Environmental Scanning Electronic Microscope study of enamel surface after using three different bleaching agents

Sahar A. M. Abd El Halim

Department of Operative dentistry, Qassim University, Saudi Arabia & Misr University for Science and Technology, Cairo, Egypt. s_saeed03@yahoo.com

Abstract: The purpose of this study was to evaluate the effect of 20% Carbamide peroxide (CP), 10% Carbamide peroxide (CP) and 25% Hydrogen peroxide (HP) on surface roughness of enamel. **Materials and methods:** Six young healthy dogs were used in this study. Six dogs were included in this study. Sixteen teeth in each dog were selected (upper and lower incisors and canines) for bleaching. Group(1):Two dogs were supposed for bleaching of upper &lower incisors with 20% CP Opalescence; Group(2): Two dogs were supposed for bleaching of upper &lower incisors with 10% CP Opalescence; Group(3): Two dogs were supposed for bleaching of upper &lower incisors with 25% HP Zoom2; Group(C): Control, no bleaching treatment (upper & lower canines in each dogs). Selected teeth on each dog were scaled and polished with a rubber cup. After this step, bleaching agents were applied for groups 1,2,3 for eight hours daily for two weeks. After the dogs were sacrificed, the teeth were stored in normal saline 0.9%. Enamel of labial surface of teeth was tested for surface roughness (Ra,um) using Environmental Scanning Electronic Microscope. Data were statistically analyzed using ANOVA followed by Duncan's Multiple Range Test using SAS program. **Results:** No significant differences were found among the bleaching groups (1-2-3). However, all bleaching agents produced a significant increase in the mean surface roughness of enamel compared to the untreated control group (C) (p<0.05).**Conclusion:** Different concentration of Carbamide peroxide and Hydrogen peroxide can promote alteration on enamel surface.

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Key words: Bleaching agents, Surface roughness, Enamel.

1. Introduction

Bleaching is a chemical process using oxidizing compounds that decolorize the intrinsic tooth pigments. As bleaching proceeds, teeth continually lighten. The effects of bleaching agents on human enamel have been analyzed by various methods and techniques, including, Raman spectroscopy ,scattering, and laser-induced fluorescence e.g., scanning and transmission electron microscopy, Mahringeret al.,(2009).

Discoloration of permanent anterior teeth is an aesthetic problem, which requires effective treatment. During the last decade, tooth bleaching has undergone great development, Moraes et al.,(2006);Marjaneh et al.,(2007) .There is a wide range of bleaching products and techniques that can be used to successfully lighten the shade of teeth. In the past, in-office vital tooth bleaching techniques used pre-operative acid etching, strong chemical oxidizing agents and heat to promote tooth whitening, Corcoran and Zillich (1974);Arens et al.,(1979). Oxidizing agents that included high concentrations of Hydrogen peroxide solutions (30–35%) were indicated for professional use only. As a result of these bleaching techniques, enamel has been shown to present superficial alterations, Titley et al., (1988); McGuckin et al.,(1992); Collinsa et al., (2004) and a reduction in the Calcium/Phosphorus (Ca/P) ratio, Rotstein et al., (1996). Tooth whitening with Carbamide is one of most popular dental procedures, and it is also one of the least understood. The available information on safety and biological properties of peroxides for tooth whitening indicated minimal risks with appropriate use of the bleaching products, Haywood et al., (1991); Marshall et al.,(1995); Li(1996). Hydrogen peroxide is widely used for whitening discoloured teeth. Carbamide peroxide (CP) is a prevalent commercial dental bleaching agent slowly releasing hydrogen peroxide under moist conditions. The main advantage of CP is that it can be used in dental offices and also by the patient at home or in a combination of both approaches, Kihn et al.,(2000);Cimilli and Pameijer(2001);. Fu et al.,(2007).

Bleaching methods have been developed and peroxide compounds at different concentrations are used for tooth whitening procedures, Arens et al.,(1972); Clark and Hintz(1998); Fu et al.,(2007).

Bleaching procedures have gained popularity with patients and dentists as conservative techniques to

lighten natural teeth in order to improve the harmony of the smile. However, the exposure of tooth hard tissues to bleaching agents can result in micro-structural changes in the enamel surface, Basting et al.,(2001); Worschech et al.,(2003) .

Evidence demonstrated that enamel presents structural changes when exposed to 10% Carbamide peroxide, compromising its composition and morphology, Haywood VB, Heymann (1989);Akal et al.,(2001) . However, there is little information about the effects of whitening products that present different peroxide concentrations, formulations and application protocols on enamel surface.

The purpose of this study was to evaluate the effect of 20% Carbamide peroxide (CP), 10% Carbamide peroxide (CP) and 25% Hydrogen peroxide (HP) on surface roughness of enamel.

2.Materials and Methods

2.1.Materials:

2.1.1.Samples;

Six young healthy dogs, aged between one and two years old with an average weight of 10 kg were used in this study. Sixteen teeth in each dog were selected (upper and lower, right and left first and second and corner incisors and canines) for bleaching.

Group(C): Control, no bleaching treatment (upper & lower canines in each dogs).

Group(1): Two dogs were supposed bleaching of upper &lower incisors with 20% CP Opalescence (ultra Dent Products Inc, South Jourdan, UT,USA)

Group(2): Two dogs were supposed for bleaching of upper &lower incisors with 10% CP Opalescence (ultra Dent Products)

Group(3): Two dogs were supposed for bleaching of upper &lower incisors with 25% HP Zoom2 (Discus Dental Product Inc, 13-2595 122706-USA)

N=24

2.1.2.Drugs:

The animals were pre-medicated with Atropine sulphate at a dose of 0.05-0.1mg/kg body weight, subcutaneously and Xylazine hydrochloride at a dose of 1 mg/kg body weight, intramuscularly. Ten minutes later, the anaesthesia was induced and maintained by using Thiopental sodium, at a dose of 5mgm/kg of 2.5% solution intravenously.

2.2.Methods:

2.2.1.Scheme of the work:

Selected teeth on each dog were scaled and polished with a rubber cup, after that in groups 1,2,3 bleaching agents were applied tested in accordance with the manufacturers' recommended procedure for eight hours daily for two weeks. Adequate measures were taken to minimize the pain or discomfort to the animals. Animals of all groups were supplied a diet composed of fresh vegetables, powdered milk and water ad libitum. The experimental procedure was conducted in compliance with ethical principles for animals' research as reviewed and approved by institutional guidelines of Kasr Alainy animal and experimental laboratory (Faculty of Medicine, Cairo University).

Dogs were sacrificed by using an overdose (0.5 grams) of 10% solution of Thiopental sodium intravenously. The jaws were immediately dissected free, and the teeth were separated from the jaws by the use of saw. Roots were removed and enamel fragments (5mm X 5mm X 2.5mm) were obtained from the middle one third of the buccal surfaces of crowns with a diamond saw (Isomet 1000, Buehler Ltd., Lake Bluff, IL, USA) under water lubrication. Each enamel fragment was stored in normal saline 0.9% for 24 hr and then tested for surface roughness (Ra,um) using Environmental Scanning Electronic Microscope (Quanta200-FEI-collected at Nether land).

2.2.2.Statistical analysis:

Data were statistically analyzed using ANOVA followed by Duncan's Multiple Range Test using SAS program.

3.Results:

The mean surface roughness (Ra, um) values and standard deviation, for all tested groups after 8 h for 14 consecutive days are displayed in table (1) & Fig.1. No significant differences were found among the bleaching groups (1,.0.00.2,03), on the other hand Zoom25% HP showed the least mean surface roughness (154.86), while the Opalescent 20%Cp showed the highest mean value (172.31). However all bleaching agents produced a significant increase in the mean surface roughness of enamel compared to the untreated control group ($p<0.05$).

Table (1): Descriptive statistics and test of significance for surface roughness of the tested bleaching agents.

Bleaching agents	Mean	S.D.	Min.	Max.	dt
Control group (no bleaching)	121.56	30.39	71.34	234.33	b
Opalescence 20% CP	172.31	19.32	114.08	297.12	a
Opalescence 10% CP	160.54	33.72	90.50	291.34	a
Zoom 25% HP	154.86	32.90	95.29	288.13	a

S.D.= Standard deviation. dt= Duncan's Multiple Range Test.

Means with the same letter within each column are not significantly different at $p\leq0.05$.

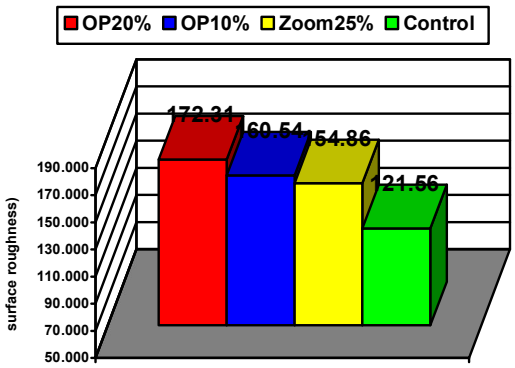

Fig. (1): Mean surface roughness of different bleaching agents and control group.

Environmental scanning Electronic Microscope (Quanta 200) image for all groups were showed in Fig.2-4-6-8. Enamel surface roughness of all groups which described by ESEM software program (XT Dolument for image analysis) were showed in Fig. 3-5-7-9.

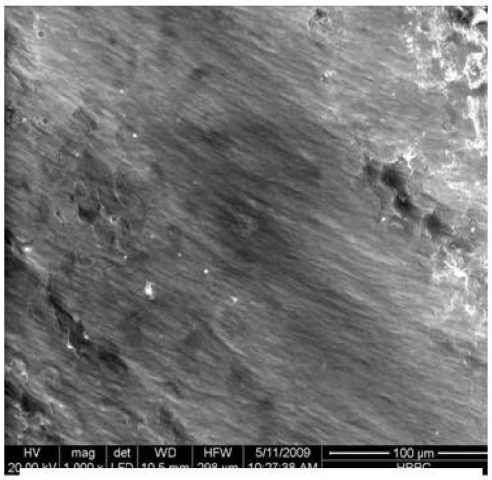

Fig2. Enamel surface of an unbleached (control group) (ESEM Image)

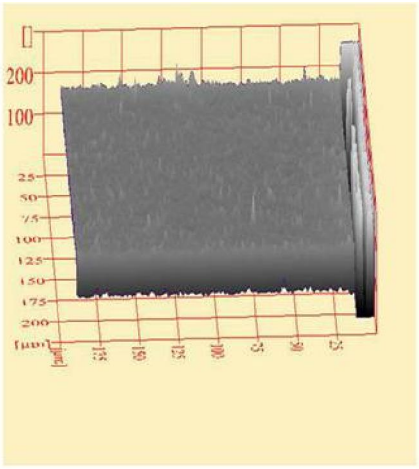

Fig.3. E. Surface roughness of an unbleached (Control group)

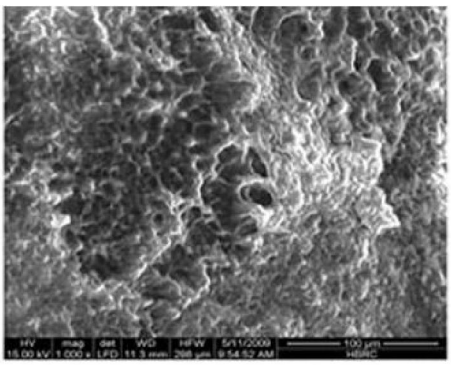

Fig.4. Enamel surface exposed to 20% Opalescence CP(ESEM Image))

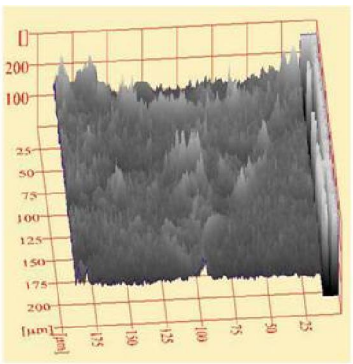

Fig.5. E. Surface roughness after exposed to 20%CP

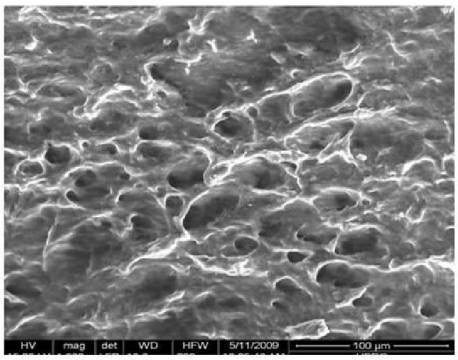

Fig.6. Enamel surface exposed to 10% Opalescence CP (ESEM Image)

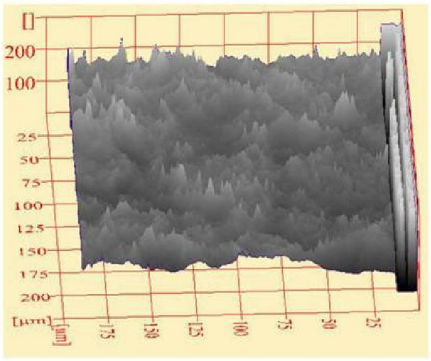

Fig.7. E. Surface roughness after exposed to 10% CP

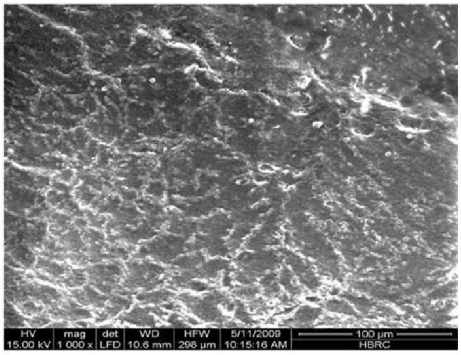

Fig.8. Enamel surface exposed to Zoom 25% HP (ESEM Image)

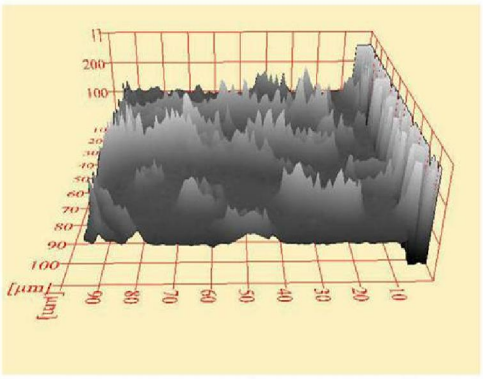

Fig.9. E. Surface roughness after exposed to 25% HP

4. **Discussion:**

This in vivo study revealed that surface roughness of enamel for all groups (CP10%- CP20%- HP25%) has no significant differences. However all bleaching agents produced a significant increase in the mean surface roughness of enamel compared to the untreated control groups.

Bleaching agents with Hydrogen peroxide are believed to lighten the discoloured tooth structure through decomposition of peroxides to give unstable free radicals. These free radicals breakdown the large pigmented molecules in enamel into smaller, less pigmented molecules through either oxidation or reduction reactions, Kelleher and Roe (1999);Nlu ey al.,(2004).

The mechanism of the action of bleaching agents is thought to be due to the ability of hydrogen peroxide to form oxygen free radicals that interact with adsorbed colored organic molecules and oxidize these macromolecules and pigmented stains into smaller and lighter molecules, Seghi and Denry(1992).

Oxidizing agents that included high concentrations of Hydrogen peroxide solutions (30–35%), has been shown to present superficial alterations of enamel, Li (1996); Cavalli et al.,(2001) ;Basting et al.,(2001); Maryline(2008) and reduction in the Calcium/Phosphorus (Ca/P) ratio. Maryline(2008) Enamel surface in contact with high concentrated Carbamide peroxide gels provided morphologic changes, causing increase in the surface roughness with etching-like appearance, Cavalli et al.,(2004)

Tooth whitening with Carbamide peroxide is one of most popular dental procedures. Carbamide peroxide (CP) dissociates into Hydrogen peroxide and urea when in contact with saliva at oral temperatures. Peroxide can diffuse through enamel

and dentin due to its low molecular weight. Hydrogen peroxide degrades into oxygen and water; urea degrades into ammonia and carbon dioxide. A general concern is expressed regarding possible weakening of the tooth structure, Swift and Perdigao(1998); Kelleher and Roe (1999);Justino et al.,(2004);. Moraes et al.,(2006). Other studies showed that some carbamide peroxide formulations caused calcium dissolution from enamel, Cimilli and and Pameijer(2001).Urea is a by-product of such bleaching agents and has been shown to be able to remove enamel proteins and related mineral elements, Nainar and Clarkson(1994) attacking the core or intra-prismatic area and producing porosities at prism surfaces. [(3)] A significant decrease in Ca and P concentration after Carbamide peroxide bleaching can also produce morphological alterations in the most superficial enamel crystallites, - Perdigão et al.,(1998);OLTU and GU8 RGAN(2000).

The 10–15% Carbamide peroxide solutions dissociated into 3–5% Hydrogen peroxide and approximately 7–10% urea. Despite the low Hydrogen peroxide concentration released, at-home tooth-whitening materials were used over prolonged periods and for extensive application time. This raises concerns of possible damage to the hard tissue that is exposed to the highest concentration, Cavalli et al.,(2004).

The daily application of Carbamide peroxide for 8 h for 14 consecutive days used in the current study simulated home-applied vital bleaching technique, Cavalli et al.,(2001).

Conclusions

This *in vivo* investigation showed that the surface roughness can be affected depending on the bleaching agent. Different concentration of Carbamide peroxide and Hydrogen peroxide can promote alteration on enamel surface.

5.References:

Akal N, Over H, Olmez A, Bodur H. (2001):
Effects of carbamide peroxide containing bleaching agents on the morphology and subsurface hardness of enamel. J Clin Pediatr Dent;25:293

Arens D.E, Rich J.J. and Healey H.J.(1972):
A practical method of bleaching tetracycline-stained teeth. *Oral Surgery, Oral Medicine and Oral Pathology* .; 34:812–817.

Arends J, Jongebloed W.L, Goldberg M. and Schuthof J, (1984)
Interaction of urea and human enamel. *Caries Research* **18**, pp. 17–24

Basting RT, Rodrigues AL, Serra MC. (2001):
The effect of 10% carbamide peroxide bleaching material on microhardness of sound and demineralized enamel and dentin *in situ*. Oper Dent;26:531-9.

CAVALLI V, ARRAIS C. A. G, GIANNINI M & AMBROSANO G. M. B.(2004):
High-concentrated carbamide peroxide bleaching agents' effects on enamel surface. Journal of Oral Rehabilitation 31; 155–159

Cavalli V, Giannini M, Carvalho RM.(2004): Effect of carbamide peroxide bleaching agents on tensile strength of human enamel. Dent Mater; 20: 733-9.

Cavalli V, Reis AF, Giannini M, Ambrosano GMB.(2001):
The effect of elapsed time following bleaching on enamel bond strength of resin composite. Oper Dent;26:597-602.

Cimilli H. and Pameijer C.H, (2001):
Effect of carbamide peroxide bleaching agents on the physical properties and chemical composition of enamel. *American Journal of Dentistry*. 14, pp. 63–66

Clark DM, Hintz J. (1998):
Case report: in-office tooth whitening procedure with 35% carbamide peroxide evaluated by the Minolta CR-321 Chroma Meter. J Esthet Dent.;10:37.

Collinsa LZ, Maggiob B, Gallagherb A, Yorkc M, Scha¨fera F. (2004):
Safety evaluation of a novel whitening gel, containing 6% hydrogen peroxide and a commercially available whitening gel containing 18% carbamide peroxide in an exaggerated use clinical study Journal of Dentistry.;32: 47–50

Corcoran J.F and Zillich R.M.(1974):
Bleaching of vital tetracycline stained teeth. *Journal of the Michigan Dental Association.* ; 56: 340–343.

Fu B, Hoth-Hannig W, Hannig M.(2007):
Effects of dental bleaching on micro- and nano-morphological alterations of the enamel surface .Am J Dent.;20:35–40.

Haywood VB, Heymann HO. (1989):
Nightguard vital bleaching. Quintessence Int.;20:173.

Haywood VB, Heymann HO. (1991):
Nightguard vital bleaching: how safe is it? Quintessence Int;22:515-23.

Justino LM, Tames DR, Demarco FF. (2004):
In situ and *in vitro* effects of bleaching with carbamide peroxide on human enamel. Oper Dent;29:219-25.

Kelleher MG, Roe FJ. (1999):

The safety-in-use of 10% carbamide peroxide for bleaching teeth under the supervision of a dentist. Br Dent J; 87: 190-194.

Kihn PW, Barnes DM,Romberg E, Peterson K. A(2000):
clinical evaluation of 10 percent vs. 15 percent carbamide peroxide tooth-whitening agents. J Am Dent Assoc.;131:1478–1484.

Li I. (1996):
Biological properties of peroxide-containing tooth whiteners. Food and Chemical Toxicol; 34: 887-904.

Marjaneh G, Sara A, Alireza M.(2007):
Effect of Different Time Periods of Vital Bleaching on Flexural Strength of Bovine Enamel and Dentin Complex. The Journal of Contemporary Dental Practice, 8; 1-6

Mahringer C, Fureder M, Castner M, Vitkov L, Hannig M, Kienberger F, Schilcher K. (2009):
Examination of Native and Carbamide Peroxide-bleached Human Tooth Enamel by Atomic Force Microscopy. Ultrastructural Pathology,; 33:189–196.

Marshall MV, Cancro LP, Fischman SL.(1995): Hydrogen peroxide: A review of its use in dentistry .J Periodontol;66:786-796.

Maryline Minoux.(2008):
Vital tooth bleaching Biologic adverse effects- A review. QUINTESSENCE INTERNATIONAL.; *39*: *645–659.*

McGuckin R.S, Babin J.F. and Meyer B.J. (1992):
Alterations in human enamel surface morphology following vital bleaching. *Journal of Prosthetic Dentistry*.; 68 :754–760.

Moraes R.R. Marimon J.L.M. Schneider L.F.M. Correr Sobrinho L. Camacho G.B. Bueno M. (2006):
Carbamide peroxide bleaching agents: effects on surface roughness of enamel, composite and porcelain. Clin Oral Invest .; 10: 23–28

Nainar S.M. and Clarkson B.H, (1994), :

8/28/2011

Fluoride profile in mature unerupted enamel following removal of surface organic material. *Caries Research* **28** pp. 83–86.

Nlu N. U, Obankar F. K. C¸ A, Altino Z C. & F. O.(2004):
Effect of home bleaching agents on the microhardness of human enamel and dentin. Journal of Oral Rehabilitation; 31: 57–61

OLTU U8 & GU8 RGAN S. (2000):
Effects of three concentrations of carbamide peroxide on the structure of enamel. *Journal of Oral Rehabilitation* 27; 332–340

Perdigão J, Francci C, Swift E.J, Ambrose W.W. and Lopes M, (1998):
Ultra-morphological study of the interaction of dental adhesives with carbamide peroxide-bleached enamel. *American Journal of Dentistry* 11, pp. 291–301.

Rotstein I, Danker E, Goldman A, Heling I, Stabholz A. and Zalkind M..(1996):
Histochemical analysis of dental hard tissues following bleaching. *Journal of Endodontics* .; 22: 23–26.

Seghi R.R. and Denry I., (1992):
Effects of external bleaching on indentation and abrasion characteristics of human enamel. *Journal of Oral Rehabilitation*. ; 71:1340–1344.

Swift Jr EJ, Perdigao J. (1998):
Effects of bleaching on teeth and restorations. Compend Contin Educ Dent.;19: 815-820.

Titley K, Torneck C.D. and Smith D. (1988): The effect of concentrated hydrogen peroxide solutions on the enamel surface morphology of human tooth enamel. *Journal of Endodontics*; 14: 69–74.

Worschech CC; Rodrigues JA; Martins LRM; Ambrosano GMB.(2003):
In vitro evaluation of human dental enamel surface roughness bleached with 35% carbamide peroxide and submitted to abrasive dentifrice brushing Pesqui. Odontol. Bras. ;17: 15-24