International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2016): 79.57 | Impact Factor (2015): 6.391

In Vivo Mechanical and Histological Evaluation of the Effect of Vacuum Storage and Ultra Violet Light Treatment of Titanium Implants on Osseointegration

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Abstract: ***Background: Titanium implant is widely used in dentistry because of its extraordinary biocompatibility and mechanical properties. To increase bone–implant connection and provide early loading after placement, implant is stored in different storage medium and treated with UV light. Both of them are applicable methods to increase the bioactivity of titanium and overcome the biological aging. This study was designed to assess the effect of vacuum storage method and air storage with and without UV light treated of Cp Ti implant mechanically and histologically. Materials and methods: Titanium screws were acid etched and prepared in four different modes using different storage methods (air or vacuum and, with or without UV treatment. The implant screws were stored 4 weeks and then implanted into rabbit's tibia for histological and torque removal examinations. Results: There was a significant increase in mean value of torque removal test for screws air treated with UV light, vacuum and vacuum treated with UV light (14.5 ,13.8 ,17.92 N.cm respectively after 2 weeks, and (21.5333, 22.5 ,26.47 N.cm respectively after 4 weeks) compared with air storage screws (8.4 N.cm after 2 weeks, and 11.4 N.cm after 4 weeks). Histological examination showed bone formation mineralization and maturation increased with advancing time for air treated with UV light, vacuum and vacuum treated with UV light screws. Conclusion: vacuum storage method and air storage with UV light treated methods of Cp Ti implant had great effect in increasing the osseointegration than air storage methods, while the vacuum storage and treated with UV light have synergistic effects on the biological properties of aged titanium surfaces.***

Keywords: dental implant, vacuum, UV light storage, mechanical and histological analysis

1. Introduction

Dental implants have become a popular restorative choice and the major factor for this success is osseointegration [1]. osseointegration can be effected by many factors like the biomaterial used as an implant, storage methods, type of machining, surface texture, surgical procedure, bone quality and quantity and prosthesis design [2].

Titanium and titanium alloy is one of the biomaterial that used in dental implant. Due to its properties like lightness, tolerance and not toxic and mechanical properties make it desirable for used it in the oral cavity as implant [3].

Titanium surfaces undergo a progressive change in their biologic characteristics after processing, resulting in a significant decrease in osseointegration capability. The vitro bioactivity of 4-week-old titanium surfaces showed only 20%–50% of the levels of attachment, settlement, and proliferation of osteogenic cells versus new surfaces. [4-7], finally the bone-implant osseointegration become less than 50% after storage of the titanium implants for 4 weeks, [4]. Thus, the storage method of implant after manufacturing till to implant in the oral cavity is critical in maintaining the titanium bioactivity.
Organic impurities (hydrocarbons) adsorb on to titanium from the atmosphere (air), water, and cleaning materials and solutions. [8]. Gradually, titanium surfaces became covered by these hydrocarbons [4]. Such accumulations of hydrocarbons were found on aging titanium surfaces regardless to surface topography [5].

This research was aimed to study the osseointegration of commercial pure titanium screws stored in air or vacuum condition with/without UV light treatment mechanically and histologically.

2. Materials and Methods

Screw preparation:
Sixty-four screw shaped implants were machined from the Cp Ti bar using lathe machine. The screw length was 8mm (3mm flat part and 5mm threaded part) and 3 mm in diameter. The height and width of the pitch was 1mm to fit the screwdriver during insertion and removal. [9].

Storage methods for the samples
Two storage methods were used:
1) Vacuum storage methods: implant screws were placed into sealed vacuum package (nylon polyethylene, Turkey) with average area of 75 Cm² and thickness 0.5 mm. at a vacuum condition of (-1 Bar) by using an automatic vacuum pump (German), and stored for 4 weeks before implantation into rabbits as shown in figure (1A).

2) Air storage methods: by using sterilizing pouches (China) with average area of 80 Cm² (at temperature 25C ± 2, humidity 50 % ±5). The implant screws were stored for 4 weeks before being implanted into rabbits as shown in figure (1B).

After packaging of the screws for the air or vacuum storage method, they were sterilized by gamma radiation and then stored for the intended period of time for each group.

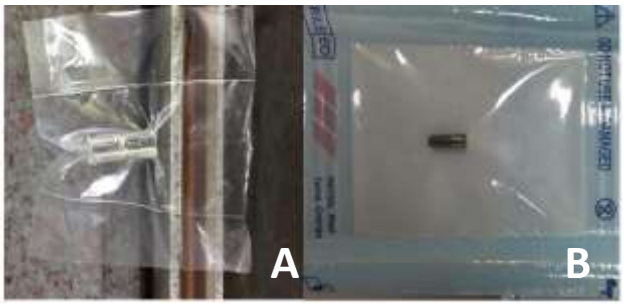

Figure 1: A: Screw stored in vacuum package, B: Screw stored in air

UV- light sample treatment:
Titanium screws (modified air and modified vacuum group) were treated with UV light source for 15 min. with wavelength 360 nm and 250 nm by a single source of a UV lamp (SANKYO DENKI, Japan), immediately before implantion into bone, **[**10**]**.

Grouping the implant samples
Sixty-four screws were divided into two groups according to the test performed:

48 Screws for mechanical test (torque removal test) were divided into:
1) Air group: 12 screws (6 screws for each healing interval - 2 and 4 weeks).
2) Modification Air group: Exposed to UV light at the time of implantation, 12 screws (6 screws for each healing interval - 2 and 4 weeks).
3) Vacuum group: 12 screws (6 screws for each healing interval - 2 and 4 weeks).
4) Modification Vacuum group: Exposed to UV light at the time of implantation, 12 screws (6 screws for each healing interval - 2 and 4 weeks).

16 screws for histological test were divided into:
1) Air storage: 4 screws (2 screw for each interval - 2 and 4 weeks).
2) Modification air group: Exposed to UV light at the time of implantation, 4 screws (2 screws for each healing interval - 2 and 4 weeks).
3) Vacuum group: 4 screws (2 screws for each healing interval - 2 and 4 weeks).
4) Modification vacuum group: Exposed to UV light at the time of implantation, 4 screws (2 screws for each healing interval - 2 and 4 weeks).

Sixteen healthy adult male New Zealand rabbits weighing 1.5 - 1.75 kg (10-12 months of age) were used. Three days before operation, subcutaneous ivermectin injection (0.2 ml) was given to eradicate parasite infection. Intramuscular injection of an antibiotic (ceftriaxone) was given once daily (0.5ml) for 3 days to avoid any infection.

The rabbits were divided into two groups for 2 and 4 weeks healing periods, 8 rabbits in each group. Two screws were implanted in both tibiae of each rabbit. General anesthesia was given to the animal by intramuscular injection of xylazine (0.7 ml/kg Body weight) and ketamine 10% (0.5 ml/kg Body weight).

Both tibiae were shaved and the skin was disinfected with alcohol and iodine. The incision was made on the medial side, the skin and fascia were reflected, and blind dissection was made to the muscle to expose the medial side of the tibia bone. A round bur of 1.3 mm in diameter was used for hole preparation. Two holes were made with a 1cm distance between them. The penetration was done by intermittent pressure at a rotary speed of 800 rpm and reduction ratio of 16:1, and continuous irrigation with normal saline for cooling. Then the holes were enlarged gradually with fissure burs to 2.8 mm. The air storage screw was implanted in the first hole and fixed in place via a screw driver. The air modified screw was set in the second hole, this was for the left tibia, while in the right one the first hole was implanted with the vacuum storage screw and the second hole was implanted with the modified vacuum screw. Suturing of muscle's fascia was done with resorbable polydioxanone suture and the skin was sutured with silk suture. An antibiotic (ceftriaxone 0.5ml per day) was given for five days postoperatively to exclude infection.

1. Mechanical testing (Torque test)
At end of healing intervals (2 and 4weeks) torque measurement was performed by digital torque meter (TQ-8800, Taiwan) after supporting the bone to prevent any movement that may affect the test accuracy. After the screwdriver of the torque meter was engaged in the slit of the implant head, a torsional force was exerted for unscrewing the implant and the value was measured in Newton/ centimeters (N.cm).

2. Histological testing
At day of scarification bone around the implant was cut by a disc cutter via prosthetic engine with straight hand piece (Marathon motor, Korea) with slow speed of rotation and normal saline irrigation. Bone-implant block was obtained by cutting about ½ cm away from the implant screw. The blocks were prepared for routine histological examination.

3. Results

Mechanical testing
The removal torque values of the implant screws after the 2 weeks are shown in (Table 1). After this period, vacuum with UV light treated implants needed the highest torque values to remove them (mean value 17.92 N.cm), vacuum and air

storage with UV light treated implants needed high torque values to remove them (mean value 13.8 -14.5 N.cm), while air storage implants needed lesser torque values (mean value 8.4 N.cm).

Table 1: Mean torque values of all implant groups for both healing periods (N.cm)

Types	Time	N	Mean	S.D.	S.E.	Min.	Max.
Air only	2Weeks	6	8.4	± 0.73212	1.79332	6.40	11.50
	4Weeks	6	11.4	± 2.4956	1.01882	8.3	14.40
Air+UV	2Weeks	6	14.5	± 1.19024	2.91548	11.00	18.50
	4Weeks	6	21.5333	± 2.95409	1.20600	17.5	25.00
Vacuum	2Weeks	6	13.8	± 1.00167	2.4535	10.50	17.00
	4Weeks	6	22.5	± 2.68328	1.09545	19.00	26.00
Vacuum + UV	2Weeks	6	17.92	± 2.2106	0.902474	15.62	21.87
	4Weeks	6	26.47	± 3.40373	1.38957	22.50	31.00

In the Multiple Comparisons of healing periods for the test groups using ANOVA the air storage test group showed an insignificant difference with time while for all the other groups the difference was highly significant (Table 2).

Table 2: Multiple Comparisons of healing periods for the test groups using ANOVA

Test groups	Healing periods				ANOVA statistics		
	2 Weeks		4 Weeks				
	Mean	SE	Mean	SE	F	df	Sig.
Air only	8.400	1.084	11.400	1.084	3.832	1	0.057
Air+ UV	14.500	1.084	21.533	1.084	21.064	1	0.000
Vacuum	13.800	1.084	22.500	1.084	32.230	1	.000
Vacuum +UV	17.920	1.084	26.470	1.084	31.128	1	.000

In the Multiple Comparisons of subgroups within groups using Least Significant Difference (LSD), there was a highly significant difference at $p<0.01$ for the 2 and 4 week healing periods when the air storage group was compared with the other groups, while there was an insignificant difference at $p > 0.05$ for the two healing periods when the vacuum storage screw group was compared with air storage treated with UV light. In the comparison of the vacuum storage screw treated with UV light with the vacuum storage screw group and the air storage screw treated with UV light a significant difference at $p<0.05$ was observed for the two healing periods, except when air storage treated with UV was compared with vacuum UV, where there was a highly significant difference (Table 3).

Table 3: Multiple Comparisons of subgroups within groups using Least Significant Difference (LSD)

Healing periods	Test groups	Test groups	MD	SE	Sig.
2 Weeks	Air only	Air+ UV	-6.100	1.532	0.000
		Vacuum	-5.400	1.532	0.001
		Vacuum +UV	-9.520	1.532	0.000
	Air+ UV	Vacuum	0.700	1.532	0.650
		Vacuum +UV	-3.420	1.532	0.031
	Vacuum	Vacuum +UV	-4.120	1.532	0.010
4 Weeks	Air only	Air+ UV	-10.133	1.532	0.000
		Vacuum	-11.100	1.532	0.000
		Vacuum +UV	-15.070	1.532	0.000
	Air+ UV	Vacuum	-0.967	1.532	0.532
		Vacuum +UV	-4.937	1.532	0.003
	Vacuum	Vacuum +UV	-3.970	1.532	0.013

4. Histological Findings

A. Two weeks after implantation

1- Air storage screws

The histological feature of air storage group in the thread area after two weeks of implantation shows bone deposition, osteoblasts, and fat cells. (Figure 2).

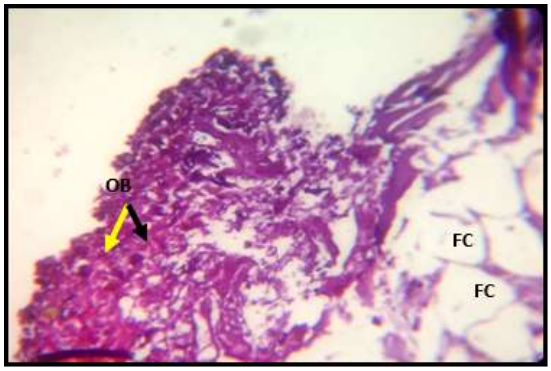

Figure 2: Microphotograph view of air storage group in thread area shows bone deposition, osteoblast (OB) and fibrous tissue and fat cell (FC) (H and E stain) X40.

2- Air treated with UV CP Ti implant screws after 2 weeks

After 2 weeks of implantation of screw stored in air and treated with UV light, its histological view shows thread area filled with marrow tissue, and fat cells, (Figure 3).

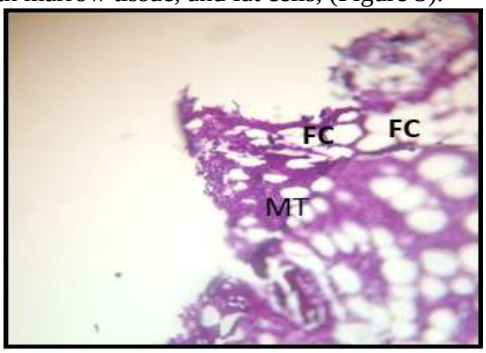

Figure 3: Histological view of air storage treated with UV light group after 2 weeks shows thread area filled with marrow tissue (MT) and fat cell (FC), H&E stain X20.

3- Vacuum storage screws

Histological feature of vacuum storage group shows screw space and threads that follow the shape of the screw with deposition of new bone at peripheries osteoblasts and fat cells (Figures 4).

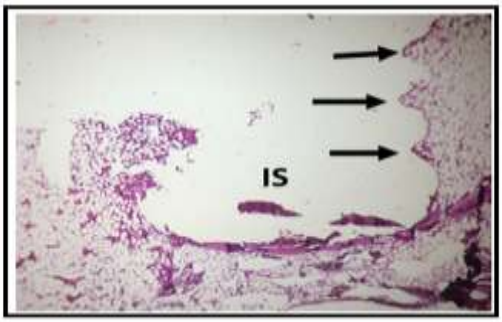

Figure 4: View of 2 weeks healing periods of vacuum storage group shows implant space (IS) and thread regions (arrows) H&E X10

4- Vacuum treated with UV screws

Histological findings of vacuum treated with UV implants after 2 weeks' duration shows bone formation at thread region with osteocytes (Figures 5).

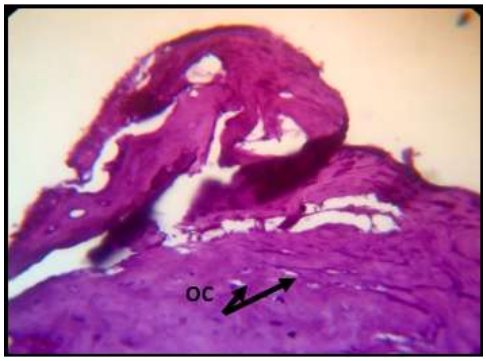

Figure 5: View of vacuum treated with UV implants after 2weeks shows immature bone in thread region filled with osteocytes (OC). (H & E stain) X40.

A. Four weeks after implantation

1- Air storage screws

The histological view of air storage screw after four weeks shows deposition of bone at periphery of the thread site with numerous fat cells in marrow tissue (Figures 6).

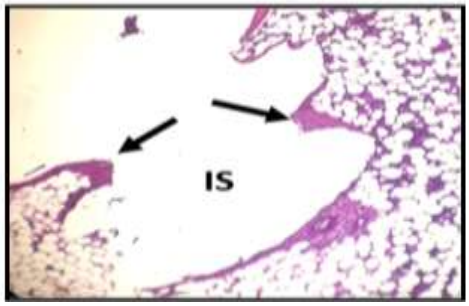

Figure 6: View of air storage group after 4 weeks shows implant space (IS) shows bone deposition at apex of threads (arrow) and fat cell(FC). H&E X10

2- Air treated with UV screws

After 4 weeks of implantation of screw that stored in air and treated with UV light, the histological view shows thread region filled with mature bone enclosing osteocytes and arranged around harversian system (Figure 7).

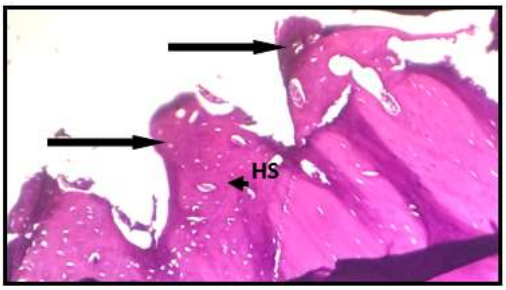

Figure 7: Histological section of a thread (arrows) of air treated with UV light storage group after 4 weeks duration shows mature bone enclosing osteocytes(OC) surrounding harversian system(HS). H&E X20

3- Vacuum storage screws

Histological feature of vacuum storage group shows mature bone at thread region and haversian system surrounded by osteocytes (Figures 8).

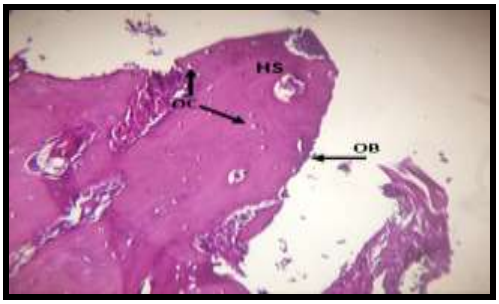

Figure 8: Magnified view of vacuum storage group shows mature bone at thread region, osteocytes (OC) arranged around haversian system (HS) and osteoblast(OB). H&E X20

4- Vacuum treated with UV screws

Histological view of vacuum treated with UV screws after 4 weeks duration shows mature bone at thread region. Osteocytes embedded in bone and arranged around harversian system (Figure 9).

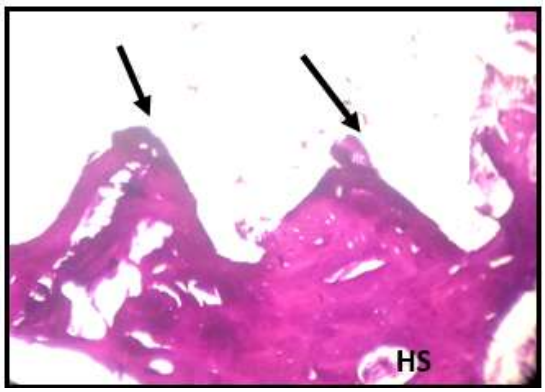

Figure 9: View of 4 weeks duration of vacuum treated with UV light storage group shows thread region (arrows), osteocyte (OC) arranged around harversian system (HS). H&E X10

5. Discussion

In vivo experiment

The rabbit is convenient for the study because it reaches skeletal maturity around 6 months of age, rapid cortical bone remodeling allows for evaluation of osseointegration of dental implants as early as 6 weeks compared with 18 weeks in human [11]. The age of the animals that used in this study was from 10-12 months thus assuring complete closure of proximal tibia epiphysis, as stated by Pearce et al. [12].

Mechanical tests

Effect of storage methods on torque removal value after 2 and 4 weeks of implantation

The storage method significantly influenced the physicochemical properties and bioactivity of the titanium surface. When the screws were stored in air, that meant they were in contact with air and the organic impurities like hydrocarbon from air adsorbed to the surface of the screw [13, 14].

The adsorption of the hydrocarbon on the surface limited the activation of the bioinert titanium surface and the bioactivity and osseocoductivity of titanium surface decreased by time. [4, 15, 16] Titanium surface wettability (hydrophilicity) decreased with increase of the surface hydrocarbons. [17]

Most studies stated that hydrophilicity enhanced cell adhesion, proliferation and differentiation, and bone mineralization at an early stage [18,19]. According to the Cassie- Baxter regime [20], the presence of air entrapment in the micropores on hydrophobic surfaces resists the contact of the solution which could have a negative effect of the surface contact area in the rough surfaces, inhibiting protein adsorption [21]. The adsorption of proteins on implant surfaces is essential because it can affect the early biological response of the surrounding microenvironment, which has an effect on the healing process as well as the final clinical outcomes of implants [22,23].

Rivera-Chacon et al. [24], proved this point by finding that increased cell attachment and proliferative capacity occurred on titanium surfaces with more fibronectin adsorption, [24]. Results of this study revealed that screws stored in air had a hydrophobic surface with lower torque mean than screws that were stored in vacuum (away from air contact). It was demonstrated that the biomechanical strength between bone–implant connection decreased with time of storage till it was less than 50% after storage of the implant screw for 4 weeks in air [4].

Hydrophilic surfaces promote titanium to interact with cells, biological fluids, and tissues [25,26]. Super hydrophilic implants can optimize the osseointegration even more as they have proved useful to magnifying the area of bone-to-implant contact and strengthen mechanical fixation in the early healing processes of at least the first 4 weeks after implantation [26]. This may explain the present findings where the vacuum storage Cp Ti screws recorded a higher mean value of torsional force than air storage screws after 2 and 4 weeks of implantation. This means vacuum storage allowed more bone formation in which bond strength of the bone-implant interface was increased and was in agreement with some previous researches, which showed increased protein adsorption on hydrophilic specimens [27,28].

The vacuum storage of the screws increased the torque removal value and the wettability which prevented the hydrocarbon accumulation from the air and increased the osseointegration.

Effect of UV light (photo functionalization) on torque removal value after 2 and 4 weeks of implantation

In this study the vacuum storage Cp Ti screws treated with UV light recorded a higher mean of removal torque value than air storage screws that treated with UV light after 2 and 4 weeks of implantation. This means vacuum storage treated with UV light increased bone-implant interface.

After storage for 4 weeks in air and vacuum, mean values of torque removal test of vacuum was more than air, but after treated the screws with ultra-violate light there was increase of mean values for both screws.

The values of the torque test of the screws stored in air and treated with UV light increased. This was due to the effect of UV light which could effectively clear the hydrocarbons. This finding was supported by a previous study which stated that the osteoblast activity is influenced by the degree of hydrocarbon contamination on titanium implants and the hydrocarbon decomposition before implant placement may increase the biocompatibility of titanium, [29].

Highly significant difference between mean values of torque removal test between 2 and 4 weeks was detected in this study, except the implant screw that stored in the air only showed non-significant differences. This may be due to increase in osseointegration in the vacuum, air treated with UV and vacuum treated with UV groups than air groups and these findings agreed with Hayashi et al. (2014) [29].

High significant difference in mean values between the air group and others, which may be due to hydrocarbon accumulation on the screw surface and decrease in osseointegration, while the other groups prevent the hydrocarbon by preventing the air from contact with screw by vacuum or could be related to the UV light effect.

6. Histological Findings

Histological examination of bone sections after 2 weeks duration showed thin bone trabeculae at thread regions rimmed by osteoblasts and numerous osteocytes entrapped into bone, in air treated with UV light, vacuum and vacuum treated with UV light groups while in air storage group the

results showed fibrous tissue and fat cells at threads regions, in agreement with Shukur, 2015,[30]

Regarding storage in vacuum and air treated with UV light groups bone deposition was detected only at the apex of thread region indicating that degree of bone deposition and maturation varied between groups could be due to either the method of storage or the used UV light.

Mature bone enclosing osteocytes regularly arranged around harversian system was detected with vacuum treated with UV light group and this may due to syrngestic effect of uses of vacuum and UV light together which seems to be confirmed by the results of mechanical test (torque removal test).

At 4 weeks duration, the histological findings of air treated with UV light, vacuum and vacuum treated with UV light groups showed dense mature bone at the thread regions with osteocytes appeared regularly distributed around harversian system in the thread regions, and this is may be due to prevention of the effect of hydrocarbon on the titanium surface. Concerning histological finding of the air storage group, bone deposition was noticed only at peripheries of threads which agrees with result of a study conducted by Waheed, 2013.[31].

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