

THE USE OF OZONE TO LIGHTEN TEETH. AN EXPERIMENTAL STUDY

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ABSTRACT

Tooth-whitening agents are available for therapeutic use in the dental office or at home. However, whitening more severe stains, such as those caused by systemic ingestion of tetracycline, constitutes a challenge. The aim of this study was to evaluate, in an experimental model of growing rats, the efficacy of using ozone to lighten tetracycline-stained incisors.

At weaning, male Wistar rats ($n=40$) were randomly assigned to one of three groups. Two control groups, C_{21} and C_{60} ($n=8$, each) were used to document the usual age-related color. The third group ($n=24$) received 0.25 g% of oxytetracycline (O) until 60 days of age. These rats were subsequently divided into three further groups: O_0 , O_3 and O_5 ($n=8$, each). These rats were anesthetized; O_3 and O_5 groups received ozone application to the lower incisors for 3 (group O_3) or 5 minutes (group O_5), respectively; while O_0 did not receive the ozone treatment. Teeth were then photographed and the incisors from the control (C_{60}) and treatment groups (O_0 , O_3 and O_5) were cut, and

compared to a standard color guide (there were eight shades numbered 0 to 7, lightest to darkest) to assess the hue visually. The teeth were then placed in phosphoric acid to quantify the color by spectrophotometry. The data (mean \pm SD) were analyzed by One-Way Analysis of Variance (ANOVA) followed by Tukey's test or Dunnett test. The visual observation, analyzed blindly by one investigator, showed that O_3 and O_5 groups had diminished yellowing of the teeth as compared to the untreated O_0 group ($P<0.001$). The color quantified by spectrophotometry also detected significant differences among groups ($O_3<O_0$, $P<0.01$; $O_5<O_0$, $P<0.001$ and $O_5<O_3$, $P<0.01$). C_{21} and C_{60} were significantly different among groups ($P<0.001$). This is the first experimental study to show that ozone can be successfully used for lightening the yellowish tinge of tetracycline-stained rat incisors. Further studies are required for its potential use in the dental clinic.

Key words: ozone, tetracycline, bleaching-agent.

USO DEL OZONO PARA BLANQUEAR DIENTES. ESTUDIO EXPERIMENTAL

RESUMEN

Los agentes blanqueadores dentales están disponibles para tratamientos que se realizan en el consultorio odontológico o en el domicilio. Sin embargo, aclarar manchas severas, como las causadas por la ingestión sistémica de tetraciclina, constituyen un desafío. El objetivo de este estudio fue evaluar en un modelo experimental de ratas en crecimiento, la eficiencia del uso de ozono para aclarar los incisivos oscurecidos por el uso de tetraciclina. Ratas macho Wistar al destete ($N=40$) fueron asignadas aleatoriamente a uno de tres grupos. Dos de ellos grupos controles, C_{21} y C_{60} ($N=8$, cada uno), para documentar el color habitual de los incisivos, correspondiente a la edad del animal. El tercer grupo ($N=24$) recibió 0,25 % de oxitetraciclina (O) hasta los 60 días de edad. Entonces, el grupo O se dividió aleatoriamente en tres grupos O_0 , O_3 y O_5 ($N=8$, cada uno) y las ratas se anestesiaron. Los grupos O_3 y O_5 recibieron en los incisivos inferiores la aplicación de ozono durante 3 y 5 minutos, respectivamente; mientras que O_0 no recibió tratamiento. Los incisivos de C_{60} , O_0 , O_3 y O_5 fueron fotografiados. Luego se cortaron y se contrastaron con una guía estándar de ocho colores

(ordenados de 0 a 7, desde el más claro a más oscuro) para cuantificar visualmente el color de los incisivos. Luego, se colocaron en ácido fosfórico para cuantificar el color por espectrofotometría. Los resultados (media \pm SD) se analizaron por medio de ANOVA y prueba de Tukey o Dunnett ($\alpha=0.05$) para determinar el efecto del tratamiento. El análisis visual de las imágenes mostró que los grupos O_3 y O_5 disminuyeron el color amarillo intenso respecto a O_0 . Dicha diferencia de color fue evaluada a través de la guía (G) y cuantificada mediante espectrofotometría (E). Según G, la mayor diferencia de color respecto a C_{60} fue para O_0 ($P<0.001$), disminuyó en O_3 ($P<0.001$) y aún más en O_5 ($P<0.01$). De acuerdo a E, $O_3<O_0$, $P<0.01$; $O_5<O_0$, $P<0.001$ y $O_5<O_3$, $P<0.01$. C_{21} y C_{60} resultaron significativamente menores por ambos métodos ($P<0.01$). Este primer estudio experimental evidencia que el tratamiento con ozono puede aclarar los incisivos de rata tratadas con tetraciclina. Se requieren estudios adicionales para su uso en la clínica odontológica.

Palabras claves: ozono-tetraciclina- agente blanqueador.

INTRODUCTION

Tooth-whitening agents are available for use in the office or at home. The demand for tooth bleaching dental treatments is increasing as patients wish to improve their appearance¹. The efficacy of tooth whitening is a major concern in dental practice, since its cosmetic result is immediately noticeable², though it may not be the only aspect involved in good facial appearance.

However, whitening more severe stains, such as those caused by systemic ingestion of tetracycline, constitutes a challenge. A number of tooth whitening agents and methods have been described in the literature for the treatment of tetracycline-stained teeth. Different bleaching agents have been utilized, e.g. carbamide peroxide³ or hydrogen peroxide plus carbamide peroxide⁴. There are also variations in the way these agents are applied, such as exposure time, e.g., months of carbamide peroxide treatment⁵, or concentration, e.g. 10%, 15% or 20% carbamide peroxide⁶. Additionally the mode of activation or methods of application of the whitening compound may differ⁷⁻⁹.

In dentistry, ozone has proved to be successful in the treatment of root caries¹⁰, non-cavitated fissure carious lesions¹¹⁻¹³, early carious lesions in teeth¹⁴, dental surgery or following tooth extraction processes^{15,16} and reduction of pathogenic micro-organisms of carious dentine¹⁷⁻¹⁹. Ozone has also been used to whiten teeth in individuals with dental sensitivity and mucousal ulcerations²⁰; in those who consume tobacco, coffee or chocolate; and in those who have extrinsically stained teeth due to brown-colored melanoidins or chlorhexidine use²¹. However, ozone has not been used to bleach intrinsically stained teeth. The aim of this study was to evaluate the efficiency of ozone to lighten tetracycline-stained incisors in an experimental model of growing rats.

MATERIALS AND METHODS

This study was performed in accordance with the Code of Ethics in Research of the University of Buenos Aires and the United States National Institutes of Health Guide for the Care and Use of Laboratory Animals. The protocol was approved by the University of Buenos Aires.

At weaning (21 days of age), 40 male Wistar rats from the animal laboratory of the Department of Biochemistry, Faculty of Dentistry, University of Buenos Aires, Argentina, were studied. The rats

were housed in galvanized cages with meshed floors in order to maintain hygienic conditions and to avoid coprophagia. They were exposed to a 12-h light, 12-h dark cycle throughout the study. Room temperature was maintained at $21 \pm 1^\circ\text{C}$ with a humidity of 50-60%. Rats were fed a rodent stock diet *ad libitum*.

Animals were randomly assigned to one of three groups. Two control groups ($n=8$, each) (C_{21} and C_{60}) remained untreated in order to document the spontaneous evolution of the color of the jaw incisors at age 21 and 60 days, respectively. Tap water was provided *ad libitum*. At 21 days of age, when the rats were weaned (group C_{21}), their lower incisors were cut and saved to determine the baseline color. At age 60 days, the lower incisors from the C_{60} group were photographed and cut in order to assess their color as described below.

The third group of rats (O , $n=24$) received 0.25 g% of oxytetracycline (Holliday, Scott) in drinking water and were fed *ad libitum* from the time of weaning to age 60 days.

At 60 days of age, group O was randomly divided into three further groups ($n=8$, each) O_0 , O_3 or O_5 . The rats were anesthetized with ketamine (70 mg/kg) and xylazine (13 mg/kg), administered intraperitoneally. The anesthesia persisted for at least 60 minutes and allowed sufficient time to administer the ozone application.

O_0 was given no ozone treatment while in groups O_3 and O_5 , ozone was applied to the jaw incisors for 3 (group O_3) or 5 minutes (group O_5), respectively. The ozone system used for the application was the HealOzone 2130C (KaVo Dental, Biberach, Germany). This is a self-contained device that produces ozone at a fixed concentration of 2100 p.p.m. ozone $\pm 5\%$ at a flow rate of 615 cc min⁻¹.

Visual Analysis

Once the experimental period was completed, the lower incisors (C_{60} , O_0 , O_3 and O_5) were photographed (Digital Camera Nikon D80, lens AF-S UR Micro-Nikkor 105 mm f/2.86. Nikon Corporation, Japan and electronic flash Macro Sigma EM-140D6, Carl-Zeiss, Rodermark, Germany) to assess the color of the teeth.

The visual evaluation was carried out using a modification of the Vita Classic shade guide (Vita Classic, Lumin Vacuum Shade Guide, Vita Zahnfabrik H. Rauter GmbH & Co.KG, Germany), which includes

shades arranged by value, and was developed for human teeth. In the present study, the shades were modified to conform to the range of colors of rat teeth, ranging from the slightly yellow color of the rats at weaning (C_{21}) to the darkest yellow tinge available. There were eight shades numbered 0 to 7, lightest to darkest, using a standard color guide. The color difference was calculated by subtracting the tab number corresponding to the experimental group (O_0 , O_3 and O_5) from that of C_{60} (paired by age). The data were analyzed statistically.

The color of the teeth of each rat was determined by a single investigator, in order to avoid individual variations in the estimate. The colors of the incisors were analyzed blindly as the investigator did not know the source of the teeth of the rats in each of the groups studied.

Spectrophotometric Analysis

The incisors of C_{21} , C_{60} , O_0 , O_3 and O_5 groups were cut and prepared for quantitative assessment of tooth color. They were placed in one milliliter of 37% phosphoric acid. The solutions, tested in duplicate, as well as the blank solution (37% phosphoric acid) were vortexed and then incubated at room temperature for 1 hour. In order to obtain the best readability and accuracy, the scale was set to read zero absorbance with a blank. Each sample was transferred to a cuvette for reading at wavelength 425nm (spectrophotometer Metrolab-1600 plus, Metrolab Argentina). This peak was chosen because it was the most prominent when the solutions were scanned for absorption from 400nm to 800nm. The data were presented as mean absorbance units (AU) \pm standard deviation, where absorbance is a measure of the quantity of light absorbed by a sample, which is proportional to the amount of chromophores present.

Statistical analysis

Color difference by age (experimental groups minus C_{60}) obtained from visual assay was analyzed by One-Way Analysis of Variance (ANOVA) followed by Tukey's test.

Comparison of readings between control (C_{60}) and experimental O_0 , O_3 or O_5 groups were performed using a One-Way Analysis of Variance (ANOVA) followed by Dunnett test. In order to detect differences between the five groups, One-Way Analysis of Variance (ANOVA) followed by Tukey's test was used. The significance level was set at 5%. The Sta-

tistical Product and Service Solutions for Windows 9.0 (SPSS, Inc., Chicago, IL) were used for statistical analyses.

RESULTS

At weaning, the lower incisors acquired a slight yellow color and by 60 days of age, this color was more intense, as expected ($P < 0.001$)²². After tetracycline treatment (age 60 days), the lower incisors in the O_0 group were dark yellow-orange. The results of the visual observation of tooth color showed that the ozone procedure lightened the pigmentation of the incisors of groups O_3 and O_5 . The teeth of groups O_3 and O_5 were lighter than those of group O_0 but darker than those of C_{60} (Fig. 1).

Additionally, using the color scale developed from the standard shades, the color difference between C_{60} and O_0 , O_3 and O_5 was significantly higher for O_0 (5.25 ± 0.46) and decreased from O_3 (3.25 ± 0.50) to O_5 (1.75 ± 0.39) ($p < 0.01$); with significant differences between O_5 and O_3 ($p < 0.01$). C_{21} numbered as zero was significantly different among groups ($p < 0.001$).

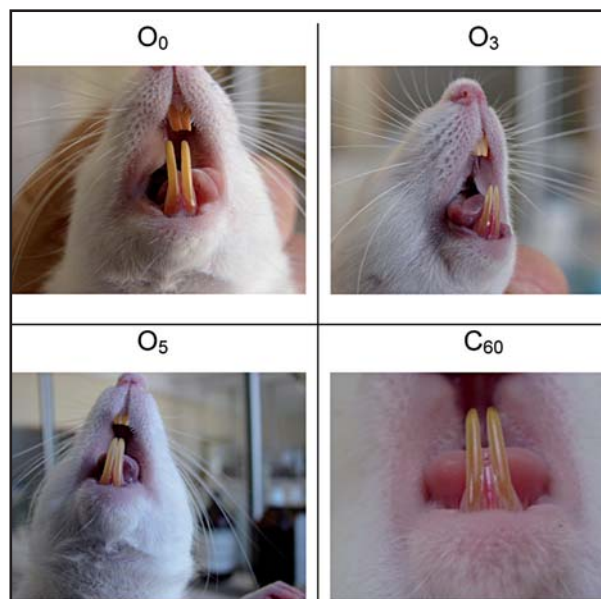


Fig. 1: Color comparison of the lower incisors shown in photographs of one rat of each group tested.

C_{60} : Rat 60 days of age to document the natural age-related color (yellow).

O_0 : Rat 60 days of age that received oxytetracycline from weaning. The antibiotic used in this study stained teeth yellow-orange.

O_3 : Rat 60 days of age that received oxytetracycline from weaning and ozone treatment for 3 minutes.

O_5 : Rat 60 days of age that received oxytetracycline from weaning and ozone treatment for 5 minutes.

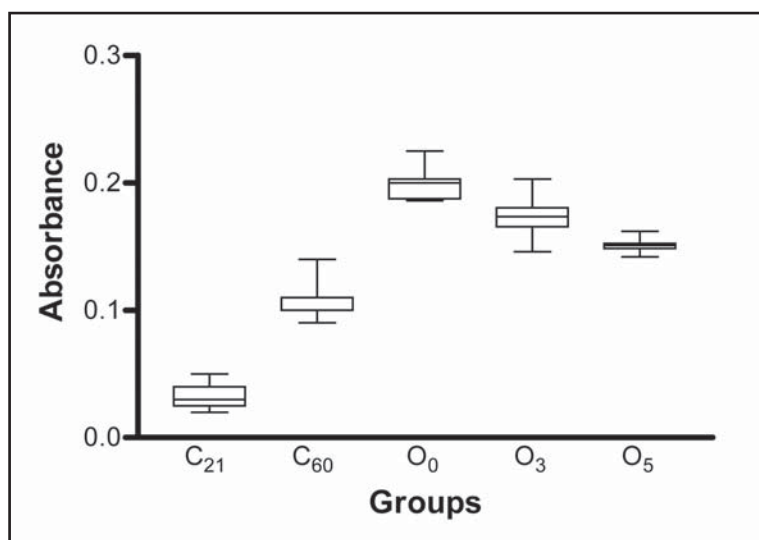


Fig. 2: Color quantification of the lower incisors by spectrophotometric analysis. The box and whiskers graph show the absorbance values of the different groups. C₂₁ and C₆₀: control rats color of lower incisors to document the natural age-related color at 21 and 60 days.

O₀= experimental group at 60 days of age that received oxytetracycline from weaning and remained ozone untreated.

O₃ and O₅= experimental groups at 60 days of age that received oxytetracycline from weaning and ozone treatment for 3 or 5 minutes, respectively.

Data were statistically significant according to one-way analysis of variance and a) Tukey's test a posteriori: *** $P < 0.001$, C₂₁ and C₆₀ differed from O₀, O₃ and O₅.

** $P < 0.01$, O₀ vs. O₃

*** $P < 0.001$, O₀ vs. O₅

** $P < 0.01$, O₃ vs. O₅.

b) Dunnett test a posteriori. C₆₀ was considered as a control group:

** $P < 0.01$, C₆₀ vs. O₀

** $P < 0.01$, C₆₀ vs. O₃

** $P < 0.01$, C₆₀ vs. O₅

The color quantified by spectrophotometry showed significant differences between groups C₆₀ and O₃, O₅ and O₀; C₆₀ was lower than experimental groups ($P < 0.01$). Additionally, differences were observed for O₃, O₅ and O₀ groups (O₃ < O₀, $P < 0.01$; O₅ < O₀, $P < 0.001$). Five minutes of ozone exposure resulted in better lightening (O₅ < O₃, $P < 0.01$). C₂₁ color was significantly lower than all groups ($p < 0.01$) (Figure 2).

DISCUSSION

This is the first experimental study designed to evaluate the use of ozone to lighten tetracycline stained incisors. The whitening response to ozone of the lower incisors of the rats was impressive, clearly evident by visual evaluation. After three minutes of ozone exposure, the incisors were whitened and the tetracycline staining was reduced to a more suitable

color. Furthermore, after five minutes' treatment the incisors continued to lighten. The quantitative spectrophotometric analysis of tooth color confirmed the visual impression of tooth whitening. Three minutes of ozone exposure reduced the yellow color by 28%, while five minutes achieved a 56% reduction. The mechanism of this process may involve the ozonation of double bond systems, which contribute to the chromophoric properties of the product²³.

Most of the clinical studies that have used bleaching agents to whiten teeth have been based on visual assessments; this methodology is subjective and potentially influenced by a number of factors. In the present study, the blind use of a standard visual color guide allowed quantification of differences in color of the incisors as natural evolution with advancing age as well as with the ozone treatment employed. The quantitative spectrophotometric assessment of tooth color confirmed the differences detected visually and provided further details of the degree of coloring among groups.

The specific model selected for assessing tooth color and the response to ozone application was the rat incisor which, unlike most teeth, is continually

erupting. This characteristic makes these teeth suitable for evaluating the whitening properties of ozone. Despite some small differences between rat and human incisor dentine²⁴, a close resemblance in enamel morphology has been established²⁵. Additionally, the relatively thick enamel layer in the mandible incisor makes it suitable for chemical and biochemical analyses²⁶.

It has been shown that rat incisors start out white in the young rat, but by age 21 days (initial time of the present study) the upper incisors have a slight yellow tinge. By 25 days these teeth are distinctly yellow while the lower incisors have acquired a slight yellow shade. By 38 days of life, these colors are more intense, though the upper incisors remain with more color than the mandible teeth. The relationship between more pigmented upper incisors

and less pigmented lower incisors remains true throughout the rat's life. In adult rats, the upper teeth are dark yellow-orange and the lower incisors are yellow²⁷. Since the color of the rat incisors changes throughout life, we performed baseline biochemical measurements, at weaning (C_{21}) and at the end of the experimental period (C_{60}). In the experimental groups given the antibiotic in the drinking water, we demonstrated that their teeth were stained yellow-orange in group O_0 .

Tetracycline interferes with odontogenesis by attaching irreversibly to calcified tooth structures²⁸. It has been suggested that the color is derived from photo-oxidation of tetracycline molecules bound within the tooth structures²⁹. In infants and children, administration of tetracycline may cause not only permanent staining of teeth, but enamel hypoplasia and decreased linear skeletal growth³⁰. Although it is known that certain types of stains can be eliminated by a number of methods³¹; tetracycline staining is more difficult to bleach³². Conventional whitening of tetracycline-stained teeth may require longer sessions at the dental office^{4,33} due to the resistance of tetracycline stains to bleach³⁴.

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REFERENCES

- Braun A, Jepsen S, Krause F. Spectrophotometric and visual evaluation of vital tooth bleaching employing different carbamide peroxide concentrations. *Dent Mater* 2007;23:165-169
- Miranda CB, Pagani C, Benetti AR, Matuda F da S. Evaluation of the bleached human enamel by scanning electron microscopy. *J Appl Oral Sci* 2005;13:204-211.
- Haywood VB, Leonard RH, Dickenson GL. Efficacy of six-months nightguard vital bleaching of tetracycline-stained teeth. *J Esthet Dent* 1997; 9:13-19.
- Kugel G, Aboushala A, Zhou X, Gerlach RW. Daily use of whitening strips on tetracycline-stained teeth: Comparative results after 2 months *Compend Contin Educ Dent* 2002; 23:29-34.
- Leonard RH Jr, Van Haywood B, Caplan DJ, Tart ND. Nightguard vital bleaching of tetracycline-stained teeth: 90 months post treatment. *J Esthet Restor Dent* 2003;15:142-152.
- Matis BA, Wang Y, Eckert GJ, Cochran MA, Jiang T. Extended Bleaching of Tetracycline-Stained Teeth: A 5-Year Study. *Oper Dent* 2006;31:643-651.
- Wang XD, Chen SL, Yu JB. Clinical survey of a combined in-office cold light bleaching and nightguard vital bleaching system for tetracycline stained teeth. *Hua Xi Kou Qiang Yi Xue Za Zhi* 2008;26:409-412.
- Matis BA, Wang Y, Jiang T, Eckert GJ. Extended at-home bleaching of tetracycline-stained teeth with different concentrations of carbamide peroxide. *Quintessence Int* 2002; 33:645-655.
- Shin DH, Summitt JB. The whitening effect of bleaching agents on tetracycline-stained teeth. *Oper Dent* 2002;27: 66-72.
- Baysan A, Lynch E. The use of ozone in dentistry and medicine. Part 2. Ozone and root caries. *Prim Dent Care* 2006; 13:37-41.

An important advantage of the ozone procedure utilized in our experimental model was the short time needed to achieve successful bleaching. Future studies will be needed to elucidate the ozone effects on morphology and/or substance of the enamel that could result from a limited application to the teeth. Other bleaching agents that have been used to treat tetracycline-stained teeth have been shown to produce morphological alterations in the enamel or on the sub-surface of enamel³⁵. Carbamide peroxide bleaching induces surface erosion, depressions, porosity and increased depth of enamel grooves and partial removal of enamel prisms. However, most bleaching agents differ from ozone since they are acidic, which is not favorable to enamel, dentin and cement².

CONCLUSION

This is the first study to demonstrate that tetracycline-stained incisors can be successfully lightened by the use of ozone. A simple, safe and non-invasive ozone treatment would provide an alternative therapeutic agent to current methods; however, further morphological assessments will be required for its use in the dental clinic.

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11. Huth KC, Paschos E, Brand K, Hickel R. Effect of ozone on non-cavitated fissure carious lesions in permanent molars. A controlled prospective clinical study. *Am J Dent* 2005;18(4):223-228.
12. Baysan A, Beighton D. Assessment of the ozone-mediated killing of bacteria in infected dentine associated with non-cavitated occlusal carious lesions. *Caries Res* 2007;41:337-341.
13. Schmidlin PR, Zimmermann J, Bindl A. Effect of ozone on enamel and dentin bond strength. *J Adhes Dent* 2005;7:29-32.
14. Lanata EJ. Intervención sin invasión con ozono. In: Alfaomega Grupo Editor Argentino S.A., editors. *Atlas de Operatoria Dental*, Buenos Aires: Editorial Alfaomega S. A. 2008;46-66.
15. Stubinger S, Sader R, Filippi A. The use of ozone in dentistry and maxillofacial surgery: A review. *Quintessence Int* 2006;37:353-359.
16. Agrillo A, Sassano P, Rinna C, Priore P, Iannetti G. Ozone therapy in extractive surgery on patients treated with bisphosphonates. *J Craniofac Surg* 2007;18:1068-1070.
17. Baysan A, Whiley RA, Lynch E. Antimicrobial effect of a novel ozone- generating device on micro-organisms associated with primary root carious lesions in vitro. *Caries Res* 2000;34:498-501.
18. Lanata EJ. Tecnologías emergentes en Operatoria Dental. In: Grupo Guía, editors. *Operatoria Dental. Estética y Adhesión*. Buenos Aires: Editorial Grupo Guía S.A. 2003;251-266.
19. Gonçalves Cardoso M, Dias de Oliveira L, Yumi Koga-Ito C, Olavo A, Cardoso J. Effectiveness of ozonated water on *Candida albicans*, *Enterococcus faecalis*, and endotoxins in root canals. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2008;105:e85-e91.
20. Ilzarbe IM. Nuevo método para blanqueamiento de dientes vitales mediante gases hiperoxidantes naturales. *Revista Maxillaris* 2000;25: año III.
21. Holmes J, Grootveld M, Smith C, Claxson A, Lynch E. Bleaching of Components Responsible for Extrinsic Tooth Discoloration by Ozone *J Dent Res* 82 Spec Iss A abstract number 0615, 2003. (www.dentalresearch.org)
22. Halse A. Location and first appearance of rat incisor pigmentation. *Scand J Dent Res* 1972;80:428-33.
23. Friedman SM. Bases bioquímicas de la aplicación del ozono. En: Alfaomega Grupo Editor Argentino. *Atlas de Operatoria Dental, Estética y Adhesión*. Buenos Aires: Editorial Alfaomega S.A. 2008;4:123-130.
24. Manton DJ, Bhide R, Hopcraft MS, Reynolds EC. Tooth Mousse may be applied concurrently with the bleach, and not reduce bleaching effectiveness *Aust DentJ* 2008;53:128-132.
25. Moinichen CB, Petter Lyngstadaas S, Risnes S. Morphological characteristics of mouse incisor enamel. *J Anat* 1996;189:325-333.
26. Robinson C, Kirkham J, Brookes SJ, Bonass WA, Shore RC. The chemistry of enamel development. *Int J Dev Biol* 1995;39:145-152.
27. Addison WH, Appleton JL. The structure and growth of the incisor teeth of the albino rat. *J Morphol* 1915;26:43-96.
28. Guggenheimer J. Tetracyclines and the human dentition. *Compend Contin Educ Dent* 1984;5:245-248.
29. Mello HS. The mechanism of tetracycline staining in primary and permanent teeth. *J Dent Child* 1967;34:478-487.
30. Baker KL. Tetracycline induced tooth changes. Part 5. Incidence in extracted first permanent molar teeth: a resurvey after four years. *Med J Aust* 1975;2:301-304.
31. Joiner A. The bleaching of teeth: A review of the literature. *J Dent* 2006;34:412-419.
32. Haywood VB. A comparison of at-home and in-office bleaching. *Dent Today* 2000;19:44-53.
33. Haywood VB. Historical development of whiteners: clinical safety and efficacy. *Dent Update* 1997;24:98-104.
34. Haywood VB, Leonard RH, Nelson CF & Brunson WD. Effectiveness, side effects and long-term status of nightguard vital bleaching. *J Am Dent Assoc* 1994;125:1219-1226.
35. Hegedüs C, Bistey T, Flora-Nagy E, Keszthelyi G, Jenei A. An atomic force microscopy study on the effect of bleaching agents on enamel surface. *J Dent* 1999;27:509-515.