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Application of laser modified polyether ether ketone in dental implantoloy in vitro and in vivo

A Thesis Submitted to the Institute of Laser for Postgraduate Studies, University of Baghdad in Partial Fulfillment of the Requirements for the Degree of Master of Science in Laser / Dentistry

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بسم الله الرحمن الرحيم

﴿ وَمَا تَوۡفِيقِيٓ إِلَّا بِٱللَّهِۚ عَلَيۡهِ تَوَكَّلۡتُ وَإِلَيۡهِ أُنِيبُ ٨٨ ﴾

صدق الله العلي العظيم

سورة هود : الآية ﴿ ٨٨ ﴾

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Abstract

Background: Dental implant is a unique treatment modality to substitute missing dentition. Polyetheretherketone (PEEK) could replace titanium as dental implant, but its surface need to be modified to improve healing in bone-implant interface to enhance osseointegration for successful healing and decreased healing time.

Aim of the study: In vitro study to estimate the preferable Fractional CO_2 laser parameters to induce surface modification of PEEK surface by using light microscope, scanning electron microscope, EDS, AFM and contact angle test. In vivo study to evaluate the effect of Fractional CO_2 laser treatment of PEEK implant screws on implant-bone interface after 2 and 6 weeks following implantation in rabbit femur bone by torque removal test and histological examination.

Material and method: PEEK discs were prepared with (10mm diameter and 2mm thickness) and irradiated by Fractional CO_2 laser in different power, pulse duration, spot distance and number of scans to induce surface modification . The surfaces of the discs were examined by light microscope, scanning electron microscope (SEM), surface roughness test was done by Atomic Force Microscope (AFM) to figure out the changes on the surface roughness .Wettability of the modified and non-modified surface of the PEEK was evaluated using contact angle test. In vivo study, twelve male *New Zealand rabbits* were chosen as implantation sites. Forty eight screws were implanted in femur bones, two screws in each femur. Mesial one was Fractional CO_2 laser treated PEEK implant, and the parameters of laser radiation were (6W power, 0.8ms pulse duration, 0,4mm distance between spots ,and single scan). While distal implant was commercial pure titanium screw. The rabbits were divided into two groups according to the healing periods of each implant materials two and six weeks. Eight screws were tested for torque removal

after each healing periods. Four screws of each group of implant material were used for histological examination including both healing periods.

Results: In vitro study helped to select the preferable laser parameters which gave modified surface with roughness, crack and carbonization free surfaces and improved wettability. In surface roughness the result indicate significantly increasing in the roughness, the average surface roughness(Sa) increased from 42.571nm to 109.02nm after laser irradiation. Also wettability was significantly increased by decreasing the contact angle from 80.81° to 60.25° . There was significant difference in mean values of torque removal for the Fractional CO_2 laser treated PEEK implants (4.5Ncm) in comparison with titanium implants (6.3Ncm) after two weeks, while there was no significant difference in values of torque removal after 6 weeks which was (11.8Ncm) for PEEK and (12.3Ncm) for titanium. Histological examination in both implants materials, indicated that time was positive factor improved bone formation and osseointegration.

Conclusion: Fractional CO_2 laser treated PEEK implants had notable influence in the improvement of the biocompatibility and osseointegration compared to commercial titanium implants.

List of Contents

No.	Contents	Page No.

List of Tables

List of Figures

List of Abbreviation

Symbol	Term
2D	Two dimension
3D	Three dimension
ADA	American dental association
AFM	Atomic force microscopy
Al_2O_3	Aluminum oxide
Ar	Aragon
CAD-CAM	Computer-aided design and computer-aided manufacturing
CO_2	Carbon dioxide
Cp Ti	Commercially pure titanium
Cr-Co	Alloy of cobalt and chromium
CW	Continuous wave
E	Energy
E.T.	Exposure time
EDS	Energy-dispersive X-ray spectroscopy
Er, Cr: YSGG	Erbium, chromium: yttrium-scandium-gallium-garnet
Er:YAG	Erbium: yttrium-aluminum-garnet
F	Frequency
GPa	Gega Pascal
HA	Hydroxyapatite

Symbol	Term
He-Ne	Helium-Neon
J	Joule
LST	Laser surface texturing
Ms	Millisecond
N.cm	Newton centimeters
Nd:YAG	Neodymium-doped yttrium aluminum garnet
Nm	Nanometer
Nm	Nanometer
P	Power
PEEK	Polyetheretherketone
PVDF	polyvinylidene difluoride
Sa	Average surface roughness
SEM	Scanning Electron Microscope
Tg	glass transition temperature
Ti	Titanium
TiO_2	Titanium dioxide
Tm	melting temperature
UV	Ultraviolet
UV–IR	Ultraviolet- Infrared
WCA	water contact angle
Mm	Micrometer

CHAPTER ONE

Introduction&

Literature Review

CHAPTER ONE

LITERATURE REVIEW

1.1 Dental implant

Dental implant is a prosthetic devise inserted into the oral tissues under the mucosal layer and on\or within the bone of the jaw . Dental implant can enhance support and retention for different type of prosthesis if it was fixed or removable (Keith, 2017). Three main kinds of dental implants: periosteal, endosteal, and transosteal dental implants (The glossary of prosthodontics terms GPT.9, 2017).

1.1.1 Indications of dental implant:

A. Psychological effects of tooth loss.
B. Failure of removable prostheses.
C. Restore dental aesthetics.
D. Reestablishment of lost dental function: mastication, phonation, maintenance of space and occlusal stability.
E. Bone preservation after teeth loss .
F. Comfort and tolerance.
G. Orthodontic anchorage . (Rafael *et al.,* 2014)

1.1.2 General contraindications of dental implant treatment:

A. Uncontrolled intraoral disease or malignancy.
B. Involvement of periodontium and tooth supporting tissues .
C. Radiotherapy to the jaw bone.

D. abuse drug or alcohol.

E. Psychological disorders.

F. Cases with immunosuppression

G. Recent myocardial infarction (MI) or cerebrovascular accident (CVA) or valvular prosthesis surgery.

H. Patients with Inability of plaque control (e.g. reduced manual dexterity or mental capacity) . (Alsaadi *et al.*,2008; Rafael *et al.*, 2014).

1.1.3 Factors affect success of dental implants

The Successful Osseointegration of implant is based on the following Key factors : figure(1.1)

1. Biocompatibility.
2. Microscopic and macroscopic topography of implant surface.
3. Bone quality
4. The surgical technique.
5. Unobstructed healing phase.
6. prosthesis design and loading protocol.
7. Premature contact or parafunctional forces.
8. Infection. (Misch, 2015; Pranav *et al*., 2016; Naveen *et al*., 2017).

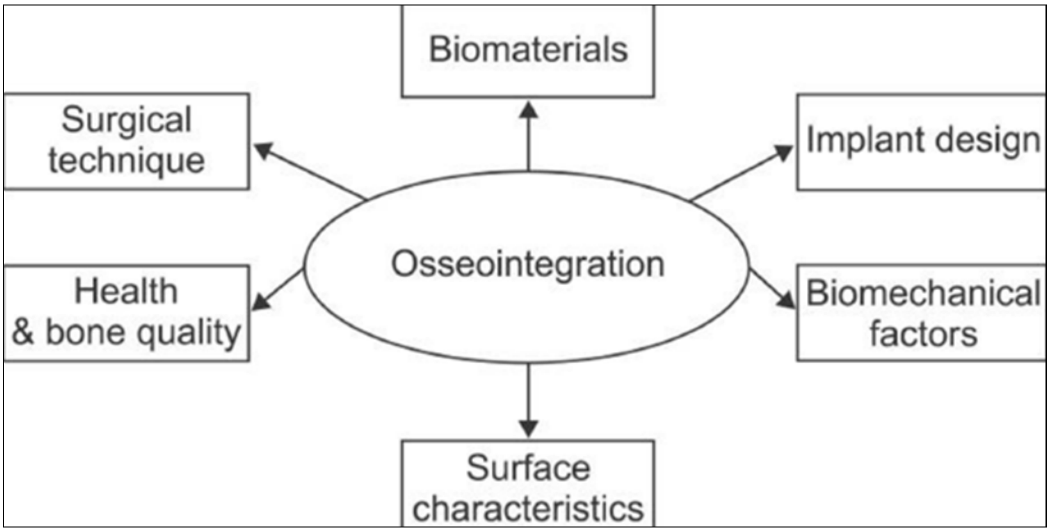


Figure (1.1): Factors that affect osseointegration
(Gaviria *et al.*, 2014).

1.2 Osseointegration:

(Osseointegration is stated to the direct structural and functional connection of the host bone tissues to the exogenous alloplastic materials without growth of fibrous tissue at the bone-implant interface. (The glossary of prosthodontics terms GPT, 2005; Mavrogenis. *et al.*, 2009). Researchers believe that osseointegration does not detected on 100% of the surfaces of the implant. It was proposed that only 30% and 95% of the implant surface showed true contact with bone according to light microscopy (Linder, 1985). Therefore, new strategies in dentistry started by introducing the biocompatible materials to jaw bone tissue stressing no evidence of rejections (Matusovits, 2009). Hence, the use/applications, size, shape, and so on are all important in detection the host response to the implanted biomaterial (Barkamot *et al.*, 2013). In spite of the material may show minimal biological response by the host; and still be considered biocompatible. Biocompatibility means: Nontoxic, Non-mutagenic, Noncarcinogenic, and Non-immunogenic. In place of poor or bad prognosis of the osseointegration, may cause a crestal bone loss and\or pocket. This cause entrapment of the bacteria that lead to inflammation and increase the amount of tissue has being lost around the implant. Therefore all efforts dedicated to have biocompatible materials and variety of surface coating techniques to stimulate the osseointegration (Chaiy *et al.*, 2008; Mavrogenis *et al*., 2009).

As a result of the body respond start by formation of a fibrous tissue (fibrosis) surrounding the surface of the implant, then progressively, encapsulated by such layer. This fibrous encapsulated implant in showed poor mechanical strength upon function when compare with osseointegrated implant (Parithimarkalaignan and Padmanabhan 2012).

Contamination of implant surfaces considered as first risk factors affect the osseointegration , this is caused rapidly by initial bacteria adhesion to subsequent biofilm formation (Lee *et al*., 2012) end up with implant loosening and eventual detachment (Zhou *et al*., 2017). As a treatment, the removal of the implant (revision surgery) is the correct decision since this fibrous tissue is impermeable to the medications (Dee *et al*., 2003).

1.2.1 Mechanisms of the Osseointegration

The mechanisms that happen through bone healing include : the activation of immunological, osteogenic and vascular sequence that happen in the bone around the implant . Thus, the stages of osseointegration around dental implants include the contribution of various cell types , cytokines and growth factors (Park *et al.,* 2000; Mavrogenis *et al.*2009).

Remodeling of the bone happens eventually for joining or reshaping of bone at the implant site, also it offers a mechanism for adaptation to stress and self-repair at bone-implant site. So, the osseointegration of implant is slow process and it can takes up to several months. However, understanding of the events that happened at both cellular and molecular levels at the bone-implant interface is required to improve the osseointegration of the implants (Hofmann *et al.*, 1997; Dimitriou and Babis, 2007). Weiss and Weiss, 2001, suggested that: there are four stages occurs in bone healing around dental implants , these stages are:

- 1st Stage name vascular stage: The appearance of angiogenesis appears in the walls of the prepared osteotomy, where the elongation occurs at the broken ends of fine blood vessels, then spreads into the spaces around the implant from the bone marrow cavities walls. Higher activity is noticed in the threaded groves or acute angles of interface geometry than the other places. These sequences happen in the early (3-7) days after surgery. After the first week, these places are quickly filled with fibroblasts, fine collagen fibers and with undifferentiated mesenchymal cells in some circumstances (Weiss and Weis, 2001; Siebersa *et al.*, 2005).

- 2nd Stage known as early bone formation stage: within two weeks of the surgical implantation procedure, Ridge-like bone with sinusoidal capillaries filled grooves can be noticed. A Bone segments -which are not continuous - at the base are adhere with a basket-like capillary networks to develop a continuous bone. This stage of initial bone formation with wound healing is known as modeling (Weiss and Weiss, 2001 ; Albrektsson *et al.*, 2003).

- 3^{rd} Stage (bone growth): The prominent finding following the fourth week of implantation is bone remodeling in the interface within the threads and the formation of woven bone . This stage essential in stability of the implant because the extensive bone resorption that detected in peri-implant cortical bone tissue before a month postoperatively (Branemark *et al.*, 1997 ; Weiss and Weiss, 2001).

- 4^{th} Stage (maturation of bone): It occurs in 6-8 weeks following surgical procedure. Hence, the bone around implants is completely formed .The lamellar compaction with an increased in callus formation is associated with remodeling processes which affect both the new bone generation and traumatized preexisting pre-implant bone tissue by the preparation of the host site. (Weiss and Weis, 2001).

1.3 Bone and bone quality:

Bone is regarded as a highly specialized connective tissue, it is consists of cells and rich extracellular matrix. Bone is a complex natural composite material, it composed of organic and inorganic materials (Smith and Hoshemi, 2006). The inorganic element form (60% -70%) of the of the bone dry weight is composed commonly of hydroxyapatite (Ca_{10} $(PO4)_6$ $(OH)_2$), this inorganic component provide the bone its hardness (Smith and Hoshemi, 2006; Wang *et al.*, 2010).

Organic part of the bone is mostly of protein which is called collagen (type I) and about 5% non-collagenous structural matrix proteins, such as bone osteocalcin, ostiopontin, sialoprotein, and proteoglycans ; besides serums proteins and growth factors , while the water is 25% (Nanci *et al.*, 2008; Wang *et al.*, 2010).

1.3.1 Classification of bone according to tissue formation:

Bone tissues can be categorized into; first, immature bone tissues (woven bone), that is characterized by irregular collagen fibers distribution . Likewise, it contains a higher ratio of osteocytes and less amounts of mineral substances. Immature bone is temporary kind of that change with time to lamellar bone. In adults this type of bone may have a pathologic basic, except in some places such as near the skull’s flat bones suture and teeth sockets**.** Second**,** mature bone tissue which is a lamellated bone characterized by regular arrangement of collagen fibers that arranged in lamellae, these lamellae are concentrically organized around avascular canal called (Haversion canal) (Hadjidaskis, 2006; Wang *et al.*, 2010).

1.3.2 Bone cells

In the typical bone there are three types of cells , these cells are:

A. Osteoblasts

Osteoblast are mononucleated cells control the new bone matrix, osteoid synthesize and then mineralization to new bone. They seen as a layer of cells covering over the surface of a new bone and it may control the ions flux into and out of bone (Florencio-Silva *et al.*, 2015). Furthermore they are responsible for hormones production like prostaglandins and alkaline phosphatase which is an essential enzyme for the mineralization of bone tissues (Pratt, 2012; Saladin, 2012).

B. Osteocyte

During formation process of the bone, some of osteoblast cells enclosed into the matrix of the bone these cells are then called osteocytes. These form about (90%-95%) of all bone cells (Nanci *et al.*, 2008). Osteocytes are responsible in matrix maintenance , homeostasis, and bone tissue formation; likewise, they act as sensory receptors to load and the other mechanical stimuli. So, osteocytes are involved in both modulation of osteoblast generation and formation of new bone (Lian *et al.*, 2003; Muhonen, 2008; Wang *et al.*, 2010).

C. Osteoclast

These cells are in charge of bone resorption because they characterized by formation of large amounts of lysosomal enzymes (acid phosphatase). So, these cells have an significant role in bone remodeling (Nanci *et al.*, 2008).

Bone quality is indicated as the amount of bone (cortical and cancellous) with their topographic relationship in which the recipient site is drilled. The poor quality and quantity of bone have been indicated as the highest risk for the impairing failure as it may be related with extreme bone resorption and damaging of the healing process in compared with higher density bones (Herrmann *et al.*, 2005).

Depending on structures and proportion of bone (compact and trabecular), the quality of bone can be categorized into four types (Ribeiro *et al*, 2010).

- **Type I:** the homogeneous cortical bones.
- **Type II:** thick cortical bones with marrow cavities .
- **Type III:** thin cortical bones with dense trabecular bone of a good strength.
- **Type IV:** A very thin cortical bone with low-density trabecular bone of a poor strength.

1.4 Main Factors Affect Osseointegration:

1.4.1 Surgical technique

The surgical procedure with least tissue violence is imperative for osseointegration. This objective depends on surgical drilling by the low speed with continuous cooling. The violent surgical technique causes the increasing of the temperature in the bone and the cells by frictional heat. Therefore, bone healing will be destroyed (Parithimarkalaignan and Padmanabhan, 2013). When the degree of temperature is more than 47 ºC for 1 min or 40ºC for 7 min necrosis will be happen. So, more care required to prevent thermal damage during a surgical drilling procedure (Friberg *et al*., 2001).

External irrigation technique with sufficient water for cooling during the procedure of drilling can keep the degree of the temperature below 47 ºC. The surface of bone can be preserved during the drilling of bone procedure by control drills speed and pressure of the drills (Sener *et al*., 2009). The using of sharp drill with cooling and gradually increased drill size to avoid high drill speed side effect (Ashly *et al*., 2003).

The undersized drilling technique was obtainable to optimize bone density and also improve primary stability (Eom *et al*., 2016). Also, both insertion and removal torques increase with using the undersized drilling technique. Therefore, the undersizing insertion site with is more essential in the case of the bone with low density (Elias *et al*., 2012).

1.4.2 Main characteristics of an implant biomaterial

In order to obtain sufficient osseointegration, the material properties of biomaterials used for construction of dental implant are of great significance, the characteristics include:

A. Modulus of elasticity: Implant material with modulus of elasticity relatively similar to bone (18 GPa) were selected to decrease stress at bone implant interface and reduction of implant movement.

B. Shear, compressive and tensile strength: An implant material should have good tensile and compressive strength avoiding distortion or fracture and improve the stability of the implant also uniform stress transfer from the implant to bone increase shear strength.

C. Yield and fatigue strength: ideal dental implant should possess high yield and fatigue strength to prevent fracture when subjected to cyclic stress.

D. Ductility: According to ADA a minimum ductility of 8% is required for dental implant. Ductility is essential in designing or shaping of an implant.

E. Hardness and Toughness: high value of hardness reduces wear of implant material and improved toughness made the implant tolerate loading and prevents fracture.

F. Flexibility in order to absorb energy from possible deformation.

G. Lightness (low density) (Wennerberg and Albrektsson, 2010; Babita *et al*., 2015).

1.4.3 Surface characteristics

(Surface tension is defined as the cohesive forces between the liquid molecules which are parallel to the surface perpendicular to a unit length line drawn on the surface, while surface energy can be defined as the energy difference between the bulk of the material and the surface of the material). As well as surface tension is recorded along a line while surface energy is recorded along an area. Surface tension and surface energy control the wettability of implant by moistening fluid, osteoblasts attachment and adhesion of proteins on implant surface. (Muddugangadhar *et al.*, 2011) .

Implant surfaces have been categorized depending on multiple factors, such as roughness and texture (Chaturvedi, 2009):

A. Implant surfaces could be divided depending on the surface roughness as: minimally rough , intermediately rough, rough .

B. Surface texture as: concave texture (by addition technique like hydroxyapatite (HA) coating and titanium plasma spraying), convex texture (using blasting or etching methods).

C. Direction of irregularities: Isotropic surfaces: have similar topography unrelated to direction; Anisotropic surfaces: have different directions and roughness.

1.5 Classification of Dental implants

Dental implants could be categoriesed:

A. Placement technique within the tissues.

B. Implant reaction with bone.

C. Type of materials used. (Misch *et al.*,2008; Babita *et al.*, 2015).

A. Placement technique within the tissues –implants classified into:

I. Endosteal, implants are subdivided into several types according to their shape, geometry and materials:

1. Cylindrical or root form implants.
2. Blade implants and the ramus frame.
3. Pin implants.
4. Disc implants.
5. Pterygoid or zygomatic implants.

II. Trans-osteal implants.

III. Subperiosteal implants.

B. Based on the capacity of the implant to stimulate new bone formation, biocompatible implant materials are classified into biotolerant, bioinert and bioactive materials (Osborn *et al.,* 1990; Meirelles, 2007).

I. Biotolerant material: It is the material have the ability to induce a distance osteogenesis; the bone will be formed but not in contact with the host bone. The retention of implant may govern by principle of interlocking characterized by mechanical means. Example of this materials are Cr-Co alloy and stainless steel.

II. Bioinert material: exhibited contact osteogenesis; direct contact of the bone is noticed to the implanted material. The retention of the implant also depends on the interlocking principle, i.e. completely by a mechanically based anchorage. The materials included in this group are represented by alumina, carbon and titanium.

III. Bioactive materials: exhibited bonding osteogenesis; direct chemical bonding between adjacent bone and implant happen. The retention of the implant depends on both chemical bonding between bone-implant as well as mechanical interlocking. Types of materials included in this group are tricalcium phosphates, glass-ceramics and hydroxyapatite.

C. Depending on the materials utilized, the implants can be grouped into:

1- Metallic implants :Titanium, Titanium alloy, Cobalt Chromium Molybdenum alloy.

2- Non- metallic implants. (Monika *et al.,* 2015).

- Polymeric material: PEEK.
- Ceramics: Alumina, Zirconia, Glass Ceramics, Calcium phosphate.

1.5.1 Metallic material

A. Titanium

Titanium (Ti): is a chemical element and silver gray metal. It is characterized with a high-strength, light weight and low corrosion (Gonzalez and Rosca, 1999). Titanium is categorized as unalloyed (commercially pure titanium) and titanium alloys. Depending on the titanium and its impurity, the commercially pure titanium (CP Ti) is categorized into four grades. Due to their high biocompatibility, titanium and its alloys are used broadly in bone surgery as biomaterials, usually due to main two reasons. First, the mechanical properties of CP Ti are well adapted to the bone in comparison with other metallic implant materials. Second, is the titanium surface, which is covered with a thin passive layer of oxides -in different oxidation states (TiO_2, $Ti2O_3$, TiO) which is spontaneously formed on its surface and becomes resistant to corrosion and behaves as bio-inert in living tissues (Schenk, 2001; Bozzini *et al.*, 2008; Popa *et al.*, 2008).

B. Titanium alloy

It is a metallic material comprises of a combination of two or more metals (Wataha, 2002). Titanium-6Aluminum-4Vanadium (Ti-6Al-4V) alloy was developed as aircraft material, and due to its adequate resistance to corrosion and greater strength , it has been tested as an alternative for Cp Ti (Iijima *et al.*, 2003). In comparison to Cp Ti, the Al and V alloying elements made a stabilized alpha-beta microstructure, which enhance the mechanical properties (Navarro *et al.*, 2008).

Releasing of harmful ions of vanadium from the alloy lead to new titanium alloys development like niobium (Nb), tantalum (Ta) and zirconium (Zr) which they are non-toxic elements (Thair *et al.*, 2004).

Ti-13Nb-13Zr is another titanium alloy, and in comparison to Ti-6Al-4V, it has a low-elastic modulus with higher resistance to corrosion (Cai *et al.,* 2003). The higher corrosion resistance is due to less solubility of Zr and Nb than Al and V with more inert passive oxide layer on the surface (Khan *et al.,* 1999). Ti-6Al-7Nb alloy was used as the substrate for coating by (Hamad, 2007) and Ti-6Al-4V was used by (Ali *et al.,* 2014).

1.5.1.1 Disadvantages of titanium and zirconia implant:

Dental implant improved the quality of life in many patients complain from tooth loss (Turkyilmaz *et al.,* 2010). Researches indicated that the material of choice for dental implants based on commercially pure titanium, the first recognition is by Branemark at the end of (1960's) (Branemark *et al.,* 1969). In spite of well evidence- based implants made from titanium and titanium alloy, (Shapira *et al.,* 2009 and Velasco *et al.,* 2010), observe some disadvantages during use, such as hypersensitivity to titanium (Tschernitschek *et al.,* 2005; Thomas *et al.,* 2006; Muller *et al.,* 2007; Egusa *et al.,* 2008; Sicilia *et al.,* 2008).

Furthermore the gradient difference in the titanium implant's elastic moduli (110 GPa) and the bone surrounding it (≈1-30 GPa) is also was disadvantage of titanium dental implants. During transmission of load, this may cause stress in the bone-implant interface (Bougherara *et al.,* 2010; Sarot *et al.,* 2010), possibly resulting in periimplant bone loss (Frost *et al.,* 1992 and Huiskes *et al.,* 1992). Similarly, because of its light transmission absence, titanium can affect esthetic results (Yildiriim *et al.,* 2003). Especially in cases of high smile line this can cause a darkness in neck of the periimplant soft tissue with thin gingival biotype or/and recession of gingivae surrounding a titanium implant (Andreiotelli *et al.,* 2009; Aydin *et al.,* 2010). The breakdown of the oxide film on the surface of implant seen in some cases of acidic oral environments is usually end up with exposure of metal surface to electrolytes (Mouhyi *et al.,* 2012). Consequently, Ti ions and metallic ions would release to the environment, this induce immunity response (type IV reaction) (Schalock *et al*., 2012; Goutam *et al.,* 2014).

In spite of that, growing worries concerning release of metallic ions, incompatible modulus of elasticity between metals and human bone, radiopacity, and the demanding of increased number of patients looking for dental restoration totally free of metal materials (Andreiotelli *et al.,* 2009). All of that lead to the necessity to recognize another substitute (Bosshardt *et al*., 2017).

As a substitution to titanium, implants made from ceramics are recommended, first design appeared nearly 40 years ago and were made from aluminum oxide (Schulte *et al.,* 1978). Lately, dental implants of ceramics made from zirconia, and due to its biocompatibility, tooth like-color, mechanical properties and low plaque affinity, which appears to be a better convenient substitution to titanium (Ozkurt *et al.,* 2011). But because of the incidence of high fracture (D`hoedt *et al.,* 1986) , and according to Andereriotelli *et al*., on (2009) in his systematic review of the literature made conclusion that not enough sufficient scientific clinical data to recommend using of ceramic implants in clinical use routinely .

Furthermore, because of higher elastic modulus of zirconia of 210 GPa than the bone, so the stress distribution of zirconia implant to bone surrounding it, might be correlated to even higher peaks stress in comparison to titanium (Ozkurt *et al*., 2010).

To overcome these shortages of Ti , Ti alloy and zirconia , recommendation were made to use some polymeric material because of its mechanical, esthetic properties and biocompatibility with low modulus of elasticity, that is close enough to modulus of elasticity of bone (Moon et al., 2009).

1.5.2 Polyetheretherketone (PEEK):

A specific material in PAEK family (Polyaryletherketone), a group of material described as high temperature thermoplastic polymer. It is recognized now as a polymer material used in the fields of orthopedics and trauma, due to its good biocompatibility, high mechanical strength and radiolucency (Kurtz and Devine 2007; Panayotov *et al.,* 2016). Meanwhile it was considered as a substitution of metallic biomedical dental implant, as PEEK neither forms by-products nor release ions or corrode and degrade (Liao 1994; Corvelli *et al.,* 1997; Kurtz *et al*., 2007; Ziebart *et al.,* 2013).

PEEK has high temperature stability (exceeding 300°C) and high mechanical and chemical resistance. It has been extensively investigated and successfully used in, trauma, neurosurgical and craniomaxillofacial procedures, dental and cardiovascular applications, joint replacements, anterior cruciate ligaments repair (Maharaj *et al*., 1993; Toth *et al*., 2006; Kurtz *et al*., 2007; Cotic *et al.,* 2015; Kersten *et al.,* 2015). And it is biocompatible material with 3.6 GPa modulus of elasticity , its modulus can be modified by reinforcing by various methods and materials, for instance, for reaching 18 GPa modulus of elasticity, same as to that of cortical bone (skinner *et al* 1988; moon *et al*., 2009).

1.5.2.1 PEEK composition

PEEK polymer consists of a linear aromatic rings linked by ketone or ether linkages, with number and order of these linkages, with little or no branching (Attwood *et al.,* 1981). PEEK is a two-phase, semi-crystalline polymer can be up to about 40% crystalline (Atkinson *et al.*, 2002), although 30 - 35% is much ideal, these crestallinity depending on the manufacturing process. The result is morphology of a two phase, containing of crystalline regions dispersed in amorphous polymer. The two-phase model has applied successfully to describe the PEEK’s dentistry (Blundell, 1983). PEEK has an aromatic molecular backbone with combinations of ketone (–CO–) and ether (–O–) functional groups between the aryl rings (Figure1.2). PEEK has high stability, low density (1.32 g/cm^3), insolubility, and a low elastic modulus (3–4 GPa) (Skinner, 1998).

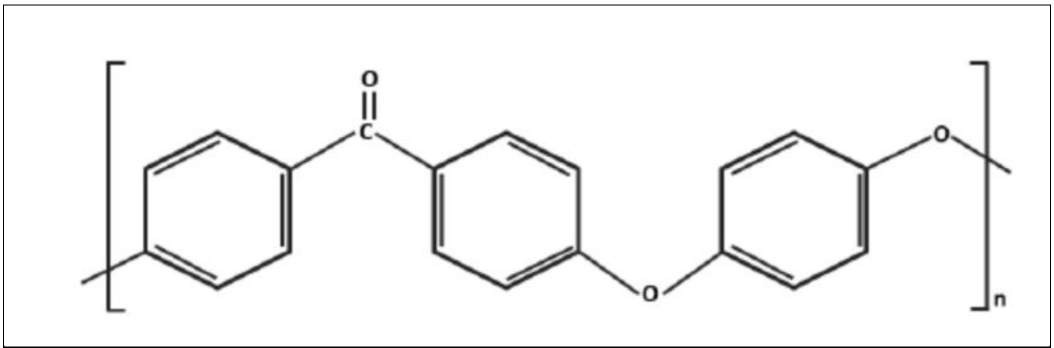


Figure (1.2): Chemical structure of polyetheretherketone (PEEK) (Kurtz & Devine, 2007).

1.5.2.2 Mechanical properties of PEEK

The PEEK has specific mechanical properties are usually related to the microstructure (i.e. crystallinity, size of the crystals and their perfection). Microstructure development of PEEK is controlled by the different thermal history (Reitman *et al.,* 2012). The mechanical performance of PEEK is attributed to: strength, stiffness and toughness (Jones *et al.,* 1985). PEEK is also described as a visco-elastic material, therefore load on time (i.e. creep) and temperature have direct effect on material stiffness. However, in-vivo where the body temperature is 37°C, it was found that elastic behavior of PEEK was relatively insensitive to temperature (Rae *et al.*, 2007). PEEK normally have high thermal stability and good mechanical performance. The glass transition temperature (Tg) of PEEK is of 143°C and a the melting temperature is (Tm) of 343°C considered high (Béland, 1990). Furthermore, Adding carbon or glass will increase the modulus from 3–4 GPa to 18 GPa to resemble bone, or 150 GPa to resemble Titanium (Williams *et al*., 1987).

1.5.2.3 PEEK medical applications

In the orthopedic medicine, PEEK is used to construct spine cages for vertebral fusion, also it is used as craniomaxillofacial implants such as skull plate in some patients (Kulkarni *et al*., 2007; Camarini *et al.*, 2011). PEEK has a lower strength when compared to metal alloys. However the good wear resistance and radiolucency of PEEK are the source criteria for using PEEK in many orthopedic applications (Cutler *et al*., 2006; Nieminen *et al.*, 2008; Ponnappan *et al.*, 2009). Also PEEK as many polymers, it is possible to use it to create complex designs by processing or reinforced with fibers considering mechanical requirements in the applications (Kurtz *et al.*, 2007).

Adding of carbon fibers to PEEK mix improved the tensile strength of PEEK to double for discontinuous fibers while when continuous fiber was used in reinforcement, PEEK was close to metal alloys in some mechanical properties (Green *et al*., 2012).

Radiolucency is one of the benefits of the use of PEEK in medical devices and can be imaged by X-ray, CT scan, or MRI without any distortion for the visualization of fusion desired in comparison to the conventional titanium (Ti) and stainless-steel materials used in these applications (Wenz *et al*., 1990 and Katzer *et al*., 2002). Because of PEEK chemical stability and no any breakdowns products release during use it was considered for long term use in a body (Wenz *et al*., 1990 and Katzer *et al*., 2002) and proven biocompatibility.

Additional required property of polyetheretherketone is the tendency to engineer the modulus of elasticity of polyetheretherketone to more close up matching to that of other materials, such as bone lead to the reduction in bone resorption risks (Chivers *et al*., 1994; Khoury *et al.*, 2018).

1.5.2.4 Applications of PEEK in dentistry

Polyetheretherketone (PEEK) is a synthetic, tooth colored polymeric material that has been used as a biomaterial in orthopedics for many years (Toth *et al*., 2006; Pokorny *et al*., 2010). PEEK has white color and excellent mechanical properties, hence it has been proposed for other prosthodontics applications such as fixed prostheses (Schmidlin *et al*., 2010) and removable prostheses (Costa-Palau S *et al*., 2014).

The effects of surface modification of PEEK have been studied for bonding with different luting agents (Schmidlin *et al*., 2010 and Kern *et al*., 2012) and extracted teeth (Uhrenbacher *et al*., 2014). Additionally, PEEK can also be used an esthetic wire for orthodontic. Compared to other polymers, such as polyether sulfone (PES) and polyvinylidene difluoride (PVDF), PEEK orthodontic wires are capable to deliver higher orthodontic forces but at a cross section of that similar to metallic wires such as cobalt–chromium (Co–Cr), titanium–molybdenum (Ti–Mo) and nickel– titanium (Ni–Ti) (Maekawa *et al*., 2015).

One method for obtaining the emergence profile in areas around dental implants was shown by (Becker, 2012), who used a provisional abutment made of PEEK, evaluation of soft and hard tissue responses to titanium and provisional PEEK abutments, and reported that no significant difference between PEEK and Ti was found in soft- and hard-tissue responses in 3 the months after the provisional abutment (Koutouziz *et al.,* 2017).

Also PEEK used for removable partial denture frameworks and dental implant components because is flexible, strong, shape-stable, biocompatible polymer (The glossary of prosthodontics terms GPT.9, 2017). Due to these unique physical and mechanical properties, PEEK is considered as a promising material for dental applications. Additionally, it can be sterilized repeatedly and shaped by machining and heat contouring to fit the bone contour (Barton *et al*., 1996).

Also the biofilm formed on the PEEK surface is lower or equal to the Ti or zirconia abutment materials, and PEEK healing abutments do not give an increased probability for recession of soft tissue and marginal bone loss through initial healing period. (Koutouzis *et al*., 2011; Hahnel *et al.,* 2015).

1.5.2.5 PEEK as a dental implant

PEEK is a thermoplastic polymer with a high-performance has the ability to replacing components of metallic implant in the field of orthopedics (Maharaj *et al*., 1993; Liao *et al*., 1994) and traumatology (Kelsey *et al*., 1997; Corvelli *et al*., 1997). Also, PEEK implants can be used for the constructions of calvarias bone (Hanasono *et al.,* 2009). With such findings that made suggestions for PEEK to be substitution for titanium as dental endosseous implants' material (Schwitalla *et al*., 2013).

Unmodified PEEK is a bioinert material, and shows a water-contact angle (CA) of 80–90 degrees, which is close to being a hydrophobic value in nature (Huang *et al*., 2001; Nieminen *et al*., 2008; Qahtani *et al.,* 2017).

Modified PEEK can have improved hydrophilicity, which leads to increased cellular proliferation because the wettability of the biomaterial and the dental implant surface influences the interaction between the material and the surrounding physiological environment (Wenz *et al.*, 1990).

Interfacial free energy (surface energy) is a physicochemical property of the material surface and much important in implant surfaces. It is related to the wettability of the material and typically it measured by estimating water contact angle (WCA), i.e., the angle formed by the interface liquid with a solid surface. Hydrophilic material has a good harmony with water, i.e., high surface energy. With this material water extends over material surface made the contact angle low. While in hydrophobic i.e., low surface energy, water would not stretch out and forming a spherical cap on the material surface which increase the contact angle (Figure1.3). It is shown that, more hydrophilic substrates (i.e., with high surface energy, low contact angles) promotes significantly the adhesion and spreading of cells as compared to in hydrophobic materials (i.e., with low surface energy, high contact angles). (Riveiro, 2018)

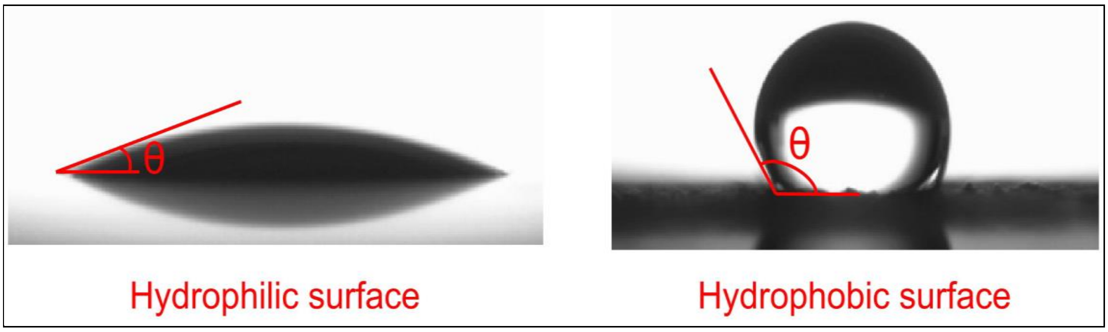


Figure (1.3): Water contact angle for hydrophilic for different surfaces. (Riveiro, 2018).

Although PEEK has lower osteoconductivity than titanium (Rabiei 2013), Nano scale surface modification with hydroxyapatite deposition, titanium deposition increasing the surface roughness, chemical modifications, and incorporation with bioactive properties (TiO2) and hydroxyfluoroapatite (Wang *et al.,* 2011; Wu, *et al.,* 2012; Wang 2014), can improve the biocompatibility of PEEK to reach early osseointegration. Furthermore, modified PEEK exhibits significantly superior tensile properties than pure PEEK (Najeeb *et al.,* 2015). PEEK has also been coated with other bioactive materials by using plasma spraying (Sandler *et al.,* 2002; Suska *et al.,* 2017), spin-coating (Barkamot *et al.,* 2013), plasma gas etching (Waser *et al.,* 2014), electron-beam deposition (Randolph *et al*., 2006).

Although PEEK it is still known as bioinert due to poor response of the surrounding tissue, this limited PEEK potential applications. Several methods have been recommended to overcoming this problem, it is either incorporation of bioactive materials such as hydroxyapatite (HA) and titanium dioxide (TiO_2) into PEEK composite or surface modification methods which also either : direct surface modification or deposition methods. Surface modification is a range of techniques which change the material surface characteristics but not disturb the bulk material properties. surface treatment techniques such as laser surface modification, coating with the bioactive material, and wet chemical treatment (Converse *et al*., 2007; Wu *et al*., 2012; Zhao *et al.,* 2013).

Among the methods used to solve polymers shortages is using of a bioactive surface coating (as titanium dioxide, hydroxyapatite or bioactive glass, etc.), or by mixing both, the polymers with bioactive materials (e.g., hydroxyapatite, bioactive glass, calcium silicate) (Tanner 2010; Bosco *et al*., 2012; Ma and Tang 2014; Durham *et al*., 2016).

The main problems of coatings are: to ensure a correct adhesion to the polymer surface or degradation of the coatings, or the need for complex and time-consuming chemical steps. Additionally, mixing of polymers with bioactive materials can significantly decrease their mechanical properties. Surface modifications have focused on multifunctional properties such as improving biological activity, avoiding bacterial infection, or modulating inflammation which are necessary functions for physiologic osseointegration, table (1.1) (Spriano *et al.*, 2018), and it is better choice to increase material bioactivity without affecting its several advantages.

Table (1.1): Some processing techniques help to modify polymeric biomaterials. (Riveiro A, 2018).

Technique	Processing rate	Processing steps	Chemical products	Treated area	Cost
Photolitography	Fast	Several	Yes	Large	High
Electron beam litography	Slow	One	No	Small	High
Ion beam litography	Slow	One	No	Small	High
Atomic force microscopy	Slow	One	No	Small	High
Soft lithography	Fast	Several	Yes	Large	Low
Chemical vapor deposition	Fast	One	Yes	Large	High
Laser texturing	Fast	One	No	Large	Medium

Abundant groups are employed to improve the bioactivity of polyetheretherketone by adding hydroxyapatite (Wang 2014), others made an attempt to use oxygen plasma to modify the surface energy

(Hamodi 2018), whereas some researchers used porous PEEK to facilitate cellular ingrowth (Toristich *et al.,* 2017).

1.6 Laser surface modification

Laser is a high energy photon source which can create modifications in surface roughness and wettability of the polymers. Surface treatments using lasers is widely recommended due to their low cost, high resolution, high-operating speed and essentially, lasers do not affect the main properties of implant's material. Therefore, scientists and researchers considered lasers as interested tool to improve the surface energy of the implant materials (Comesa˜na *et al*., 2010). Some surface treatment technique able to modify the surface chemistry of PEEK (Laurens *et al.,* 2000) .Furthermore, in another study used UV irradiation found out enhancement of the wettability of the dental implant surface and formation of polar groups such as hydroxyl, carboxyl and peroxide groups (Gittens *et al.,* 2014).

1.6.1 Laser basics

Laser is a light with specific properties. Light is an electromagnetic energy that exists as tiny particles called photons, move in space as waves. The term Laser is an acronym for Light Amplification by Stimulated Emission of Radiation (Donges and Reinhard 2015).

1.6.2 Components of laser system:

1. The optical cavity (resonator): It forms the laser cavity and has two mirrors, one at each end. They cause the bouncing of the photons to stimulate the release of more photons; therefore, one of the mirrors

has high reflectance, while the other has less reflectance. This stimulation process may cause increase in temperature; therefore, heat must be controlled by using cooling systems (Malik and Chatra 2011).

2. Active medium: It may be gas (CO_2), solid (Nd:YAG), or liquid, placed in the cavity. It is the source of the photons, and it amplifies the photon chain reaction that results from stimulated emission when its atoms are excited (Malik and Chatra 2011).
3. Pumping medium: It is the energy source that applies energy to pump the atoms in laser excited state. It may be flash lamb strobe device, electrical circuit, or others. Figure(1.4).

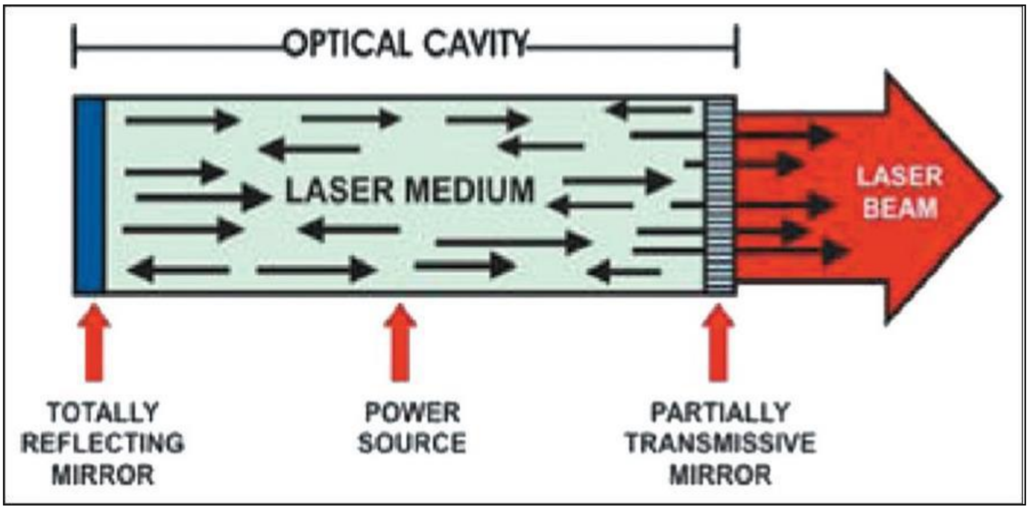


Figure (1.4) : Laser optical cavity (Elavarasu *et al*., 2012).

4. Delivery system: Is a light weight attachment or instrument that conducts the laser beam to the target material or tissue from the laser cavity. There are four delivery system types (depending on laser wavelength) (Parker 2007)

a. Fiber optic.
b. Articulated arm.
c. Flexible hollow waveguide.
d. Direct beam from the laser devise.

And contain another auxiliary components such as:

A. Cooling system: It is usually the bulkiest component of the laser system. Heat generation is a by-product of the lasing process, so it is very necessary . Air or water are considered as a coaxial cooling systems (Parker 2007).

B. Control panel: The control panel is one of laser device components that adjust the laser parameters, wavelength changing in a multi laser instruments and sometimes allows to print the parameters during clinical use (Parker 2007).

1.6.3 Laser Operating Modes

I. **Continuous wave (CW):** Such as Argon ion $(Ar)^{+3}$ and Helium-Neon (He-Ne) lasers. It is meaning that laser peak power and average power are matching (without fluctuations) (Coluzzi and Convissar 2007).

II. **Pulsed mode:** Pulsed laser is produced by the using of pulsed pumping source such as flash-lamp for active medium excitation .In medical applications, Pulse mode avoids the high risk of thermal damage to the tissue and permits cooling between each pulse without a great temperature raising of the tissue (Krauss *et al.,* 2010).

When the continuous-wave laser beam passes through opening and closing the shutter, the gated pulse mode is generated with producing of peak powers around 10 - 50 time the original CW power (Coluzzi and Convissar 2007).

III. **Q-Switched mode:** Q-switching or gain pulse formation is a technique to produces high energy pulses with low pulse repetition rate and short pulse duration by putting an attenuator inside the laser cavity. Q-switching technique could be achieved by active methods such as electro optical and optoacoustic switches or by passive method such as saturable absorbers (Renk 2012).

IV. Mode locking: A train of narrow equal spaced pulses are achieved by using the mode locking technique. As a Q-switched technique the mode lock could be produced by active or passive methods. This technique is recently used in modern refractive surgery (Miserendino *et al*., 1995).

1.6.4 Important terms in laser:

- **Laser Wavelength λ (m):** It is the length of the light wave, or the shortest distance at which the wave pattern usually repeats itself. In laser studies, micrometer and nanometers were used to measure the wavelength.
- **Laser Frequency υ (Hz):** Is the number of times that the wave oscillates per second or per meter.
- **Pulse Duration (pulse width):** It is the time of the single pulse in pulsed laser.
- **Spot Size:** It is the diameter of the beam of laser radiation. It influences the concentration of photons in the target.
- Pulse Repetition Rate: It is the number of pulses per second.
- **Power Density (Intensity) (Irradiance) W/cm^2:** It is the concentration of photons in a unit area, or the power of laser beam divided by its cross section.
- **Energy Density (Fluence) J/cm^2:** It is the total energy delivered by laser on a unit area during an expose time.
- **Peak Power (P peak):** It is the maximum amount of power that the laser single pulse delivers to matter.

P peak = energy of single pulse / pulse duration.

- Average Power (P ave): It is the amount of energy released over the period of the cycle.

  P ave = energy of single pulse * Pulse Repetition Rate (PRR) (Deepika 2013).

1.6.5 Characteristics of Laser

1- **Coherence.** Every wave of laser light is identical in physical shape and size. This referred to that the frequency of all the waves of photons are identical, producing a focused electromagnetic energy with specific form. (Robert and Convissar, 2011). Temporal or longitudinal coherence is referring to the closeness in phase of several portions of the laser frequency bandwidth. While the closeness in phase of different spatial portions of the beam after the beam has propagated a certain distance is referred to as spatial or transverse coherence (William and Silfvast, 2003).

2- **Collimation:** All the waves are traveling in a certain direction and hence they are all parallel to each other and travel for long distance. Lasers produce the most collimated light with the smallest divergence angle (Robert and Convissar, 2011).

3- **Monochromaticity:** Laser light is generated only as one color. This color can be either visible or invisible to the human eye. Monochromaticity refers to how pure in color (frequency or wavelength), the laser beam is, as all the photons have the same wavelength (William and Silfvast, 2003).

4- **Focusability:** It is the ability of focusing the laser beam to be precisely to a very small spot size . Transverse electromagnetic mode (00) can produce a laser light with the smallest beam diameter, nearest to the dimension of the wavelength of the laser (Robert and Convissar, 2011).

5- **Brightness:** It referred to the high concentrations of energy when the laser beam is focused on a small spot area. It is rises from the high degrees of collimation of the laser as it passages through spaces keeping its concentration. (Catone and Alling, 1997).

1.6.6 Fractional CO_2 laser

A CO_2 laser of wavelength 10600 nm with an advanced technique referred to as a Fractional CO_2 laser. Fractionated lasers deliver energy in parallel vertical columns of multiple microscopical thermal spots termed microscopic treatment zones (MTZs), while the distance between spots remains intact and untreated (Ahrari *et al.*, 2013; Hantash *et al.*, 2007). The concept of fractional photothermolysis(FP) was introduced in 2003 (Huzaira *et al.*, 2003), and then FP became commercially available for clinical use in 2004 (Geronemus, 2006).

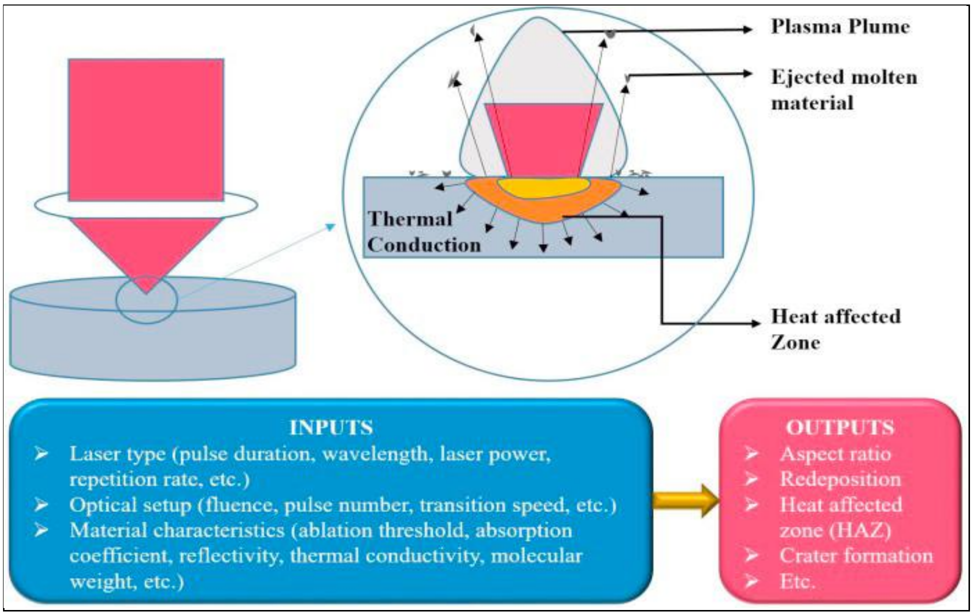
Studies have shown that fractional delivery may be higher to the traditional uniform delivery of heat due to the fact that higher irradiation within the columns results in more effect. This can be reached without increasing the power of the optical laser source, also due to increased surface-to-volume ratio of the (MTZ) with larger safety margins (Huzaira *et al.*, 2003).

The use of Fractional CO_2 laser may have several advantages in dentistry, especially when surface treatment is of concern. The precise irradiation area can be determined by the apparatus and the laser irradiates multiple zones in the target area with predefined space between them. As a result a more homogenous etching pattern can be achieved, also restriction of the manual movement of the laser handpiece during conditioning. In addition, a lesser amount of thermal damage to the underlying area compared to that happens with conventional CO_2 laser (Ahrari *et al*., 2013).

1.6.7 Laser ablation

Ablation is the top-down process happens by focusing a laser beam onto a substrate to removing materials, occurs only when sufficient energy absorbed by the material to be melted or vaporized (Ravi-Kumar *et al*., 2019). It is a combination of vaporization and melt expulsion. Figure (1.5).

After absorption of focused laser beam , the electrons of the substrate are excited by laser photons (Brown & Arnold 2010). This excitation lead to generation of heat by absorbing photon energy, which is consistent with Beer Lambert's law (Ahmed *et al.*, 2016) . This law states that the amount of absorption of light is dependent on the intensities of the light and thickness of the materials.



Figure(1.5): The mechanism at the laser-material interface (Ravi-Kumar *et al.*, 2019).

This phase conversion occur in a sequences of steps. By the absorption of the laser photons the initial heat produced and results in the formation of a melting pool at the laser-substrate interactions zone. Due to the incoming pulses the temperature of the material is further increased and the melt pool reaches the vaporization state (Von der Linde &Sokolowski-Tinten, 2000).

During vaporization high pressure is produced, which is also called a recoil pressure, it pushes molten materials from the pool where it is ejected (Hoffman, 2015). The ejected materials are concern due to its redeposition on the interaction zone or on the substrate. (Singh *et al.*, 2005; Tangwarodomnukun *et al.*, 2015) . At the laser-substrate interaction zone, the liquid reaches an explosive liquid vapor phase transition stage by further increasing the temperature (Bulgakova & Bulgakov 2001). The molten material resolidification lead to geometric changes in the ablated features of the substrate . According to laser and materials properties such as fluence, wavelength, pulse duration absorption coefficient and reflectivity, the mechanism of ablation can be purely thermal, chemical or a combination of both.

Photochemical ablation happens due to breaking of the covalent bonds in the chains of polymer by the energy of the UV photons. While Photothermal ablation occurs by excitation of the electrons by photons of UV irradiation to be thermalized which then results in the breaking of the bonds of polymer. So, while studying the laser-material interaction, it is essential to consider some important phenomena. These phenomena include the magnitude of light energy absorption and the time scale of the laser pulse.

At normal intensities, the linear absorption is occur and follows Beer Lambert’s law. But, at ultra-short timescale, nonlinear absorption occur and becomes intensity dependent. The direct ionization of the bound electrons of the materials can be done by large absorption coefficient and due to high-intensities. Thus, it is important to characterize the laser type and to predict the mechanism that occur (Ravi-Kumar *et al*., 2019).

Laser ablation of polymers depends on many factors such as laser wavelength (LaHaye, 2013) , repetition rate (Burns & Cain, 1996) , fluence (Okamuro, 2010) , and pulse duration (Chichkov, 1996) . Initially, for a system of laser, two features are necessary to laser ablation, the directionality and monochromaticity of the beam. The monochromaticity is the wavelength of all radiated light is identical.

1.6.8 Laser texturing

It is direct treatment of polymeric biomaterials by a laser beam, (LST; laser texturing, laser structuring, or laser patterning). This technique have many advantages ; and the main one is the ability of one step alteration the surface roughness and modification of the polymeric surfaces at a macro-, micro-, and nano-size scale with a high spatial and temporal resolution (Lippert, 2004). Furthermore, this process happen with non-contact nature and easily avoided contamination of the workpiece ; this is additional advantage for biomedical applications by sterilization of the implant can be guaranteed. Also, Another advantages are the high speed during processing, and large areas could be treat. The roughness modification also leads to the change in the wettability (or surface energy) of polymers (Riveiro *et al.*, 2018).

1.6.9 Process Fundamentals

Laser surface texturing (LST) is one of the simplest methods for modification of the surface topography and selective removal of the material is achieved (Etsion, 2005) . The focused laser beam is directed to the materials surface; then, the topmost layer absorbed the laser radiation (Figure 1.6). Heating of the material happen by the absorbed energy, reaching the melting, or even the vaporization temperatures. If the photons of the laser radiation are sufficiently energetic, e.g., UV-laser, they are able to breaking the chemical bonds, and then changing the surface chemistry of the polymers. Therefore, thermal and/or photochemical processes can be changing the surface of polymers:

- Thermal processes: Thermalization of the optical energy in the surface of the polymer causing increasing in the temperature of the material, this leading to melting or vaporization ,which is resulting in modification of the surface roughness (Tan *et al.*, 2015).
- Photochemical processes: High energy of the laser photons can cause direct breaking the molecules of the irradiated surface. This process is responsible for the chemical surfaces modification . So, the ultraviolet (UV) lasers are the most commonly employed ones due to the requirement of high energy photons (Wong *et al*., 2001).
- Photophysical processes: in this mechanism, thermal and photochemical process jointly influencing produce the effect (Bäuerle *et al.*, 2013). So, both surface roughness, and chemistry can be simultaneously modified.

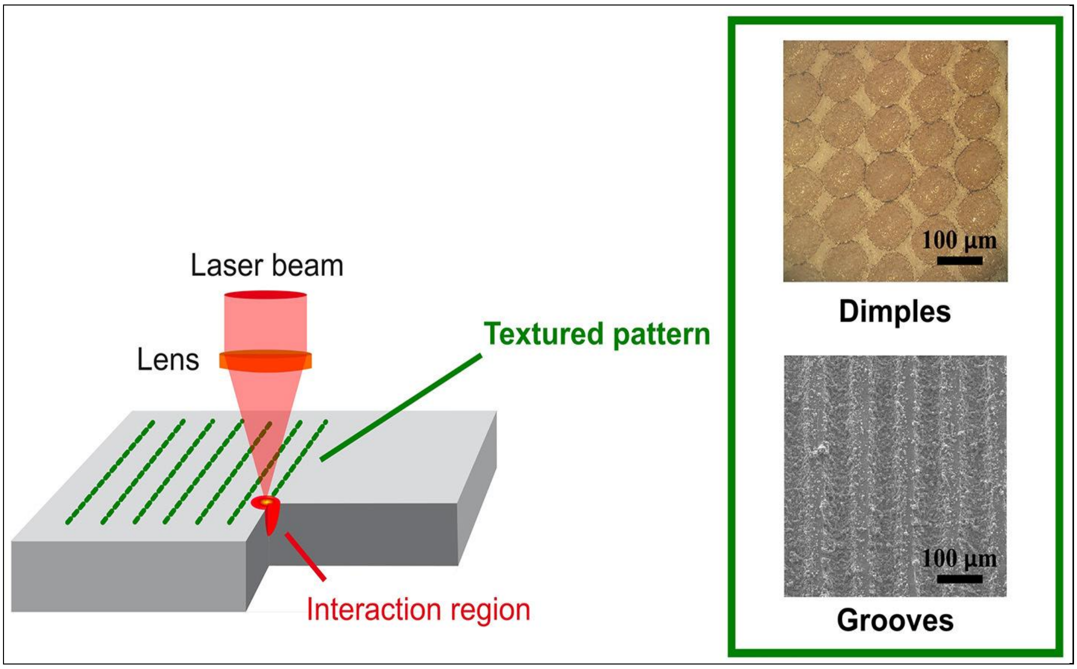


Figure (1.6): Schematic of the principle of operation of laser surface texturing (LST) (Riveiro A, 2018).

LST effect can create regular or irregular patterns of bumps, dimples, and (linear or non-linear) grooves as shown in Figure (1.6) (Etsion, 2005). If the laser beam melted the surface of the material, bumps or dimples can be formed due to projections formation, depressions or because of foaming of the materials.

The UV–IR spectral range can be produced a patterns in biomedical polymers by using continuous-wave (CW) or pulsed laser radiation (Makropoulou *et al.*, 1995; Serafetinides *et al.*, 2001). It depends on many parameters in addition to the nature of the polymer used to produce the desired surface pattern and mechanism (e.g., photo-thermal or photo-chemical ablation of the material, laser swelling or bumping, laser grooving, etc.) (Eaton *et al.*, 2012; Bityurin, 2014; Roa *et al.*, 2014) .

Polymers structure determine its absorption characteristics, but this affected by fillers or additives present in its structure . IR radiation tends to produce thermal ablation or melting of the polymers, while UV radiation is tends to ionize and decompose polymers without substantial melting. This laser radiation can also modify the surface chemistry of polymers, the polar component of the surface energy can be greatly increased, and the wettability of polymers can be promoted (Lawrence 2001).

1.7 Aim of the study

The present study aims to:

1. Estimation of suitable parameters of Carbon dioxide laser for surface treatment of PEEK material to be used as dental implant .
2. Study the effect of Fractional CO_2 laser surface treatment of polyetheretherketone using light microscope, scanning electron microscope, EDS, AFM and contact angle test.
3. Evaluation of the effect of Carbon dioxide laser surface treatment on osseointegration of PEEK implant by torque removal test on after (2 and 6) weeks implantation in rabbit tibia in comparison to commercially pure titanium.
4. Study the responses of bone healing of Fractional CO_2 laser treated PEEK implants by histological examination of bone after (2 and 6) weeks after implantation in rabbit tibia in comparison to commercially pure titanium.

CHAPTER TWO

Materials and Methods

CHAPTER TWO

MATERIALS AND METHODS

This chapter includes a description of the sample preparation, grouping, irradiation procedure, materials and equipment used in the present study with the method(s) used to perform the study.

2.1. A. List of Materials used in this study:

Materials	Company and origin
Ceramill PEEK 98X20 N	(JUVORA dental innovations, UK
Ethanol absolute 99.8%.	Sigma-Aldrich Company, Germany
Titanium bar grade 2, 6mm in diameter	Baoji Jinsheng, China
Nitric acid (HNO3) 70%	Sigma Aldrich, Germany
Hydrofluoric acid (HF) 48%	Honeywell Fluka, Germany
Ketamine10%, Xylazine 20%	Kepro, Holland
Sodium chloride solution (Normal saline)	0.9% /India
Antibiotics: Injection of ceftriaxone 1g	Julphar/ U.A.E

2.1.B. List of Equipments

Equipments	Company and origin
(CAD)-(CAM)/Computer Aided Design Computer Aided Manufacturing system	Sirona /Germany
Clamp holder with vertical arm	
Digital ultrasonic cleaner	NEW TREND/China
Digital Venire	TOPEX 150 mm /China
Fractional CO_2 laser system	CO_2 fractional laser Brouchure / JHC118/China
Laser protective eyeglasses	
Polishing machine	mopea/160E/China
Light-microscope	BX51/OLYMPUS/Korea
AFM	AA3000/Anstgrom Advanced.Inc./USA
Contact angle measuring device	Creating Nano Technologies Inc., Taiwan
Scanning electron microscope	Hitachi S4700 EDAX ApolloX Genesis software
Customize made holder to fix the PEEK sample in place.	Iraq
Polydioxanone suture	(3/0 absorbable, reverse cutting, Demetech/ England
Silk suture	3/0 Non-Absorbable, ½ circle curved cutting/ China
Disposable syringes (5ml)	China
Surgical towels, gauze and Cotton	China
Latex surgical gloves and masks	Malaysia
Shaving machine	China
Scalpel handle with blades	China
Screwdriver	China
Tissue forceps	Italy
Flap reflector (Italy)	Italy
Needle holder	Pakistan
Drills with diameters	(1.3 mm, 1.82 mm and 2.31 mm) (South Korea)
Autoclave	Wisow, China
Engine	Saeshin, South Korea
Digital torque meter	TQ-8800/ Taiwan
Scissors	Italy

2.2 Methods

2.2.1 In vitro experiments:

2.2.1.1 Sample preparation:

Ceramill PEEK 98X20 N (JUVORA dental innovations, UK) block figure(2.1A) was used to prepare substrate . By CAD- CAM system , the PEEK block was cut into discs with (2 mm thickness and 10 mm diameters) figure(2.1B). For having a uniform smooth surfaces and standardization , the discs were smoothen by using silicon carbide paper of 500 grits by rotating polishing machine at 200 rpm for one minute. Cleaning of the PEEK discs was done by using ultrasonic device, this help to remove the debris might be attached to the discs surfaces.

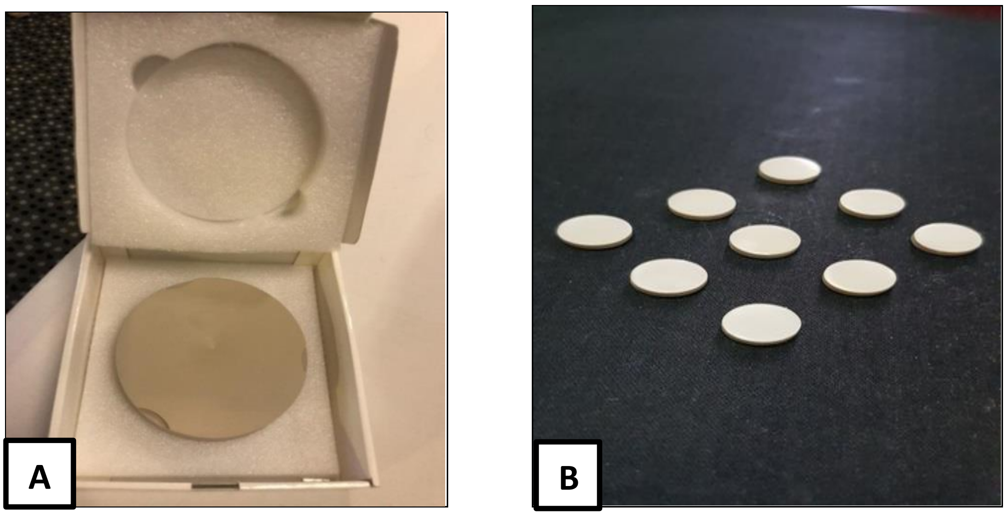


Figure (2.1): PEEK A) block B) discs.

2.2.1.2 Laser irradiation:

Laser irradiation was done at the Institute of Laser for post graduate studies/ University of Baghdad. Fractional CO_2 laser system was used for surface treatment of PEEK samples. A clamp with vertical arm was used to stabilize the head (handpiece) of laser system directed perpendicularly during laser irradiation. The process accomplished at a fixed distance between the PEEK sample surface and the tip of the hand piece (50 mm from the lens). The laser energy was delivered in the fractional mode and a circular area of 10 mm diameter. Figure (2.2A,B and C).

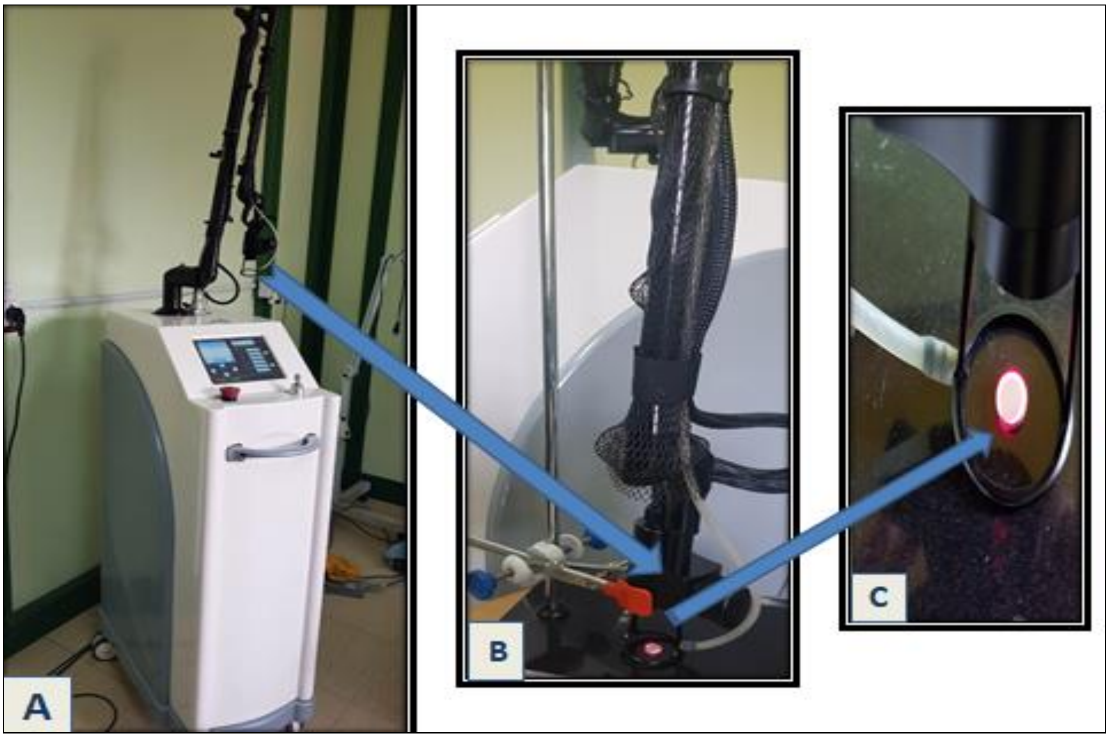


Figure (2.2) A: Fractional CO_2 device. B: Stabilized articulating arm of the device C: PEEK disc ready for laser irradiation.

2.2.1.3 Pilot study:

Different parameters were tested to study their effect on PEEK, therefore several trails were made considering these parameters. Effect of these variables were evaluated by laser irradiation then using the following tests:

I. **Light microscope:** Surfaces of the PEEK discs were examined microscopically at (10,20,40X) magnification powers using light microscope with digital camera . Figure(2.3).

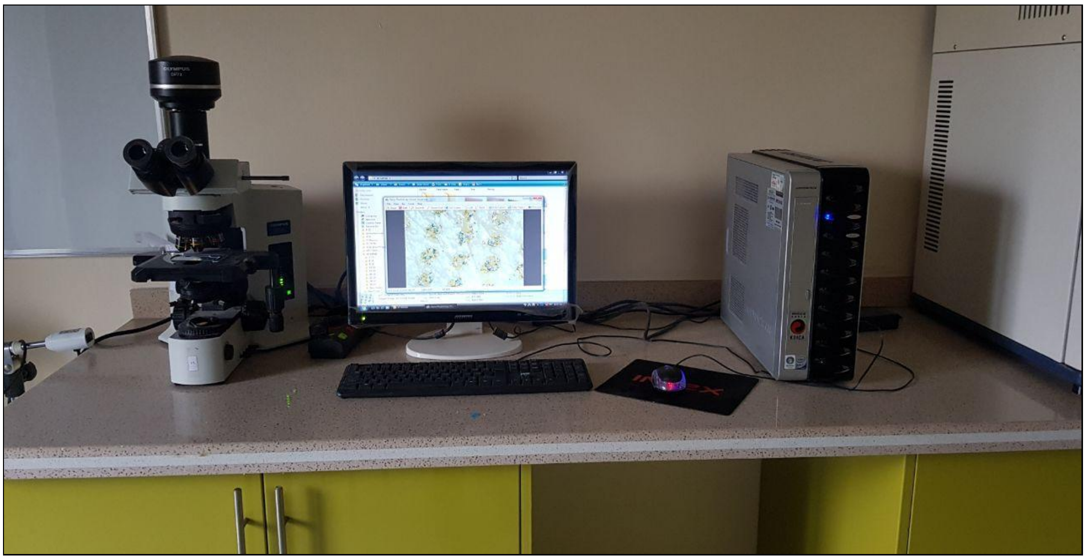


Figure (2.3): Light microscope.

II. **Scanning electron microscope (SEM):** Laser irradiated samples were scanned by SEM (Oxford instruments, UK) to see the difference on the surface topography . This test done in Production Engineering and Metallurgy Department –University of Baghdad . Figure(2.4)

Figure(2.4): Scanning electron microscope.

III. **Energy-dispersive X-ray spectroscopy (EDS):** PEEK samples were scanned by (EDS) (Oxford instruments, UK) for analyzing the surface of PEEK and to calculate the percentage of the elements (carbon) on the surface of the sample. Figure(2.4) .

IV. **Contact angle:** The wettability test was conducted using Contact angle measuring device , and using drop of normal saline .The size of the drop was 6.89 micro liter and the distance between the needle and the sample was 4 mm. The specimen was placed on adjustable table and dropper was used to dispense the drop figure(2.5)

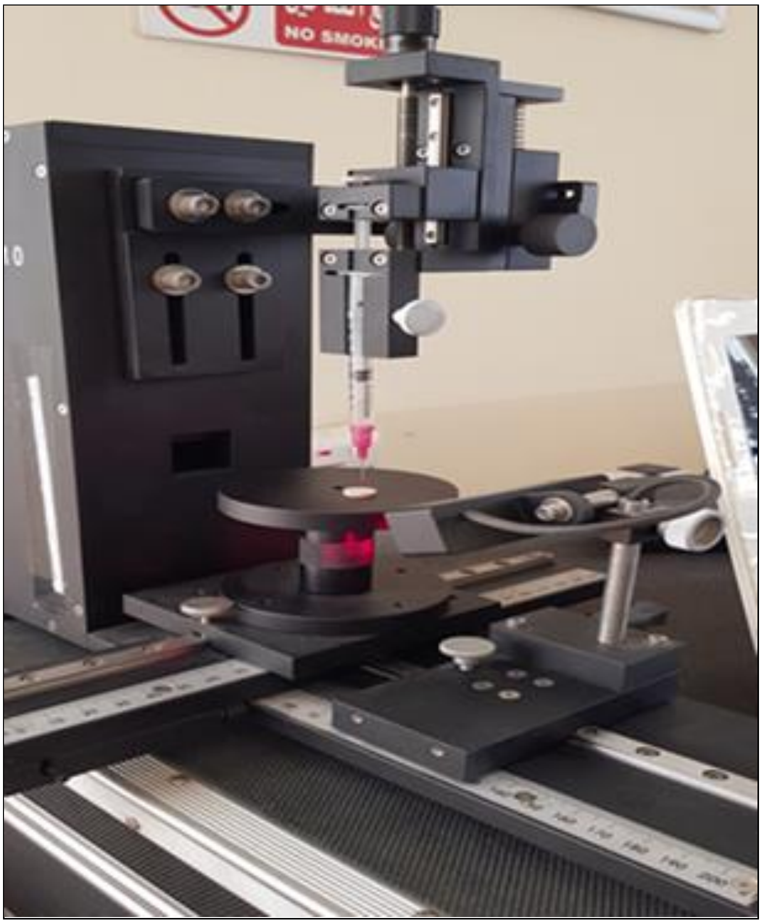
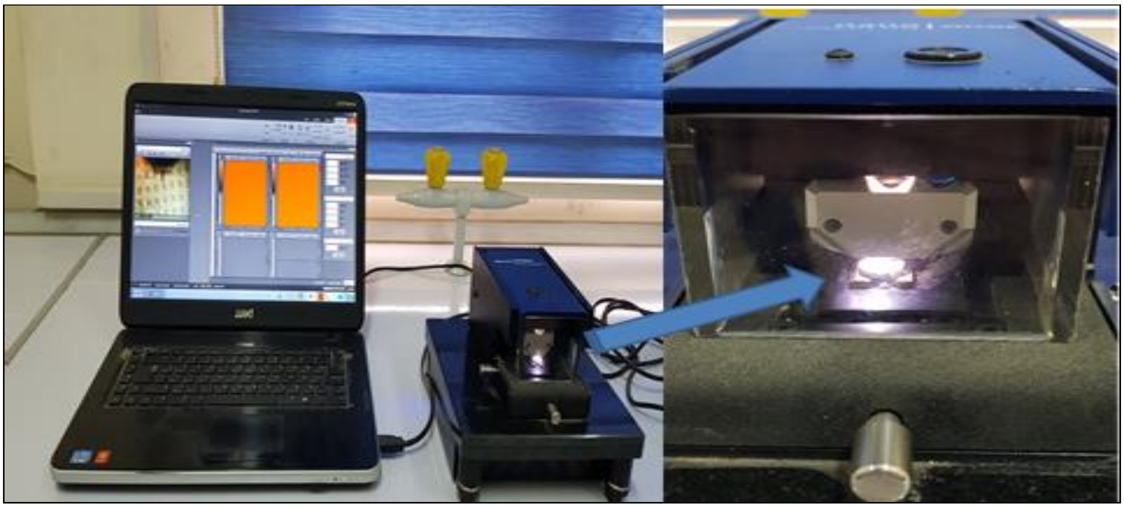


Figure (2.5): Sample tested for wettability

V. Surface roughness measurement(AFM): Surface roughness was assessed by Atomic Force Microscope (AFM), as shown in Fig (2.6). The diamond tip of the device with a scanning rate of 3 Hz was passed through the surface of the samples in a contact mode. The average surface roughness (Sa) was determined for non-irradiated specimen and other irradiated specimens those which determined according to the microscopical examination.



Figure(2.6): Atomic force microscope (AFM).

Laser parameters in PEEK irradiation

1. The powers were used (2,4,6,8,10,12W) with minimum distance between spots 0.2mm to ensure maximum coverage of laser effect, and minimum duration 0.2 , there was no effect .
2. Pulse duration was also tested starting from 0.2ms then increased gradually (0.4,0.6ms) to increase the energy per pulse, and there was no effect up to 0.8ms the effect was appeared
3. Considering the result from previous trials an attempt carried out regarding distance ,duration and scans. Then decision was made to

increase the distance between the spots to reduce to reduce thermal effect.

4. To test the effect of the number of scans, another trials were made regarding same parameters. two scans with different interval (10,20,40) seconds to enhance heat dissipation and redaction of thermal damage.

Data collected from the pilot study was analyzed to decide the best parameters planes to be used in the in vivo study .It's clear that increase the power over 6 W lead to carbonization and change the chemistry of the material which may affect its biocompatibility. Furthermore reduction in the distance between spots also increased the chance of carbonization due to enhance the thermal effect within the material. So the most preferable parameters used in vivo study were (6W, 0.4mm distance, 0.8 ms duration and one scan).

2.2.2 In vivo experiments

2.2.2.1. Implant preparation

I. Method:

Twenty four screws were prepared from PEEK block and twenty four screws shaped implant were prepared from the titanium rods, CAD-CAM machine was programed to cut the screws. The screws length were 8mm (3mm flat part and 5mm threaded part) and 3mm in diameter, figure(2.7), on the top surface of flat part, a slot was made to engage the screw driver during insertion and torque measurement, figure (2.8) (Hamad, 2007).

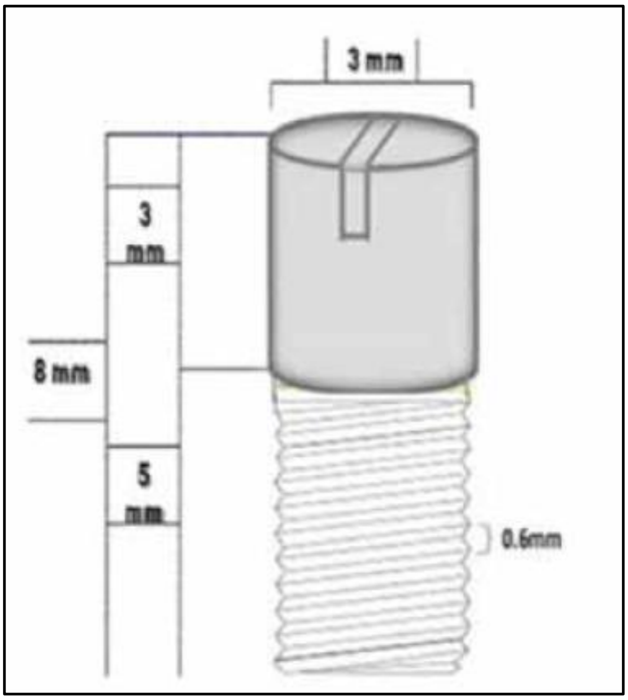


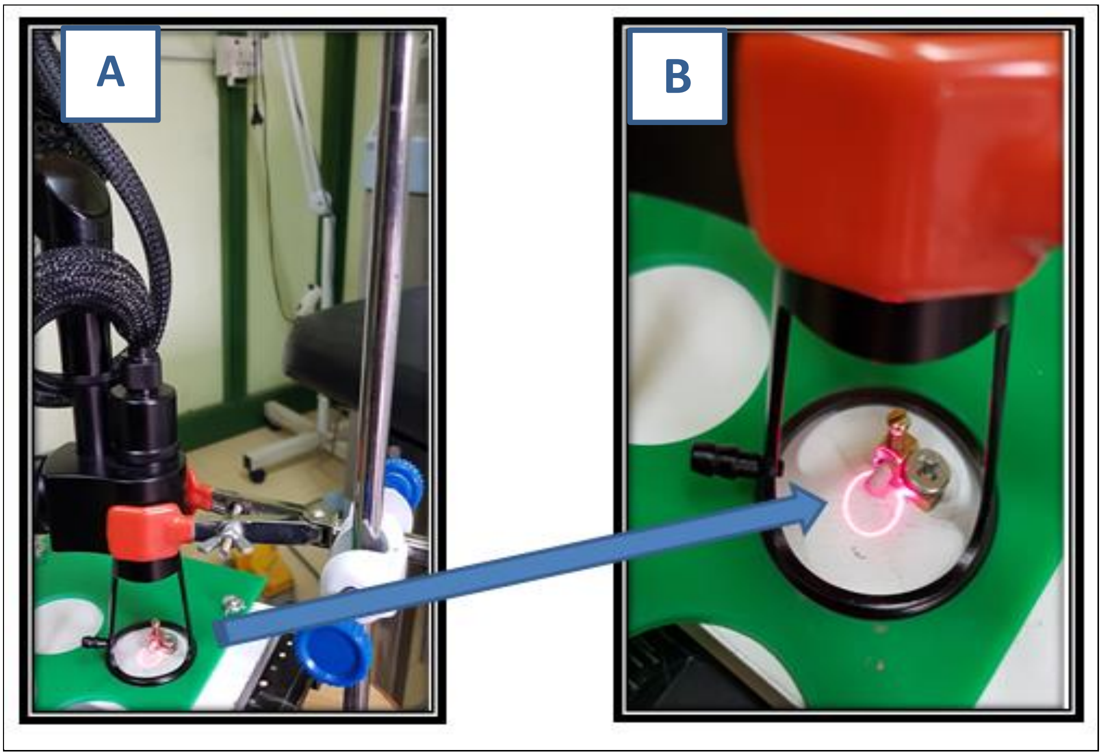
Figure (2.7): Screw implant design.

Figure (2.8): Titanium and PEEK screws.

To remove all the contamination from the Ti screws, they were immersed in a solution constitute of (3ml nitric acid (HNO_3), 1ml of hydrofluoric acid (HF) and 6ml distal water). This step was followed by ultrasonic cleaning for all PEEK and titanium screws (Jani, 2014). Figure(2.9).

Figure(2.9): Ultrasonic cleaner.

Then PEEK screws were irradiated by fractional CO_2 laser with the parameters which selected in the pilot study .The irradiation performed by using custom made holder to fixed the screws in stable position and distance from the source of laser during procedure of irradiation. Figure (2.10A,B).



Figure(2.10): A) Handpiece of fractional CO_2 laser device fixed to vertical arm B) PEEK screw fixed by custom made holder.

2.2.2.2 Sterilization:

Every 2 screws were put in airtight plastic sheets (Figure 2.11 A and B), then sterilized by autoclave sterilizer by using plastic option ($121C^{\circ}$, 15 Bar, 20min), titanium screws and all the instruments were autoclaved at 121 C° and 15 bars for 30 minutes (Tekin *et al*., 2018). Figure (2.12).

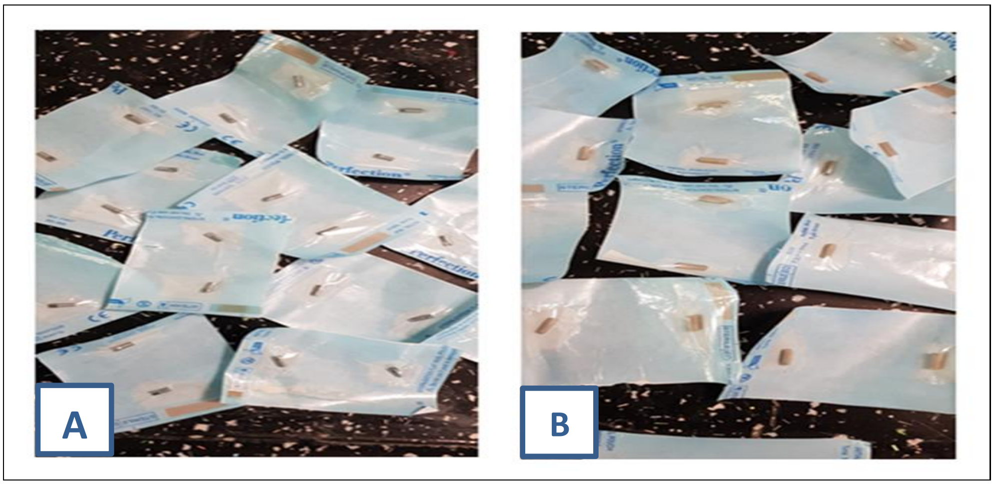


Figure (2.11): Screws A)Titanium B) PEEK.

Figure (2.12): Autoclave used in sterilization.

2.2.2.3 Sample grouping:

The overall animals (12 rabbit) were grouped into two groups according to the healing interval and sample collection (2 and 6 weeks) . Each group consisted of 6 animals, every femur of each animal was implanted with two screws (one PEEK and one titanium).The samples for histological study were collected from 2 animals from each group , whereas the other 4 animals were sacrificed for torque removal test.

Forty eight screws were divided into two groups according to the test performed:

- 32 PEEK and titanium screws for (torque removal test): were divided into:
  - **I.** Control group: 16 titanium screws (8 screws for each interval 2 and 6 weeks).
  - **II.** Experimental group: 16 PEEK screws (8 for each interval 2 and 6 weeks).
- 16 PEEK and titanium screws for histological test: were divided into:
  - **I.** Control group: 8 titanium screws (4 screws for each interval 2 and 6 weeks).
  - **II.** Experimental group: 8 PEEK screws (4 screws for each interval 2 and 6 weeks). Figure (2.13).

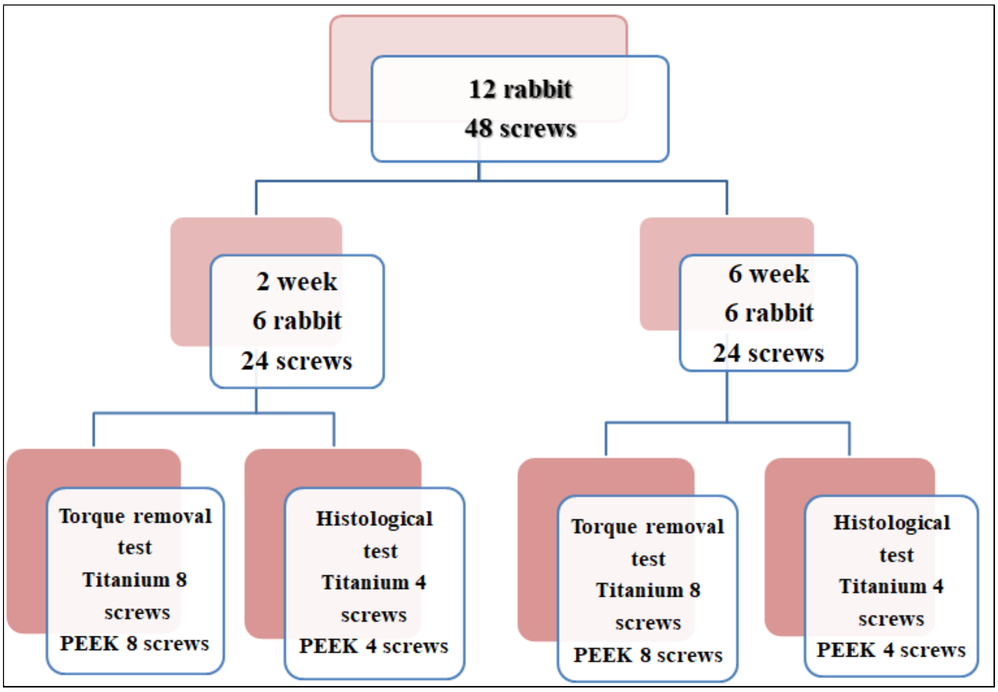


Figure (2.13): Sample grouping and distribution of the screws in vivo experiment.

2.2.2.4 Experimental animal description

Twelve adult male of New Zealand Albino rabbits (weight 1.5 -2 kg) 12-14 months of age were including in this study. The rabbits were kept in cages and fed with various pellets with protein, jet and carrot. (figure2.14). The animals were conditioned in the animal house for one week before date of surgical operation ,three days before the operation, Ivermectin injection (0.2 ml/Kg) was given subcutaneously to eradicate the internal and external parasites. Intramuscular injection of an antibiotic (ceftriaxone) was given once daily (0.5ml/Kg) for 3 days to avoid any infection can be happen (Lenney *et al.,* 2011).

Figure (2.14): Rabbits in cages.

2.2.2.5 Implantation and Surgical Procedure

I. Preparation

Both femurs were shaved from outer side and skin was cleaned with povidone iodine .In order to determine the amount of dose required for antibiotic and general anesthesia, the animals were weighed before surgical operation.

All the surgical operation were done at aseptic condition and sterilized equipment in the operating room of the privet animal care office. Figure(2.15).

Figure (2.15): Some of the instrument used in the surgical operation for the rabbits.

II. Anesthetic protocol:

To anesthetize the animals , ketamine hydrochloride (1 ml/kg) and xylocaine 2% (1 ml/kg) was given intramuscularly (Azzawi *et al*, 2018).

III. Surgical technique:

After the induction of general anesthesia the animal positioned with the lateral side of the thigh region faced to the surgeon, figure (2.16).

Figure(2.16): Prepared site of operation (rabbit femur).

Disinfection of the surgical field with 2% iodine, cover all the area with sterile drapes except the surgical site. Figure(2.17).

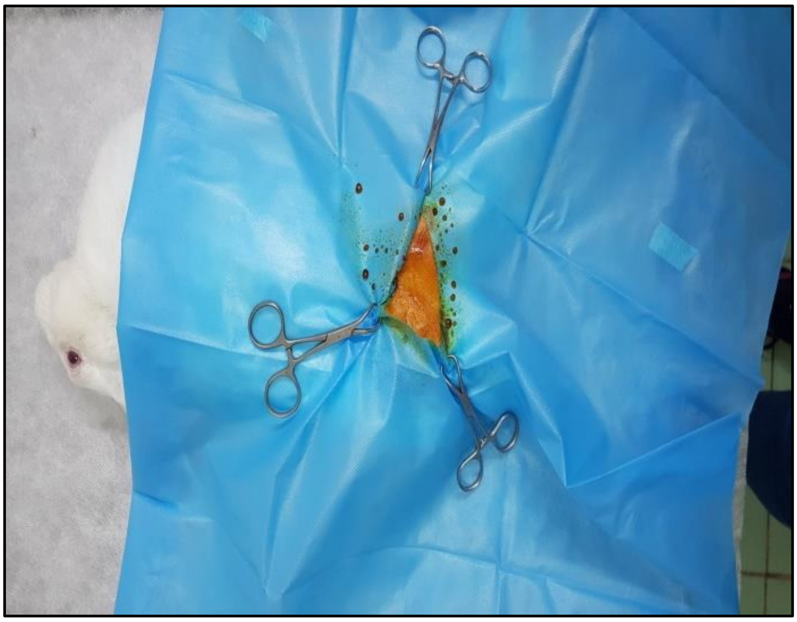


Figure (2.17): Disinfection of the surgical field with 2% iodine.

Three centimeters length of skin was sharply incised with the subcutaneous tissues, the skin and fascia were reflected also a dissection of the muscle was made to expose the distal side of the femur bone. Figure (2.18) .

Figure (2.18) : Femur bone exposure after skin and muscle reflection.

First access to the implant site was done by penetrating the cortical bone using round bur of 1.3 mm diameter . In the same way two drills were done with 1 cm apart. To perform implant site drills , a rotary instrument set at 800 rpm speed was used, the slow speed and profound irrigation was essential to prevent heat generation, figure (2.19). Final hole diameter was 2.5 mm according to Jani, 2014, the enlargement was done gradually using fissure bur .The drilling was made by using the implant surgical engine.

Figure (2.19): Distilled water irrigation during hole drilling .

The implant samples were removed from the plastic sheet and placed in the holes via torque meter that fitting the screw slot up until 5mm of the screw (threaded part) was totally embedded into the bone tissue and test out for stability .Figure (2.20).

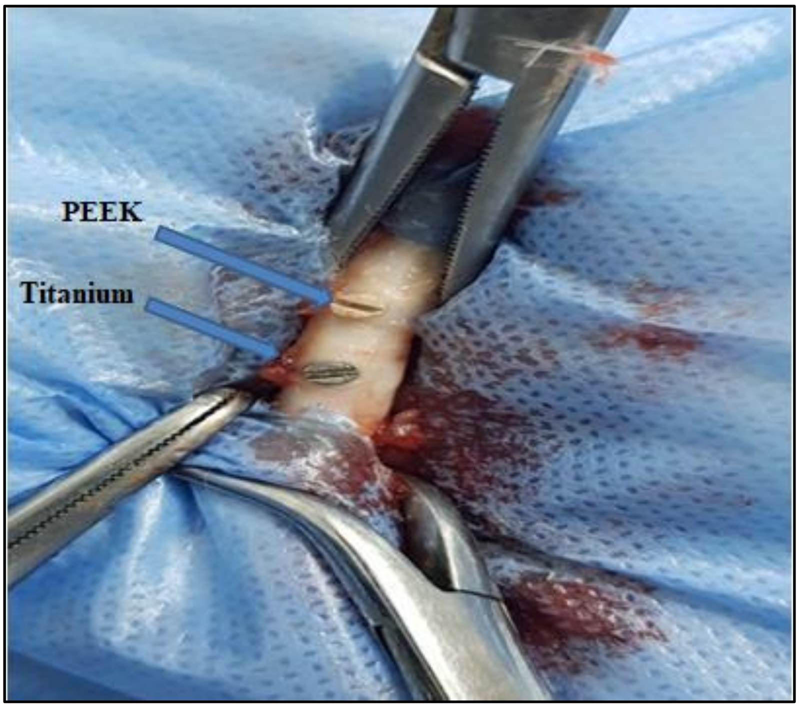


Figure (2.20): Titanium and PEEK screws in there prepared sites.

In this study, the size of the holes that made in the bone were slightly smaller than the size of the implant screws in order to obtain surgical fit. All titanium screws inserted medially while peek screws placed in the distal holes. The stitching of muscles was made with absorbable catgut suture 3/0 as shown in figure (2.21), while a silk suture 3/0 was used to stich the skin. (figure 2.22).

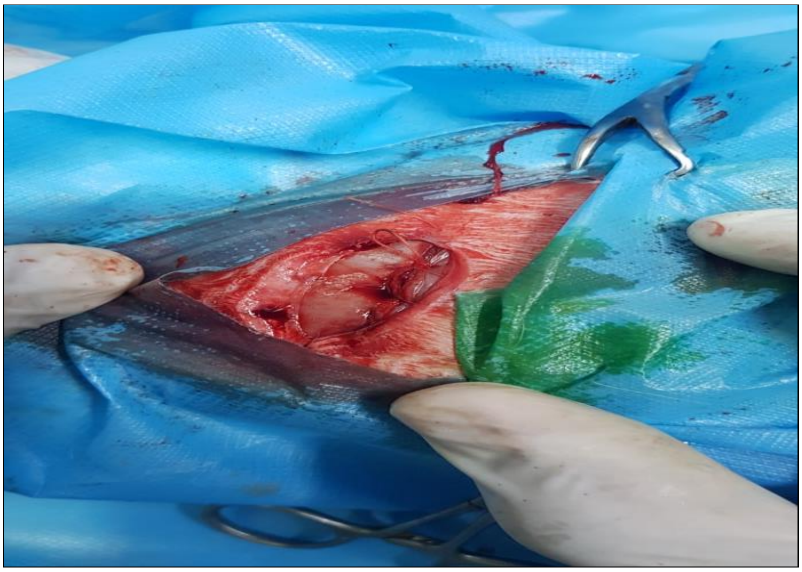


Figure (2.21): The stitching of muscles.

Spraying of the operation site was carried out with local antibiotic (Oxytetracycline spray). Systemic antibiotic for postoperative care (Ceftriaxone 1g, 0.5ml/kg body weight) once daily was given for 3 days after surgery. Then the rabbits were followed for 2 and 6 weeks. The surgical operations was done in private research veterinary unite.

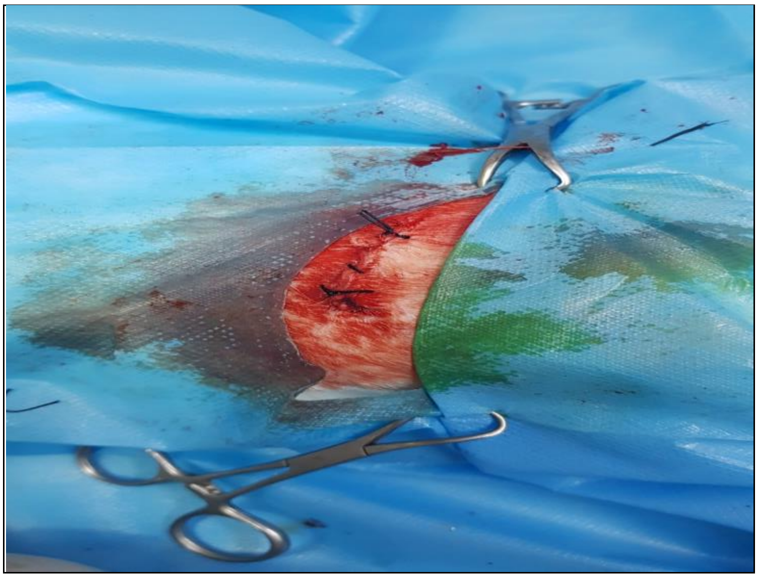


Figure (2.22): Skin stitching (after completed suturing).

2.2.2.6 Torque measurement:

For the purpose of this study and to have accurate measurements of removal torque of screws, a digital torque meter (TQ-8800/ Taiwan) with range of 0.1 to 147.1 N.cm was used to test torque value. Figure(2.23). The rabbits were given anesthesia as recommended in the implantation phase , with same way and dose.

The incision was done medially to the first incision of implanted femur with reflection of skin, fascia and muscle for exposing the implant. By using two hand metal instruments, the implants were checked for stability to assess possibility of failure.

The bone was well supported while digital torque meter attached to the implanted screws to ensure no movements during testing that may affect the accuracy of the test. After engagement of the torque meter screw driver with slit of the implant head, a torsional force was applied for unscrewing the implant and the value was measured in Newton centimeters (N.cm). This step was performed after 2weeks and 6 weeks after implantation .

Figure (2.23): Torque meter device.

2.2.2.7 Histological sample preparation:

For histological evaluation , according to the healing periods and materials four screws were examined . Scarifying of the animals was done by isoflurane general anesthesia over dose .

Disc and mandrel used to separate bone blocks with implants , under continuous irrigation with normal saline and slow speed cutting . Bone-implant blocks were obtained by cutting about 1 cm away from the implant screws. Figure (2.24 A,B,C).

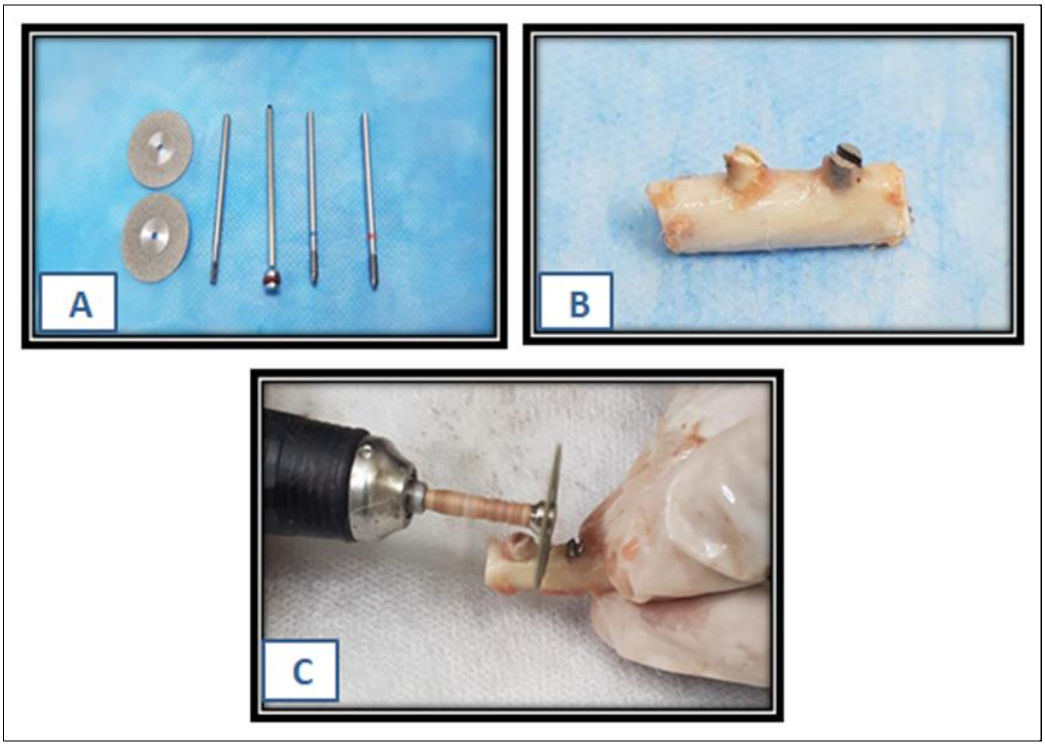


Figure (2.24): A) Disc and mandrel. B) bone implant block after cutting. C) Cutting of histological sample.

Sample preparation for histological analysis was done according to Carson, (2007):

1. The blocks of implants screws were stored in 10% formalin for at least three days for fixation.

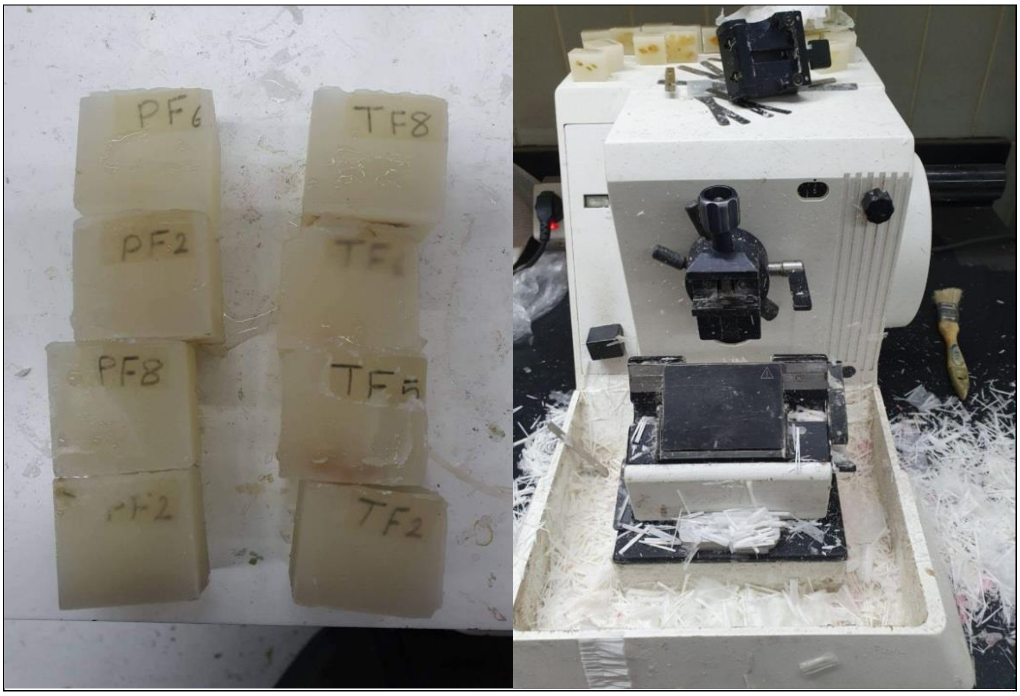
2. After fixation with formalin, bone-implant block was washed slowly in running water, and left in 8% formic acid that changed every day until complete decalcification.

3. Bone-implant block was checked for decalcification completely when the block could be penetrated by a needle to the deepest part, which it took 2 weeks for complete decalcification.

4. The implant (screw) was removed gently from the bone bed.

5. The bone was cut in cross section (horizontally) by new sharp scalpel.

6. The spacimen was put in a small closed basket and then in a running water for one hour for washing.
7. After that, the spacimen was dehydrated by putting it in a dish of alcohol with increasing the concentration percentage (70%, 80%, 90% and 100% alcohol). The specimen remains in each concentration for 60 minutes.
8. Then the specimens were passed through three changes of xylene for one hour and put in a jar of melted paraffin into an oven with temperature of 58- 60℃ for half an hour. Subsequently, the paraffin wax will take the place of the xylene in the tissue. The specimens were placed in the center of paraffin block. Figure(2.25A).
9. The paraffin block was adapted to microtome to create a series of sections with thickness of (4-5) µm, and put on a slide. Figure(2.25B)
10. Dewaxing in xylene for (20-30) minutes and then rehydration by passing the tissues through descending concentration of ethanol alcohol (100%, 90%, 80%, 70%) for total period of 3-5 minutes.
11. The slide were then washed in distal water for five minutes .
12. In order to stain the tissue, the slide was immersed in hematoxylin and eosin and stain for ten minutes. After that, it was washed with deionized water, then a cover glass was fixed on the slide with Canada balsam.
13. By using a light microscope (BX51/OLYMPUS/Korea), photographs of the sections were taken at 10, 20 and 40 magnification.
14. The histological slide preparation was done in Human Anatomy department/College of Medicine / University of Al- Nahrain.



Figure(2.25): A) paraffin block. B) microtome.

2.2.2.8 Statistical analysis

The assessment and analyzing of the result was performed by descriptive and inferential statistics methods using IBM SPSS statistic program (version- 20.0) and Microsoft excel program (Office 2013).

1. Descriptive statistics:

A) Summary statistic of readings distribution (maximum, minimum, mean, standard deviation SD and standard error SE).

B) B-Graphs such as bar chart.

2. Inferential statistics:

Acceptance or rejection of the statistical hypotheses was determined by inferential statistics that include:

A) 95% confidence interval of the difference (lower and upper bonds).

$P > 0.05$ non-significant

$P \leq 0.05$ significant

B) The means compared by student T test.

CHAPTER THREE

Results, Discussion and Future Work

CHAPTER THREE

RESULTS

Result of this work, discussion of the result, conclusion and future work suggestions are present in this chapter.

3.1 Results

3.1.1 In vitro experiment

1. Light and scanning electron microscope (SEM):

A) Testing of different powers (2,4,6,8,10,12w) with minimum distance between spots 0.2mm and minimum duration 0.2ms were with no effect visually and microscopically. Table (3.1).

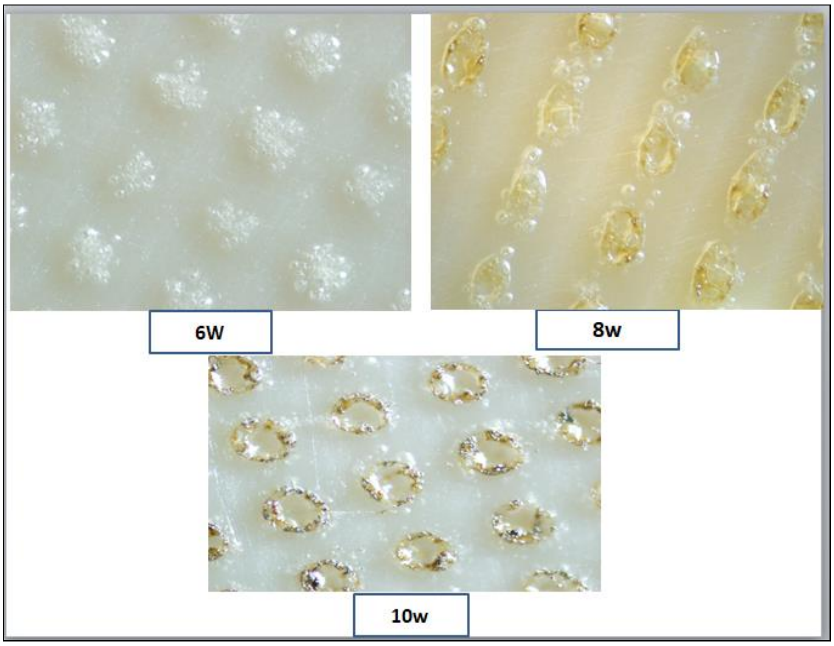
Table (3.1): Effect of different laser powers on PEEK surface using fractional CO_2 device at 0.2mm distance , 0.2ms duration and one scan.

Power W	Distance 0.2 mm	Duration 0.2 ms	No. scans 1	Effect
2				No effect
4				No effect
6				No effect
8				No effect
10				No effect
12				No effect

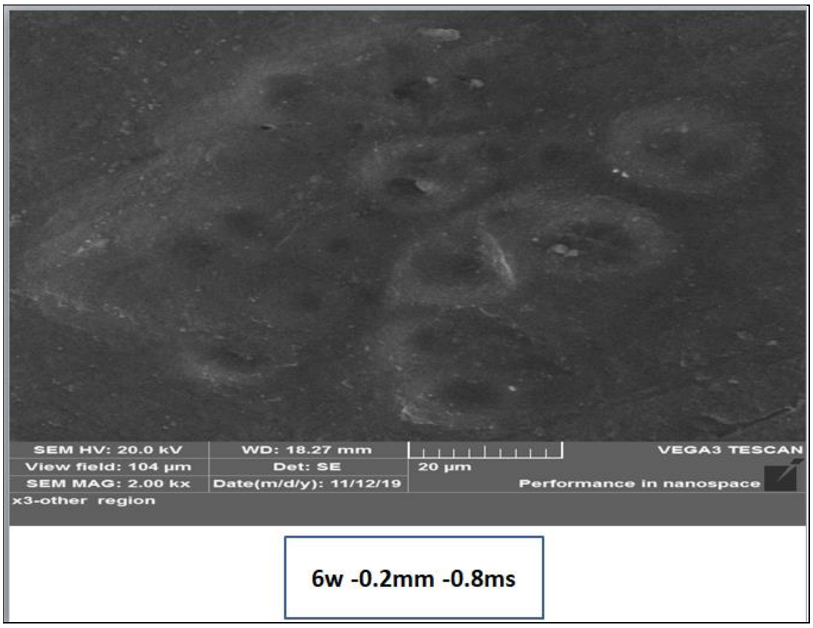
B) Pulse duration was also increased starting from 0.2ms, with short pulse duration (up to 0.6ms) there was no effect even with increasing the power, table (3.2). When pulse duration reach 0.8 ms laser effect was appeared on the surface, as shown in figures (3.1) and (3.2) which display different degrees of effect with different powers.

Table (3.2): Effect of different pulse duration in relation to the powers on PEEK surface using Fractional CO_2 laser device at 0.2mm distance and one scan.

Pulse Duration / Power(W)	0.2ms	0.4ms	0.6ms	0.8ms	Distance (0.2mm)	No . Scan 1
2	No effect	No effect	No effect	No effect		
4	No effect	No effect	No effect	No effect		
6	No effect	No effect	No effect	Good effect		
8	No effect	No effect	No effect	Sign of carbonization		
10	No effect	No effect	No effect	carbonization		
12	No effect	No effect	No effect	carbonization		
14	No effect	No effect	No effect	Carbonization		



Figure(3.1): Light microscope image of PEEK specimen treated with 0.2mm distance, 0.8ms duration , 1 scan and powers (6,8,10 w) 20X .



Figure(3.2): Scanning electron microscope (SEM) image of PEEK specimen treated with 6W power, 0.2mm distance, 0.8ms duration, 1scan.

C) Increase the distance between the spots to reduce heat accumulation and carbonization , table (3.3), as shown in figure (3.3) different powers were tested with 0.4mm distance and 1ms duration which produce different effects with sign of carbonization in some trails.

Table (3.3): Effect of laser parameters on PEEK surface at 0.4mm distance, 1ms duration and one scan.

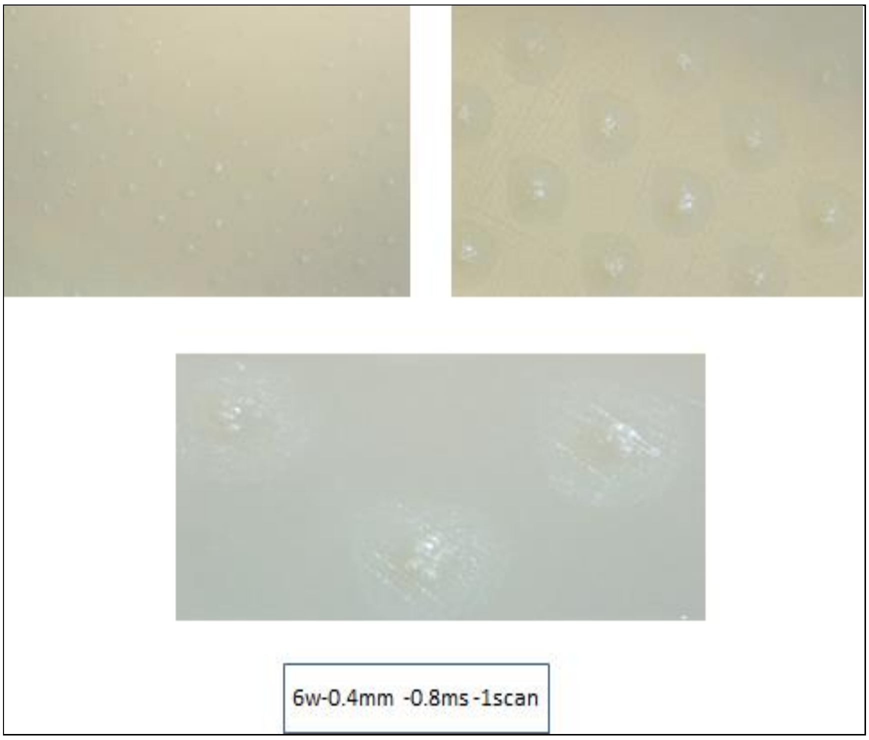
Power w	Distance 0.4mm	Duration 1ms	No .scan 1		effect
4					shallow
6					Good effect
8					carbonization
10					carbonization

Figure(3.3): Light microscope image of PEEK specimen treated with 0.4mm distance, 1ms duration,1scan for (4,6,8,10w) powers (10 X) .

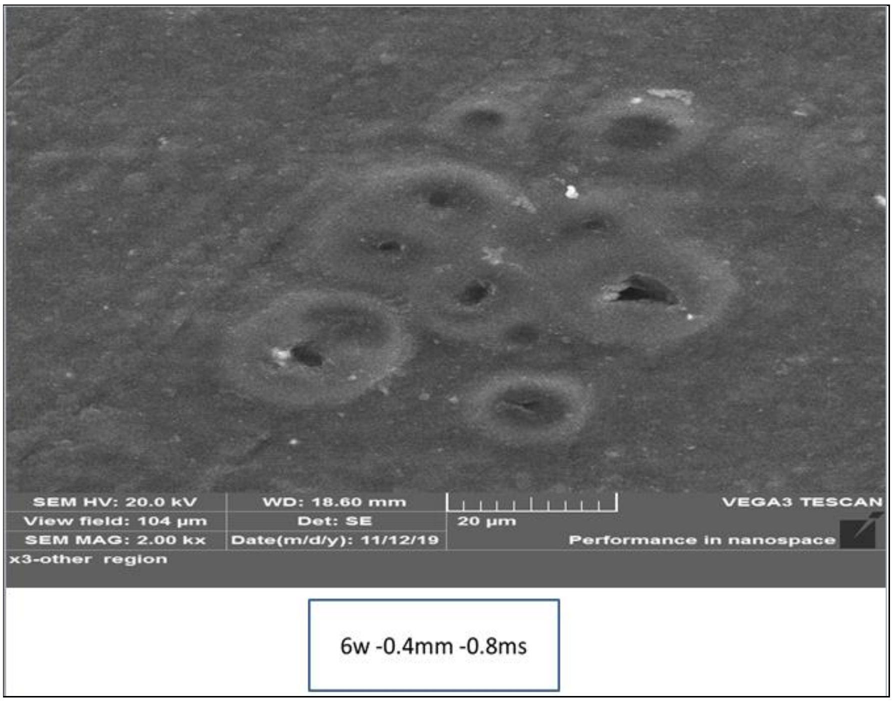
D) Then reduction in the pulse duration was attempted to optimize the criteria, including surface roughness, wettability, without carbonization or cracks table (3.4). The effect shown in figure (3.4) was for 6W power which produce interaction without carbonization as appear in SEM figure(3.5). Also(8,10W) tested with same others parameters, but the carbonization was clearly appeared microscopically, figure(3.6) and (3.7), and in SEM as shown in figure (3.8).

Table (3.4) : Effect of power on PEEK surface at 0.4mm distance, 0.8ms duration and one scan.

Power w	Distance 0.4 mm	Duration 0.8 ms	No. scans 1	effect
6				shallow
8				carbonization
10				carbonization

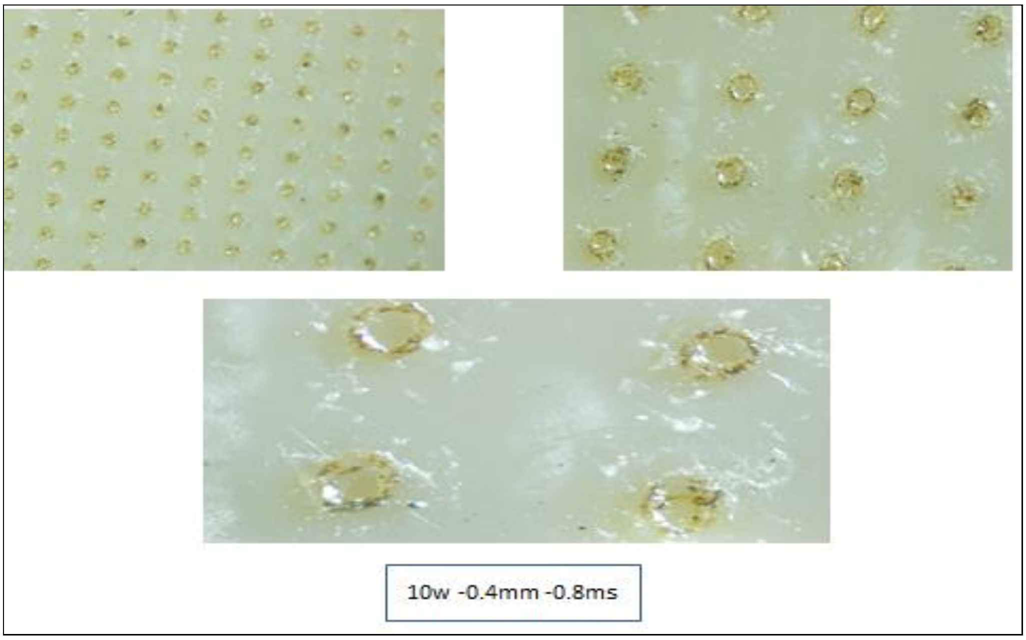


Figure(3.4): Light microscope image of PEEK specimen treated with 0.4mm distance, 0.8ms duration,1scan and 6w power (4,10, 20 X) .

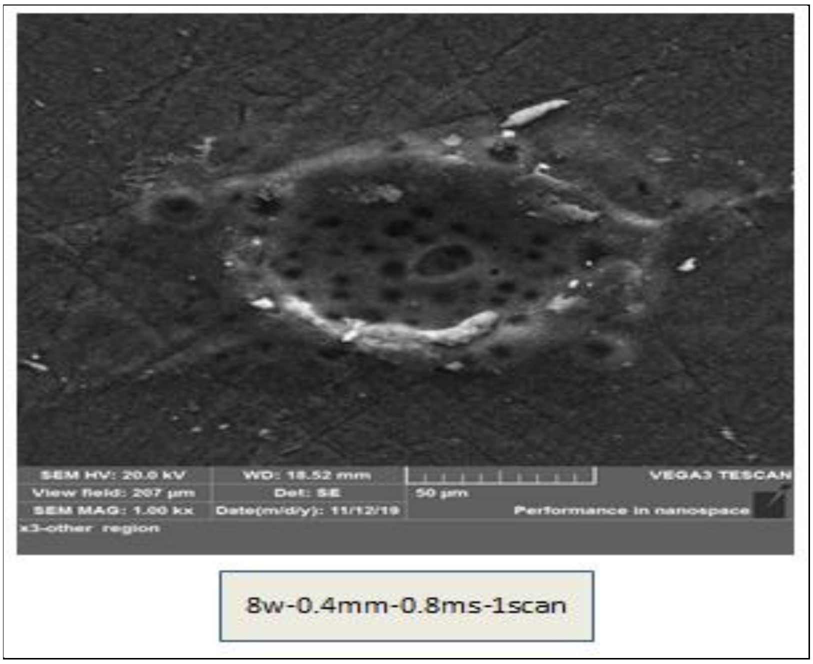


Figure(3.5): Scanning electron microscope (SEM) image of PEEK specimen treated with 0.4mm distance, 0.8ms duration, 1scan and 6W power.

Figure(3.6): Light microscope image of PEEK specimen treated with 0.4mm distance, 0.8ms duration,1scan and 8w power (4,10, 20 X).



Figure(3.7): Light microscope image of PEEK specimen treated with 0.4mm distance, 0.8ms duration,1scan and 10w power (4, 10, 20 X).



Figure(3.8): Scanning electron microscope (SEM) image of PEEK specimen treated with 0.4mm distance, 0.8ms duration, 1scan and 8W power.

1. Two scans showed cracks at the area of laser- PEEK interaction even with low power(4 W) and different intervals (10,20,40) seconds to enhance heat dissipation and reduction of thermal damage. Figure(3.9).

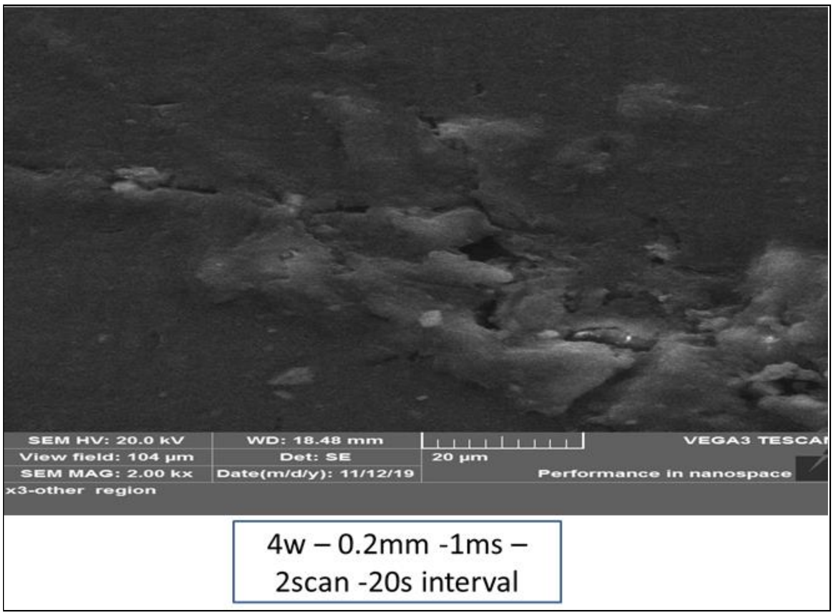


Figure (3.9): Scanning electron microscope (SEM) image of PEEK specimen treated with 0.2mm distance, 1ms duration, 2scan and 4W power.

2. Energy-dispersive X-ray spectroscopy (EDS):

When comparing the sample treated by laser without visual and microscopical carbonization showed lower carbon percentage by weight on the surface of the sample which was 85.9 , as shown in figure (3.10). While sample treated by laser with carbonization showed higher carbon percentage by weight on the surface which was 100 in the site of carbonization ,with presence of another elements which indicate change in surface chemistry of sample. Figure (3.11).

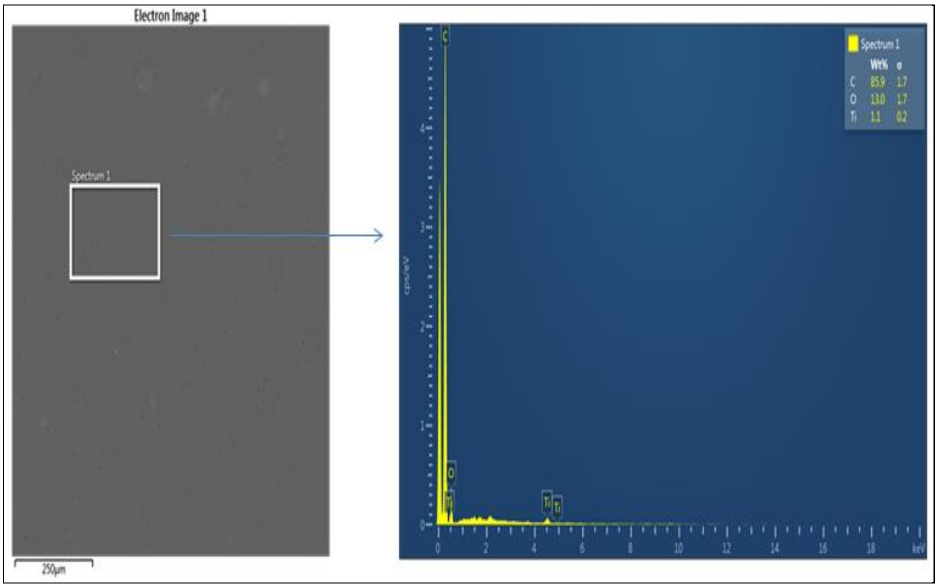
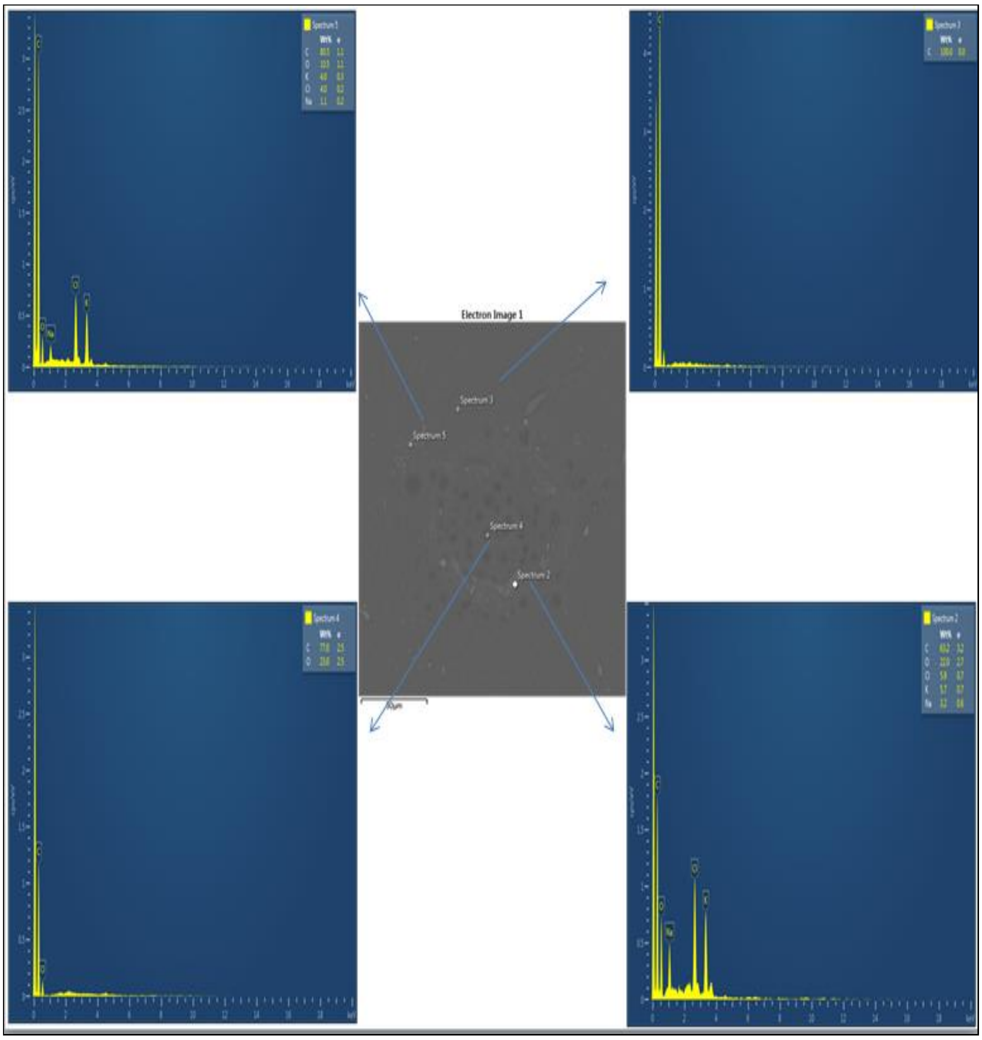


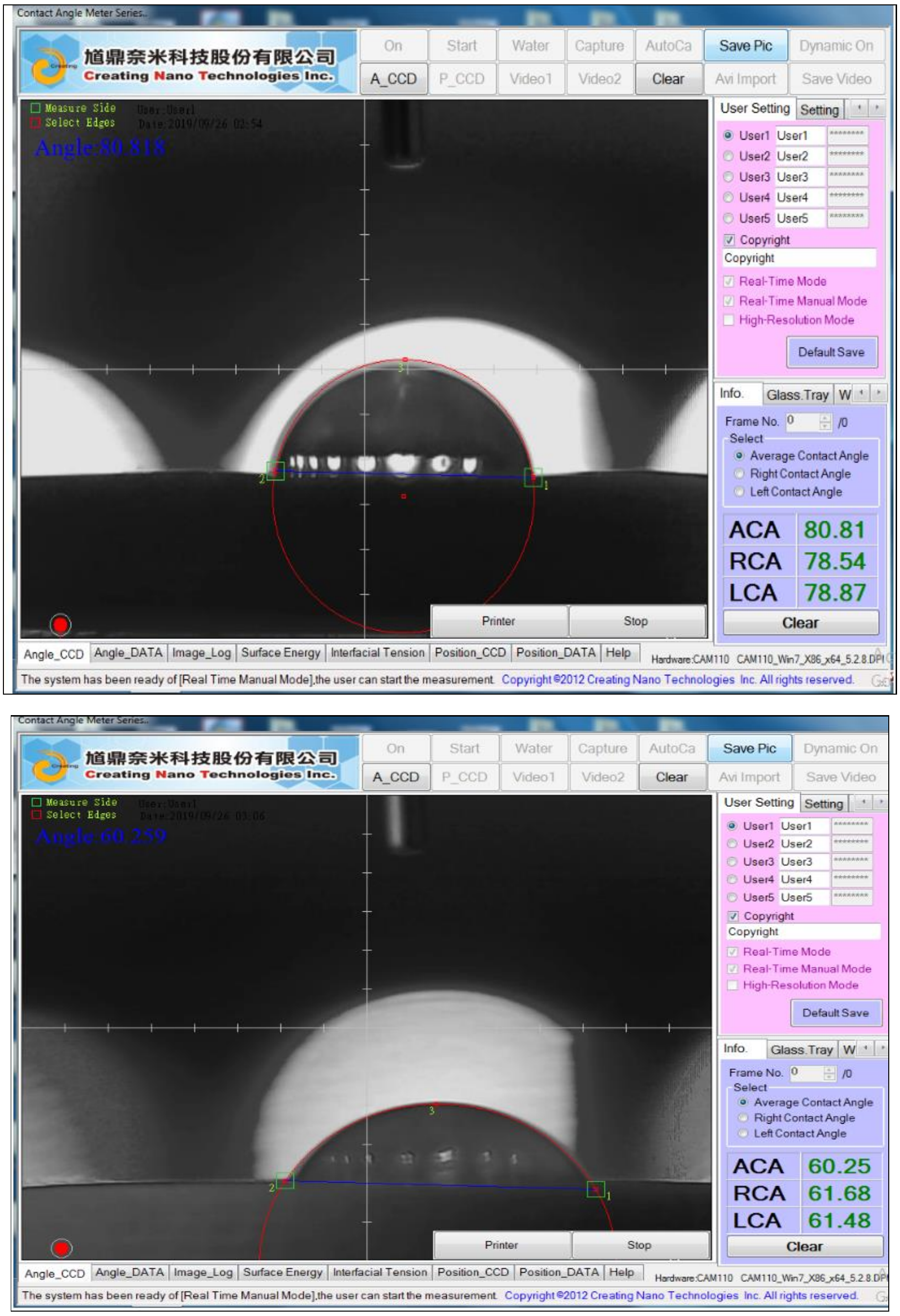
Figure (3.10): EDS of laser treated sample without carbonization effect on sample surface.



Figure(3.11): EDS of laser treated sample with carbonization effect on surface of sample.

3. Contact angle:

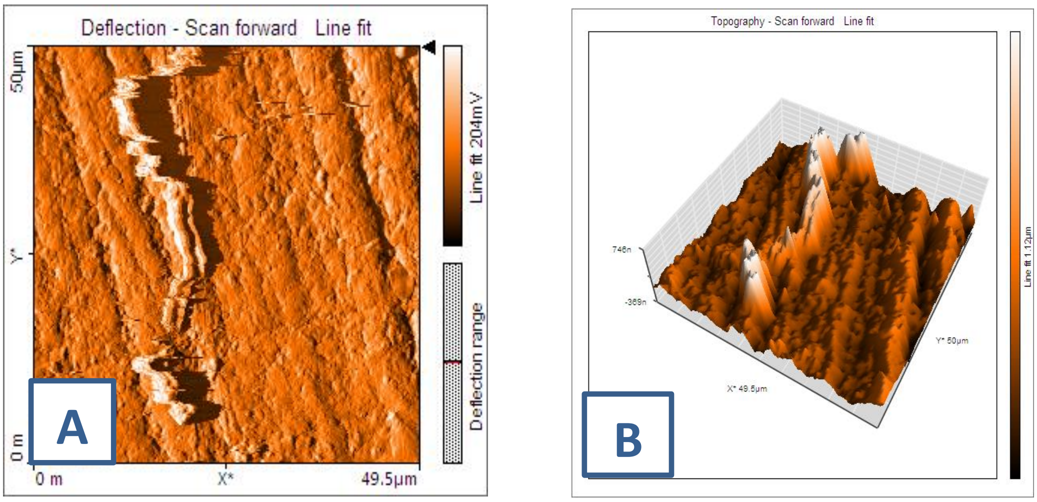
The contact angle was decreased to 60.25° for laser treated PEEK sample as compared to control PEEK sample where the contact angle was 80.81° as shown in figure (3.12).



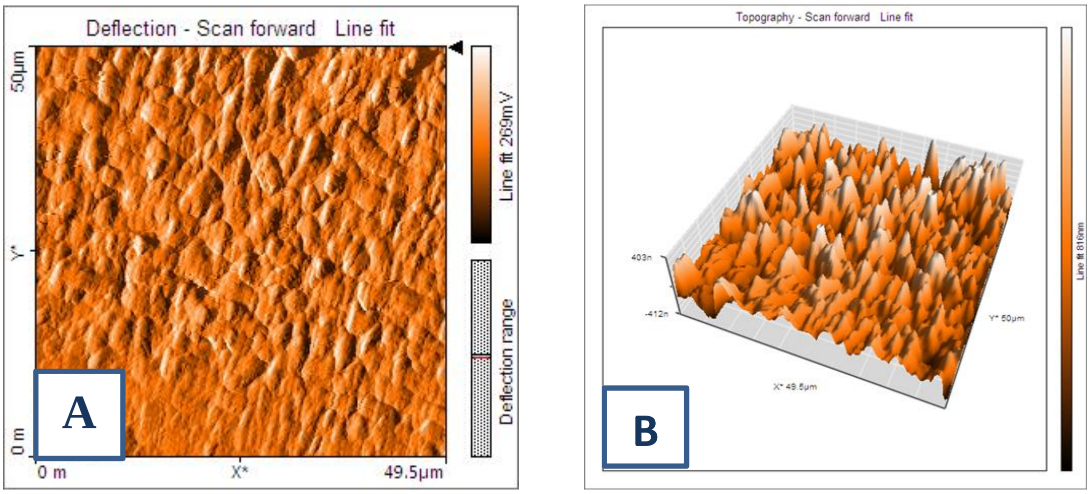
Figure(3.12): (A) Shows measure of contact angle before laser irradiation (B): Contact angle after laser irradiation. (6W , 0.4mm distance, 0.8ms duration and one scan).

4. Surface roughness measurement(AFM):

Surface roughness was assessed by atomic force microscope (AFM). The 3D roughness parameters (nm) such as; the average surface roughness (Sa) which was 42.571nm for untreated PEEK specimens, figure(3.13). While Sa for irradiated specimens were higher . The Sa of PEEK with selected parameters was 109.02 nm. Figure(3.14).



Figure(3.13): Atomic force microscope (AFM) imags, A)2D, B)3D, showed roughness of untreated PEEK spacimen .



Figure(3.14): Atomic force microscope (AFM) imags. A)2D .B)3D, showed roughness of irradiated peek spacimen (parameters:6w power, 0.4mm distance ,0.8ms duration and one scan).

Table (3.5) showed different laser parameters and their effect on surface topography , it is clear that when the power increase and spot distance between spots decrease , AFM value increase . While when the power decrease and distance increase , AFM value decrease .

The specimen NO.1,2,4,5 were excluded because it is clear that the roughness is high and this reduce flow body fluid which is essential in starting of healing process , attachment of cells , and protein adsorption . It is observed that very rough surfaces are expected to follow the Cassie-Baxter regime, this model is that the presence of air pockets have a tendency to increase the hydrophobicity.

Table (3.5): Relation between surface topography of PEEK and different laser parameters.

NO.	**Power (W)**	**Distance (mm)**	**Duration (ms)**	**No. scan**	**Contact angle**	**AFM**
1	4	0.4	1	1	56	64.962
2	6	0.2	0.8	1	66	195.81
3	6	0.4	0.8	1	60	109.02
4	6	0.4	1	1	64	125.12
5	4	0.2	1	1	49	90.06
Non-Irradiated	--------	----------	----------	------	80	42.571

Finally repeated number of scans deterioration the material due to cracks seen with in the spot area even with rest intervals between scans. Therefore decision was made to use sample No.3 with parameters (6W, 0.4mm distance, 0.8 ms duration and one scan) , which recorded contact angle which is comparable to the contact angle range of titanium which was 62.43˚.

3.1.2 In vivo experiment

Clinical observation of all rabbits recovered well after surgery and they moved normally after one week that indicated toleration of the implantation. After healing period interval, at the time the animals are executed, the tissue surrounding the implant has negative clinical observation without any signs of infections. Stability of implants after each healing period was indicated by inability to remove the implant with manual force.

1. Mechanical testing

The torque removal mean value of the Fractional CO_2 laser treated PEEK and titanium implants after 2 and 6 weeks are shown in the table (3.6) and figure (3.15).

Mean torque value of PEEK after 6 week of healing period was (11.875Ncm) which improved than torque value after 2 weeks (4.5 Ncm).

While the mean torque value of Titanium implants after 6 week of healing period was (12.375 Ncm) which developed than torque value after 2 weeks (6.375 Ncm) .

Table (3.6): Descriptive statistics of torque removal of PEEK and titanium implant of 2 and 6 weeks healing periods (Ncm).

Materials	Time	N	Mean (Ncm)	Sta. Deviation	Sta. Error Mean
PEEK	2 Weeks	8	4.5000	.75593	.26726
	6 Weeks	8	11.8750	2.47487	.87500
Titanium	2 Weeks	8	6.3750	1.06066	.37500
	6 Weeks	8	12.3750	1.59799	.56497

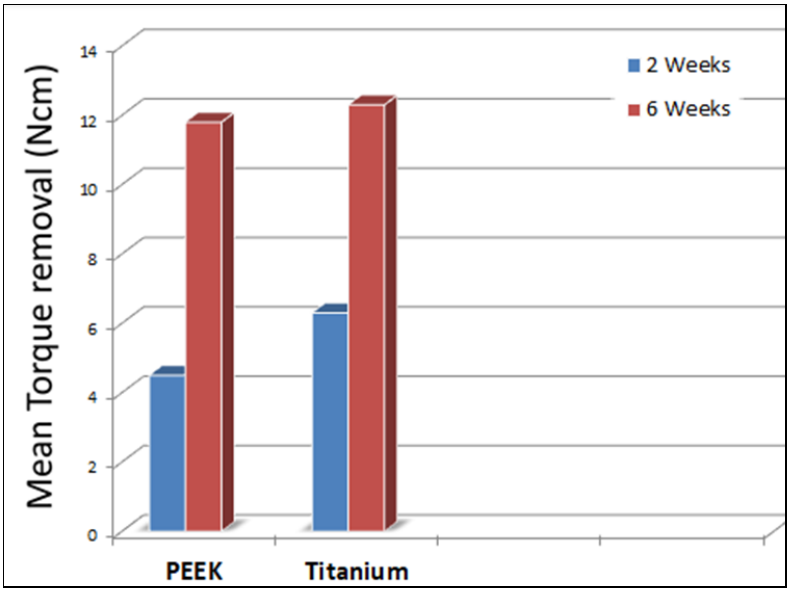


Figure (3.15): Indicate increased torque value after 2 and 6 weeks interval for both PEEK and Titanium.

To test the effect of time for both implants materials, student t-test was used. The result indicate highly significant difference between 2 and 6 weeks healing interval in both implants materials. Table (3.7).

Table (3.7): Student t-test evaluate the effect of time in each implant material.

Materials	t- test	df	Sig.
PEEK	-8.061	14	.000
Titanium	-8.848	14	.000

Student t-test was used to evaluate the effect of material which indicate significant differences between PEEK and Titanium after 2 weeks while the comparison after 6 week was non-significant as shown in table (3.8).

Table (3.8): Student t-test evaluate the effect of materials (PEEK and Titanium) in each time interval.

Intervals	t- test	df	Sig.
2 Weeks	-4.072	14	.001
6 Weeks	.480	14	.639

2. Histological finding:

Divided into response of osseous tissue to titanium and PEEK implants

A) Response of osseous tissue to titanium implant

1) Titanium group After 2 weeks interval

At the bone- titanium implant interface after 2 weeks interval , histological examination showed the transformation of the connective tissue into ossification center, in which bone forming cells can be recognized, these cells producing the acidophilic matrix at the borders of ossification center, figure (3.16 A&B), then at some regions bone spicules can be observed that appeared scattered within the site of implant, these are of variable shapes and sizes intervening the highly vascular connective tissue , they showed weak connection to each other and weak integration to the original bone surface (figure3.17). Each bone spicules made by bone matrix with lacunae occupied by osteocytes, and at the bone surface osteoblasts can be seen forming single row of cells, figure (3.18).

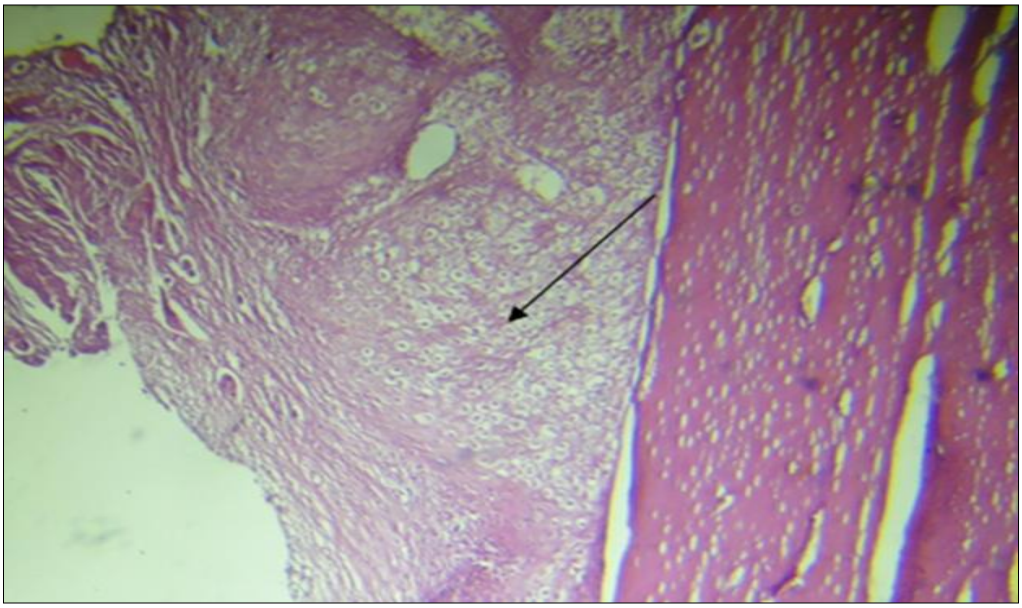


Figure (3.16A): Development of ossification center at the connective tissue at the site of implant (black arrow) .H&E,X10.

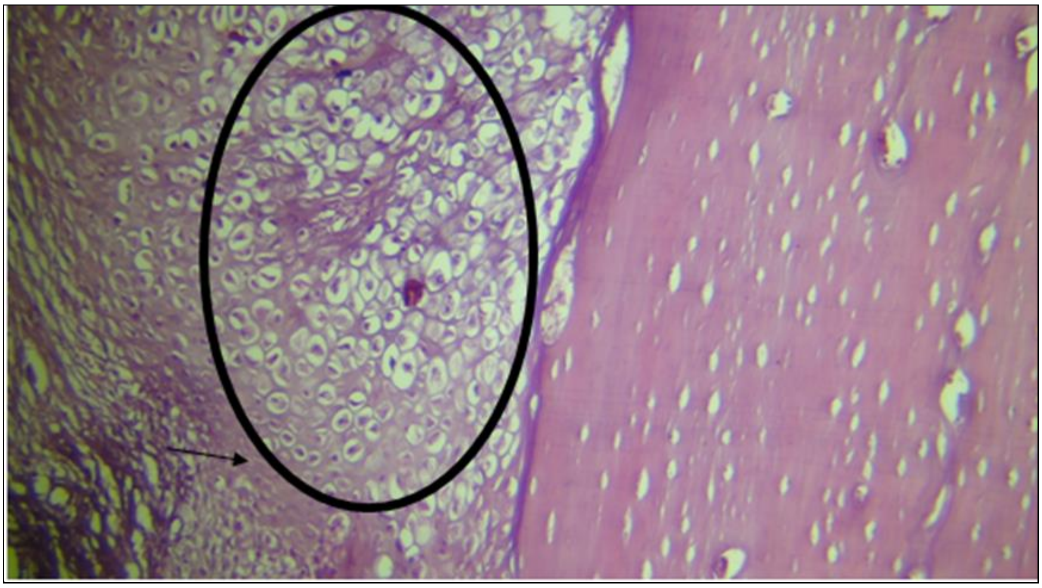


Figure (3.16B): The ossification center at the connective tissue at the site of implant (oval shape) with the formation of new matrix (black arrow). H&E, X40.

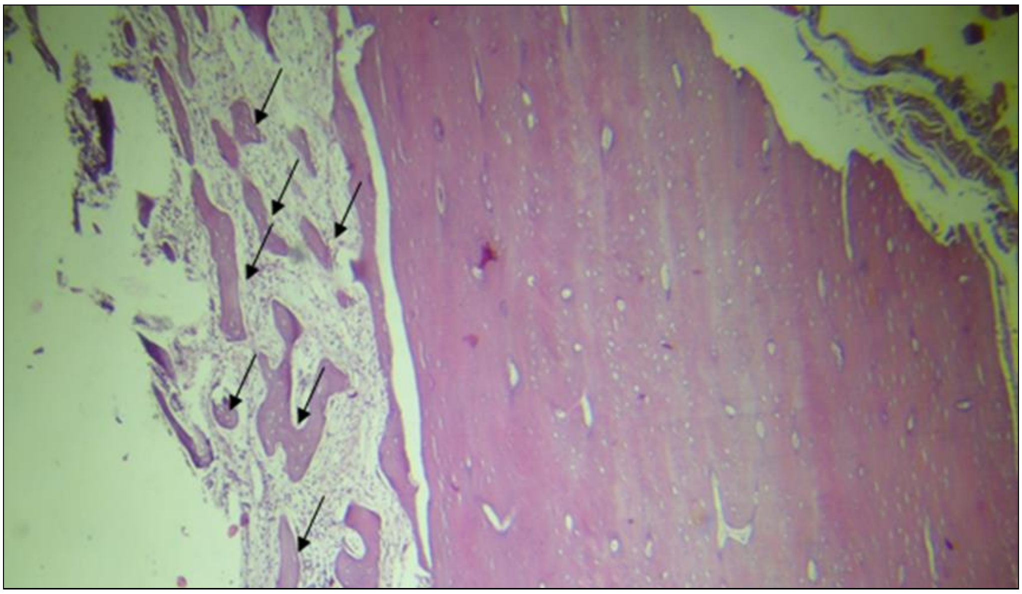


Figure (3.17): Bone spicules at site of implant are small and not connected to each other and separated from the original bone. H&E, X10.

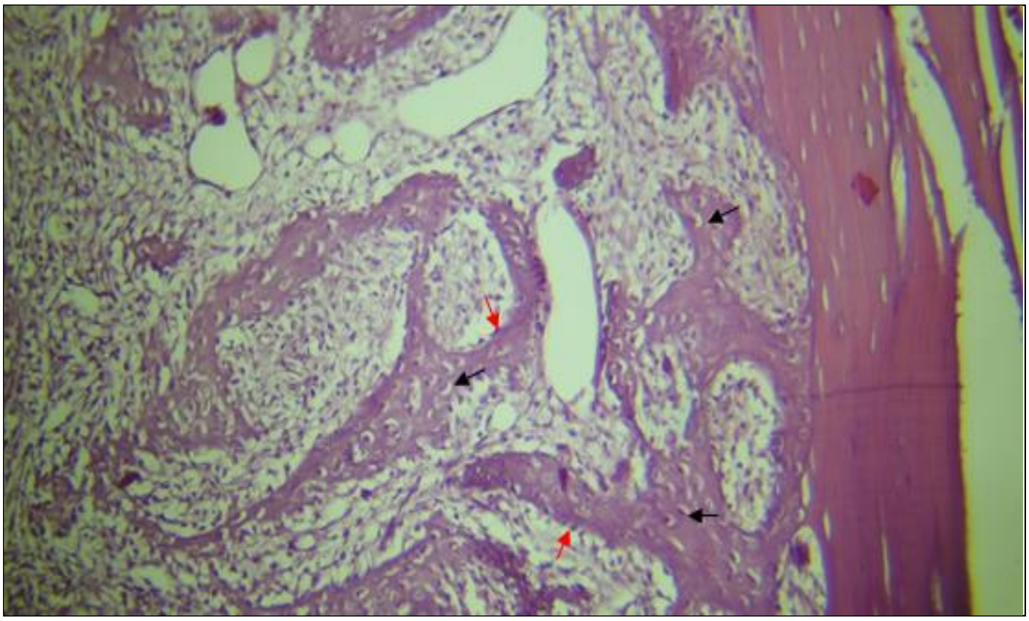


Figure (3.18): Bone spicules at site of implant with osteocytes in lacunae (black arrow), and osteoblasts at bone surface (red arrow). H&E,X10.

2) Titanium After 6weeks interval:

At this interval bone spicules are more well developed showing more profuse matrix that filled with lacunae occupied by osteocytes with considerable osseous integration to each other, but slight increase in the integration to original bone surface, figure (3.19)and reduction of vascular connective tissue at the site of implant, figure (3.20).

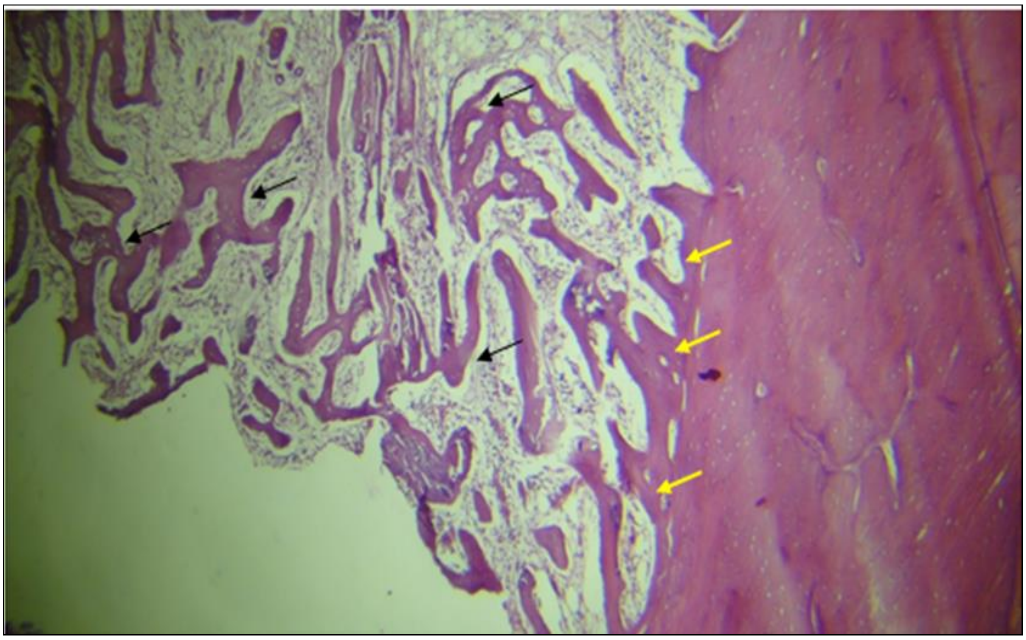


Figure (3.19): Bone spicules are connected to each other at some regions (black arrows) and the integration to original bone (yellow arrow). H&E,X10.

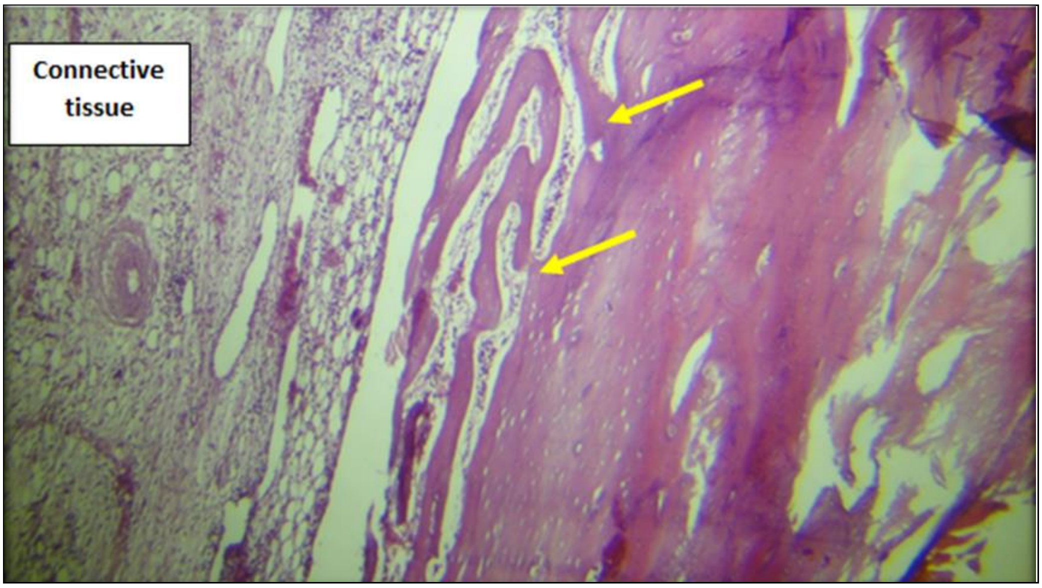


Figure (3.20): Bone spicules with weak integration original bone (yellow arrows) with wide area of vascular connective tissue. H&E, X10.

B) Response of osseous tissue to PEEK implant:

1) PEEK After 2weeks interval

With PEEK implant at 2 week interval the connective tissue at the site of implant showed osseous transformation at various stages of development ranges from presence of ossification center to more differentiated bone spicules at the osseous implant interface, figure 3.21A,B), these early spicules showed weak integration to original bone, figures (3.21B) (3.22).

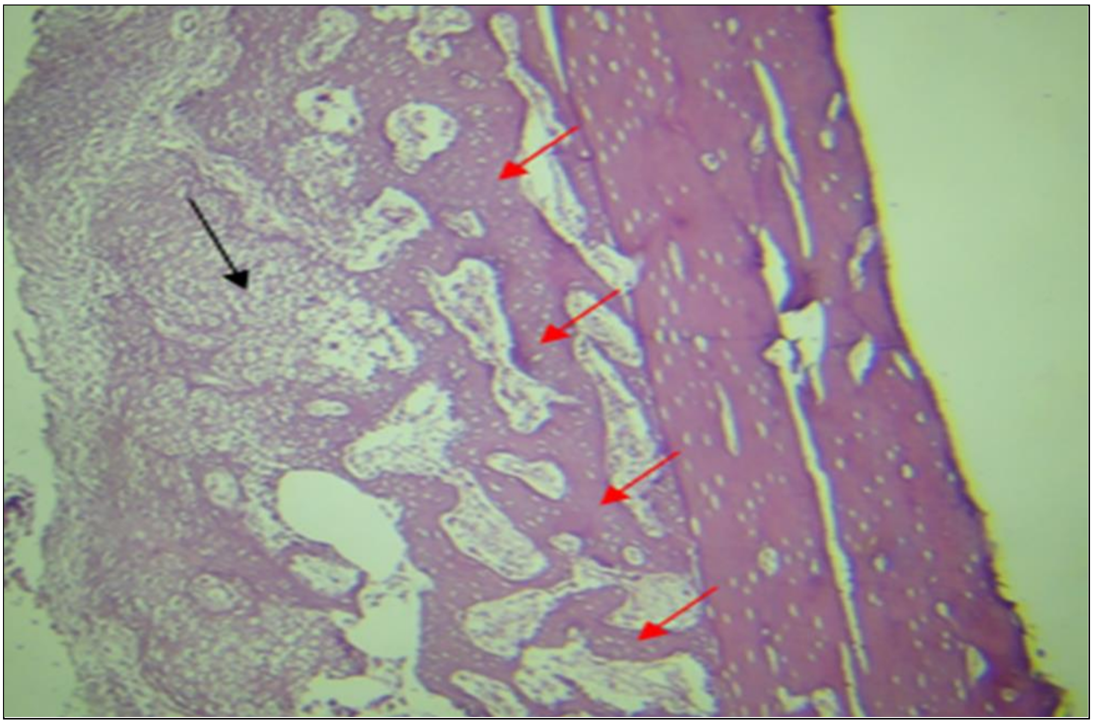


Figure (3.21A): Development of ossification center at the connective tissue at the site of implant (black arrow), with presence of bone spicules (red arrows). H&E, X4.

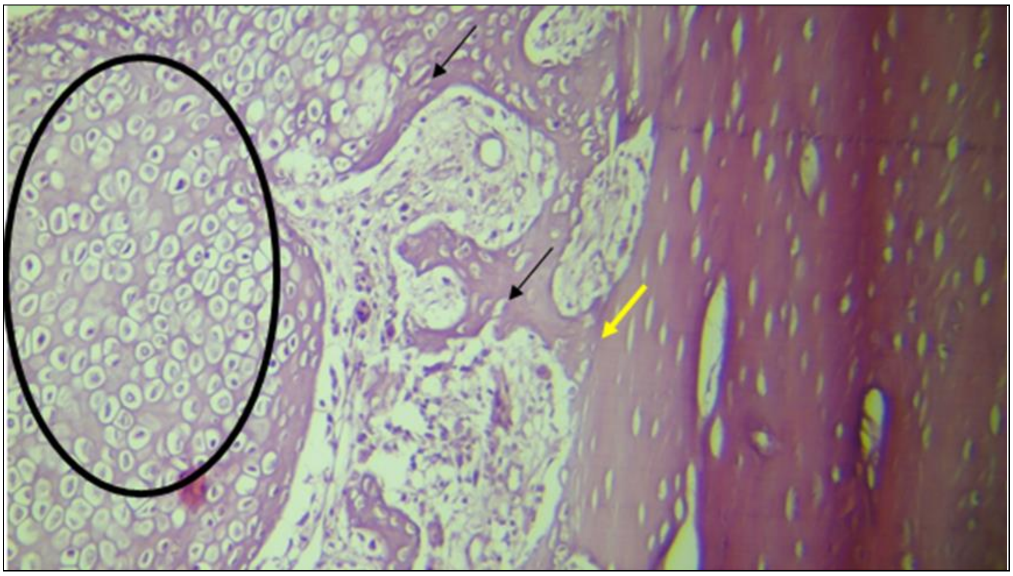


Figure (3.21B): The ossification center at the connective tissue at the site of implant (oval shape) with the formation of bone spicules (black arrows) with weak integration at bone –implant interface (yellow arrows). H&E,X10.

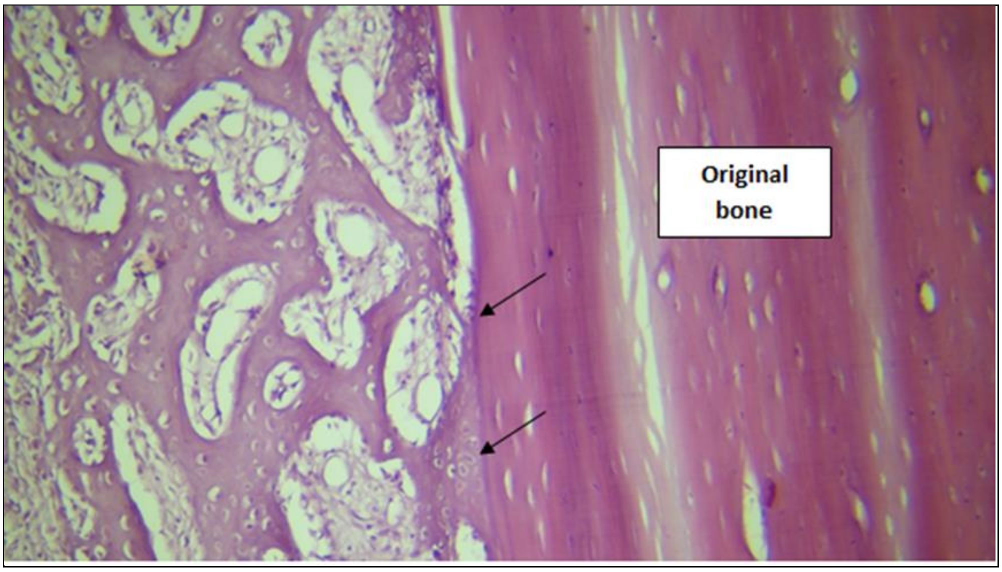


Figure (3.22): Bone spicules with weak integration to the original bone (black arrows). H&E, X40.

2) PEEK after 6weeks interval

At this stage bone spicules are more developed forming mature matrix with lacunae occupied by Osteocytes with wide areas of connection to each other, and at the same time wide area of integration to osseous tissue at bone –implant interface, figure (3.23). Connective tissue is largely reduced in amount at the site of implant aiding into more osseous implant integration, figure (3.24).

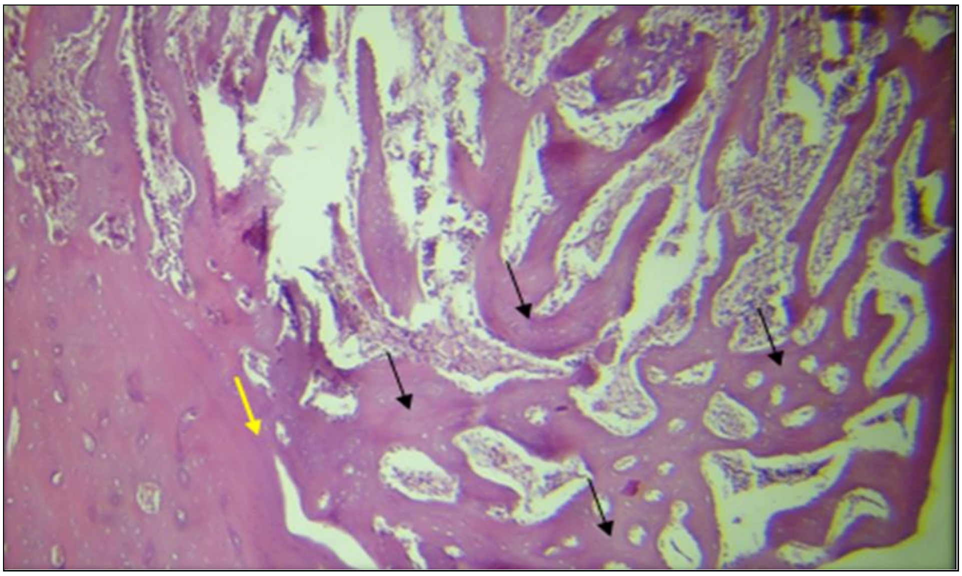


Figure (3.23): Bone spicules are more integrated to each other (black arrows), and to the osseous surfaces (yellow arrows). H&E.X10.

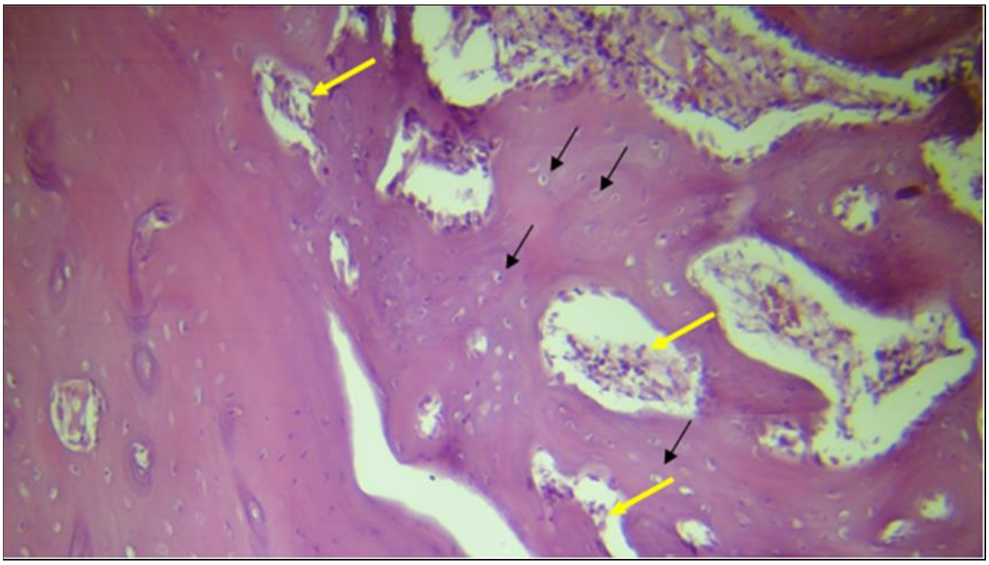


Figure (3.24): Bone spicules of mature matrix with osteocytes in lacunae (black arrows), and reduction of intervening connective tissue (yellow arrows). H&E.X40.

Osseointegration involve wide area of contact between the newly developed bone from ossification centers at the site of the implant to the surface of original bone, this can be seen as lamellar integration and diminish in the intervening connective tissue this can be considered as an index for implant success figure(3.25).

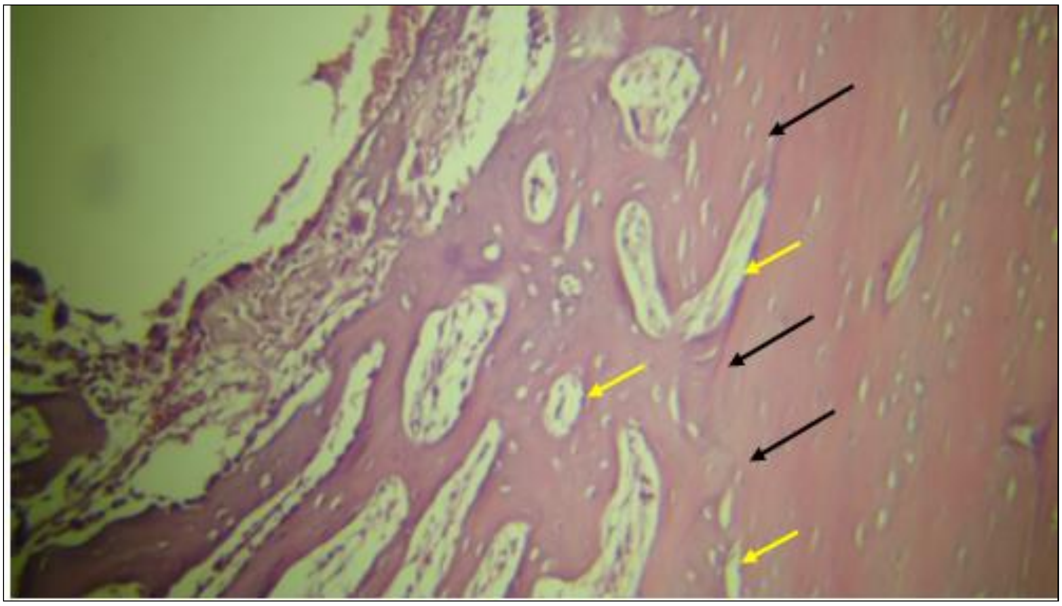


Figure (3.25): The osseointegration of new matrix to the original bone surface (black arrows), and reduction of intervening connective tissue (yellow arrows).H&E.X40.

Discussion

3.2 Discussion

Using of polyether ether ketone (PEEK) in dental implant and orthopedic applications now is highly recommended due to the suitability of its modulus of elasticity which is relatively close to that of the bone, in addition to its biocompatibility property, and the radiolucency (Al-Masi *et al.,* 2016).

Polyether ether ketone (PEEK) has excellent physical and chemical properties, as highly strength or stiffness , good fatigue and fracture toughness properties. Furthermore it suitable for most methods of sterilization, easy manipulation and forming by using machining or molding , corrosion resistant, and a compared density to the human tissues (McKenzie *et al.,* 2004 and Kurtz *et al.*, 2007).

PEEK still classified as hydrophobic material due to its much reduced reaction with the surrounding tissue , in spite of its highly properties, this possibly cause restriction in its applications (Kurtz *et al.,* 2007). The bioinert properties of Polyether ether ketone mean soft tissues growth instead of bone growth around the PEEK implant (Poulsson *et al.,* 2014).

Studies have shown that PEEK can be easily modified by incorporation of other materials like : hydroxyapatite, carbon fibers , and titanium (Najeeb *et al.,* 2016). The mechanical and physical properties of PEEK is similar to bone and dentin, so increasing the bioactivity of PEEK without disturbing their mechanical properties is a foremost challenge (Gonçalves *et al.,* 2010 and Gittens *et al*., 2011).

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3.2.1 Laser ablation

Using of lasers in dentistry increased in last several years with introduction of soft-tissue diode lasers, which are portable, cost-effective, and reliable. Lasers have several clinical uses in dental implantoloy : improving the pre-surgical, surgical, post-surgical, and prosthetic phases of modern implant dentistry (Romanos *et al*., 2013).

When the surface irradiated by a focused beam of laser radiation , the polymers electrons are excited, this excitation result in heat generation due to absorption of laser photon energy. Therefore, selection of suitable laser type and parameters to preform surface modification and roughness is very important considering the material , so in this study the wavelength which was used is 10600nm according to Lawrence (2001) who state the IR radiation tends to produce thermal ablation or melting of the polymers.

According to Ravi-Kumar *et al*., (2019) who approved the ablation happens only when the material absorbs sufficient energy to be melted or vaporized. Therefore, power less than(6 W) was not enough to induce ablation. So, the power was increased up to (6W), and the energy (4.8mj) to have an effect was detected microscopically .

When the power was increase , the absorbed energy increased too, so localized carbonization area surrounding the spots was resulted. This due to low thermal conductivity of PEEK which is (0.25W/(m.k) . Hence, heat build-up during irradiation is rapid and heat dissipation is low (Steven , 2012) .

The pulsed laser is preferred because the diffusion of heat into the polymers is avoided, and the thermal damage is negligible close to the radiated area, compared to CW operation mode creates patterns with high thermal affect around the laser-treated area and low quality. Therefore, formation of debris can be avoided (Momma *et al.*, 1997).

Laser surface treatment diameter significantly influencing by pulse width (Uchtmann, 2016). Smaller pulse duration results in more vaporization and less melting, while higher melting can cause a large heat affected zone and redisposition due to expulsion, non-uniformity and cracks (Ravi-Kumar *et al.*, 2019).

The pulse width increased up to (0.8ms) until reach desired effect. From the equation (3.1), it can be seen that, for a fixed pulse peak power, as the pulse width increases, energy per pulse is increased accordingly.

$E = t\mathrm{P} \times P\mathrm{P}$ …….. (3.1)

where; E is the pulse energy, $P\mathrm{P}$ is the peak power, and $t\mathrm{P}$ is the pulse width. In general, longer pulse results in larger diameter and the deeper effects (Webb 2010; Yang *et al*., 2016). Above (0.8) pulse width carbonization was appear due to increase the energy and accumulation of heat , lead to a larger heat-affected zone, specifically with longer pulse durations(Ravi-Kumar *et al.*, 2019).

In addition to the parameters of the laser source, material properties such as thermal conductivity (Pham *et al.*, 2002), and melting point which is >280°C for polymers (Tekin *et al.*, 2018), so the distance between laser spots was 0.4mm to overcome heat accumulation problem and in the same time ensure maximum surface coverage by laser effect.

Number of scans is another variable which was also tested .This resulting in cracks as observed by SEM was seen in two scans samples even in low energies. This could due to melting and resoldifecation after each scan, so it was excluded in this study.

3.2.2 Surface wettability

The four primary factors that affect the biocompatibility between biomaterial and cell contact are surface compositions , surface energy, roughness of the surface, and surface topography (Schwartz and Boyan, 1994). Knowing that PEEK is a hydrophobic material, having exceptional biomechanical properties, there is a further need to improve its bioactivity for application in dental and orthopedic fields (Gaggl *et al*., 2000).

The surface wettability can be modified by numerous methods , such as Fractional CO_2 laser treatment because laser treatment can be used to target specific areas and provide different treatments at different positions with higher precision , restriction manual movement of the hand piece during operation , and the pattern which can be achieved is homogenous. (Ahrari *et al.,* 2013).

After treatment with laser the materials surface contact angle was changed dramatically, the contact angle was reduced to 60.25˚ for laser treated PEEK sample as compared to untreated PEEK sample where the contact angle was 80.81˚, which indicate increase in hydrophilicity and this agreed with result of (Guo *et al.,* 2016).

Wettability results agreed with Wilson *et al.,* (2015) who concluded that analysis of the contact angle showed a reduction in water contact angle with increasing laser power intensity, and the derived surface free energy increased consequently. Different methods of surface modification enhance wettability this is agree with Hamodi (2018) and Mahdi (2019).

For blood coagulation the hydrophilic surfaces are better than hydrophobic surfaces, so surface of dental implants have been modified, with high rough and hydrophilic surfaces of implant show better osseointegration than conventional ones. Adsorption of proteins such as fibronectin and vitronectin on the dental implants surfaces could stimulate adhesion of cell and bone osseointegration (Grassi *et al.*, 2006).

3.2.3 Surface roughness:

Technologies for material surfaces processing and modification have been developed quickly. Laser is always used as a new method to modify materials surface due to its ability in modification and high efficiency and accuracy.

In dental implantoloy the surface physical properties of dental implant materials, should has a certain degree of roughness which is essential for the formation of bone implant interface (Gue´hennec *et al.*, 2007).

Primarily, rough surface helps in the adherence or adsorption of the essential minerals and proteins. Then the subsequent steps as adhesion, migration, proliferation, differentiation, protein synthesis, and mineralization of osteoblasts on the surface of the implanted material were also enhanced due to the roughness of the surface (Kenar *et al.*,2013). Furthermore increased roughness of the surface can extend the contact area between implant materials and bone making formation of locking effect at the bone implant interface. Finally, bone implant interface can be improved help in enhancing the bonding properties (Deyneka-Dupriez *et al* ., 2007) (Deng *et al* ., 2015).

Guo *et al.*, (2016) study the PEEK with SEM and considered wettability and surface roughness are important factors may control cell adhesion mechanism and characteristics of implants and measured them to found roughness of the material can directly affect the binding interface between the material and the bone. SEM observation in this study reveals many porous and concave structures and they increased when increase the pulse duration, number of scans and laser power. The efficient binding area enlarges when the material surface roughens; rough surfaces are more attractive to cells and proteins (Jimbo *et al.*, 2011, Bartold *et al.*, 2011).

The average surface roughness (Sa) in this study which was 42.571nm for untreated PEEK specimens, while Sa for irradiated specimens were higher than it, the Sa for selected parameters was 109.02

nm . Rough implant surfaces lead to quicker osseointegration due to new bone formation and reduction in healing period this agree with conclusion of Aparicio *et al*., (2011) .

Multilayer microstructures of a material can be produced using laser processing, with uniformly distributed on surfaces or pattern of the surfaces may also help in roughness increase of the materials, this probably enhance the wettability which may stimulate better biological activity (Nuutinen *et al.*, 2013).

As stated the surface roughness and wettability are considered to be the most govern factors affecting cell adhesion characteristics and implant success. Wettability can be expressed by the contact angle. Better wettability can be described by smaller contact angle which in turn suggests better cell adhesion. Researches indicated that materials have a range of roughness, could have lower contact angle. Furthermore, the biological activity can be increased (Riveiro *et al*., 2012).

3.2.4 Torque Removal:

One of the methods to evaluate the osseointegration is measurements of torque removal of screws, a digital torque meter was used. After 2 weeks, Fractional CO_2 laser treated PEEK implants recorded higher torque removal value (4.50N.cm), as compared to result of Hamodi, (2018) who recorded much less torque value for removal (1.4 N.cm) for untreated PEEK. Same result was obtained by Koch *et al.*, (2010)when he compared the bone –implant contact values of PEEK and titanium implants, and the PEEK was observed to have the lowest values.

Similarly after 6 weeks of the healing period, the laser treated PEEK implants recorded higher torque removal value (11.88 N.cm), while for the untreated PEEK implants at the same period recorded a less amount of torque to be removed (5.8 N.cm), as approved by Hamodi

(2018) . This indicate that laser treatment using Fractional CO_2 laser improved surface roughness and osseointegration which have better torque removal value.

After two weeks titanium implants torque removal were (6.38 N.cm), so was significant differences in with titanium and treated PEEK this could attributed to biocompatibility , surface passivity and surface characteristic of titanium which enhance better result in this period . And could regarded to a biofilm formation of PEEK with a rougher surface topography showed increased this layer this as concluded by Barkarmo *et al*.,(2019).

Many reports demonstrated that the roughness of the surface of titanium implants influence osseointegration rate via the speed and amount of formation of bone tissue at the interface. In comparing of the behavior of different types of cell on materials reveals that they are affected by roughness of the surface (Healy *et al.,* 1996).

After six weeks titanium implant torque removal was (12.37), so there was non-significant differences in torque removal value between Titanium and treated PEEK . These results agree with Koch *et al*., (2010); and Schwitalla *et al*., (2013), they were proposed that there is no significant difference between the PEE osseointegration and conventional implant materials such as zirconia and titanium .

Rough surface, increase wettability present at the interface could be the reason for the activation which could have continuous effect up to 6 weeks and may be more (Deligianni *et al.,* 2001; Lamers *et al.,* 2010; Mendonça *et al.,* 2010).

The properties of the surface of biomaterials are fundamental to the cells response at biomaterial interface, influencing the growth and quality of new bone tissue formation (Von der *et al*., 2010).

Further investigation was required to estimate the causes of osseointegration rate of titanium in comparison with modified PEEK, (Mishra and Chowdhary, 2019).

3.2.5 Histological findings

Histological analysis is an important method helps evaluate implant stability or success, this method can be performed at any step during implantation, as stated by Atsumi *et al.,* (2007). The formation of new bone trabeculae can be illustrated clearly by histological analysis in the tested groups with active osteoblast and osteocytes. Also, it clear from the research data that there was accepted normal inflammatory reaction observed during the periods of experiment. This in agreement withYang *et al.,* (2009) research.

Two weeks after the implantation, the bone spicules made by bone matrix with lacunae occupied by osteocytes, and at the surface of the bone osteoblasts can be seen .The woven bone formation began in this period .

An osteoid tissue with numerous bone and progenitor cells around, the bone marrow showed active blood vessels, which indicate the beginning of new bone formation These findings are supported by the work of Lins *et al,* (2003) and Cooper (2003). However, titanium implant showed thicker bone trabeculae than modified PEEK, which implied primary bone stimulation, due to passivity, oxide layer and biocompatibility of Ti (Bozzini et al., 2008).

Microscopical observation after 6 weeks of implantation shown that the newly bone developed and increase osseous integration to the old bone in two groups .

Implant osseointegration affected by surface characteristics of implants, like micro- and nano-roughness and chemical composition of implants (Mendonca *et al*., 2009).

Osteoblasts represent higher rate of differentiation and mineralization of matrix and higher growth factors production in the presence of rough substrates (Cooper *et al*., 1998)

Berglundh *et al*. in (2003) showed that histological features after 6 weeks was characterized by the formation of new bone and beginning of maturation.

The histological results in this study showed formation of new bone around implants without fibrous encapsulation or inflammatory reaction during the experimental intervals in both implant materials and both durations of the implantation. The result of this study agree with the findings of **(**Hussein 2015, Hamad *et al.*, 2018). Trabecular features and cells around dental implants assess bone quality (Jemat *et al.*,2015).

The newly formed bone included woven bone often combined with both parallel-fibered and lamellar bone. Large areas of this newly formed bone were characterized by the occurrence of primary and secondary osteons. Similar results was seen by (Habibovic, 2005) .

The presence of bone cells such as osteoclasts and chondroclasts coincides with blood vessel invasion, and the formation of new capillaries, is expected in the development, remodeling and repairing of most tissue including bone and the resorption of mineralized matrices are essential events for bone morphogenesis and growth (Baron, 1996).

Conclusions and Suggestions for future Studies

3.3 Conclusions

1. Fractional CO_2 laser treatment is a appropriate method for increase wettability and changing the PEEK surface topography for biological applications.
2. Torque removal value indicated a significant difference between treated PEEK in comparison to titanium after 2 weeks of healing period.
3. Torque removal value between titanium and treated PEEK showed non-significant differences at six weeks of healing period .
4. Histologically, Fractional CO_2 laser treated PEEK implant had a well-tolerated by bone after 2 and 6 weeks implantation with features of new bone and osseointegration .
5. Wettability test indicate decrease contact angle for laser treated PEEK and increase wettability .
6. AFM test indicate significant increase in roughness of treated PEEK .

3.4 Suggestions for future Studies

1. Evaluation of osseointegration of PEEK when using different parameters for Fractional CO_2 laser treatment such as power, pulse duration, intervals between pulses, distances between spots and numbers of scans .
2. Studying the effect on osseointegration of another laser type other than Fractional CO_2 laser to modify PEEK surface like UV laser and make comparison between there osseointegration .
3. Studying other types of surface modifications like coatings the surface of PEEK by laser with different materials and its effect on osseointegration.
4. Studying the effect of different PEEK implant geometry and laser treatment on osseointegration.
5. Test the effect of Fractional CO_2 laser on osseointegration for longer implantation periods .
6. Study the effect of laser surface roughness of PEEK on osseointegration in comparison with acid etching treatment or other methods .

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جمهورية العراق

وزارة التعليم العالي والبحث العلمي

جامعـــة بغـــــداد

معهد الليزر للدراسات العليا

تطبيق بولي ايثر ايثر كيتون المعدل بالليزر في زراعة الاسنان خارج وداخل الجسم الحي

رسالة مقدمة الى

جامعة بغداد معهد الليزر للدراسات العليا / لاستكمال متطلبات نيل شهادة
ماجستير علوم في الليزر / طب الأسنان

من قبل

ناهدة زاهر كامل

بكالوريوس طب وجراحة الفم والاسنان 2007

اشراف:

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1442هـ 2021م

الخلاصة

الخلفية: تعتبر زراعه الاسنان علاجا بديلاً فريداً من نوعه لتعويضات الاسنان المفقودة. مادة البولي ايثر ايثر كيتون بأمكانها ان تحل محل التيتانيوم في زراعه الاسنان، لكن سطحها يحتاج الى تطوير لكي يزيد تكوين العظم في منطقه التقاء الزرعه والعظم وتَحسين التماسك العظمي لتوفير شفاء سليم وتقليل فترة الشفاء.

أهداف الدراسة: (في الدراسة المختبرية) لتقدير أفضل عوامل لليزر ثاني اوكسيد الكاربون المجزء لحث تطوير سطح ماده البولي ايثر ايثر كيتون باستعمال المجهر الضوئي، المجهري الالكتروني الماسح ، وطاقه تشتت الاشعه السينيه الطيفي ، مجهر القوه الذرية لمعرفه التغيرات في خشونة السطح وتم قياس زاوية التماس (التبلل) .(في الدراسه المجراة) لتقييم تأثير ليزر ثاني اوكسيد الكاربون المجزء لمعالجة سطوح مسامير الزرعات في مادة بولي ايثر ايثر كيتون على منطقة التقاء العظم بالزرعه بعد اسبوعان وست اسابيع بعد الزراعه في عظم الفخذ للارنب عن طريق إزالة عزم التدوير التحايل النسيجي .

المواد والطرق:أقراص من مادة البولي ايثر ايثر كيتون حُضرت (قطر الدائره عشرة مللي متر وسمكها اثنان مللي متر) وشُععت بليزر ثاني اوكسيد الكربون المجزأ بمختلف الطاقات ،مدة النبضة، المسافة بين البقع وعدد المسحات لاحداث خشونه في السطح .تم فحص الاقراص بواسطة المجهر الضوئي والمسح المجهري الالكتروني وتم فحص خشونة السطح بواسطة مجهر القوه الذرية لمعرفه التغيرات في خشونة السطح. وتم قياس زاوية التماس (التبلل) للسطوح المطوره وغير المطوره عن طريق قياس زاوية التماس. في الدراسة المجراة تم إختيار إثنا عشر ارنباً ذكراً نيوزلندياً من اجل الزراعة.زرعتان في كل فخذ .القريبة كانت المعامله بليزر ثاني اوكسيد الكاربون بالعوامل التاليه (طاقة ٦ واط، مدة النبضة ٠،٨ مللي ثانية، المسافة بين البقع ٠،٤ مللي متر ومسحة واحدة. قُسمت الارانب الى مجموعتين حسب مدة الشفاء لكل مادة الى اسبوعين وستة اسابيع . ثمان زرعات تم فحصها بعزم التدوير بعد كل فترة شفاء . أربعة زرعات من كل مجموعه لكل فترة شفاء استخدمت للتحليل النسيجي.

النتائج :ساعدت الدراسة المختبريه على اختيار افضل عوامل الليزر التي اعطت سطح خشن معامل ،خالي من التكسر والكربنة اضافة لتحسين التبلل. في خشونه السطح تشير النتيجه الى زيادة كبيرة في الخشونة وارتفع متوسط الخشونة من ٤٢،٥٧١ الى ١٠٩،٠٢ كان .هناك اختلاف في القيم لمتوسط عزم التدوير بالنسبة لزرعات البولي ايثر ايثر كيتون المعامله بثاني اوكسيد الكاربون المجزأ (٤،٥) مقارنةً مع زرعات التيتانيوم (٦،٣)بعد اسبوعين . بينما لم يكن هناك فرق في قيم ازاله عزم التدوير بعد ستة اسابيع التي كانت(١١،٨) لماده بولي ايثر ايثر

كيتون و (١٢،٣) للتيتانيوم. كشف الفحص النسيجي عن زيادة نمو العظم لكلا المادتين المزروعتين وكان الوقت عاملا ايجابيا لتحسين النمو والالتحام العظمي.

الاستنتاج : معاملة زرعات بولي ايثر ايثر كيتون بليزر ثاني اوكسيد الكاربون المجزأ كان له تأثير ملحوظ على تحسين التوافق الحيوي والاندماج العظمي مقارنةً بزرعات التيتانيوم .