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Early And Late Impact of Diode LASER Activated Bleaching on Ultrastructure And Mineral Content of Surface Enamel in Rabbits

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Abstract: *The study directedto qualitatively analyze both early and late effect of Diode LASER activated bleaching on enamel surface of rabbit's teeth.*
Materials and Method*: Fifteen male rabbits were divided into three groups; control and two experimental. LASER bleaching gel was applied upon the labial surfaces of rabbits` incisors then activated by Diode LASER940nm.Teeth extracted after 24hrsin groupI and after one month in groupII. Examination of groups performed by Scanning Electron Microscope with Energy Dispersive X-ray Analyzer. Results; both experimental groups expressed structural and chemical alteration of enamel surface. Conclusion; Laser activated bleaching can cause irreversible denaturation of protein matrix and disturbance of chemical profile on rabbits' surface enamel. There is evidence of enamel surface weakness after LASER bleaching.*
Keywords*: Diode LASER, Enamel, Bleaching, SEM.*

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I. Introduction

Perfect smile has become one of the most common dental services offered to patients. Enamel bleaching is one of the most applicable techniques to obtain nice smile. To the food and drug administration (FDA), bleaching the first whitening of these beyond their natural color, whereas whitening restores the natural tooth color. Then the bleaching is concerned with three moving away any colorants in tooth structure by means of oxygen radicals (Fornainieet al., 2013)One of the most hallmark technologies in dental bleaching is LASERs. LASER activated bleaching was introduced and approved by FDA in 1996 (Kumar et al., 2014). LASER bleaching has the advantage as it acts as a jump-start for resistant difficult stains such as tetracycline and fluorosis(Al-Karadaghi et al., 2016). LASER can enhance bleaching by photo- oxidation of colored molecules in teeth or by interaction with components of the bleaching gel through photochemical reaction. LASER provides additional energy for more rapid breakdown of hydrogen peroxide into its components. This serves to increase and speed the release of reactive oxygen species into the stained tooth surface. Moreover, LASER heats the bleaching solution quickly and efficiently then dissipated quickly (DeMooret al., 2015).
Addition of coloring agent to the bleaching gel is mandatory for absolute absorption of LASER (DeMooret al., 2015).

Among different types of LASERs, Nd: YAG (1,064 nm), Er:YAG (2,940 nm), Co2 (10,600 nm) and DIODE (940 nm). Diode LASER (940nm, 7w)is advocated for bleaching because it is not absorbed by water and hydroxyapatite of the tooth structure (DeMooret al., 2015).It was reported that Diode LASER can reduce the time of bleaching without tooth surface modification in Scanning Electron Microscope (SEM)vitro study (Dostolova et al., 2004)Concerning the chemical structure of enamel, it was concluded that no significant change in chemical component of LASER activated bleached enamel (Cesar et al., 2009). While, Berger et al. results showed a considerable loss of calcium after LASER bleaching (Berger et al., 2010).Another study of low power Diode LASER bleaching on bovine teeth concluded that professional bleaching using Diode LASER could prevent loss of mineral content of enamel and maintain its crystalline structure compared with bleaching without LASER activation (Son et al.,2012). For human teeth, Coceska et al., 2016, reported significant loss of calcium and phosphate of enamel after LASER activated bleaching. They also found some loss of enamel structure with evidence of erosive areas and shearing off enamel rods in this invitro study.
Morphological analysis with SEM of labial surface of human impacted third molar teeth using Diode LASER bleaching showed minor to mild porous enamel surface (Kemalogluet al., 2014).

From the former literature, few data was available regarding the effect of Diode LASER bleaching on enamel surface under normal vital oral condition, as most of the previous studies performed upon extracted

teeth(Nematianaraki,2015). Therefore, the present study directed to analyze qualitatively both early and delayed effect of Diode LASER activated bleaching on enamel surface of rabbit's teeth.

II. Aim of The Study

The Present Study Aimed To Qualitatively Analyze Both Early And Late Effect Of Diode Laser activated bleaching on enamel surface of rabbit's teeth, using Scanning Electron Microscope and Energy Dispersive X-ray Analyzer.

III. Materials And Method

3.1 Animals

Fifteen male NewZealand rabbits selected from the animal house of Faculty of Agriculture, Al-Azhar University. Their average weight ranged between 2.5 to 3 kg and aged 4-4.5 months. All animals previously vaccinated against scabies and coccidiosis. The rabbits were placed in clean cages and fed a specific diet Ad-labium about 150gram daily. They left for two days before the experiment to settle down.

The study proposalwasapproved by the Research Ethics Committee (REC), Faculty of Dental Medicine for Girls, Al-Azhar University, Cairo, Egypt

3.2 Materials

LASER bleaching gel; BIOLASE: It contains hydrogen peroxide of 38% concentration.

Diode LASER unit; EPIC DIODE LASER.At LASER unit of Misr University for Science and Technology, 6th of October City, Egypt.

3.3Grouping

The animals equally divided into three groups; control and two experimental. The control group received no treatment while both experimental groups received LASER bleaching. The first experimental group examined after 24 hours after LASER application while the second experimental group examined one month after LASER application.

3.4 Anesthesia

Rabbits were anesthetizedby intramuscular injection of tranquillizer Xylazine HCL (Xylajet, the Egyptian co. for chemicals and anesthetic solution of Ketamine HCL (Ketmar, Amoun pharmaceutical company, 10thof Ramadan City, Egypt).A dose of 0.5 ml of 10% XylazineHCL(2-3 mg/kg) was injected to the experimental rabbits of group one and two. Then after 10 minutes, a dose of 50 mg / kg of animal weight of Ketamine HCL injected. This combination allowed duration of 20-30 minutes of anesthesia. The same protocol was repeated when needed (Attia-Zouair,Adawy and Khedr,2010).

3.5 Procedures

First, the surface of each tooth in upper and lower incisors gently cleaned with brush and dried by air spray. A layer of 1 mm thickness of laser bleaching gel applied over the incisor teeth using a small application tip.Biolase system was adjusted at 7W power, 200 Joule and continuous wave mood, according to the manufacturer instructions.LASERdirected to the labial surfaces of incisors at a distance of 1mm, withoutcontacting the gel for 30 seconds.After 10 minutes, another laser application performed for 30 seconds.The bleaching gel removed and cleaned with water and air spray.The procedure repeated for second time.

3.6Methods of evaluation

Scanning Electron Microscopic Examination

Rabbits sacrificed according to the experimental periods. Extraction of teeth done and then placed in this that water in labeled jars. Labial surfaces of teeth were sputter coated with gold in a vacuum evaporator. Then, examined by SEM 5500 LV, Japan Electronic Optics, at the Regional Center of Mycology and Biotechnology at Al-Azhar University, Cairo, Egypt.

Energy Dispersive X-ray (EDXA) Analysis

For each tooth, five points with randomly selected and the percentage of elemental levels of carbon, phosphorus and calcium in weight measured at enamel surface. This analysis performed using EDXA analysis with a count rate of 1800-2000 counts/second, at the Regional Center of Mycology and Biotechnology at Al-Azhar University.

Statistical analysis

Descriptive statistics of elemental content in weight percent in rabbit's incisor enamel surface normally, 24hours after, and 30 days after exposure to laserbleachingwastabled. Student's t-test was done to compare between different groups.

IV. Results

SEM Results

Control

Scanning electron micrograph of Normal unbleached enamel surface revealed smooth enamel surfaces. Numerous wave- like ridges ran continuously over the entire labial surface then become more apart incisally. This feature represents the typical morphology of the perikymata (Fig.1).Numerous small pits werescattered all over the enamelsurface.In between such a small pits, smooth elevated areas weredetected (Fig. 2). Fine scratches observed on the enamel surfaces.

Group I

SEM examination of group I reveled altered surface enamel when compared to control group. The classical perikymatic configuration was faintly preserved (Fig. 3). Most of the specimens appeared with marked wide porosities and erosive areas over the enamel surface (Fig. 4) .Well defined focal areas of smooth glazed enamel were detected incloseproximity to the eroded parts (Fig. 5).

Group II

SEM examination of group II showed affected surface structure of enamel. It revealed nearly lost perikymatic pattern, irregular enamel surface with few pits of enamel rod endings (Fig. 6).
Small erosive pits detected scattered all over the enamel surface interlacing with divergent cracks. Well-defined large area of hyalinized molten enamel with fish scale appearance at its margin also existed (Fig. 7 and 8).

Fig1.SEM micrograph of normal enamel showing ridges of perykimata (arrows).
Org.mag.: 1000, 30kv.

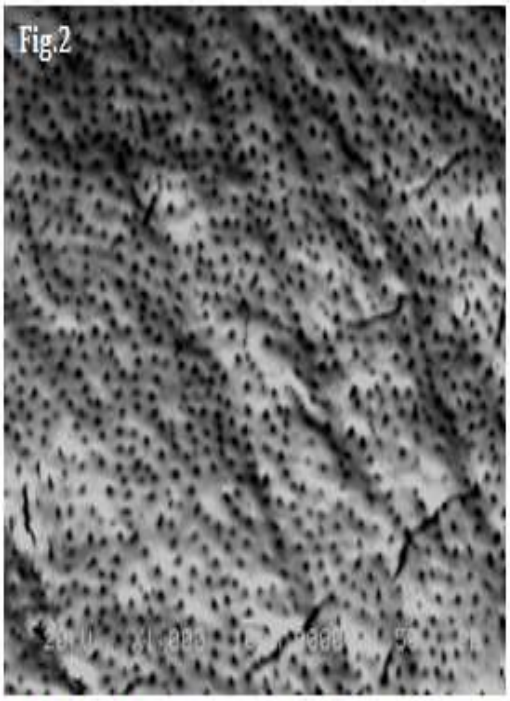

Fig 2.SEM micrograph of normal enamel showing numerous small pits (prism endings) and in between smooth elevated interprismatic substances.
Org.mag.: 1000, 20kv.

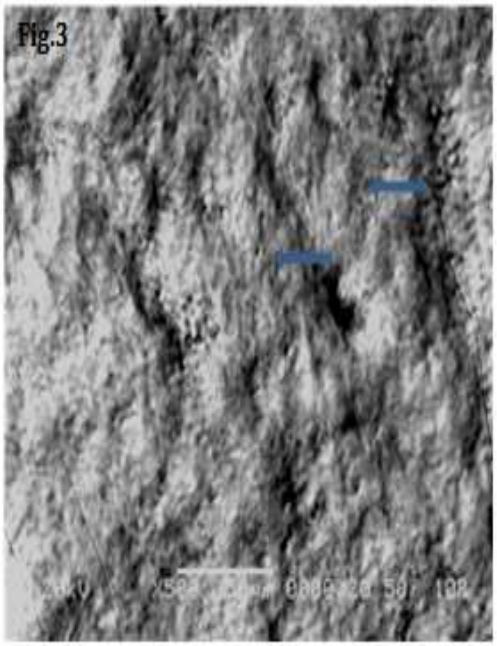

Fig 3.SEM micrograph of group I showing faint perikymatic ridges (arrows).
Org.mag.: 500, 20kv.

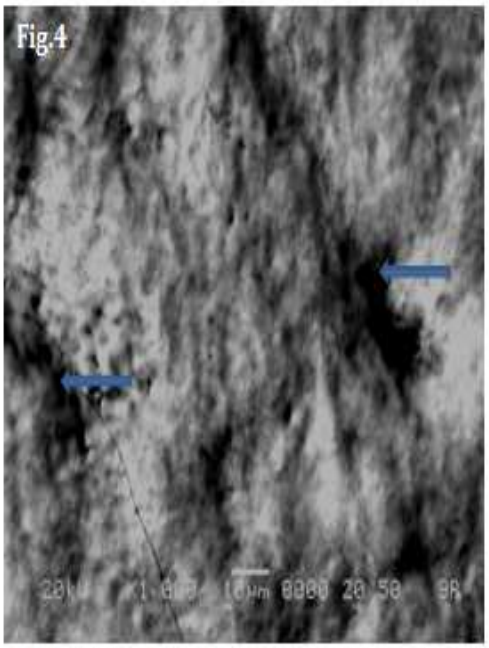

Fig 4.SEM micrograph of group I showing multiple wide erosive areas (arrows) and disintegrating prism endings.
Org.mag.: 1000, 20kv.

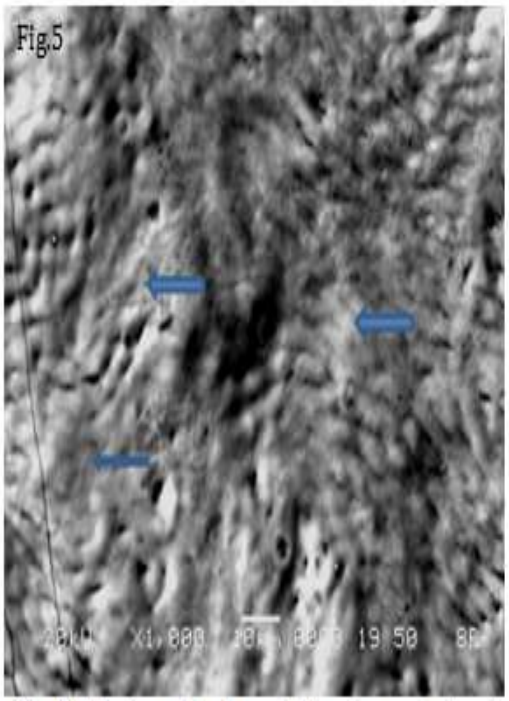

Fig 5.SEM micrograph of group I showing smooth glazed enamel (arrows) in close proximity to eroded areas.
Org. mag.: 1000, 20kv.

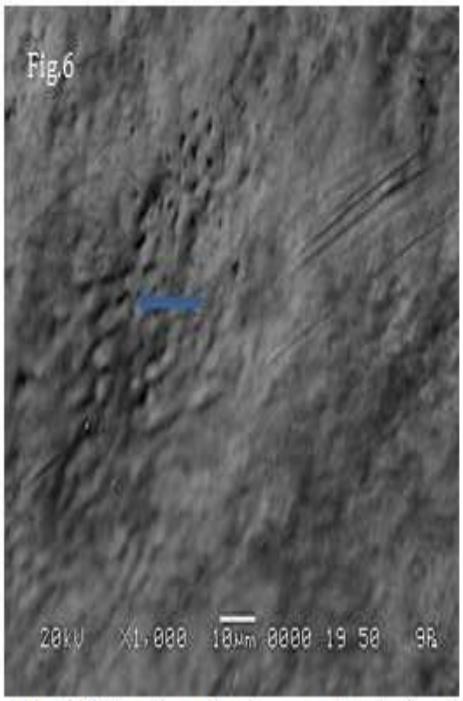

Fig 6.SEM micrograph of group II showing lost perkymatatic ridges and few enamel prism endings (arrow).
Org.mag.1000, 20kv.

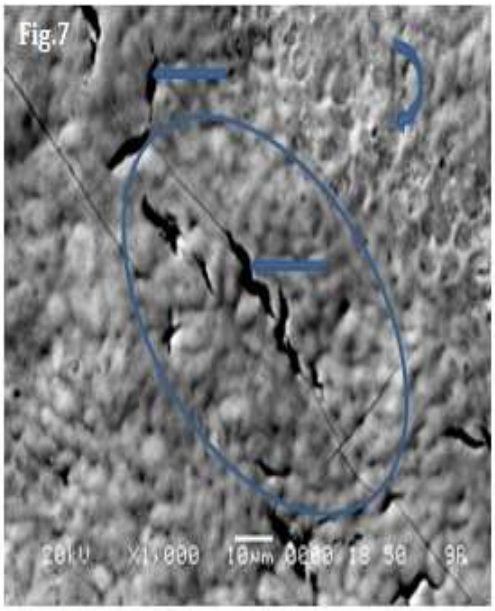

Fig7.SEM micrograph of group II showing large area of molten enamel(oval), multiple cracks (arrows)and fish scale appearance at the periphery (curved arrow). Org.mag.1000,20kv.

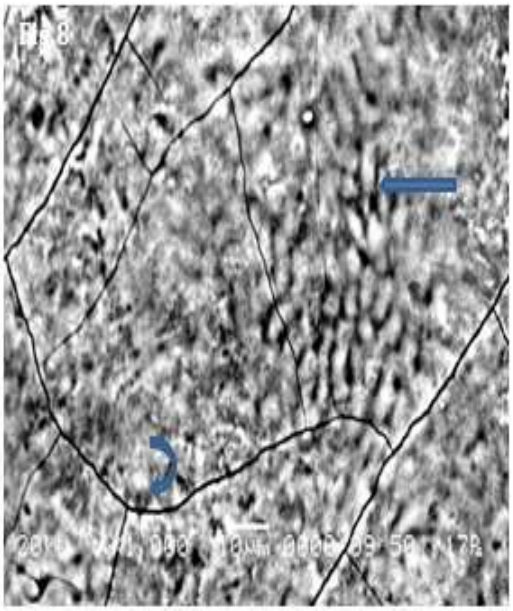

Fig8.SEM micrograph of group II showing fish scale appearance (arrow)and multiple cracks (curved arrows). Org.mag.10000, 20kv.

Energy Dispersive X-ray (EDXA) Analysis

Descriptive statistical analysis of calcium,phosphorus and carbon on enamel surface tabled (Tables 13)and t-tests between each two of the three groups postulated(Tables 4-6).Calcium results revealed significant decrease in weight percent on enamel surface 24 hrs after exposure to laser bleaching. While the difference between the percentage of control and after 30 days was non-significant (p=0.38). The difference between calcium percent after 24hrs and 30 days was significant (p=0.035)Phosphorus results showed insignificant decrease in weight percent after 24hrs (p=0.57) and insignificant increase from 24hrs to 30 days (p=0.83).The net result through one month was insignificant decrease in phosphorus percentage (p-0.72).

Carbon results showed dramatic decrease after 24hrs of exposure to laser bleaching (p=0.000000047).Moreover, there is insignificant increase in weight percent from 24hrs to 30 days (p=0.25), so the net result from control to 30 days is highly significant decrease in carbon percentage (p=0.00000015).

Table 1: Descriptive statistics of Ca (% Wt) in rabbit incisor enamel surface before, 24hours after, and 30 days after laser exposure:

	n	Mean	SD	Median	Mode	Range	Min	Max
Control	10	33.35	3.56	33.2	N/A	10.4	27.7	38.1
24 hours	10	28.51	2.98	27.95	N/A	9.5	23.9	33.4
30 days	10	31.91	3.64	31.9	N/A	10.7	26.1	36.8

Table 2: Descriptive statistics of P (% Wt) in rabbit incisor enamel surface before, 24hours after, and 30 days after laser exposure:

	n	Mean	SD	Median	Mode	Range	Min	Max
Control	10	20.26	1.61	20	N/A	4.6	18.1	22.7
24 hours	10	19.85	1.59	19.5	N/A	4.5	17.7	22.2
30 days	10	20	1.56	19.7	N/A	4.5	18	22.5

Table 3: Descriptive statistics of C (% Wt) in rabbit incisor enamel surface before, 24hours after, and 30 days after laser exposure:

	n	Mean	SD	Median	Mode	Range	Min	Max
Control	10	18.09	0.79	18.2	N/A	4.5	15.7	20.2
24 hours	10	15.04	0.74	15.15	N/A	4.2	12.7	16.9
30 days	10	15.27	0.73	15.4	15.5	4.1	13	17.1

Table 4: t-test of Ca (%Wt) in rabbit incisor surface between each two of the three groups:

	t value	t (critical)	p value
Control VS 24 hours	3.3	2.1	0.004*
Control VS 30 Days	0.89	2.1	0.38
24 hours VS 30 days	-0.28	2.1	0.035*

Table 5: t-test of P (%Wt) in rabbit incisor surface between each two of the three groups:

	t value	t (critical)	p value
Control VS 24 hours	0.57	2.1	0.57
Control VS 30 Days	0.37	2.1	0.72
24 hours VS 30 days	-0.21	2.1	0.83

Table 6: t-test of C (%Wt) in rabbit incisor surface between each two of the three groups:

	t value	t (critical)	p value
Control VS 24 hours	8.96	2.1	0.000000047*
Control VS 30 Days	8.3	2.1	0.00000015*
24 hours VS 30 days	-1.18	2.1	0.25

V. Discussion

Tooth bleaching has become one of the most important and demanding oral care branches. Tooth bleaching activated by Diode LASER recently applied in dental office under the supervision of dental practitioners (Sari et al., 2015). Diode laser was chosen in bleaching activation due to its analgesic and minimal thermal effect on dental pulp(Sari et al., 2015).Accordingly, this study aimed to evaluate early and delayed biological impact of Diode LASER bleaching on dental enamel morphologically and chemically.This study conducted upon rabbits under normal oral conditions. Their enamel was typically exhibited the same morphological criteria as that of human, so they represented a useful model for the experiment. The examination time was 24 hours and one month after LASER activated bleaching in order to detect early and delayed alterations, in addition to get an idea about whether these changes are reversible or irreversible.Scanning electron microscope provided a concise baseline data and reduced confusion between different experimental groups. Normal enamel surface of rabbits' teeth showed classical surface structures like perikymatic ridges considered as a guideline for enamel surface preservation. It also showed enamel prism endings, whichappeared as numerous small pits covering the enamelsurface.Their presence denotes young, healthy and intact enamel. The smooth elevated areas in between enamel prism ending noted as interprismatic substance of great organic content and low in organic substance (Fig.1and 2).

In Group I , (Fig. 3,4 and 5), some morphological features were altered such as fainting of perikymata, appearance of multiple erosive areas. Focal areas of glazed enamel spotted. These results denote affection of both prismatic and interprismatic structure of enamel due the action of bleaching gel and LASER activation. However, the interprismatic parts (multiple erosive areas) likely more affected than the prismatic part. The reason may be due to its higher organic content or due to its action as a channel for hydrogen peroxide(Park et al., 2008).This goes with Jiang et al., 2008, who reported that organic matter of enamel greatly affected by bleaching agent when activated by LASER-induced fluorescence. Meanwhile, focal areas of enamel glazing may refer to reduction of protein matrix or its fragmentation into inter-rod enamel channels (Elfallah et al., 2015).

In group II, Altered morphological features were detected after one month of LASER exposure, (Fig. 6,7 and 8). Generally, the molten enamel surface, cracking and fish scale appearanceobviouslydetected, which reported by many researchers. De Paula et al.(2009) and Anaraki et al.(2015) reported insignificance in surface roughness after laser bleaching while Kemaloglu et al.(2014) and Coceska et al.(2015) reported slight increased surface porosity and evidence of erosion and shearing of enamel rods after laser bleaching. Reduction of erosive bits detectedingroup II might be referred to the action of salivary remineralization after onemonth of laser activation. However, the fish scale appearance was marked at the same areas denoting permanentaffection of inter-prismatic region. Multiple cracks may reflect the weakened enamel surface after one month of laser exposure.

The chemical profile results of rabbits' surface enamel after laser application, showed significant decrease of calcium weight percent on surface enamel after 24hrs. This is in agreement with Coceska et al.(2015) who reported significant loss of calcium and phosphorus of enamel after laser bleaching. Moreover, this loss of calcium almost restored after one month as its percentage was significantly increased. This may be referredto the remineralizationeffect of oral environment.Phosphorus results reported insignificant decrease after 24hrs but it also compensated by insignificant increase through one month. The net result was insignificant decrease in phosphorus weight percentage, which goes with previous studies reported disturbance in both organic and inorganic enamel component due to photothermal reactions (De SantAnna et al., 2009).Other researchers mentioned pyrophosphate formation as enamel change after laser application (Hicks et al., 2003) and (Westerman et al., 2004).Carbon results expressed highly significant decrease even after one month, thismay referred to dehydration and loss of carbonate content due tophotothermal and photochemical effects of laser application(Kato et al., 2006).

VI. Conclusions

1- Laser activated bleaching can cause irreversible denaturation of protein matrix and disturbance of chemical profile of surface enamel in rabbits.
2- Evidence of enamel surface weakness obtainedafter LASER bleaching.

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