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## **Enhancement the Biological Behavior of Titanium Dental Implants by Laser Pulses Treatment**

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**Abstract:** The long-term success of dental implants largely depends on rapid healing with safe integration into the jaw bone. Geometry and surface topography are crucial for the short and long-term success of dental implants. This work aim to enhancing clinical performance of titanium dental implants by laser Pulses treatment to provide bone in a faster and improved osseointegration process. The results show that using different manufacturing processes (machining and powder technology) produced topographical differences. The topographical change observed from powder technology method was more than the machined one. Also Strong titanium oxide layer was observed after laser pulsed resulted in improving surface roughness and topography and it was the method of choice for complex surface geometries providing energy focused on one spot especially in the inside of implant thread. The release of Ti ion rise in first three days and after that released of Ti ions begins to stabilize after laser treatment. Finally, the histological view of implant samples after 4weeks of implantation, showed active bone formation in all implant surface which give clear indication of tissue acceptance and the appearance of mature bone was observed after laser treatments at short implantation periods.

**Keywords:** dental implants, osseointegration, powder technology, laser pulsed, histological view.

### **Introduction**

The long-term success of dental implants largely depends on rapid healing with safe integration into the jaw bone. Geometry and surface topography are crucial for the short and long-term success of dental implants. Implant surfaces have been developed in the last decade in a concentrated effort to provide bone in a faster and improved osseointegration process. Osseointegration, defined as a direct structural and functional connection between ordered, living bone and the surface of a load-carrying implant, is critical for implant stability, and is considered a prerequisite for implant loading and long-term clinical success of endosseous dental implants. Osseointegration of titanium implant surfaces is dependent upon both physical and chemical properties (Johansson et. al., 2005). Surface properties of biomaterials are important parameters influencing cellular reactions towards artificial materials. The properties of dental implant surfaces are extremely important in influencing the healing process leading to osseointegration and ultimate clinical success of the implant. Surface morphology modulates the response of cells to a dental implant, and surfaces with defined microstructures may be useful for enhancement of the stable anchorage (Al Hasani, 2020).

These modifications may subsequently influence conformational changes in the structures and interactive natures of adsorbed proteins and cells. Furthermore, within the complexities of an in vivo environment containing multiple protein and cellular interactions, these alterations may differentially regulate biological events. Modifications to the implant surface chemistry may lead to alterations in the structure of adsorbed proteins and have cascading effects that may ultimately be evident at the clinical level (Kilpadi et. al., 1994).

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The laser technology for surfaces preparation already has numerous industrial applications. This process results in titanium surface microstructures with greatly increased hardness, corrosion resistance, a high degree of purity with a standard roughness and thicker oxide layer. Biological studies evaluating the role of titanium ablation topography and chemical properties showed the potential of the grooved surface to orientate osteoblast cell attachment and control the direction of ingrowth. A number of laser-based techniques have been applied (Al Hasani, 2020). Besides the prompt intense heating of the surface, excimer laser illumination may further enhance the sterilising effect in consequence of the high dose in the UV range. The laser technique has several advantages, add no chemicals and can be used in routine manufacturing. Only the valley and parts of the flank of the implant threads was laser treated while the remaining part was left as-machined (Cooper, 2000). The idea behind this design is that the flank portion of the implant thread, which might have the higher risk to expose to the Microorganism and plaque, is characterized by a relatively smooth surface to minimize the incidence of peri-implant, whereas the valley part of the implant threads has the rougher surface (Frenkel et. al. 2002). Manufacturers recommend the use of Nd: YAG laser for soft tissue periodontal procedures such as debridement of diseased epithelial linings, gingivoplasty, crown lengthening, vestibuloplasty, gingivectomy and reduction of drug-induced gingival hypertrophy (Cochran, 1999; Eriksson et. al. 2001). Many of these procedures involve removal of soft tissue in areas adjacent to teeth which may have amalgam restorations in close proximity to the working area. Accidental laser exposure to such restorations is a distinct possibility. With the development of modern medical lasers; laser therapy has gained an increasing role in the wide spectrum of treatment modalities. Also in oncology, laser techniques have become interesting alternatives in radical tumor resection and to palliative tumor treatment methods. Due to the great variability of induced tissue reactions from microsurgical precise coagulation and cutting to voluminous coagulation or tumor vaporization, the Nd: YAG laser is the most important surgical laser (Bauerle, 2000; Palmquist, 2010).

The possibility of transmitting its light through flexible fibers allows wide variation of applications and tissue effects. Even if this laser is mostly known for its ability of volume coagulation, which is due to its large optical penetration depth, it shows a reaction depth which can be varied in the widest range of all medical lasers by using the appropriate parameters (Cernavin et. al., 1999; Daikuzono et. al., 1985). Many research focus on improvement of titanium surface by laser treatment In 2003 M. Bereznaia et al. modified the titanium surface by excimer laser irradiation of titanium samples in order to improve their surface characteristics so as to facilitate biointegration, and to enlarge the effective interfacial area of bone–implant contact, holes were ablated by laser pulses of ns or sub-ps length. X-ray photoelectron spectroscopy analysis of the laser-polished titanium surface revealed that laser treatment led to a decrease in surface contamination and in thickening of the oxide layer (Bereznaia et. al., 2003). In 2006, Milan Trtica et al. used the interaction of an Nd:YAG laser, operating at 1064 or 532 nm wavelength and pulse duration of 40 ps, with titanium implant. The surface damage thresholds were estimated to be 0.9 and 0.6 J/cm<sup>2</sup> at wavelengths 1064 and 532 nm, respectively. The titanium implant surface modification was studied by the laser beam of energy density of 4.0 and 23.8 J/cm<sup>2</sup> (at 1064 nm) and 13.6 J/cm<sup>2</sup> (at 532 nm). Generally, both laser wavelengths and the corresponding laser energy densities can efficiently enhance the titanium/implant roughness. This implant roughness is expected to improve its bio-integration (Trtica et. al., 2006). In 2008 Rafael Silveira Faeda et al. evaluated by using a biomechanical test, the force needed to remove implants with surface modification by laser (Nd:YAG) in comparison with implants with machined surfaces. Surface characterization showed that a deep and regular topography was provided by the laser conditioning, with a great quantity of oxygen ions when compared with the MS (Faeda et. al. 2009).

## **Method**

### **Samples Preparation**

Preparation of implant samples involved using two different methods in order to make comparison between them and to study the effect of manufacturing process on the surface roughness which may affect the biological behavior of the implant in the body by elimination of healing period (rapid osseointegration). The first method involved the use of commercial pure titanium rod with high purity (99.8 %). While the second method included the use of powder technology in order to produce the implant screws. In this method the raw material used was pure titanium powder (from Aldrich Chemical Company) purity (99.8%). the compaction process accomplished by hydraulic press machine with maximum applied load 15 ton for five minutes and then the load released gradually. The samples were then removed carefully from the die resulting compacted rods with 20 mm in diameter were then sintered in a furnace (local manufacturing) under controlled atmosphere using argon gas at 1000°C for two hours. After the sintering process the resulting samples with diameter (20 mm) then converted into a pin shape with diameter (3mm) using a wire cutting machine. The final implants screws were obtained by

machining the titanium pins using lathe. The final implant samples whether produced from commercial pure Ti powder or rod have the dimensions; 2.8 mm in diameter ,8mm in length.

### Laser Pulses Surface Treatment

The titanium implant surface was activated by using laser pulses. The technique generates short pulses of light of single wavelength, providing energy focused on one spot. It is rapid, extremely clean, and suitable for the selective modification of surfaces. The Nd:YAG system was employed type (Nd:YAG LASER ,made in Korea maximum power 1000mJ,pulse duration 6ns). The implant Samples were irradiated by focusing the laser beam using a quartz lens of 12 cm focal length. During the irradiation process, the laser was operated in the fundamental transverse mode with power energy 1000mJ, pulse duration 6ns, at frequency 2Hz and wave length 1064nm. The angle of incidence of the laser beam with respect to the surface was 90°. The irradiation was carried out in air, at standard relative humidity. Finally, the resulting implant samples were organized as shown in (Table 1).

Table1. Implant samples numbers

Sample	Number
Sample produced from Ti powder	1
Sample produced from Ti rod	2
Sample produced from Ti powder treated with laser	3
Sample produced from Ti rod treated with laser	4

### Testing and Characterization

Experimental work also involved implant testing and characterization that have been done after laser surface treatment procedure to examine implant samples and to ensure the safety performance of the implant inside the body in which the implantation process have been done, the characterization involves; microstructure observation using scanning electron microscope (SEM), surface chemical composition analysis using Energy-dispersive spectroscopy EDS. The EDS device was coupled with SEM and the inspection was done at the same time of SEM observation. the topographic change after the surface treatments as well as the amount of roughness using Atomic Force Microscope (AFM), ion release test. The metal ion release from the implant material occurs as a direct consequence of the corrosion process. Release of metal ions can cause local and systemic health problems, due to the ions diffusion through the whole body. The ion release of titanium implant samples was performed by static immersion test in which The samples were exposed to a corrosive solution with minimum relative motion between samples and solution. Each sample was placed in a separate container with a medium that simulates biofluids. Hank'ssolution. The samples incubated at 37°C using a water path and thermostat. The immersion time used was 7 days; certain amount of solution was removed every day to conduct the release of ions. The type and concentration of ions released from the metallic implant materials was determined using Inductively Coupled Plasma optical emission spectrometry ICP-OES. Finally, vivo testing in which twenty males of New Zealand rabbit weighing 2-2.5 kg were used. The age of them was from 10 -12 months. The rabbits were kept in standard cages and had free access to tap water, and fed with standard pellets. Before the implantation surgery the implants were sterilized by placing each two implants in a single air tight plastic sheet then the implants were autoclaved at 121°C and 20 bars for 30 minute, also all instruments were autoclaved at 121°C a20 bar for 30 minute.



Figure1. The tibia bone

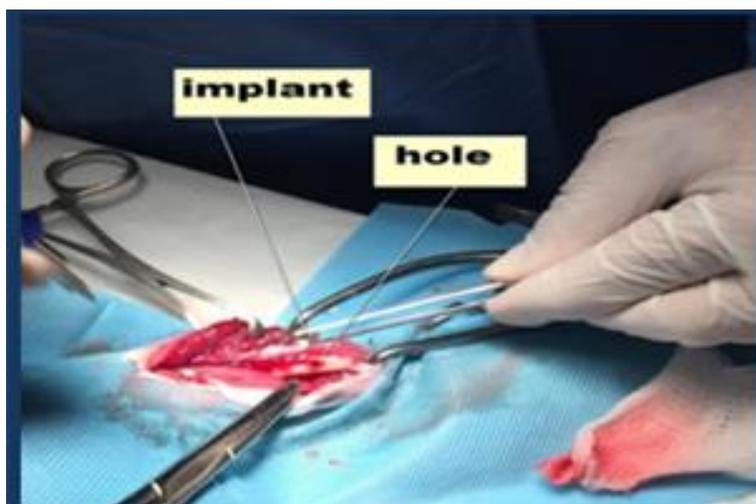


Figure2. Hole in bone.

Anesthesia was induced by intramuscular injection of Ketamine hydrochloride 50mg (1ml/kg body weight) Xylazine 20 % (0.1 ml/kg body weight). The surgery was performed under sterile and gentle surgical technique. Both tibias of each rabbit were shaved from the inner side and the surgical towels were placed around the operation site, then the skin was cleaned with alcohol and iodine then a piece of cotton was damped with iodine and left on the shaved skin for five minutes. Incision was made on the medial side of the legs about 3 cm in length to expose tibia bone the skin and fascia were reflected the periosteum was carefully reflected as shown in (Figures1, 2).

Drilling was done using round bur with intermittent pressure and continuous cooling of normal saline at a rotary speed of 1500 RPM and reduction torque 16.1. The enlargement of the hole was made gradually with spiral drill from 2.2 mm to 2.5mm till a hole with about 2.7mm was obtained as shown in (Figure 2). The operation site was cleaned with copious amount of saline to remove bone sheared then the implant was removed from the plastic sheet and placed in holes with slight spiral movement until 5mm was completely inserted in bone as shown in (Figure 3) finally histological testing with optical microscope have been done.

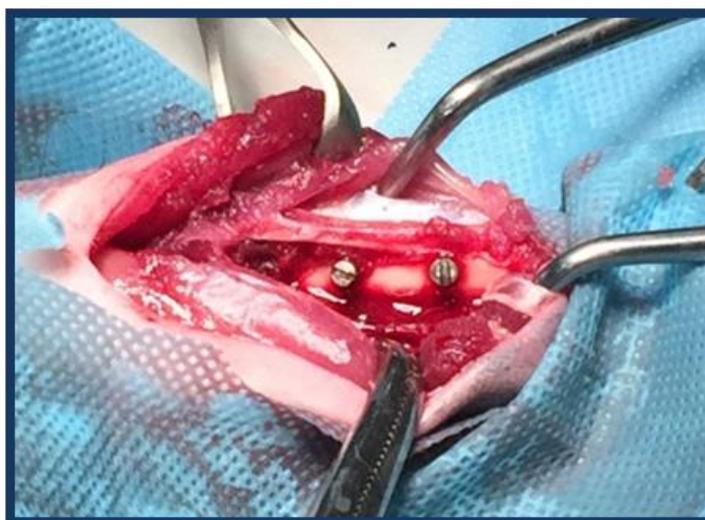


Figure3. Implants in tibia.

## Results and Discussion

### Microstructure Characterization

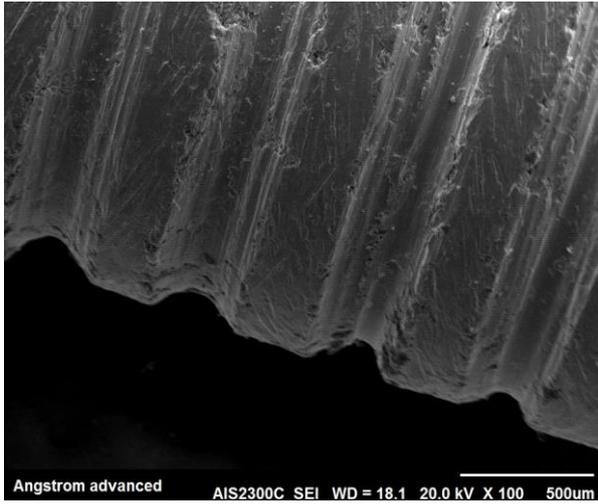


Figure 4. SEM image of sample 1.

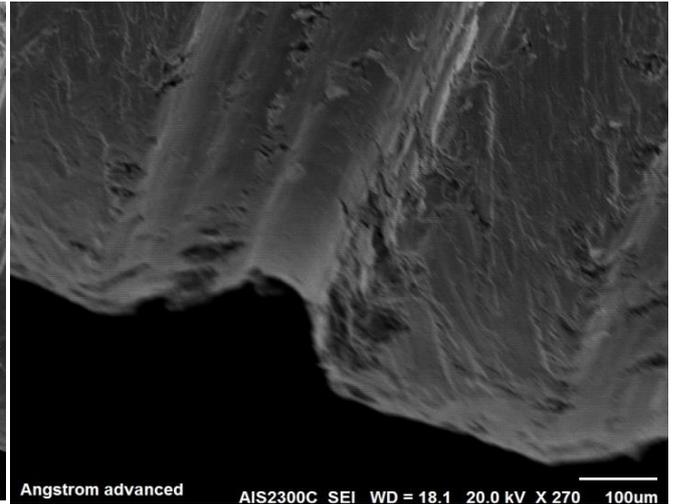


Figure5. SEM image of sample 1 in other magnification.

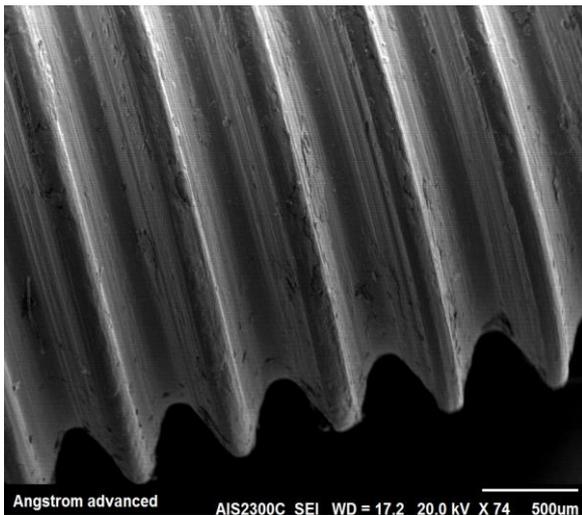


Figure 6. SEM image of sample 2.

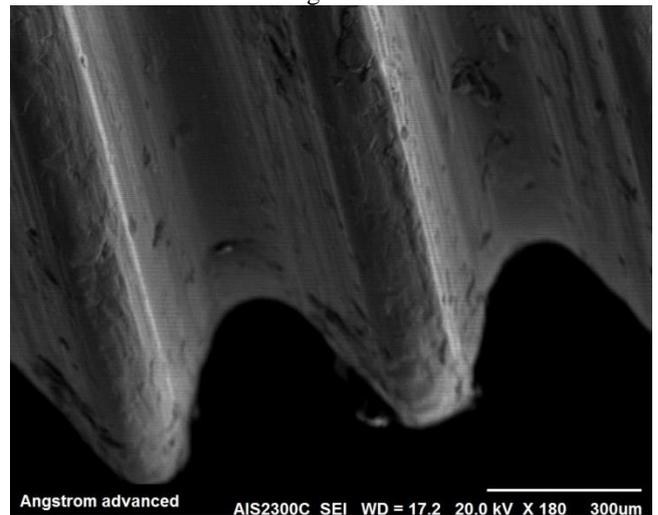


Figure 7. SEM image of sample 2 in other magnification

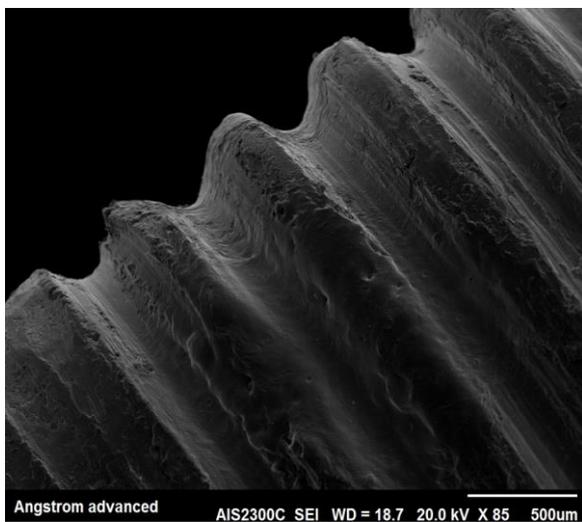


Figure 8. SEM image of sample 3.

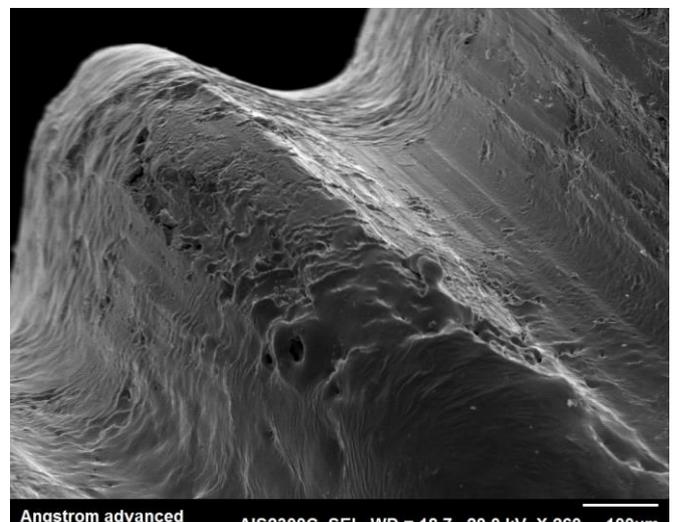


Figure 9. SEM image of sample 3 in other magnification

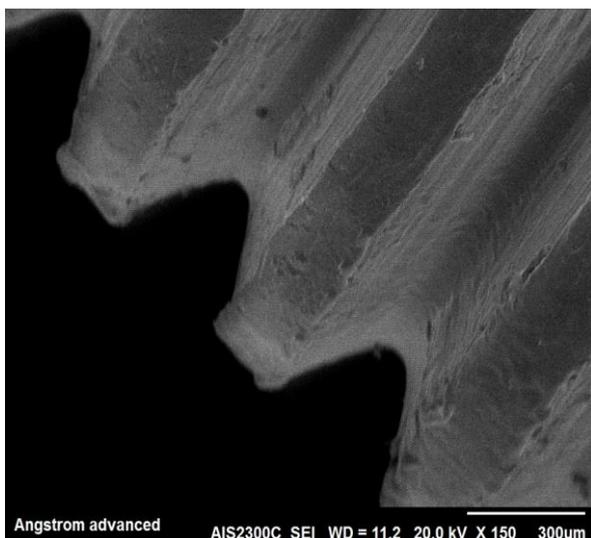


Figure 10. SEM image of sample 4.

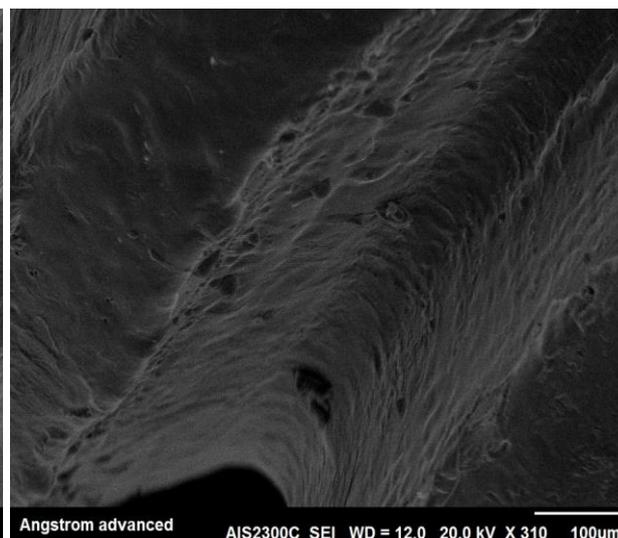


Figure 11. SEM image of sample 3 in other magnification

Scanning electron microscope was conducted implant samples, (Figures 4 and 5) show the surface morphologies of sample (1) and sample (2), these two samples differ in manufacturing process but both of them are without laser treatment. No morphological differences were observed among the implants surfaces except for some considerable morphological differences as a result of the manufacturing process. For sample (1) produced by powder technology it obvious to have a degree of porosity which result in roughen surface than sample (2) which was produced by turning. The tool marks created by the turning process made the surface anisotropic with clear directional surface irregularities. Therefore, it was possible observe that sample (2) surface similar to some commercial dental implants available from various companies. The microstructure observation of the laser group demonstrate that the laser pulses energy used ( $1\text{J}/\text{cm}^2$ ) was appropriate to make the surface damages and the laser beam created a deep and regular morphological pattern as seen in (Figures 8, 9, 10 and 11) which illustrated the SEM image of samples (3 and 4). It is clearly recognized that the laser pulses have caused remarkable surface topographical changes, From (Figures 8, 9) of sample (3) easy to distinguish the laser pulses effects on the samples surface topography than the (Figures 10, 11) of sample (4). This is because sample (3) was produced by powder technology which resulted in more initial surface roughness and topographical irregularities than the other methods. Laser pulses can be altered to be more effective to change the surface topography under given laser pulse energy. The benefit of using laser treatment for implant samples prepared by powder technology process is to facilitate the creation of microstructural surface roughness in the inner part of implant's thread. It is believed that the inner part of thread is more in bone formation than the outer part. From the microstructure observation of laser samples it is concluded that the technique is a suitable choice for complex surface geometries. The technique generates short pulses of light of single wavelength, providing energy focused on one spot especially in the inside of implant thread. It is rapid, extremely clean, and suitable for selective modifications of surfaces and allows the generation of complex microstructures/ features with high resolution.

### Energy-dispersive Spectroscopy EDS

Elemental analysis or surface chemical characterization of implanted a sample have been made before and after surface treatments using interaction of some sources of X-ray excitation and the sample by energy - dispersive spectroscopy (EDS). This test was done in order to identify the effect of laser treatments on the implant surface chemistry. EDS graph of the samples (1, and 4) in (Figures 12 and 13) show a large peak of titanium element without any peaks of other elements which refer to samples with high degree of purity and the manufacturing process will not affect or result in a change of the surface chemical composition. Elemental analysis of the titanium sample treated with laser showed that oxygen contents are high, the increasing in oxygen content implied that possible oxidation of the titanium sample took place. Presence of titanium oxide on the implant surface can be considered very desirable for many reasons, firstly the composition of the oxides, including their distribution, can resist corrosion of the implant sample secondly the titanium oxides can influence implant surface adhesion properties and finally the oxidation of a titanium implant can lead to local hardening and improvement of the wear resistance. There are some differences observed in the EDS graphs. These are due to the using of different manufacturing process that has been employed. Finally it is found that manufacturing

process (powder metallurgy) resulted in increasing oxygen content at the surface and therefore improving the oxide layer that have been formed after laser pulses.

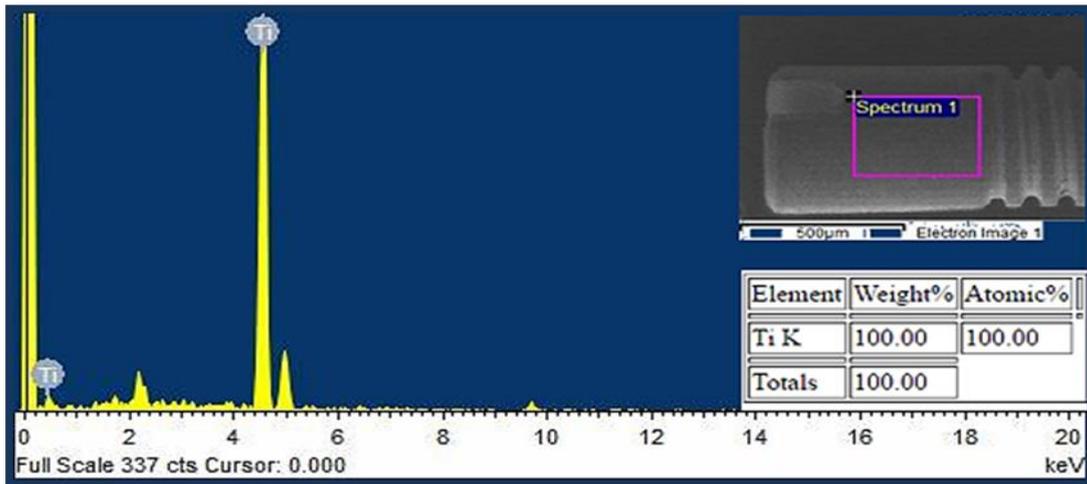


Figure12 .EDS graph of sample (1).

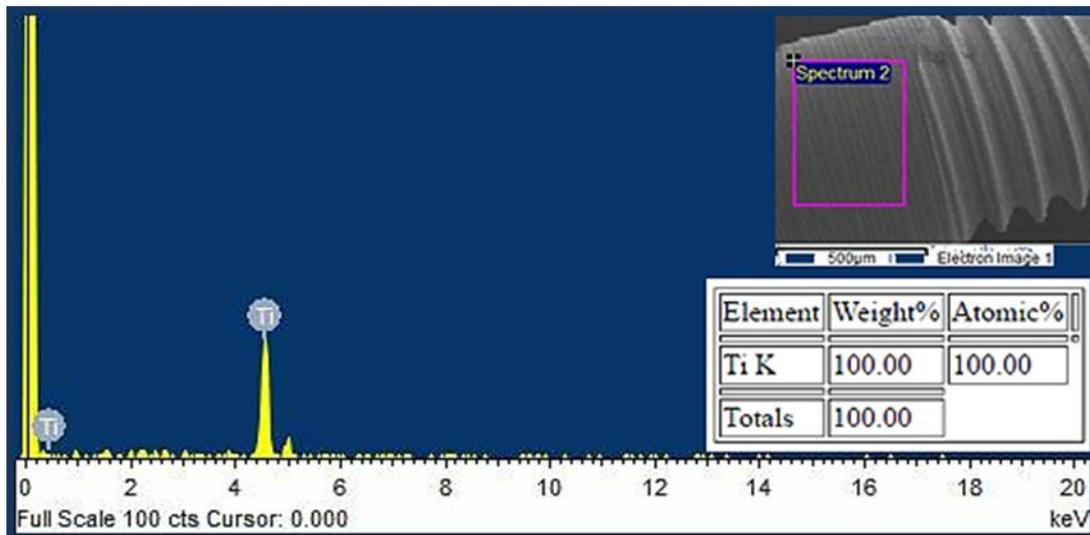


Figure13. EDS graph of sample 2.

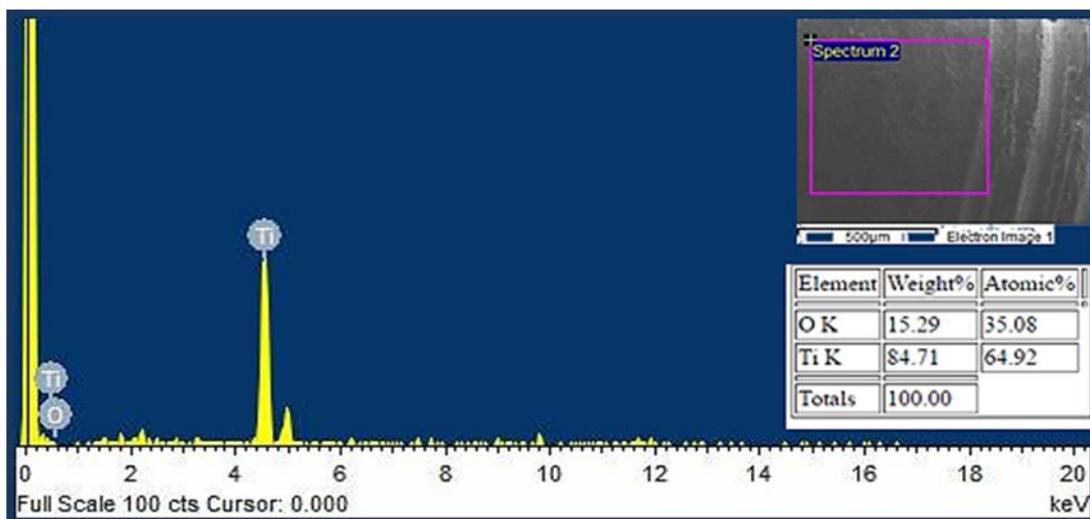


Figure14. EDS graph of sample 3

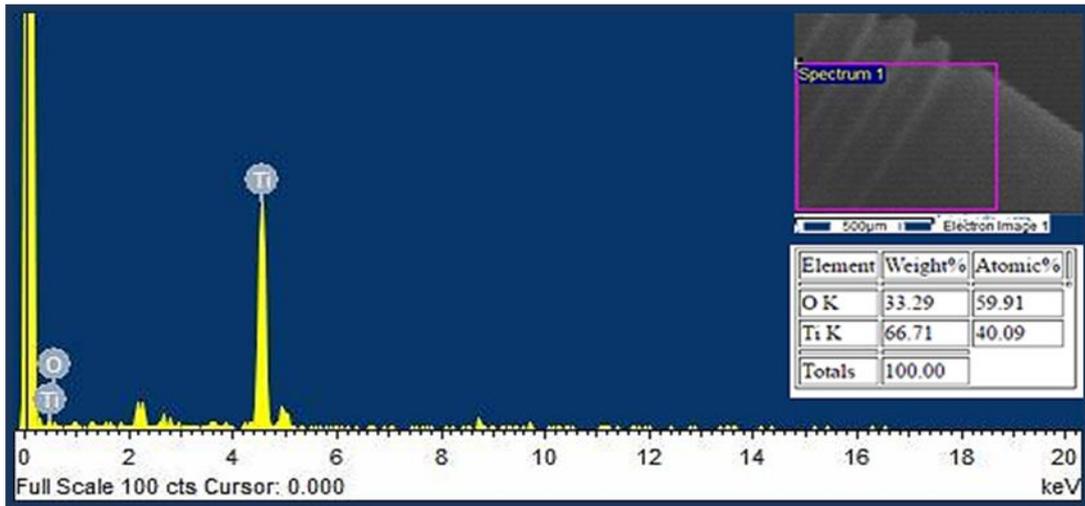


Figure15. EDS graph of sample 4

**Surface Roughness:**

Surface roughness has been identified as an important parameter for implants and its capacity for being anchored in bone tissue. Surface topography was displayed by Atomic-force microscopy (AFM) in which images of three-dimensional shape (topography) of a sample surface at a high resolution was obtained. This is achieved by scanning the position of the sample with respect to the tip and recording the height of the probe that corresponds to a constant probe-sample interaction. (Figure 16) displays the roughness value of the sample (1). This sample was produced by powder technology process without any surface treatment have higher roughness (845.36 nm) than sample (2) in (Figure 17) , produced by machining without any surface treatment (531.7nm). Hence the powder technology process produced samples with higher surface roughness compared to the machining process. The use of laser pulses will also effect on the surface roughness of implant samples as illustrated in (Figures 18 and 19) shows that the samples changed their roughness values either raised or reduced after the laser pulses. This is due to the formation of a strong oxide layer refer that all samples have the same response to the laser irradiation and the slight differences in the roughness values were due to the change in surface heating rate that resulted from the manufacturing process employed which caused a considerable change structural surface properties.

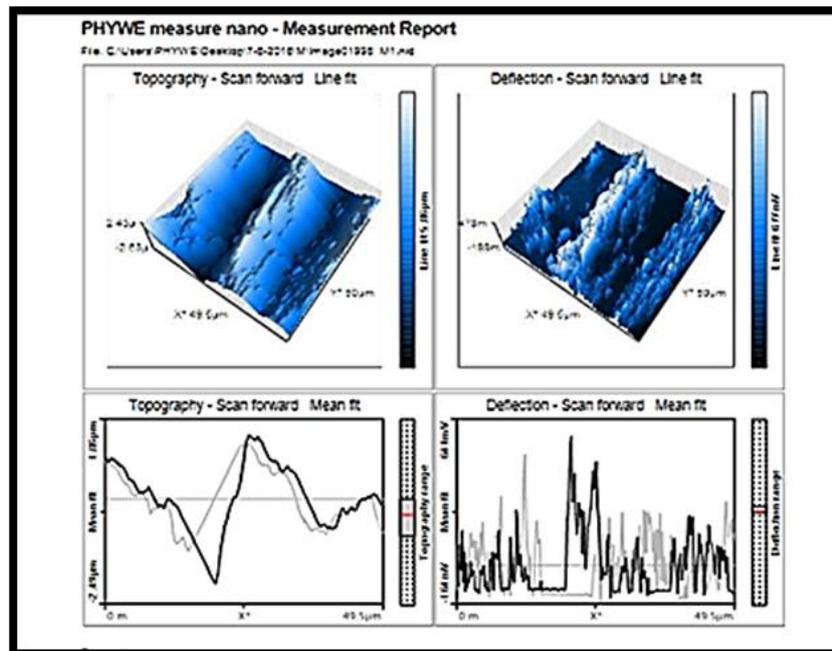


Figure 16.AFM chart of sample 1.

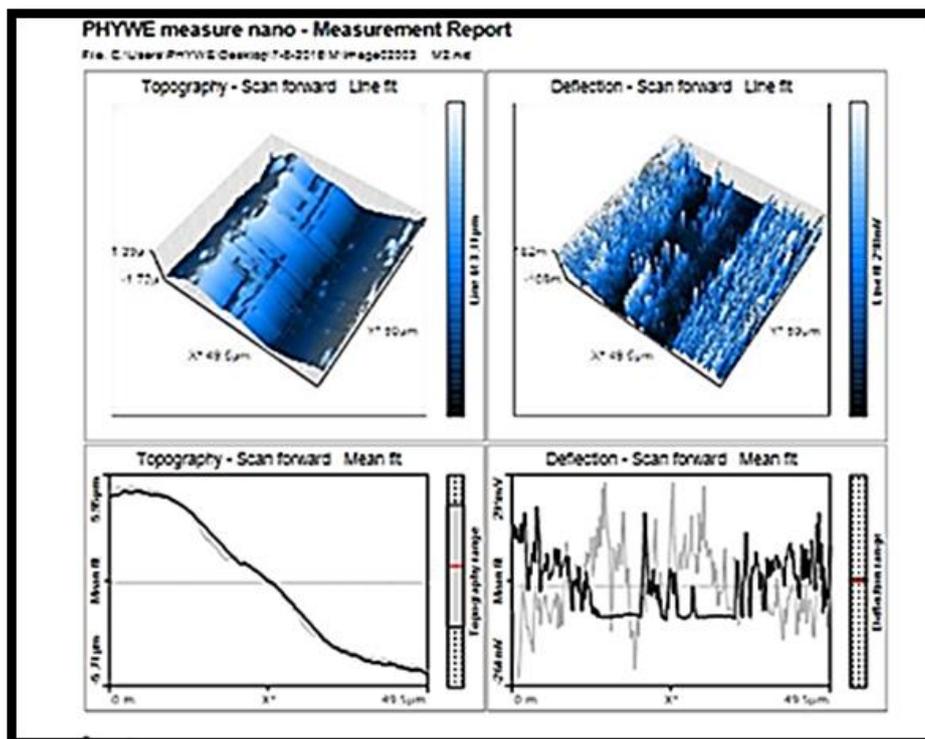


Figure17.AFM chart of sample 2.

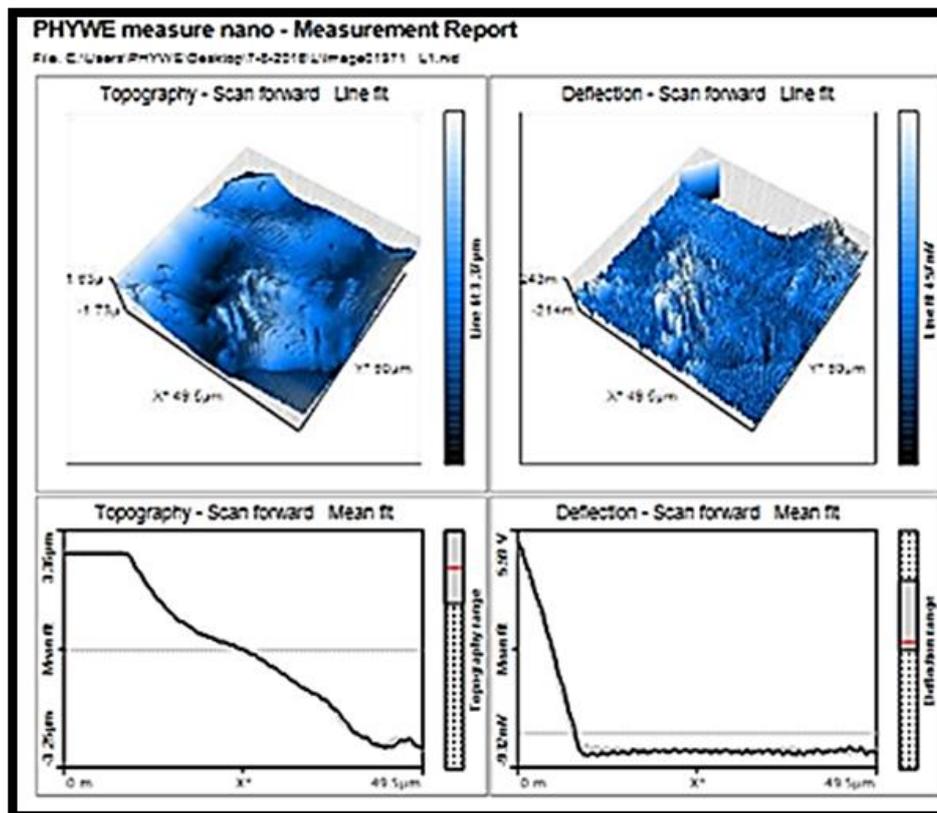


Figure 18.AFM chart of sample 3.

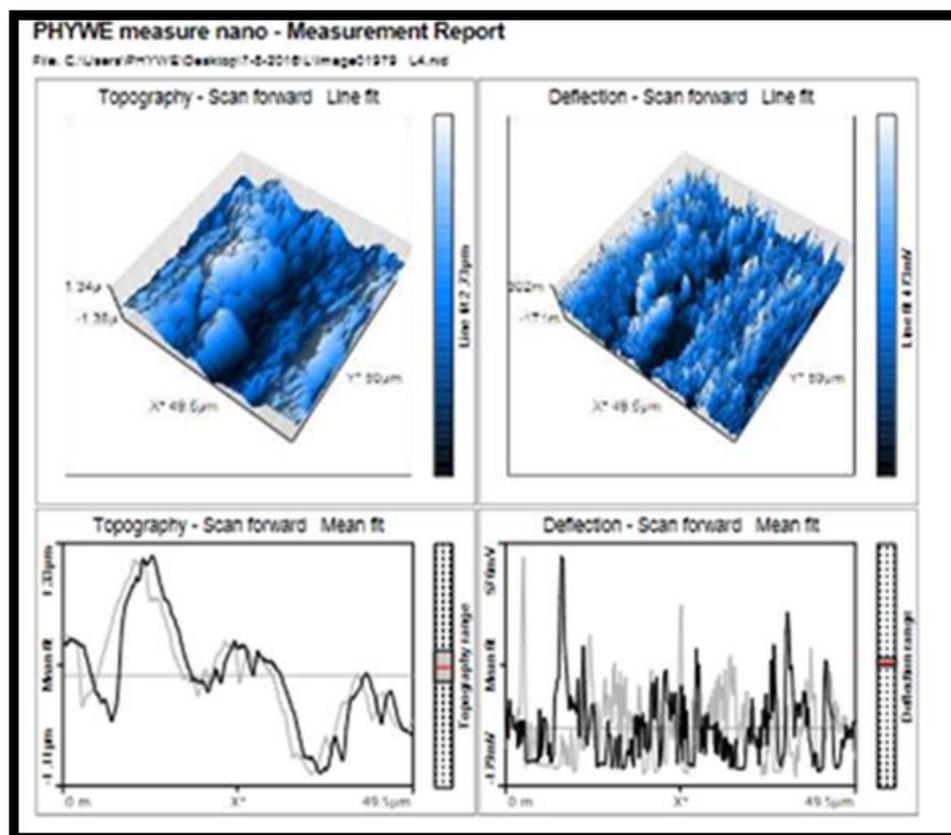


Figure 19. AFM chart of sample 4.

### Ion Release Analysis:

Ions are released from implant materials as a direct consequence of the corrosion process. Release of metal ions can cause local and systemic health problems due to the ions diffusion through the whole body. The amount of Titanium ions that have been released from all samples in Hank's solution was measured. It was found that the surface oxide films on titanium implant play an important role as an inhibitor of ion release also the regeneration time of the surface oxide film after disruption governs the amount of released ion. Low concentration of dissolved oxygen, inorganic ions, proteins, and cells may accelerate the metal ion release. It is obvious that the behavior of metal ion release into biofluid is governed by the electrochemical rule and the released metal ions do not always combine with biomolecules to show toxicity because the active ions immediately combine with a water molecule or an anion near the ion to form an oxide, hydroxide, or inorganic salt. Thus, there is only a small chance that the ion will combine with biomolecules to cause cytotoxicity, allergy, and other biological influences and this was not observed in the current titanium samples. From the results of ion release analysis, it was found that all samples in all groups have similar ion release behavior when the samples are immersed in Hank's solution for seven days as illustrated in (Figure 20), it is observed that the release of Ti ion rise in first three days and after that release of Ti ions begin to stabilized. This was due to the fact; when the metal is immersed inside the body consequently their ions begin to be released from the surface by adsorption process. At the same time these ions will combine with other molecules in the environment and desorb at other surface so that the amount of released ions is increased with increasing immersion time until the adsorption- desorption equilibrium is reached thus the amount of released ion will be fixed.

### Histological Analysis

Modifications of the implant surface have benefits regarding the response of the surrounding bone tissue, accelerating the healing process and improving the quality of the newly formed bone. Osseointegration is related to micro geometric features, such as the degree of surface roughness, and to factors such as the physical and chemical properties of surfaces. Rough surfaces were found to stimulate osteoblastic gene expression and to enhance bone formation and bone implant fixation. From The histological view of implant samples after 4weeks

of implantation, it was found that there are active bone formations in all implant surfaces which gives clear indication of tissue acceptance. (Figure 21) shows histological view of sample (1) prepared by powder technology method without surface treatment and shows new bone trabeculae (BT) filled thread area and reversal line separate between old and new bone which refers to the new bone have been formed in a thread region as shown in (Figure 22).

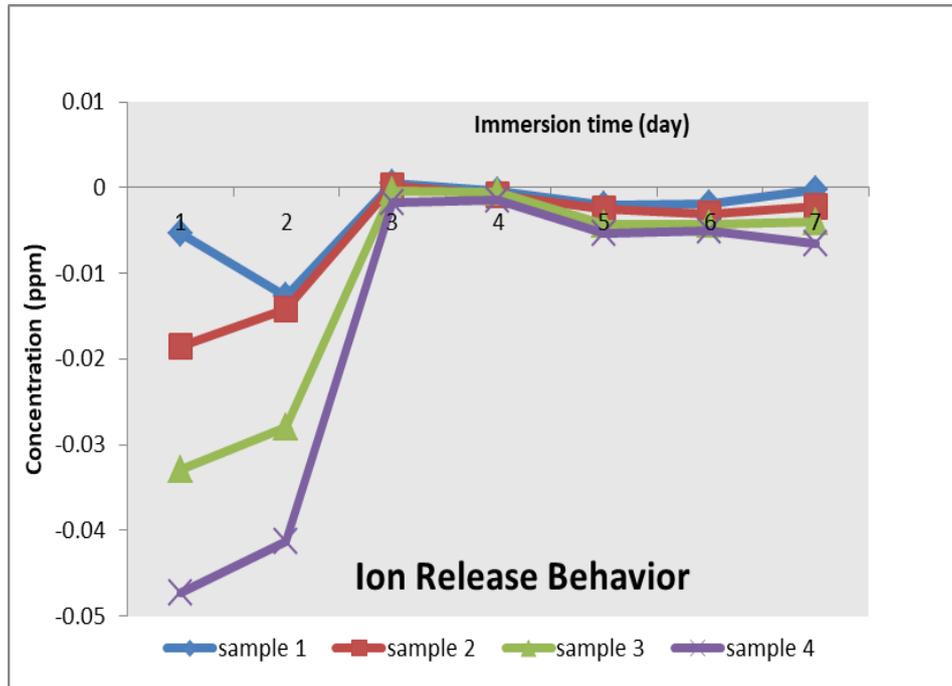


Figure.20. Release amounts of Ti ions in Hank's solution.

While in sample (2) prepared by machining process as shown in (Figure 23) more bone trabecula formation is seen with large number of osteocyte preosteocytes (POC) and blood vessels (BV) as shown in (Figure 24). This indicates that the deposition of bone was still continued and therefore the bone formation in sample (1) was faster than sample (2) due to the differences in production method. Thus the powder technology method will produce a porous surface which yielded greater bone formation than smoother one due to their larger area in contact with the bone tissue as well as it present intercommunicating porous structure allowing the formation of three dimensional osseointegration networks. When the implant sample was laser pulsed, a strong oxide layer will form on the implant surface resulting in improving the adhesion between the implant surface and bone thus good osseointegration was obtained. This was observed in samples (3) and (4). The microstructural view of sample (3) which produced by powder technology method then treated with laser show dense bone filled thread area as in (Figure 25) bone trabeculae filled thread area with large osteocytes (OC) surrounded by osteoblasts (OB), and osteoclasts (OCL). While, in sample (4) the microstructural view shows mature bone filled thread area with small osteocytes (OC), osteoblasts (OB) lined Haversian canal (HC). The appearance of mature bone in the (3) rather than (4) is due to the stability of oxide layer in sample (3) which was more than sample (4). It is largely affected by the surface nature that resulted from the manufacturing process. From the laser treatment it was found that increasing in oxide layer stability result in the improving osseointegration as well as enhancing the bone formation process.

## Conclusion

Using different manufacturing processes (machining and powder technology) produced topographical differences. The topographical change observed from powder technology method was more than the machined one. Strong titanium oxide layer was observed after laser pulsed resulted in improving surface roughness and topography and it was the method of choice for complex surface geometries providing energy focused on one spot especially in the inside of implant thread. The release of Ti-ion rise in first three days and after that released of Ti-ions begin to stabilize. The histological view of implant samples after 4 weeks of implantation, showed active bone formation in all implant surface which give clear indication of tissue acceptance and the appearance of mature bone was observed in polymer coated samples at short implantation periods

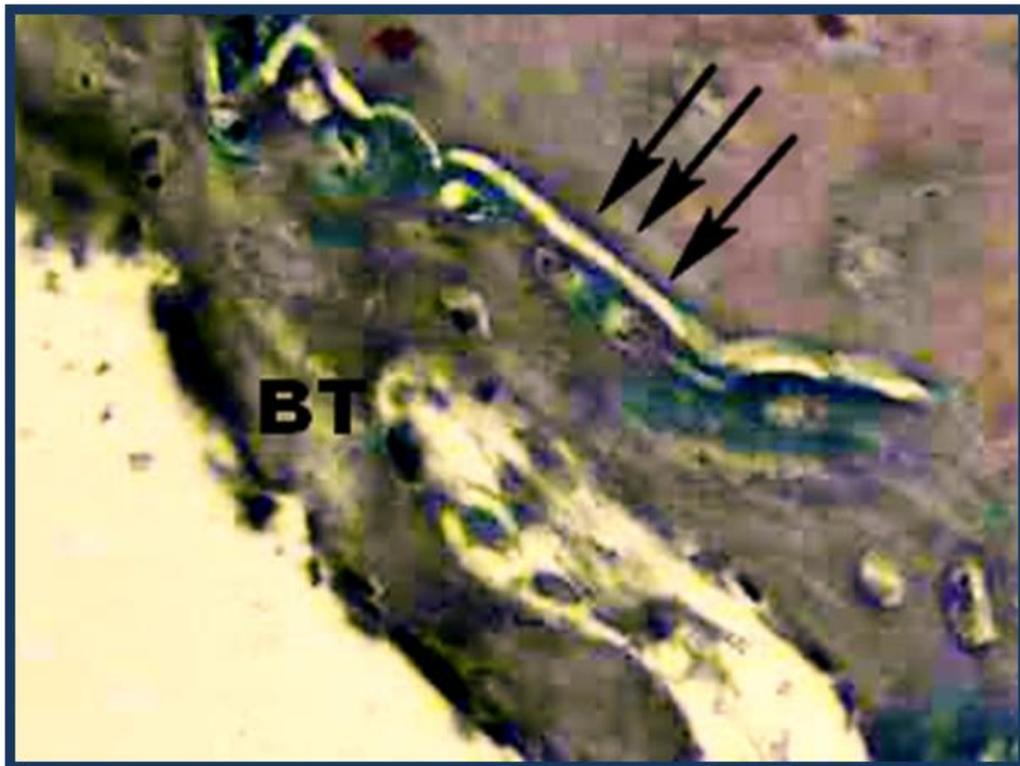


Figure21. Histological view of sample (1) show new bone trabeculae (BT) filled thread area and reversal line separate between old and new bone (arrows).H&E X10

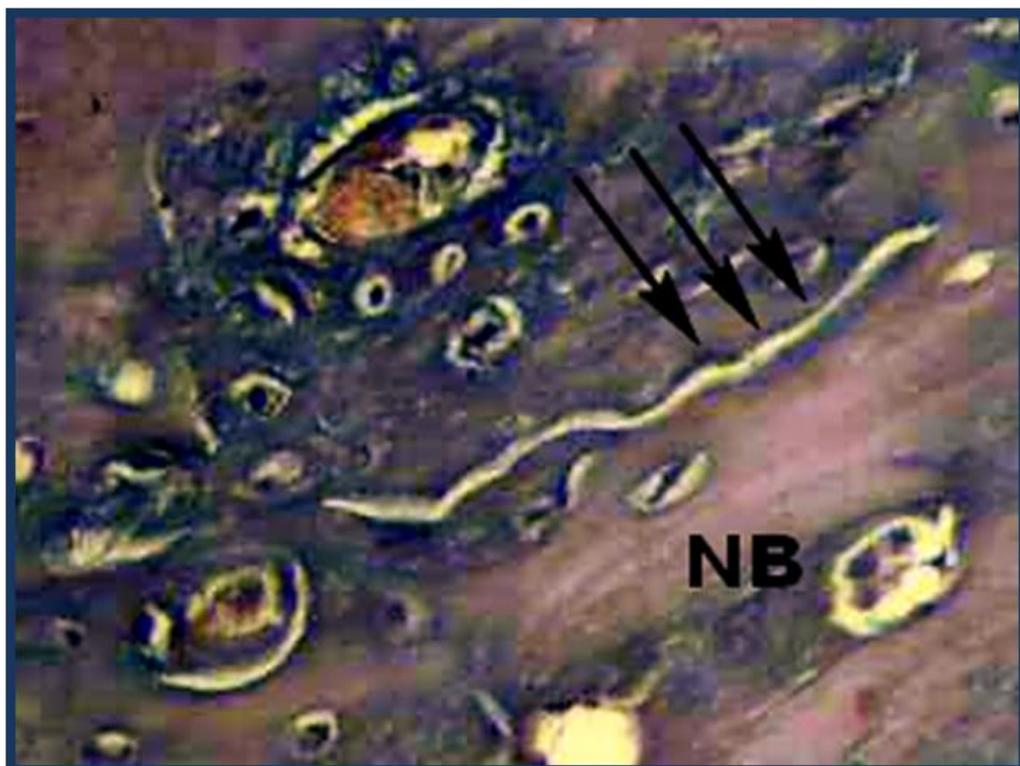


Figure 22. Magnifying view of sample (1) show new bone formation (NB) in thread region which separate from old bone by reversal line (arrow). H&E X40.

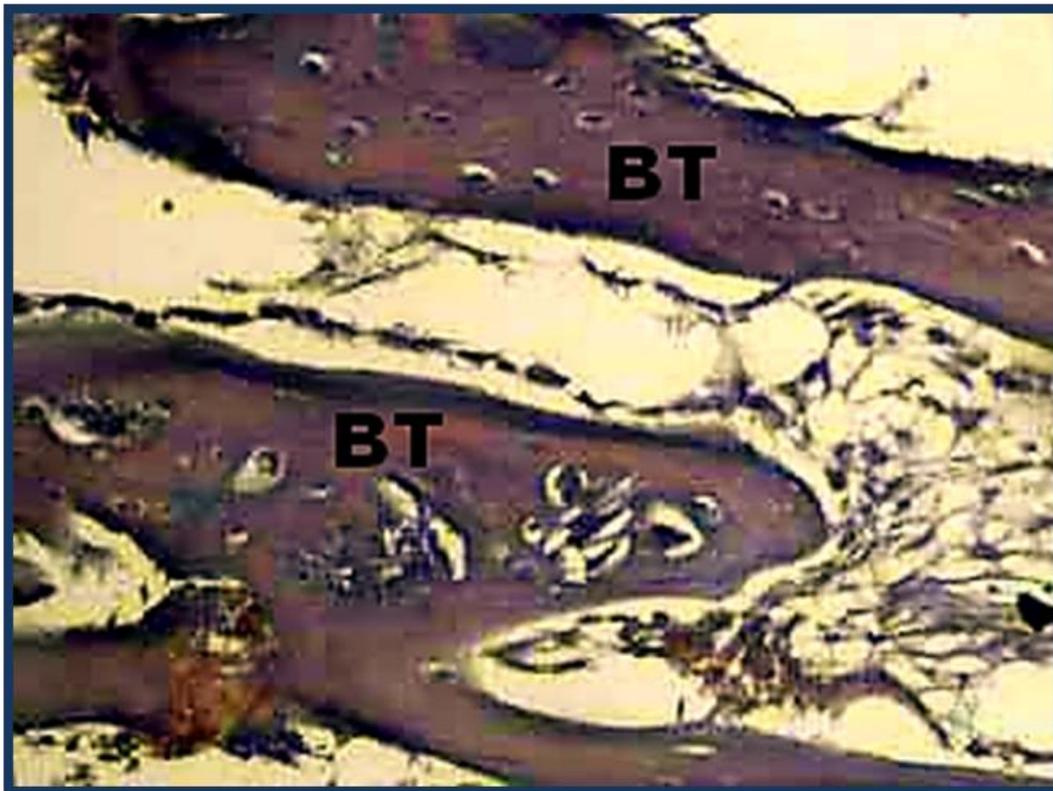


Figure 23. Histological view of sample (M4) show new bone trabeculae (BT) in thread region . H&E X20

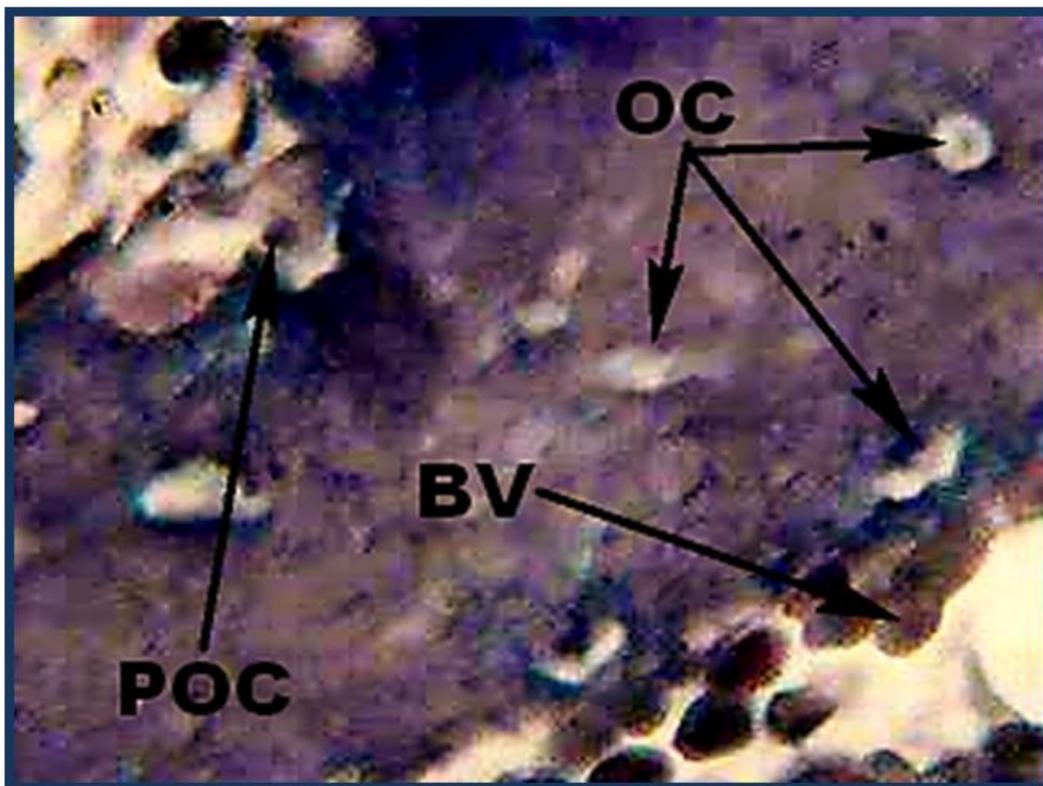


Figure 24. Magnifying view of sample (M4) show new bone formation with osteocytes (OC) , preosteocytes (POC) and blood vessels (BV) .H&E X40

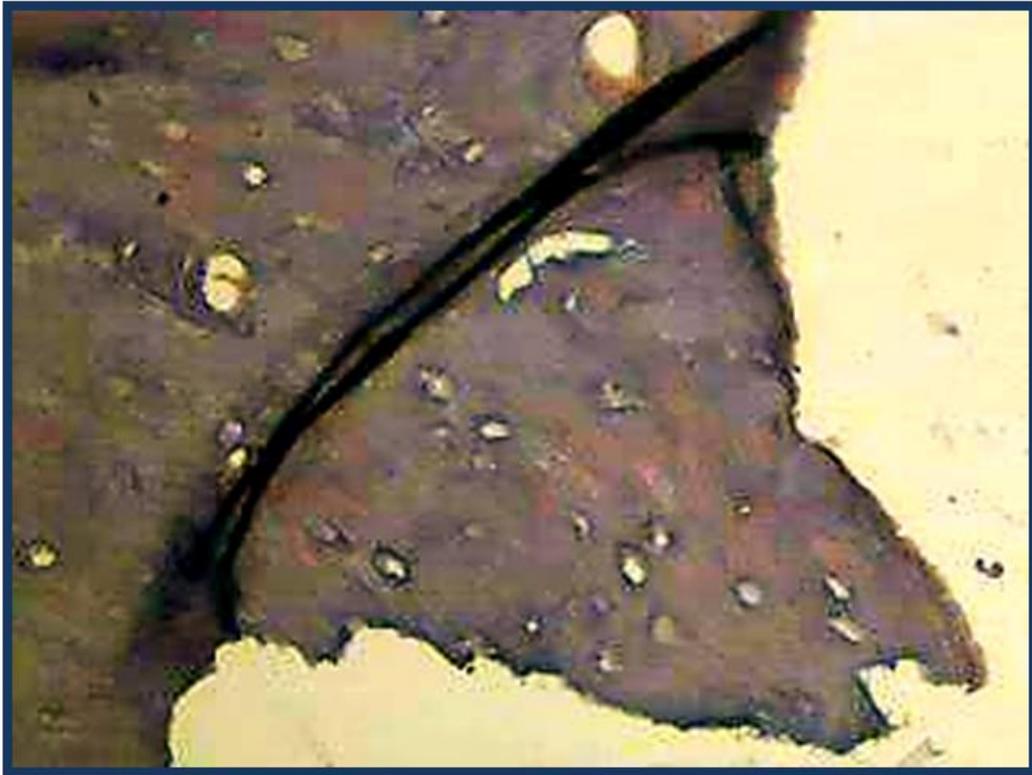


Figure 25. Histological view of sample (L1) show dense bone filled thread area .H&E X20

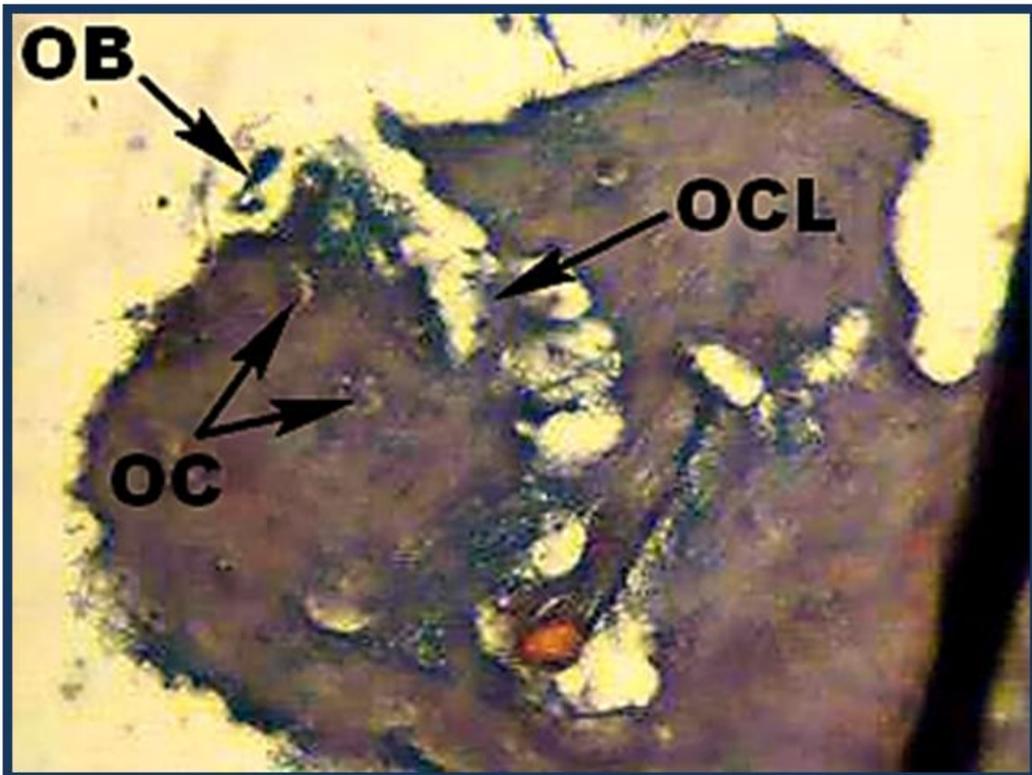


Figure 26. Histological view of sample (L1) show bone trabeculae filled thread area with large osteocytes (OC) surrounded by osteoblasts (OB) ,and osteoclasts (OCL).H&E X20



Figure 27. Histological view of sample (L4) show v .H&E X10

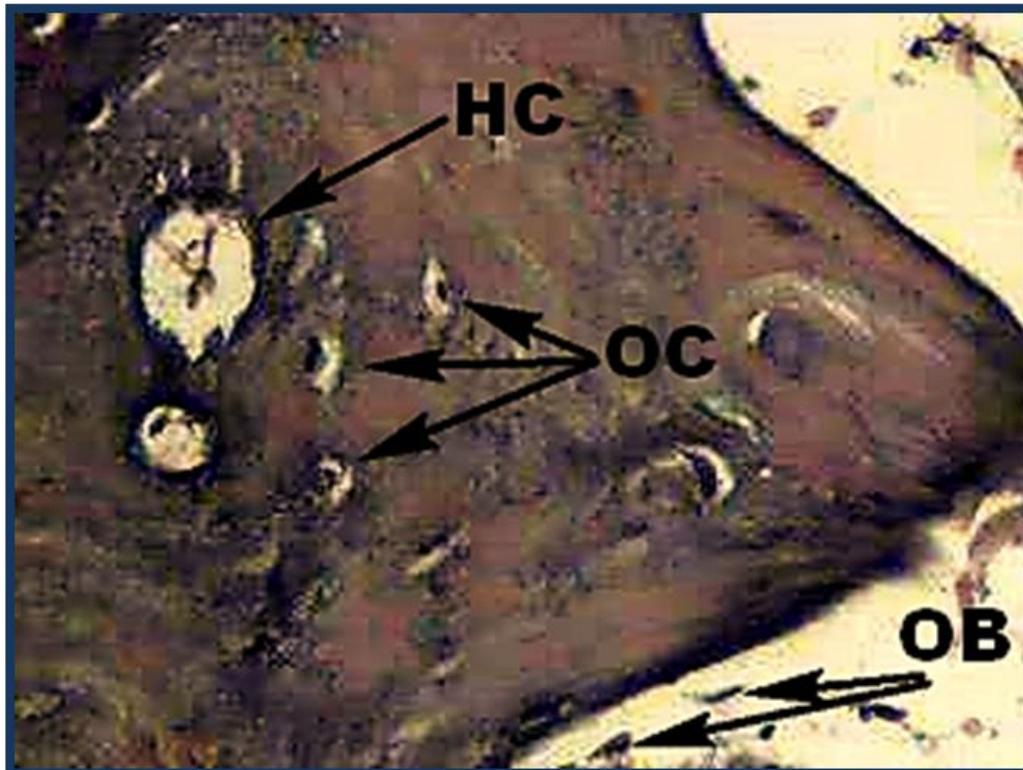


Figure 28. Magnifying view of sample (L4) show small osteocytes (OC) , osteoblasts (OB) lined Haversian canal (HC) .H&E. X20

## Recommendations

After possible results gets from the experimental work, many recommendations may be possible for future; like using laser pulse as surface activation process for other types of metallic implant, using the laser treatment to precipitate elements or oxides at the surface of implant samples.

## Scientific Ethics Declaration

The authors declare that the scientific ethical and legal responsibility of this article published in EPSTEM journal belongs to the authors.

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