

Analysis of inflammatory cells in osseointegration of CpTi implant radiated by low level laser therapy

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ABSTRACT

Background: Dental implants provide a unique treatment modality for the replacement of a lost dentition. This is accomplished by the insertion of relatively an inert material (a biomaterial) into the soft and hard tissue of the jaws, there by providing support and retention for dental prostheses. Low level laser therapy (LLLT) is an effective tool used to prompt bone repair and remodeling, this has referred to the biostimulation effect of LLLT. The Aim of this study was to evaluate the effects of inflammatory cells on osseointegration of CpTi implant irradiated by low level laser.

Materials and Methods: thirty two adult New Zealand white rabbits, received titanium implants were inserted in the tibia. The right side is considered as experimental groups and the left side considered as control groups. Low power diode laser (GaAlAs) with wave length (904nm) and (5mW) power applied with the right implants. The sample divided into four groups, eight rabbits were sacrificed at four interval 4days, 1 weeks, 2weeks, and 6weeks respectively. Histological and inflammatory analyses were done for each interval.

Results: Histological examination showed acceleration of bone formation and more rapid healing process in the screw implant with laser irradiation than in the control implant. Inflammatory analysis showed dramatic decrease with the presence of laser irradiation especially with advancing time.

Conclusion: This study illustrated that the inflammatory cells were reduced in osseointegration of dental implant treated with LLLT.

Key words: Dental Implants, low level laser therapy, inflammatory cell. (J Bagh Coll Dentistry 2015; 27(1):105-110).

INTRODUCTION

Dental implants are biocompatible screw like titanium objects that are surgically placed into the mandible or maxilla to replace missing teeth. The mechanism by which an implant is biomechanically accepted by the jaw bone is called osseointegration ^(1,2)

The clinical long-term success of the implants depends on the osseointegration and the adhesion of the soft tissues and epithelium to the titanium surfaces of the implant ⁽³⁾.

Titanium is the most wide spread metal for orthopedic implants intended for bone integration. It represents high fatigue strength comparatively low modulus of elasticity, in respect to other metals, so it is able to support loads and distribute them to bone, limiting stress shielding. Besides titanium is characterized by a thin natural oxide layer on the surface that limits ion release and reactivity, making the surface almost inert and biocompatible ⁽⁴⁾.

Several treatments have been proposed to improve and accelerate bone formation onto implant surface, among which is low-level laser therapy (LLLT) ⁽⁵⁾. LLLT Known as cold laser, soft laser, biostimulation, or photobiomodulation, it basically exposes cell or tissue to laser or low-level red or near-infrared (IR) light generated from light-emitting diode. LLLT stimulates or controls cellular function to minimize the extinction of cell or tissue, accelerates the healing of fractures, fast recovery from the damage of soft

tissue, nerve, bone, and cartilage, and relieves acute and chronic pain and inflammation ⁽⁶⁾.

MATERIALS AND METHODS

All experimental procedures were carried out in accordance with the ethical principles of animal's experimentation. Thirty-two adult male New Zealand weighing 2-3kg were used in this study. Screw CpTi implants were inserted in the right tibia of each animal (experimental side), and left tibia was used as (control side).

Low power diode laser (GaAlAs) with wave length (904nm) and (5mW) power applied with the right screw implants. The animals were divided into four groups, for 4days, 1weeks, 2weeks, and 6weeks healing intervals.

Inflammatory cells counting were done under light microscope, in eight histological sections (stained by hematoxyline and eosin stain) for each healing period and for both experimental and control groups at / magnifying power lens X40.

Score for intensity of inflammatory reaction per unit area (mm²):

- 1- Absent: or few (0-4) inflammatory cells.
- 2- Mild: average number less than 10 inflammatory cells.
- 3- Moderate: average number (10-25) inflammatory cells.
- 4- Severe: average number greater than 25 inflammatory cells ⁽⁷⁾.

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RESULTS

Histological findings (hematoxylin and eosin stain)

At 4 days duration

Control group

Screw that implant in the tibia of rabbit shows bone marrow with stromal cells Figure (1).

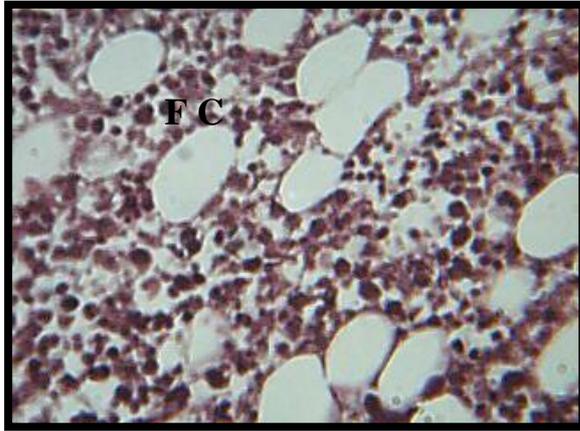


Figure 1: High magnification view of implant site shows fat cell (FC) in marrow tissue H&E X400.

Experimental group

Histological view of implant site treated with laser irradiation after 4 days of implantation revealed primitive new bone formation in which future bone is formed as embryonic bone Figure (2).

High magnification view of marrow tissue illustrates the presence of fat cells associated with presence of numerous blood islets Figure (3).

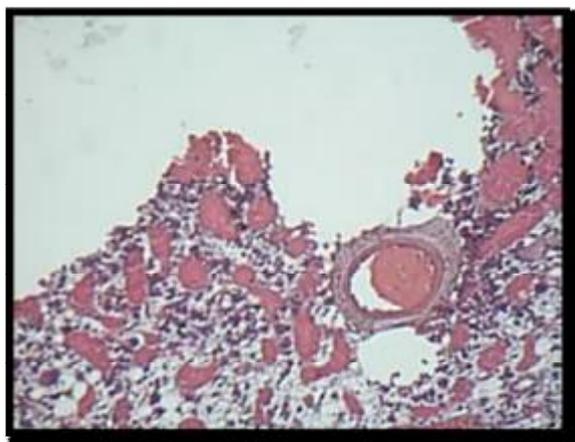


Figure 2: Microphotograph view of implant rabbit tibia shows bone marrow H&E X200.

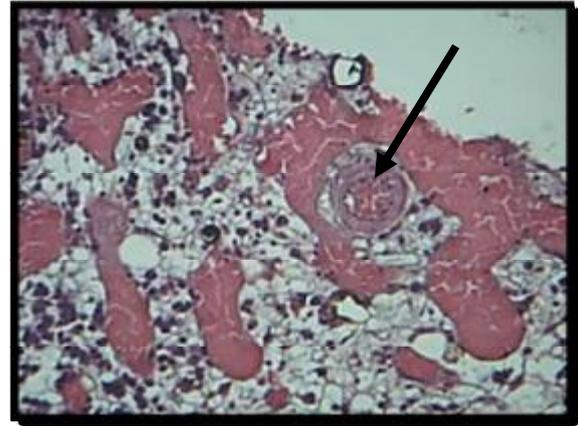


Figure 3: High magnification of previous figure (2) shows numerous blood in the marrow tissue (arrow) point to blood vessel H&E X400.

At one week duration

Control group

Screw that implanted in the rabbit tibia shows the formation of osteoid tissue, abundant blood vessels in thread region that follows the screw shape Figure (4).

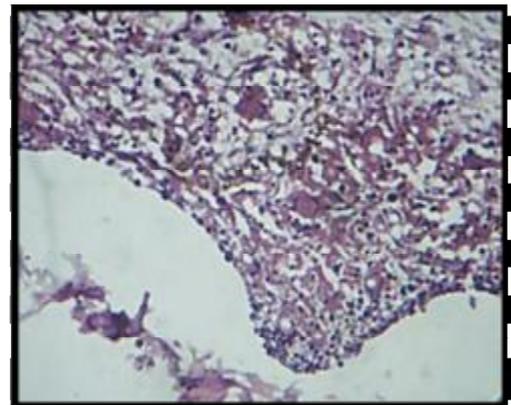


Figure 4: Microphotograph view of bone marrow in thread region in implant site (control) of one week duration shows fat cell (FC) H&E X100.

Experimental group

The histological view of implant site in rabbit tibia treated with the laser irradiation after one week duration shows the bone trabeculae, osteoid tissue formed by active osteoblast Figure (5).

Active bone marrow is indicated by active osteoprogenitor cell, osteoblast cell and by the formation of osteoid tissue Figure (6).

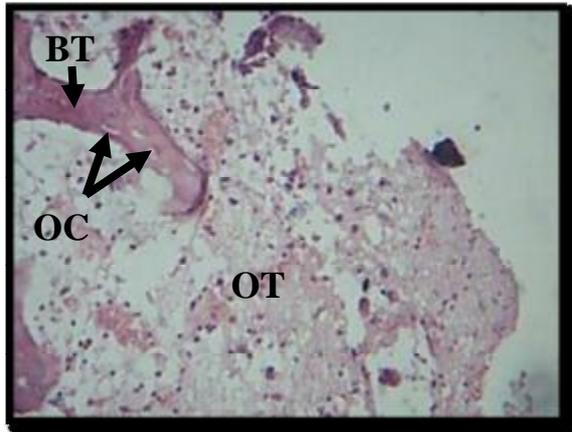


Figure 5: Microphotograph view of implant in rabbit tibia treated with laser irradiation for one week duration shows osteoid tissue (OT), bone trabeculae (BT), osteocyte (OC) H&E X200.

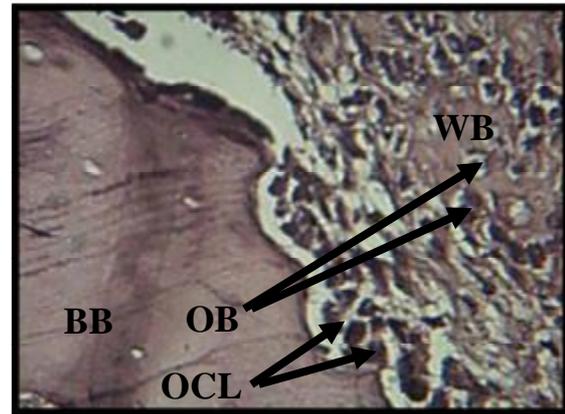


Figure 7: Microphotograph view of implant site after 2 weeks (control) shows formation of woven bone (WB) against basal bone (BB), active formative osteoblast cell (OB), and osteoclast cell (OCL) .H&EX200

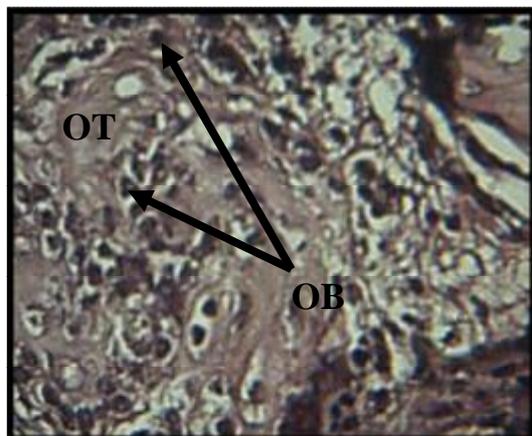


Figure 6: Microphotograph view of implant site treated with laser irradiation after one week of implantation shows osteoblast cell (OB) and osteoid bone formation (OT) H&E X400.

Experimental group

The histological view of experimental group with areas of marrow tissue shows the formation of bone trabeculae, osteoblast cell on the surface of the bone, osteocyte cell trapped inside the bone matrix cell. Figure (8, 9)

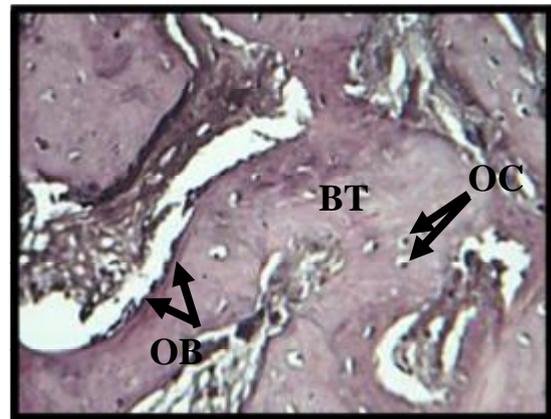


Figure 8: View of implant site of 2 weeks duration treated with laser irradiation showing bone trabeculae (BT) with the osteoblast (OB) and osteocyte trapped inside bone matrix (OC) H&E X400.

At 2 weeks duration

Control group

The histological view of implant site illustrates the apposition of woven bone against the basal bone with many osteoblast cells, osteocyte cell and osteoclast occupying Howship's lacunae Figure (7).

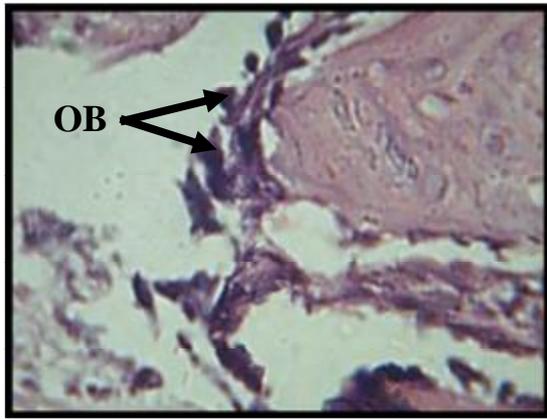


Figure 9: High magnification of previous figure (9) of 2 weeks duration showing osteoblast cells rimming the bone surface (OB) H&EX400.

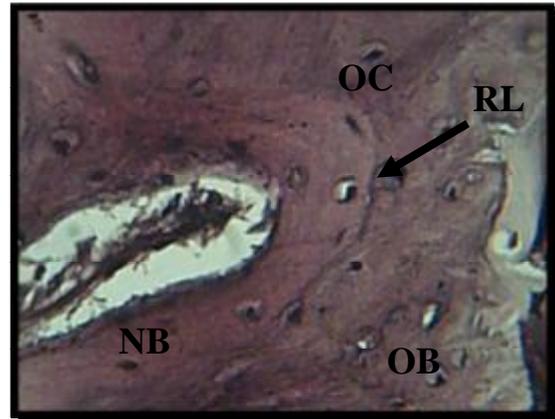


Figure 11: other figure of mature bone in implant site for 6 weeks duration treated with laser irradiation shows osteocyte cell (OC), reversal line (RL) separated between old bone (OB) and new bone (NB) H&E X400.

At 6 weeks duration

Control group

Screw that implanted in the rabbit tibia (control) for 6 weeks duration shows mature bone formation in the thread region Figure (10).

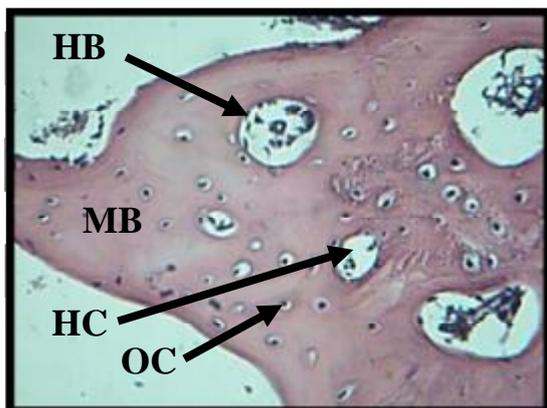


Figure 10: Microphotograph view of bone thread in implant site after 6 weeks of implantation (control) shows mature bone (IMB) with haversian bone lamellae (HB), osteocyte cell (OC) that surround haversian canal(HC) H&E X200

Experimental group

The histological picture of this group shows mature bone formation filling the thread of cortical bone area, the newly bone appear dense with regular distribution of osteocyte cell trapped inside the bone matrix, reversal line separating between old and new bone with the presence of numerous resting lines, Haversian bone lamellae are obviously seen Figure (11).

Inflammatory cells parameter

The result of the present study indicated that the majority of the inflammatory cells parameter was reported at the control group, as well as the first score and decreasing up to the last period of time after treatment in (6 weeks). There was no significant difference between mean values of the control and experimental groups at each healing period and the highest mean values were recorded at the 4 days and 1 week duration, where as after 2 and 6 weeks of implantation mean values of scores were clearly less than the first two periods.

In addition to that, the comparisons significant among different periods of times reported a non significant differences at $p > 0.05$ for both control and experimental groups throughout the healing periods (4 days, 1, 2 and 6 weeks). As shown in figure (12) (Table1).

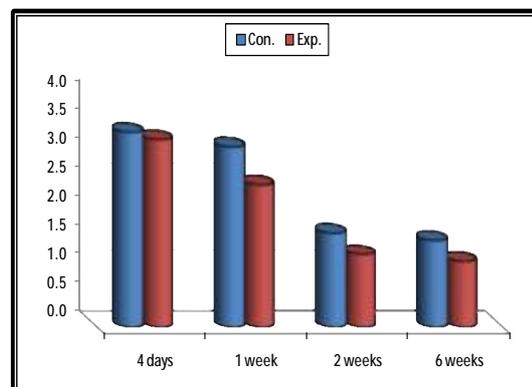


Figure 12: Cluster bar chart for mean values of scoring (inflammatory cells outcomes) distributed among different studied sources of variations.

Table 1: Distribution of the observed frequencies and percentages with summary statistics of the inflammatory cells outcomes according to different sources of variation with comparisons significant

Periods	Scores	Control				Exp.				C.S. ^(*) P-value
		No.	%	MS	SD	No.	%	MS	SD	
4 days	Score-1	0	0.00	3.375	0.744	0	0.00	3.250	0.707	0.125 NS
	Score-2	1	12.5			1	12.5			
	Score-3	3	37.5			4	50.0			
	Score-4	4	50.0			3	37.5			
1 week	Score-1	0	0.00	3.125	0.835	0	0.00	2.455	1.128	0.273 NS
	Score-2	2	25.0			2	25.0			
	Score-3	3	37.5			4	50.0			
	Score-4	3	37.5			2	25.0			
2 weeks	Score-1	4	50.0	1.625	0.744	6	75.0	1.250	0.463	0.250 NS
	Score-2	3	37.5			2	25.0			
	Score-3	1	12.5			0	0.00			
	Score-4	0	0.00			0	0.00			
6 weeks	Score-1	4	50.0	1.500	0.535	7	87.5	1.125	0.354	0.375 NS
	Score-2	4	50.0			1	12.5			
	Score-3	0	0.00			0	0.00			
	Score-4	0	0.00			0	0.00			
C.S. ^(**) P-value		F=14.848 S				F=15.615 S				-

(*) Comparison sig. based on the Kolmogorov-Smirnov test for two independent groups of an ordinal scale.

NS: Non Sig. at P>0.05 (the critical value of K-S Statistic = 0.680). (***) F – test (ANOVA) Statistics, S: Sig. at P<0.05

DISCUSSION

All animals tolerated the implantation well, no sign of gross infection, tissue reaction or any other negative clinical indications

The histological findings showed biocompatible and osseointegrative implant surfaces in all groups, with different rates of bone deposition and remodeling. The results showed accelerated growth of bone around implant in groups who were exposed to laser and this may be attributed to the followings:

- 1-Stimulation with LLLT creates a number of environmental conditions that appear to accelerate the healing of bone in vivo investigations⁽⁸⁾.
- 2- LLLT is an effective tool used to prompt bone repair and modeling post surgery, this has referred to the biostimulation effect of LLLT, it is directly dependent on the dose applied^(9,10).
- 3-The early bone maturation could be attributed to the stimulation of fibroblast proliferation through the application of LLLT as it has been reported by⁽¹¹⁾.
- 4- LLLT-related effects include stimulation of blood flow, recruitment and activation of osteoblasts, osteosynthesis, a decrease in osteoclastic activity and anti-inflammatory action could also be considered as factors that stimulate biomaterial osseointegration.

These findings were in agreement with previous findings^(9,12-18). The response of the bone to the trauma through placement the implants by an inflammatory reaction played a great role in fixation of implant at early regenerative process⁽¹⁹⁾.

Within a few hours after injury, inflammatory cells invade the wound tissue. In addition to the defense functions, inflammatory cells are also an important source of growth factors and cytokines, which initiate the proliferative phase of wound healing^(20,21). At 4 days and 1 week of healing periods there was a large number of inflammatory cells that migrated toward the site of implant while at 2,6 weeks there was marked reduction in the number of inflammatory cells that infiltrated in to the implant sites which indicates earlier acute inflammatory response and more rapid resolution resulting in earlier phase of regeneration ;which agreed with⁽²²⁻²⁴⁾ who found that LLLT was able to promote wound healing by reducing inflammation without compromising the proliferation of fibroblasts and keratinocytes.

Goldman, 1987⁽²⁵⁾ Walsh, 2003⁽²⁶⁾ found that LLLT is supposed to reduce pain, accelerate wound healing and reduce inflammatory processes. Furthermore, it enhances bone remodeling, attenuates pain and modulates the immune system.

The present study suggests for beneficial use of LLLT in practice of dentistry implantation or in other branch related to osseointegration process.

REFERENCES

- 1- Guan H, Staden RV, Loo YC, Johnson N, Ivanovski S, Meredith N. Influence of bone and dental implant parameters on stress distribution in the mandible. *Int J Oral Maxillofacial Imp* 2009; 24: 866 - 76.
- 2- Oshida Y, Tuna EB, Aktören O, and Gençay K. Dental Implant Systems. *Int J Mol Sci* 2010; 11: 1580-678.
- 3- Ghahroudi AAR, Talaepour AR, Mesgarzadeh A, Rokn AR , Khorsand A, Mesgarzadeh NN, Kharazi Fard MJ .Radiographic vertical bone loss evaluation around dental implants following one year of functional loading. *J Dentistry, Tehran Uni Med Sci* 2010; 7:55-123.
- 4- Ferraris S, Spriano S, Pan G, Venturello A, Bianchi CL, Chiesa R, Faga MG, Maina G, Verne E. Surface modification of Ti-6AL-4V alloy for biomineralization and specific biological response. Part 1, inorganic modification. *J Mater Sci Mater Med* 2011; 11: 545-53.
- 5- Peplow PV, Chung TY, Baxter GD. Laser photobiomodulation of wound healing: a review of experimental studies in mouse and rat animal models. *Photomed Laser Surg* 2010; 28: 291-325.
- 6- Kim YD. Biophysical therapy and biostimulation in unfavorable bony circumstances: adjunctive therapies for osseointegration. *J Korean Assoc Oral Maxillofac Surg* 2012; 38:195-203.
- 7- Accorinte , M , Holl and , R Reis, A Bortoluzzi, M , murata, 5, Evaluate of mineral trioxide cement as pulp capping agent in teeth. *J Endod* 2008 ; 34: 1-6
- 8- Pinheiro AL, Gerbi ME. Photo engineering of bone repair processes. *Photomed. Laser Surg* 2006; 24: 169-78
- 9- Nicola R A, Jorgetti V, Rigau J, Pacheco M T, dos Reis L M, Zangaro R A. Effect of low power Ga Al As laser (660 nm) on bone structure and cell activity: an experimental animal study. *Lasers Med Sci* 2003; 18(2): 89-94.
- 10- Yousif M, Ashkar S, Hamade E, Gutknecht N, Lampert F, Mir M. The effect of low level laser-therapy during orthodontic movement: a preliminary study. *Lasers Med Sci* 2008; 23:27-33
- 11- Yu W, Naim JO, Lanzafame RJ. The effect of laser irradiation on the release of bFGE from 3T3 fibroblasts, *Photochem – Photobiol* 1994; 59(2): 167-70.
- 12- Sennerby L, Thomsen P, Ericson EL:Early tissue response to titanium implants inserted in rabbit cortical bone. Part I light microscopic observation. *J Mater Sci Mater Med* 1993; 4: 240-50.
- 13- Mohammed IFR, Yonus NA, Al-Tatabani NG. Histopathological study of osseointegration around titanium implant post LLLT. *Iraqi J Oral Dent Sci* 2003; 2(1): 9-19.
- 14- Pinheiro ALB, Limeira F, Gerbi MEM, Ramalho LM P, Marzola C, Ponzi EAC. Effect of low level laser therapy on the repair of bone defects grafted with inorganic bovine bone. *Braz Dent J* 2003; 14(3): 177-81
- 15- Ibrahim RS. Effect of low energy laser irradiation on bone healing around intraosseous titanium implants in experimentally diabetic rabbits (morphometric and histological evaluations). A Ph.D. thesis, College of Dentistry, University of Baghdad, 2003.
- 16- Al-Talabani MAH. Effect of 904 nm diode laser with different exposure times on titanium dental implants inserted in rabbit's tibia (histological study). A master thesis submitted to the College of Dentistry, University of Baghdad, 2004.
- 17- Pretel H, Lizarelli RF, Ramalho LT. Effect of low-level laser therapy on bone repair: histological study in rats. *Lasers Surg Med* 2007; 39(10): 788-96.
- 18- Blaya DS, Pozza DH, Weber JB, Oliveria MG. Histological study of the effect of laser therapy on bone repair. *J Contemp Dent Pract* 2008; 9(6): 41-8.
- 19- Branner R, HaniaLadthaler MW, BraunFulco O. application of the laser light of low power density, clinical &experimental investigation. *Curr Prob Derm* 1988; 5:111-6.
- 20- Edmonds M, Bates M, Doxford M, Gough A, and Foster A. New treatments in ulcer healing and wound infection. *Diabetes Metab Res Rev* 16 Suppl 2000; 1: S51-S54.
- 21- Harding KG, Morris HL, and Patel GK. Science, medicine and the future: healing chronic wounds. *Br Med J* 2002; 324: 160-3.
- 22- Nascimento PM, Pinherio LB, Salgado MAC, Ramalho LMP. A preliminary report on the effect of laser therapy on the healing of cutaneous surgical wounds as a consequence of an inversely proportional relationship between wavelength and intensity: Histological study in rats. *Photomedicine Laser Surgery* 2004; 22(6): 513-8.
- 23- Gal P, Vidinsky B, Toporcer T. Histological assessment of the effect of laser irradiation on skin wound healing in rats. *Photomed Laser Surg* 2006; 24(4): 480-8.
- 24- Lajcakova K, Bobrov B, Polakova M. Effects of equal daily doses delivered by different power densities of low level laser therapy at 670nm on open skin wound healing in normal and corticosteroid treated rats: a brief report. *Lasers Med Sci* 2010; 25(5): 761-6.
- 25- Goldman LB, van Lieu N. Current laser dentistry. *Lasers Surg Med* 1987; 6: 559-62.
- 26- Walsh LJ. The current status of laser applications in dentistry. *Aust Dental J* 2003; 48: 146-55.

الخلاصة

الخلفية: إن الغرسات السنية للفك يوفّر وسيلة استثنائية لتعويض الأسنان المفقودة حيث يتم ذلك بإدخال مادة خاملة نسبياً (مادة حيوية) في الأنسجة الرخوة والعظمية للفك مما يوفّر إسناداً وثباتاً لتعويض السني. إن علاج الليزر المنخفض الطاقة أداة فعالة تستعمل لتحفيز إلى إعادة بناء العظم بعد الجراحة ، وهذا ما يسمى بالتأثير الحيوي المحفز لليزر المنخفض الطاقة. أهداف هذه الدراسة هي تقييم تأثير تعقيم الليزر المنخفض الطاقة حول الخلايا الالتهابية في مجاميع الاختبار والسيطرة وتأثيره على التئام العظم حول غرسات التيتانيوم.

طريقة العمل والمواد المستخدمة: استخدم 32 اثنان وثلاثون ارنب نيوزلندي ذكر البيض، ادخلت غرسات التيتانيوم في عظم قصبه الساق الجانب الايمن الذي يمثل مجموعة التجربة والجانب الايسر اعتبر مجموعة السيطرة. وتم تشيع الغرسات بالليزر المنخفض الطاقة (GaAlAs) بطول موجة (904nm) وقوة (5mw) للغرسات اليمنى. تم تقسيم العينة إلى اربعة مجاميع، 8ممانية ارنب ، لاربع فترات 4ايام، اسبوع واحد، اسبوعين و ستة اسابيع بالتتابع. الجري التحليل النسيجي والالتهابي لكل فترة.

النتائج: اوضحت نتائج الفحص بان تعجيل تكوين العظم وسرعة عملية الشفاء للغرسه بوجود اشعة الليزر اكثر مما في مجموعة السيطرة. التحليلات الالتهابية اظهرت انخفاض بوجود اعة الليزر خاصة بتقدم الوقت.

الاستنتاج: هذه الدراسة اوضحت بان تطبيق اشعة الليزر المنخفض الطاقة سرعت عملية التكوين العظمي والالتئام العظمي وتقليل الالتهابات.

الكلمات الرئيسية: الغرسه السنية ، الليزر المنخفض الطاقة ، الخلايا الالتهابية.