T (2005) Efficacy of different whitening modalities on bovine enamel and dentin *Clinical Oral Investigations* **90(2)** 91-97.
17. Ley M, Wagner T & Bizhang M (2006) The effect of different fluoridation methods on the red wine staining potential on intensively bleached enamel *in vitro American Journal of Dentistry* **19(2)** 80-84.
18. Luk K, Tam L & Hubert M (2004) Effect of light energy on peroxide tooth bleaching *Journal of the American Dental Association* **135(2)** 194-201.

19. Tredwin CJ, Naik S, Lewis NJ & Scully C (2006) Hydrogen peroxide tooth-whitening (bleaching) products: Review of adverse effects and safety issues *British Dental Journal* **200(7)** 371-376.
20. Sydney GB, Barletta FB & Sydney RB (2002) *In vitro* analysis of effect of heat used in dental bleaching on human dental enamel *Brazilian Dental Journal* **13(3)** 166-169.
21. Toh CG (1993) Clinical evaluation of a dual-activated bleaching system *Asian Journal of Aesthetic Dentistry* **1(2)** 65-70.
22. Adibfar A, Steele A, Torneck CD, Titley KC & Ruse D (1992) Leaching of hydrogen peroxide from bleached bovine enamel *Journal of Endodontics* **18(101)** 488-489.
23. Schiavoni RJS, Turssi CP, Carrasco LD, Pécora JD, Serra MC & Froner IC (2006) Comparative study of human and bovin enamel permeability (Abstract 085 Pa) *Brazilian Oral Research* **20** 161.
24. Li Y (2003) Tooth color measurement using chroma meter: Techniques, advantages and disadvantages *Journal of Esthetic Restorative Dentistry* **15(Supplement 1)** S33-S41.
25. Joiner A (2004) Tooth color: A review of the literature *Journal of Dentistry* **32(Supplement 1)** 3-12.
26. Baltzer A & Jinoian VK (2004) The determination of tooth colors *Quintessenz Zahntechnik* **30(7)** 726-740.
27. Torres CRG, Borges AB, Kubo C, Gonçalves S, Araújo R, Celaschi S, Giordano C & Arcas FC (2007) Mechanism action of bleaching agents. In: *Dental Bleaching with LED/LASER Hybrid Sources* São Paulo: Ed Santos.
28. Wendel A & Jacoby WB (1980) *Enzimatic Basis of Detoxification* New York.
29. Masterton WL, Slowinski EJ & Stanitski CL (1990) *Chemical Principles*, Rio de Janeiro Ed Guanabara.
30. Bishop DF (1968) Hydrogen peroxide catalytic oxidation of refractory organics in municipal wastewaters *Industrial & Engineering Chemistry Process Design and Development* **7(1)** 110-117.