Ministry of Higher Education
And Scientific Research
University of Baghdad
Institute of Laser for Postgraduate Studies

Testing and Characterization of Zirconia Dental Implant Treated by Q- switched Nd:YAG Laser (In-vitro study)

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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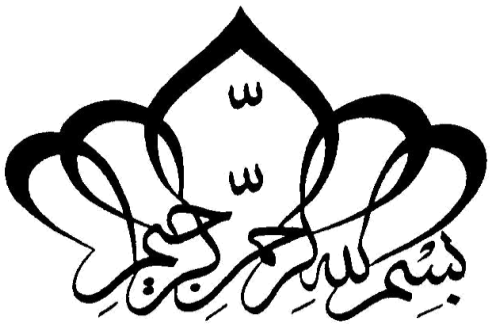

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Dedication

I whould like to dedicate my work to…

My parents, whose love , encouragement and prays of day and night make me able to get such success..

Mr. Raed Al-Shawi and Mrs. Ektifaa Al-Qaisy

My sister and Brothers..

Rula , Mustafa , Abdullah

My husband..

Laith

My two little Angels, my daughters..

Ghina and Sarah

My nephews..

Aymen and Ahmed

With my love

Sena …

ACKNOWLEDGMENT

At first , deep thanks and gratefulness to our creature **Allah** Glory be to Him for everything I get and enjoy

Many thanks to **Prof. Dr. Hussein A. Jawad**, Dean of the Institute of Laser for Postgraduate Studies, for his support during the period of research.

Special thanks with all respect to my supervisor **Asst. Prof. Dr. Basima Mohammed Ali,** Head of Biomedical applications depertment , Institude of Laser For Postgraduate Studies , for her support , encouragement and guidance.

My thanks to **Lec. Dr. Mahmood Shaker** , associate dean of the Institute of Laser for Postgraduate Studies for his great help.

Special thanks to **lec. Dr. Thaier abid Tawfiq,** (Institute of Laser for Postgraduate Studies), for great help.

My thanks to **Asst. Prof. Dr. Ali S. Mahmood,** for his support.

Thanks to Eng. **Atheer Rasheed** , for his help .

I whould like to thank all teaching staff in the Institute of laser for postgraduate studies, my **friends** and my **family** for their support.

Special thanks to **ASCo learning center** . for their assistance.

Abstract

Background The implant unit after a certain period of time (following the surgical procedure) should be connected to the surrounding bone by newly formed bone. Many factors may affect the progress of new bone formation. Among these, surface propertis including, surface roughness of an implant unit and surface wettability. **Aims of study**, enhancement of zirconia implant surface roughness by irradiation with Nd:YAG laser of (1064, 532) nm wavelengths and assessment of the modification on osteoblast cells attachment and proliferation (in vitro study).**Materials and Methods**. Zirconia block was used for samples preparation. The block was sectioned using CAD\CAM technique into discs of (7,2) mm diameter and thickness respectively. Pilot study was carried out to select the most appropriate parameters that can modify zirconia surface with minimum thermal damage. The samples were divided into three groups, A: control group(untreated), B: treated with Nd:YAG laser of 10 Hz frequency 1064 nm wavelength, energy density is 38.22 J\cm², pulse duration is 9 nanoseconds , exposure time is 7 seconds, C: treated by 2nd harmonic Nd:YAG laser of 532nm wavelength, the energy density is 19.11 J\cm², frequency is 10Hz, pulse duration is 9 nanoseconds ,exposure time is 7sec. Contact angle measurement was done for three groups. Osteoblast cells were isolated from new born rat (2-3) days age by a process of enzymatic digestion to calvarias bone. After seven days incubation period, osteoblast cells were seeded on the discs of three groups (control and two experimental). Twenty samples for each group, five samples for each period. Cellular attachment was assessed after 24 hours and osteoblast proliferation assessment was done after (3, 6, 9) days for each group. Alkaline phosphatase secretion was also assessed for three same intervals for three groups. **Results** Cellular attachment after 24 hours showed a significant difference between the control group

and other two experimental groups. The number of proliferated cells after three days incubation indicates a high significant difference between the control group and other two experimental groups. After six days interval, there was a significant difference in the number of proliferated cells between three experimental groups. High significant difference on both irradiated groups compared to control one, while, no significant difference between two experimental groups. Significant difference was seen when the number of proliferated cells after nine days was compared between three tested groups, high significant difference was seen between the two irradiated groups.

A significant difference in Alkaline Phosphatase level was seen after (24) hours culturing, high significant increase on the group irradiated by 532nm Nd:YAG laser. Contact angle has significantly increased on both irradiated groups compared to control one. **Conclusion** Q switched Nd:YAG (532 and 1064)nm wavelength laser can be safely used in modification of surface properties of zirconia implant without thermal damage using specific parameters, attachment and the number of proliferated osteoblast cells significantly increased by enhancement of implant surface roughness.

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List of abbreviations

Symbol	Term
AFM	Atomic Force Microscope
ANOVA	Analysis of Variance
CAD\CAM	Computer aided design /computer aided manufacturing
cm^2	Square centimeter
CNC	Computer numerical control
3D	Three dimensional
D MEM	Dulbecco's modified Eagle's medium,
FCS	Fetal Calf Serum
g	Gravity
HBSS	Hank Buffer salt solution
J	Joule
LSD	Least significant difference
µs	Microsecond
nm	Nanometer
Nd^{+3}	Neodymium
Nd:YAG	Neodymium-doped yttrium aluminum garnet
PBS	Phosphate buffer solution
sec	Second
SEM	Scanning Electron Microscope
SPSS	Statistical package for the social sciences
Y_2O_3	Yttrium oxide
Zr	zirconium
ZrO_2	Zirconium dioxide

Introduction

Oral implant treatment has been widely used in replacement of missing teeth partially or completely edentulous patients. The main issue in dental implant treatment is primary stability of the implant unit immediately after surgical insertion, since, it has an impact role in determination of the success rate of implant treatment. It mainly depends on bone implant contact area and engagement of cortical bone by implant surface. Moreover, the secondary stability, which represents healing process and new bone formation onto implant surface [1].

Different materials had been used in construction of implant units and the most commenlly used materials are titanium and titanium alloys. Inspite of their good mechanical properties they also had many of drawbacks. So ceramics were introduced as dental implant material and successfully considered as an alternative to titanium. [2].

Modification of implant surface roughness can accelerate healing process and improve implant biocompatibility. In addition, better functional loads distribution obtained with roughened implants compared with smooth implants. Consequently, implant surface treatment had indicated in certain cases including, low bone density and insufficient remaining alveolar bone to improve bone implant contact and enhance healing process. [3].

Different methods were used in modification of implant surface roughness and laser surface treatment is one of them.

In this chapter more details will be shown about osseo integration and its steps, cellular events and factors affecting on it.

Also, zirconia and its properties, phases will be explained next.

Finally, this chapter also contains a little about laser as a technique in surface treatment of dental implant and about laser material interaction processes.

1.1. Dental Implant

(A prosthetic device made of different types of material(s) inserted inside the oral tissues beneath the mucosal and/or periosteal layer and on or within the bone to obtain retention and support for a fixed or removable dental prosthesis; a substance that is placed into and/or on the jaw bone to support a fixed or removable dental prosthesis)[4].

Many benefits can be obtained by using dental implants in case of denture construction, better denture stability due to incorporation of implant unit into surrounding bone after healing period.On the other hand dentures can be used in place in spite of gum tissue problems. The main protocol that followed in using of dental implants is two phases which includes firstly, placement of implant unit in surgically prepared bony socket and waiting for (3-6) months for healing. Secondly loading and placement of dental prosthesis for functional and esthetic demands. Another protocol which is known as (Immediate loading), that means placement of implant unit and dental prosthesis at same time [5].

1.2. Response of bone to the placement of dental implant

The placement of implant unit into bone can result into **either Fibro integration or Fibro osseous retention** which means that the implant surface is connected to the surrounding bone by fibrous connective tissue, or **Osseo integration** which means the intimate bony contact between implant surface and surrounding bone. Consequently, masticatory loads can be successfully transferred to the surrounding bone during function [6].

1.3. The bone

The bone is highly mineralized connective tissue which has a function of withstanding mechanical loads [7]. The bone is highly dynamic organ, it undergoes resorption and new formation continuously by osteoclast and osteoblast cells respectively [8, 9]. The bone formed by deposition of matrix by osteoblast cells. The matrix is made of collagen

molecules in form of layers in between calcium and phosphate crystals are precipitated [10]. The bone mainly consists of four cellular types, osteobalsts, osteoclasts, osteocytes and bone lining cells [11, 12]. Figure (1.1). The osteoblast cells are derived from mesenchymal cells in bone marrow. They are responsible for new bone formation within several weeks of time, as well as osteocalcin and osteonectin[10], alkaline phosphatase and bone sailoprotine[13]. Differentiation of mesenchymal stem cells into osteoblast cells requires expression of specific genes in a specific periods of time[14]. Once a pool of osteoblast progenitors expressing *Runx2*(Runt- related transcription factor 2) and *ColIA1*(collagen 1) has been established during osteoblast differentiation, there is a proliferation phase. In this phase, osteoblast progenitors show alkaline phosphatase (ALP) activity, and are considered preosteoblasts. The transition of preosteoblasts to mature osteoblasts is characterized by an increase in the expression of osterix (Osx)and in the secretion of bone matrix proteins such as osteocalcin (OCN), bone sialoprotein (BSP) I/II, and collagen type I. Moreover, the osteoblasts undergo morphological changes, become large and cuboidal cells [14]. The synthesis of bone matrix by osteoblasts occurs in two main steps: deposition of organic matrix and its subsequent mineralization. In the first step, the osteoblasts secrete collagen proteins, mainly type I collagen, non-collagen proteins (OCN, osteonectin, and osteopontin), and proteoglycan including decorin and biglycan, which form the organic matrix. Thereafter, mineralization of bone matrix takes place into two phases: the vesicular and the fibrillar phases [15]. The vesicular phase occurs when portions with a variable diameter ranging from 30 to 200 nm, called matrix vesicles, are released from the apical membrane domain of the osteoblasts into the newly formed bone matrix in which they bind to proteoglycans and other organic components because of its negative charge.

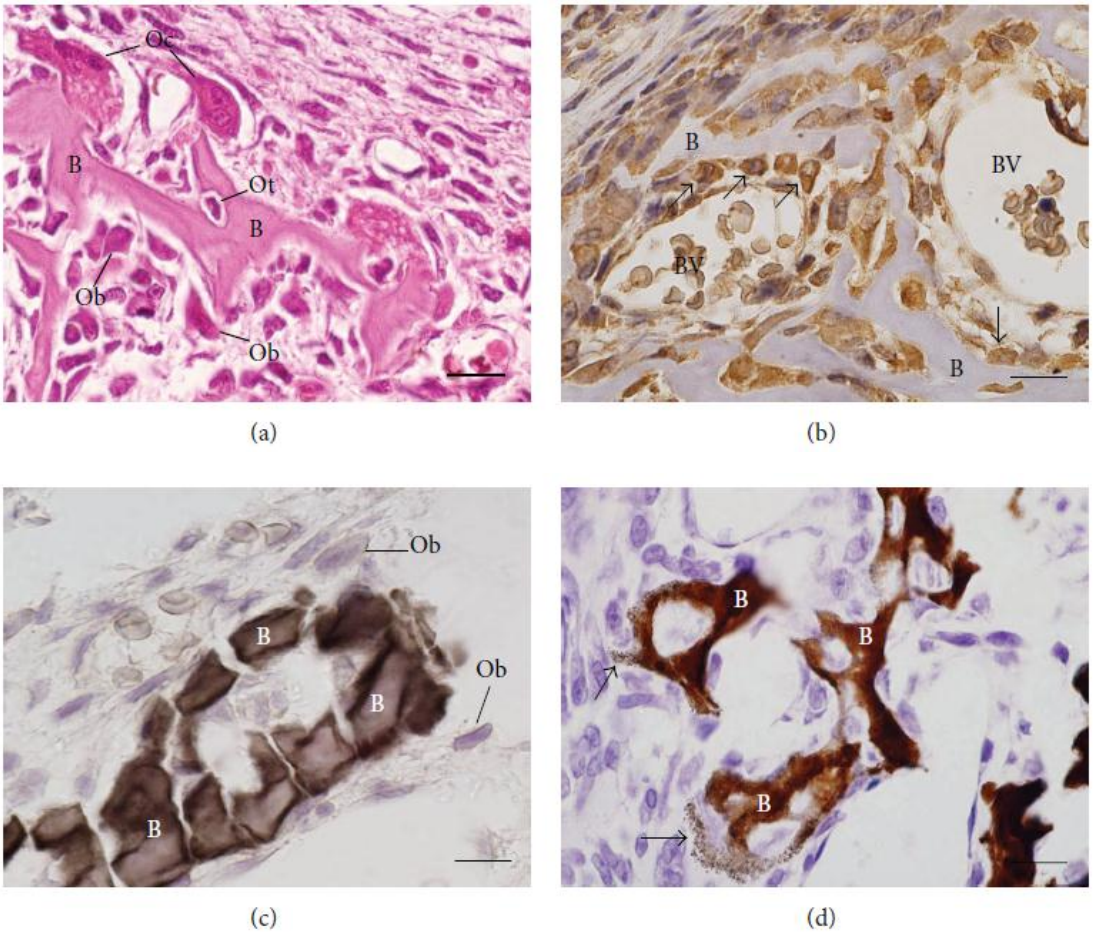


Figure (1.1) Light micrographs of portions of alveolar bone of rats. (a) HE-stained section showing a portion of a bony trabecula (B).Polarized osteoblasts (Ob) and giant multinucleated osteoclasts (Oc) osteocyte (Ot) surrounding bonematrix is also observed. (b) Section subjected to immunohistochemistry for osteocalcin detection and counterstained with hematoxylin. Osteocalcin-positive osteoblasts (arrows) on the surface of a bony trabecula (B). BV: blood vessel.(c) Undecalcified section subjected to the Gomori method for the detection of alkaline phosphatase, evidencing a portion of bone matrix (B) positive to the alkaline phosphatase(in brown/black). Ob: osteoblasts. (d) Undecalcified section subjected to the von Kossa method for calcium detection (brown/dark color).von Kossa-positive bone matrix (B); some positive granules (arrow) can also be observed on the surface of the bone trabeculae. Scale bar: 15 μm.[14].

When osteoblasts secrete enzymes that degrade the proteoglycans, the calcium ions are released from the proteoglycans and cross the calcium channels presented in the matrix vesicles membrane. These channels are formed by proteins called annexins. On the other hand, phosphate-containing compounds are degraded by the ALP secreted by osteoblasts, releasing phosphate ions inside the matrix vesicles. Then, the phosphate and calcium ions inside the vesicles nucleate, forming the hydroxyapatite crystals. The fibrillar phase occurs when the supersaturation of calcium and phosphate ions inside the matrix vesicles leads to the rupture of these structures and the hydroxyapatite crystals spread to the surrounding matrix [16].

1.4 Osteoblast model for vitro studies

Osteoblast cells are required in vitro experiments in case of determination the response of bone forming cells against biomaterial insertion inside the body [17, 18].

In vitro studies, osteoblast cells can be obtained from different approaches. Either from primary cell culture or osteoblast like cell line. Both ways can provide informative documents about cellular differentiation, proliferation and function of osteoblasts [18].

In case of primary cell culture, several techniques had been followed to obtain such cells including isolation from different skeletal organs Re.g. clavaria and long bones from rats and mice.[19,20,21].

In many previous studies cell line osteoblast cells used in vitro studies. Using such cells may have advantages and disadvantages. For instance,large cellular number is available at the same time and there is no need for waiting for cell culturing[17]. On the other hand, these cells may not provide a real behavior of cells in a certain tissue and their behavior may not be a representative to the ordinary cells. Specifically,

in the case of malignant cell lines, proliferation is non-physiological [22].

The osteoblast cells isolated from rats can be considered as an alternative for human osteoblast cells in vitro researches for determination the tissue response against biomaterials insertion[17]. The most commen method used in isolation of rat osteoblasts is enzymatic digestion by using Trypsine and collagenase enzymes. Any soft tissue ruminants specifically in periosteal layer is removed, osteocytes also declined due to poor proliferative activity [1]. The age of the selected animal may affect the cellular response against the biomaterial surface in vitro study specially through the degree of cellular maturation state. More immature osteoblast cells, higher sensitivity and better cellular response can be obtained against biomaterial surface[17].

1.5. Osseo integration

A phenomenon that results from direct newly formed bone deposition on the surface of implant unit with absence of fibrous tissue formation along implant surface. Clinically there is no implant movement under normal masticatory loads[23]

Incorporation of implant surface into surrounding bone results from sequence of cellular and protein adhesion processe which can be enhanced by modification of implant surface properties[24].

1.5.1.Stages of Osseo integration

Osseo integration is a process mainly similar to that of bone fracture healing process. It is achieved by action of different growth factors and proteins in combination with extracellular matrix excreted by bone forming cells.

Three main stages required to obtain successful Osseo integration

1. Woven bone deposition around implant which leads to incorporation of implant unit.

2. Replacement of woven bone by lamellar bone in order to withstand applied functional loads
3. Bone remodeling process after complete bone healing. [25].

1.5.2. Cellular events of Osseo integration

The procedure of dental implant placement starts with surgical preparation of localized bony area to create an implant bed, drilling during preparation considered as a trauma that stimulates bone healing process around implant[26].

The first step in healing process immediately after insertion of implant unit is formation of blood clot which fills the space between implant surface and surrounding bone. Red blood cells, platelets and macrophages are dominant cell contained in fibrin scaffold found in formed clot in this stage. Then these cells start to release cytokines and growth factors that regulate healing process including (TGF alpha, TGF beta, FGF, EGF). In this step, the main role of macrophages and leukocytes in formed blood clot is to eliminate the tissue remnants resulted from drilling process. The cleaning process is followed by destruction of the blood clot by fibrolysis to create space for next step in healing process. The created space then occupied by granulation tissue which is rich in un differentiated mesenchymal cells a round newly formed blood vessels, these cells will differentiate into bone forming cells first into pre-osteoblast cells then mature osteoblast cells[27].

Once implant surface becomes in contact with blood in implant bed, different types of proteins adsorb on it, a such adsorption process is greatly affected by surface topography of inserted implant unit[28].

All these events occur during an inflammatory phase which ends with blood clot removal by poly morphoneuclear cells and monocytes [29].

One day after implant insertion, migration of osteoblast progenitor cells starts toward implant surface and start to attach on it[30].

The direction of Cellular migration is mainly governed by fibrin scaffold, osteoblast cells reach and attach onto implant surface through fibrin scaffold, so once fibrin scaffold detached from implant surface no osteoblast cells reached and attached on the surface. For this reason, improvement of implant surface roughness plays an important role in the progress of osseointegration and reduction in healing time, because fibrin scaffold can be removed easily from smooth surfaces than rough surfaces [31].

According to the direction of bone apposition, bone formation process can be classified into contact osteogenesis and distant osteogenesis. In contact type, osteoblast cells migrate to implant surface and attach and start bone formation there. While in distant type, bone formation starts on the wall of implant bed and gradually progress toward implant surface[32].

The second stage of osseointegration is prolifrative phase, in which the mesenchymal cells differentiate into osteoblast cells and proliferate. These cells start to secret non collagenous extra cellular matrix deposited as a thin layer on implant surface. This layer act as a guide for further cellular attachment, it is composed mainly from poorly mineralized osteoid (which is a tissue formed by osteoblast cells represents the continuity of osteogenesis, rich in calcium, phosphorus osteopontin and bone sialoprotein[33]. Then woven bone arrangement and bone trabeculae start to act as bridges to anchor further cells and newly formed bone [34]

The last stage is remodeling phase which starts at the end of healing process and continue through a life in response to functional loads[35].

1.5.3. Role of Alkaline phosphatase (ALP) in osseo integration process

Alkaline phosphatase is a biomedical marker that can be considered as an indicator for osteoblast cells differentiation. It is a cell membrane

bounding glycoprotein secreted by osteoblast cells in high concentrations on their membrane during bone formation process. It has an impact role in catalization of phosphate monoester involving in bone calcification process [36]. moreover, its role in mineralization of extracellular matrix can be due to its calcium binding ability and destroying of mineralization inhibitors.[2].

ROSA A.L. and BELOTI M.M. measured ALP level in vitro study to compare between different surface roughness values of titanium discs. After 21 days following discs culturing, the level of ALP had increased on roughened titanium discs. [37].

Song Y. et al also measured ALP level in vitro study to compare between smooth and Nano porous alumina implants. The results revealed that the ALP level had increased after 12 day after discs culturing[38].

Since the ALP considered as an indicator that reflects the osteoblasts activity, ALP level was measured in many previous studies in each study it reflects a specific fact. Its level was measured by Chen et al in diabetic patients and they concluded that increased ALP level had been inversely proportioned to bone mineral density[39].

ALP also used as marker that determines the biocompatibility of an implant material. A study which was done by Han et al measured ALP level to compare the response of osteoblasts against nanohydroxyapitite/ polycaprolactone composites and polycaprolactone implant materials. They found a higher ALP level in nanohydroxyapitite/ polycaprolactone composites. Consequently better osteogenic procedure was expected [40].

ALP secretion increases specifically during mineralization of extracellular matrix and decreases when the matrix mineralization prognoses well[26 in c2]. Manjo et al measured ALP level in vitro to compare the response of osteoblast like cells against two types of

commercial titanium surfaces, Osseospeed and TiOblast. Higher ALP level was obtained in Osseospeed group.[41].

In vivo, salivary ALP level was measured by ABDLHAMEED et al pre and post surgical insertion of dental implant by using two different flap techniques. In both techniques ALP level increased significantly after surgical procedure compared with normal condition[42].

1.5.4.Factors affecting the Osseo integration success rate are

1. Biocompatibility of implant material
2. Implant design and implant surface properties
3. Implant bed condition
4. Loading condition
5. Surgical technique used in preparation of implant bed[43].

1.5.4.1. Biocompatibility

Capablility of the material of existing in harmony with the surrounding biologic environment known as a biocompatibility [4]. The biomedical material or implant material considered as a biocompatible material when it is placed in contact with living tissue without any acute or chronic inflammatory response and without prevention of healing process[44].

According to biologic response of host tissue against implant insertion, implant materials can be classified into three main types:

1. Bio tolerant material
2. Bio inert material
3. Bio active material

1.5.4.1.1..Bio tolerant material

The surface of this type releases molecules in nontoxic concentration that stimulates fibrous tissue formation around implant surface instead of newly formed apposition[45].

1.5.4.1.2. Bio inert material

This type of implant material stimulates bone formation process successfully starting from the wall of implant bed toward implant surface (slow osseointegration)[27].

1.5.4.1.3.Bio active material

The surface of this material type can be divided into…Osteoconductive, osteoproducive [46].

The surface of osteoconductive material stimulates bone formation on implant surface by releasing of bone forming molecules e.g.(Hydroxyapatites.,Tricalciumphosphatase)[45].

The surface of osteoproductive material stimulates bone formation process starting from implant surface toward wall of implant bed.such material characterized by different surface treatment to enhance surface roughness in different degrees[27].

1.5.4.2.Impact of implant surface on Osseo integration

The surface of an implant unit plays an important role in determination the biological response against implant placement[47]. Once the implant unit becomes in contact with surrounding biological tissue, the water molecules are the first species adsorb onto the biomaterials surface, then small molecules lastly, the bigger organisms adhere on. Adherence of the last entities is greatly affected by previously adhered biofilm[48].

The texture of the implant surface has an impact role on tissue response against implantation procedure. Increase surface roughness can be utilized in obtaining larger surface area of implant unit integrating with the surrounding bone during healing process. Moreover, textured surfaces have an advantage of tissue ingrowth deeper compared with smooth surfaces[49,50].

The goal of implant surface texturing is to enhance cellular response and improve bone formation process during healing period[51,52], specially

in case of poor quality and quantity of bone is available, rougher surfaces are indicated to get accepted results[53,54].

The modification of the implant surface can be achieved either by increasing surface porosity or by coating the surface with other material, both ways will increase the bone implant contact area[51].

On rough surface of inserted implant, osseo integration can be enhanced by platelets and monocytes adhesion[55].Direct attachment of osteoblast cells consequently proliferation and differentiation[56].

Better primary stability can be obtained by using rough implant due to larger surface area engaged with surrounding bone[57]. Moreover, in case of using rough implant, both contact and distant osseo integration will take place. While by using smooth surfaces,distant one will take place[58].

Surface roughness can be classified into macro, micro and nano sized surface topography[27].

Macro sized surface topography is related to implant geometry,e.g. threaded screw, solid body press-fit design. Macro sized roughness ranges from millimeters to microns(more than 10 ml). Screw threaded implants showed better functiona loads distribution to surrounding bone so better primary stability con be obtained. Consequently secondary stability. This degree of surface roughness can be utilized in providing sufficient space for new bone apposition[59]. Micro sized surface topography ranges between(1-10)micrometers, can be utilized in osseoinduction and osseocoduction. By using the implant surface as a carrier for a bio active material. Micro textured implants showed better bone implant contact when compared with machined implants[60].

Nano sized surface topography plays an important role in protein and cellular adhesion and determins the success rate of osseointegration. Better osseo integration out come can be obtained when the implant surface treated in micro and nano surface roughness range. Since on

both surfaces bone formation process is stimulated by contact osseointegration. [61,62].

1.5.4.3.Surface wettability

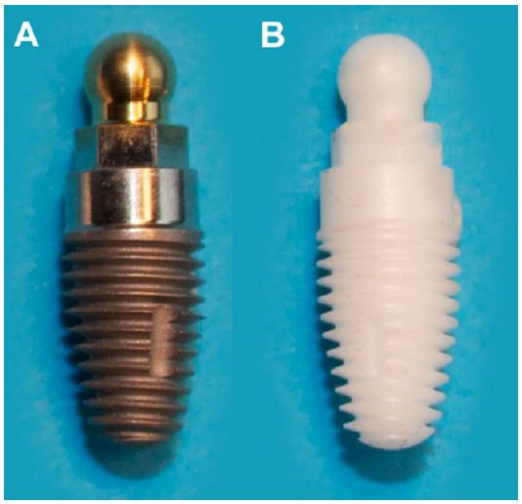
Surface wettability is one of several surface properties that determins the degree of fluid diffusion and interaction with the solid surface when they are in contact together. Specially,once the dental implant has been inserted into implant bed it will be in contact with the blood. In dental implantology, hydrophilic surfaces are preferable to be used more than hydrophobic surfaces in order to get better fluid and cellular interaction. Surface wettability is determined by contact angle measurement. Contact angle greater than 90º indicates hydrophobic surface. While contact angle less than 90º indicates hydrophilic surface and higher flattening of fluid drop on the solid surface which means higher attractive forces between fluid atoms and solid surface atoms. In dental implantology, higher surface energy means increased area for cellular attachment [5].

1.6.Zirconia implant material

Zirconium oxide (ZrO2) was discovered accidentally by a German Chemist Martin Klaproth while he was heating some germs. The first using of zirconia as a bio material was in 1970 when it used for hip joint replacement[63]. Zirconia is not found in nature as a pure state, zircon is the main source of zirconia(in which zirconia is combined with silicate oxide). Also a mineral named as Baddelyite considered as most commenly used source for zirconia extraction. Since it contains about (96.5to 98.5)% of its composition in form of zirconia[64].

Due to many drawbacks of titanium implant using, zirconia used as an alternative implant material. These titanium drawbacks which limit using such implants are, ions releasing into body fluids which leads to non specific immunomodulation and auto immunity[65], dark_grayish color which reduce esthetic outcome specially in anterior area in case of

gum recession due to chronic gingival disease[66]. Using of metallic implant simultanously with other metallic restorations results in battery like behaviore and saliva act as electrolyte. This increase corrosion rate and increase chance of sensitivity reactions. All these drawbacks were successfully overcomed or solved by using zirconia. Since the color of this material is comparable to the natural teeth color, also ceramic material doesn't subjected to corrosion and ion release. These properties made zirconia excellent alternative to titanium[67,68]. Figure(1.2) shows these two types.



Figure(1.2), two types of dental implants A, titanium implant. B, zirconia implant.[69].

1.6.1Properties of zirconia

1. Excellent strength,hardness
2. High corrosion resistance
3. It has modulus of elasticity similar to that of steel
4. It has thermal expansion coefficient similar to that of iron
5. High fracture toughness [70,71].
6. Provide good esthetic demands[72,73].
7. Minimal thermal and electrical conductivity [74,75].

1.6.2.Zirconia phases

According to atomic arrangement of zirconia and crystallographic structure, zirconia phases can be classified into

1. Monoclinic phase
2. Tetragonal phase
3. Cubic phase.(figure 1.3)

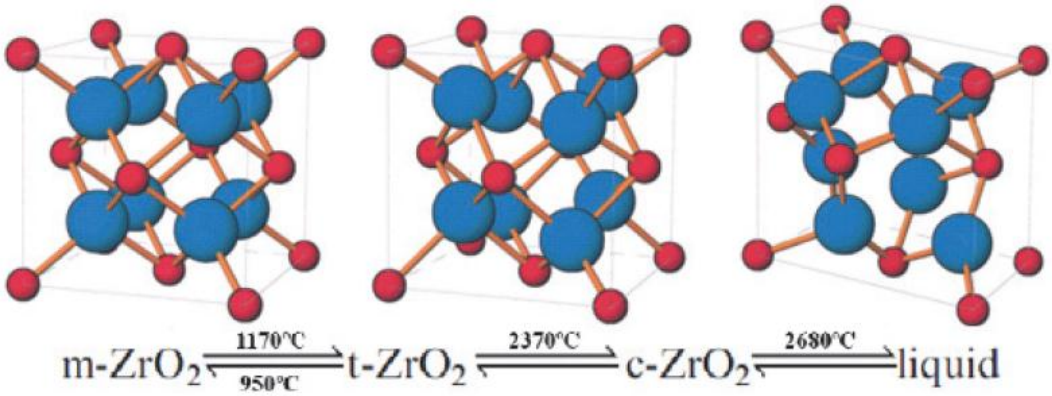


Figure (1.3),Three phases of zirconia [76].

At room temperature pure zirconia presents in monoclinic phase upto (1170) Cº. Heating of zirconia over 1170Cº up to 2370Cº results in tetragonal phase, while heating over 2370Cº results in cubic zirconia[77]

Tetragonal to monoclinic phase transformation occurs spontaneously during cooling almost at (850_1000) Cº temperature. This results in volume expansion about (3_5)%,which leads to lower mechanical properties and surface flaws and crack [78]. in order to overcome this un desirable phenomenon, the chemical composition of zirconia is modified by adding different oxides in different concentrations to get stable tetragonal zirconia at room temperature e.g. adding Yttrium oxide, calcium oxide, magnesium oxide[78,79].

Regardless the type of added stabilizer, three main types of zirconia based ceramics is most commonly used.

1. Partially stabilized zirconia. The material is composed mainly from cubic phase particles in which tetragonal phase particles are precipitated.
2. Zirconia Toughened Composites. The grains of tetragonal phase embedded in the high modulus elasticity material almost alumina used.
3. Tetragonal Zirconia poly crystals. In which all the constituent grains are in tetragonal phase.

The most commonly class used in biomedical applications is tetragonal zirconia poly crystals. Specially Yttrium stabilized zirconia (3 to 8)% of Y2O3. Due to its excellent mechanical properties including, fracture toughness and strength. Also, higher corrosion resistance, superior wear resistance compared with other ceramics. [80,81].

1.6.3.Low temperature degradation (LTD)

Low temperature degradation is a spontaneous transformation of tetragonal phase to monoclinic phase in zirconia poly crystal materials. This event results in lower mechanical properties and lower efficiency of the bio material[82].

LTD can be a result of surface micro cracks. Consequently, grains pull out results due to increased particle size which related to phase transformation. This phenomenon begins from the surface of biomaterial which is in contact with water molecules, Then, continues deeply toward material core. It had been suggested, the penetration of water molecules inside crystal lattice stimulates internal stresses formation by reduction of the oxygen positions modification of oxygen configuration around zircon (Zr) [83, 84,85].

The continuity of phase transformation from the surface to the core material can be achieved by (nucleation and growth) mechanism. In this mechanism, at grain boundaries, water derived species diffuse more,so tetragonal to monoclinic phase transformation is easier. Since,

crystalline disorders are more, the nuclei of monoclinic phase gradually increase on the surface till saturation. Finally, transformation then directs toward the material core[86].

1.6.4.Laser sintering Technique

Sintering process can be considered as a process that responsible for improvement of material strength. Zirconia normally at room temperature found in a monoclinic phase and by sintering process under a certain temperature it undergoes into tetragonal phase of better mechanical properties that able to withstand the functional loads. The conventional method for sintering is by using the sintering furnace. The sintering results and material properties in this method were mainly governed by holding time and temperature[87].

Selective laser sintering can be considered as additive manufacturing process in which laser beam used to melt and sinter the powder material. In this process, the main issue that determines procedure success is the energy that transferred to the powder material. It must be high enough to cause material melting, consequently sintering. This method was indicated in case of the high melting temperature materials sintering. Sometimes, high melting temperature materials were mixed with a certain compounds those had a low melting point. In this case, the process termed as "Indirect selective laser sintering". Laser sintering process was mainly governed by material properties including, thermal conductivity, specific heat, thermal diffusivity and optical absorptivity. Also, governed by laser beam properties including energy, wavelength and interaction time.[88].

In general, laser sintering systems consist of laser source, laser scanner, powder loading unit, building plate and gas flow controller. Laser wavelength selection must be based on a matching with the processed material absorptivity. The most common types were CO_2 laser of 10600

nm wavelength and Q- switched Yttrium laser of 1100 nm wavelength[89].

1.7. Laser material processing

Laser beam machining of different materials based on conversion of an absorbed optical energy to thermal energy, which results in heat generation on the surface of an workpeice [90,91]. The temperature rise of the substrate might be due to heating, melting, vaporization of the surface depending mainly on the intensity of incident laser beam[92].

1.7.1.Basic components of laser system

Each laser system must contain the main three following parts…

1. Active medium, in which light amplification takes place.
2. Pumping source, which is utilized for excitation of active medium constituents.
3. Optical resonator, which ensures multiple reflections through active medium.

The laser beam is generated by a physical phenomenon known as (stimulated emission). The constituent atoms of active medium can be excited by absorption of pumping energy then they return back to ground state emitting their energy in form of photons. These emitted photons will be bounced back and forth between cavity mirrors passing through active medium causing further stimulated emission for excited atoms. Finally, these bouncing photons reach the threshold to pass through output coupler mirror as a coherent, monochromatic light [91].

According to active medium, lasers can be classified into following types[93].

1. Solid state lasers.
2. Gas lasers.
3. Dye lasers.
4. Semiconductor lasers.

1.7.2. Advantages

Many advantages can be obtained by using the laser in surface texturing process including[94].

1. Chemically cleanliness.
2. Thermal affected depth can be controlled by automation of laser parameters.
3. Precise determination of heat affected zone.
4. No or less requirements for after machining process.
5. Non-contact technique and ease of automation.

1.7.3. Neodymium Yttrium Aluminum Garnet laser, Nd:YAG laser

Nd:YAG laser belongs to the family of solid state lasers. Its active medium composed of crystal rode, which is composed from Neodymium, Yttrium, Aluminum, Garnet. The active medium is pumped by using the flash lamp or diode lasers. It can be operated by continuous or pulsed mode [95].

The Nd^{+3} ions absorb the light of pumping flash lamp to be excited to higher energy level then decay to metastable energy level. Lasing process occurs by decay of excited ions from metastable state to terminal state. By cooling mechanism, Nd ions will return back to ground state.(figure 1.4)[94].

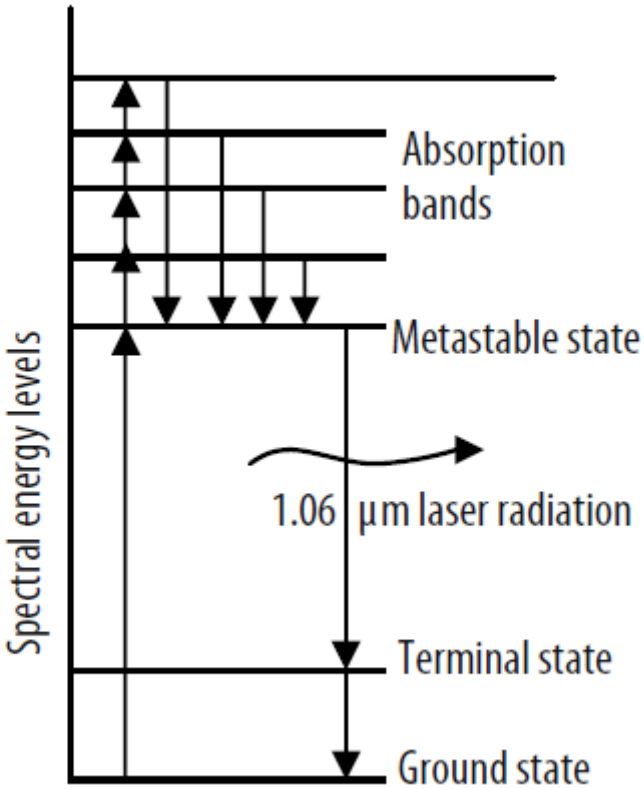


Figure (1.4) Energy levels of Nd-YAG laser.[93]

As shown in the previous figure(1.4), Nd:YAG laser is four levels system, different wavelengths can be obtained due to the energy gap between ground state and high energy levels. The prominent emitted wavelength is 1064nm. It can be obtained also in frequency doubling 532nm[96]. The laser beam is obtained depending on three main electronic processes including, absorption,spontaneous emission, stimulated emission. Whenever there is population inversion can generate energy that overcomes system losses, laser beam will be generated. Laser output can be modulated depending on the time of previous three processes[97].

1.7.4.Q-switching Technique

Is the process of giant pulse generation in which pulse length is in order of nanosecond. The peak power that obtained from Q- switched lasers are several orders of normally obtained power.

In case of Q- switching, giant pulse can be obtained by changing the Q value of the cavity, which means, how long the cavity able to store its energy. High Q value means stored energy without laser irradiation[96].

The mechanism of Q switching is based on prevention of spontaneous emission process, consequently, prevention of stimulated emission and lasing process. This situation promotes further build up of population inversion inside active medium until saturation. Once the Q switch gets off,intense power pulse is generated [97].

1.7.5.Laser Material Interaction

The laser beam interacts with the substrate material by interaction of incident photons with the basic constituent units of the material. For solid materials, the interaction of incident electromegnatic radiation will be with the outer electrons of constituent units. Such interaction may result in different phenomena depending on laser parameters and material properties including thermal and optical properties. The main laser parameters that affect laser material interaction are..[82]

1. Wavelength (λ)
2. Power (P).
3. Intensity (peak power/ Area) (I).
4. Fluence (Energy/ Area) (F).
5. Pulse duration in case of pulsed laser (Δt).

1.7.6.. Physical phenomena at laser substrate interface

When the laser beam strikes the material surface, part of it will reflects away from surface, another part will be absorbed by the material,scattering will occur too, finally, transmission through the material may occur, when the applied intensity is high and this happened with an almost transparent medium.

The most important phenomenon is absorption, because any effect in a specific workpeice occurs after absorption of incident laser beam[93].(figure 1.5)

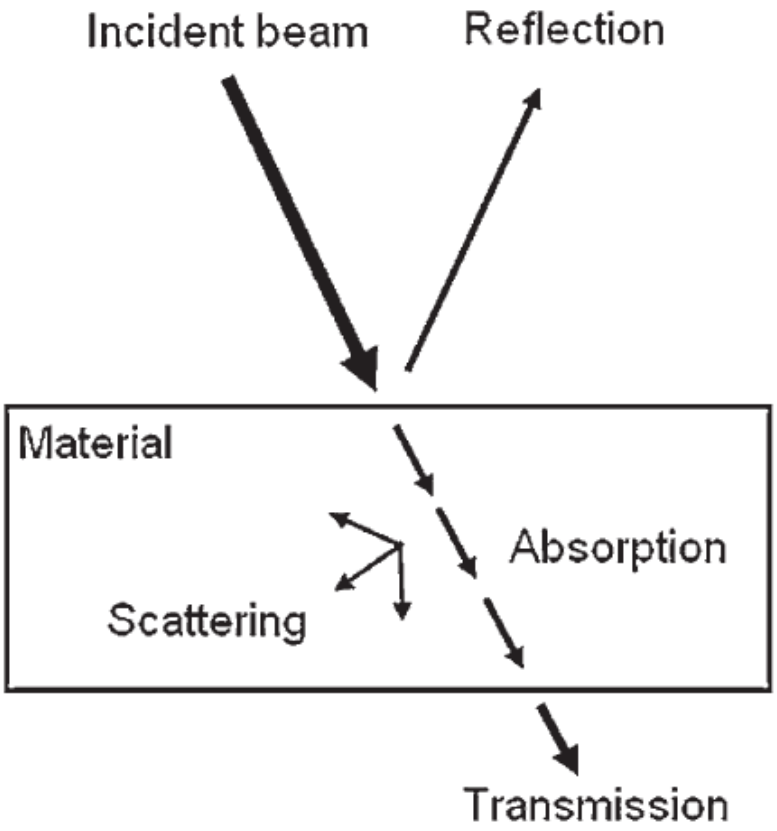


Figure (1.5), Possible interactions of laser light with material.[98]

1.7.7. Processes of laser material interaction.

Laser material interaction can be explained generally by two main processes:

1. Absorption of energy.
2. Conversion of energy (relaxation).

The first process includes the mechanism by which the incident photo-energy can be absorbed. While the second one explains the mechanism by which the absorbed energy can be transformed and redistributed to the surrounding material[99].

1.7.7.1. Absorption of energy

In general, materials can be classified into metals and non-metals depending on their electronic structure. In case of metallic materials, the absorption of an incident laser beam is achieved by free electrons resulting in excitation of these electrons. Concequentlly, increasing their kinetic energy and collisions with the surrounding electrons [100].

After a certain period of time, these excited electrons tend to return back to their stable condition converting their energy to another type of energy "thermal energy"[99].

For non-metal materials the story is completely different. The electronic structure is divided into valence and conduction bands. In normal conditions, high electrons population found in valence band and less in conduction one[101].

The incident photons must have an energy at least equal to the energy of the band gap of the material to enable the electron to jump to excitation energy level[82].

In case of photon energy is lower than the energy of band gap, multi-photon absorption takes place. In other words, cooperation of two or more photons to achieve electrons inter band transitions by using high laser intensities[99].

1.7.7.2 Relaxation process

The absorption of incident laser beam results in excitation state of material basic constituent units (specially the elactrons). After a specific period of time, these excited electrons tend to return back to their stable state depending on material properties and laser parameters. This process is termed as **Relaxation process [**101].

The outcome of relaxation process is either thermal or non-thermal. In thermal process, the collisions between the excited electrons and the lattice results in conversion of the kinetic of excited electrons into vibrational energy for lattice. This is known as thermalization (thermal

relaxation time). This type of energy conversion will continue until thermal equilibrium is reached between the lattice and excited electrons[102].

This process is greatly governed by the thermal properties of material. In other words, in metals, heat diffusion deeply into bulk material is much greater than that of insulator materials. So the depth of heat affected zone exceeds the absorption depth. On the other hand, in insulators, the penetration depth is related to absorption depth[82].

In case of non-thermal relaxation process, the energy of an incident photon is equal to the binding energy of constituent molecules. So, energy absorption leads to bond breaking releasing of binding energy in form of kinetic energy of ejected fragments[99].

1.7.8.Material response to laser irradiation

After absorption of incident laser energy, conversion into thermal energy results in different processes on material surface,heating, melting, vaporization or chemical decomposition, depending on the intensity of incident laser beam[97].

The effect of laser irradiation also depends on thermal properties of substrate (thermal conductivity, thermal diffusivity). The relation between rate of laser energy deposition and the rate of heat dissipation into core material also determines material response to laser absorption. When the rate of heat generation on material surface higher than the rate of heat transfer into core material, changes will take place in a thin superficial layer of material surface and vice versa[103].

1.7.9.Classification of laser material interaction depending on intensity.

1. Heating without phase transformation, low intensities required for such method, this process is called heat treatment. It occurs with (10^4) W/cm² intensity.
2. Melting, higher intensities are required, between (10^4_ 10^5) W/cm².

3. Vaporization which can be achieved by using high intensities greater than (10^5) W/cm² with very short pulse duration or interaction time [103].

1.7.9. 1.Heating

Incident laser beam absorption results in rising material temperature but the resultant temperature still beyond melting threshold of a specific material. Heating process causes re organization of crystalline structure of substrate [104]. Figure (1.6) explains the heating effect of laser irradiation in relation with time.

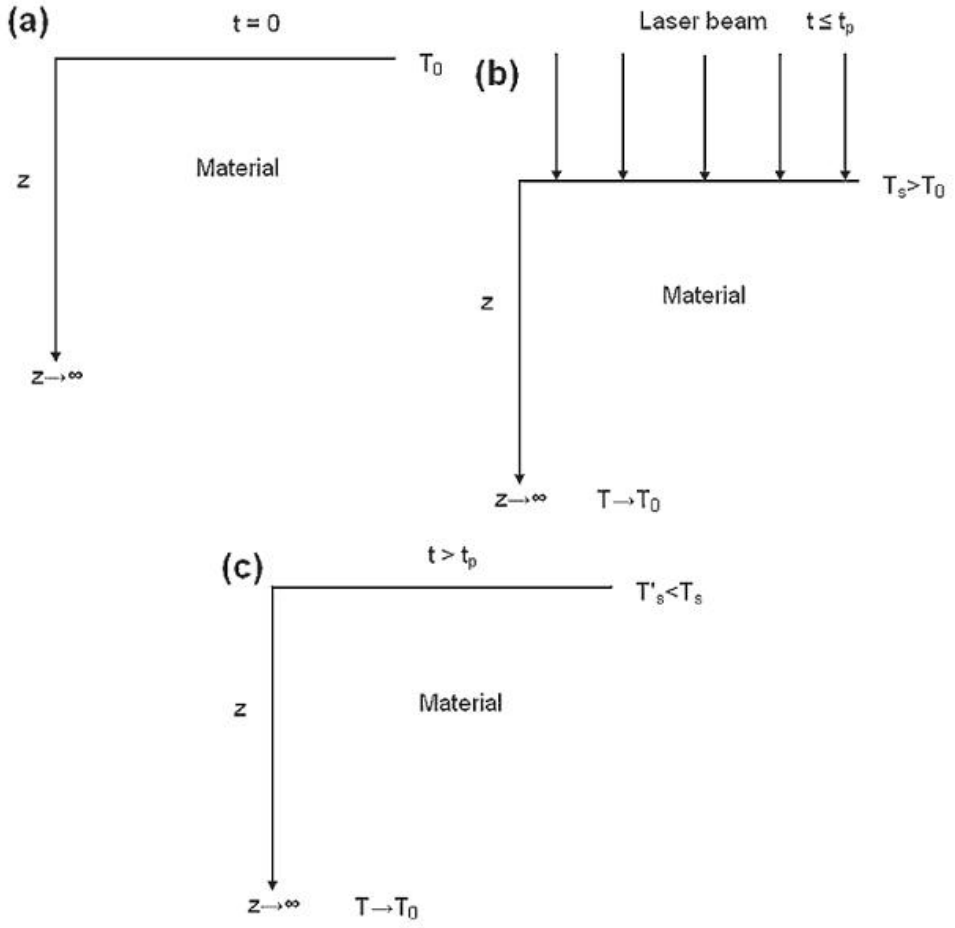


Figure (1.6), Schematic of the laser irradiation geometry and surface temperatures at various times [96].

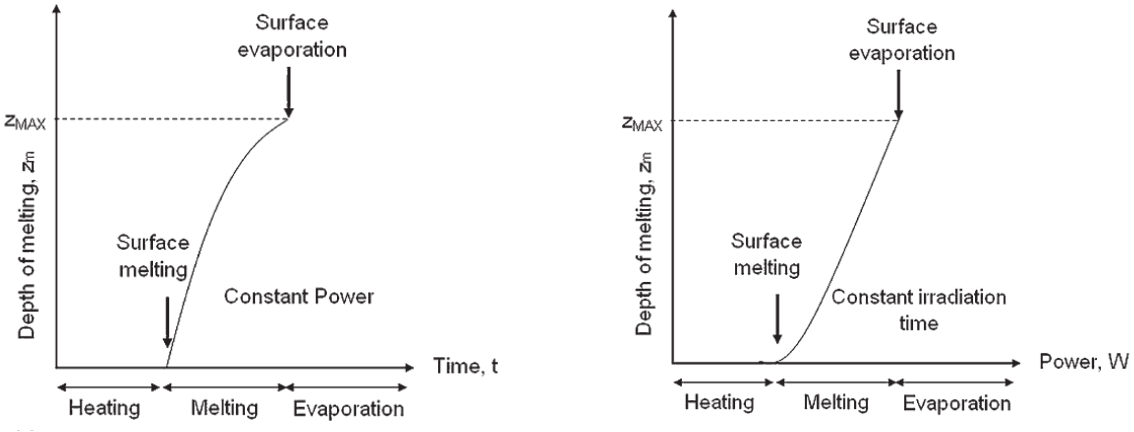
As it is shown in Figure 1.6 (a) the surface temperature before starting laser irradiation which was represented by (T_0) and (t =0) related to time while, (b) explains surface temperature rise due to laser irradiation

which was represented by $T_s>T_0$ and ($t<t_p$) which indicates times less than pulse duration. Finally (c) explains the cooling process, which starts when the pulse stops represented by ($t>t_p$) which means the time has passed the pulse duration[96].

1.7.9.2. Melting

Starting melting process on substrate surface depends on incident intensity between (10^4_10^5) W/cm², while propogation of solid – liquid interface deeply inside core material depends on interaction time. The intensity during whole interaction time must be beyond threshold of vaporization for a specific material[103].

If the heat generated on material surface after absorption of incident laser beam is high enough it will cause phase transformation from solid to liquid state[105]. As shown in figure (1.7).



A B

Figure (1.7), Schematic of the variation of depth of melting with laser irradiation time and power. [96].

As shown in figure 1.7 A, the depth of molten material increased with prolonged interaction time, and in B, increased depth was due to increased intensity.

Cooling rate and re-soldification of molten material has an impact role on the surface condition. Surface defects result from higher re-

solidification rates,while slower rates end with re crystallization and formation of grains of larger size than original[106].

1.7.9.3 Vaporization

This process occurs if the incidence laser intensity is sufficient to heat the material over its boiling threshold leading to matter removal by vaporization. When vaporization takes place, a vapor-liquid interface initiates and moves inside the material with matter removal from the area above this interface[107].

1.7.10.Ablation

Is a process of material removal directly by absorption of laser energy without passing through thermal processes (heating, melting), figure (1.8). The most important parameters that affect onset of ablation process are intensity, wavelength and pulse duration (in case of pulsed laser). [108].

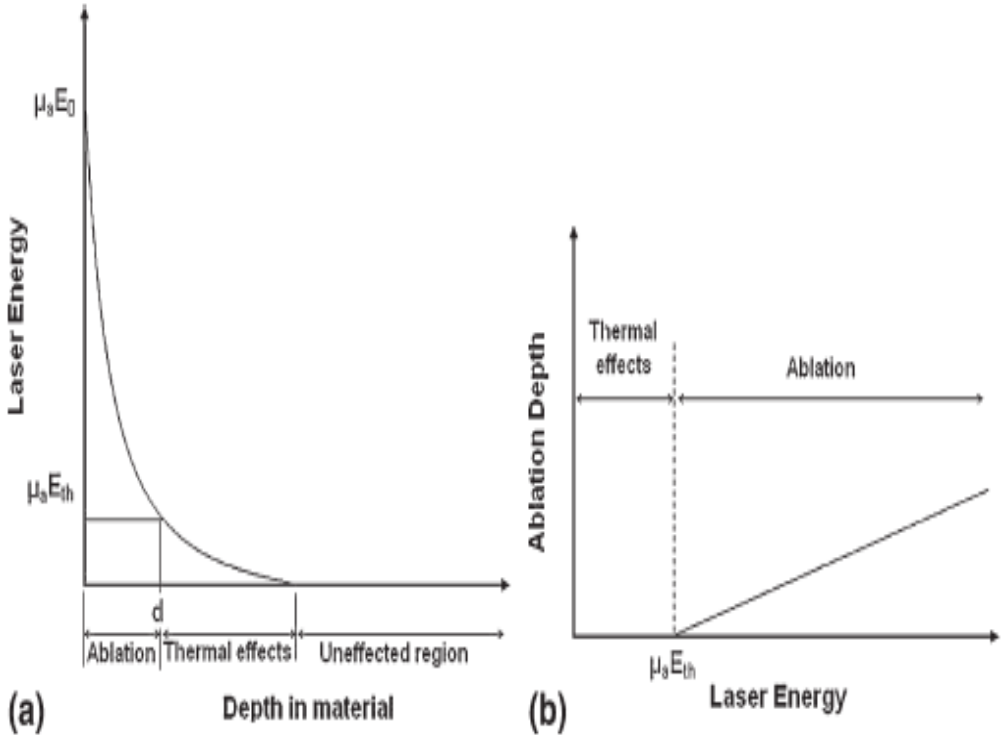


Figure (1.8), Blow-off model of laser ablation: (a) distribution of absorbed laser intensity in the depth of material and (b) variation of depth of ablation with laser energy [96].

The mechanism of material removal by ablation process can be classified into photo-thermal and photo-chemical ablation depending used parameters [109].

1.7.10.1. Mechanisms of ablation

Ultra short pulse duration lasers can achieve the process of ablation, because they able to generate great intensities on the substrate surface. So greater precision can be achieved in the surface micro machining process [110]

In case of photo thermal ablation, the absorbed photo energy is converted to thermal energy which is enough to excite the constituent molecules of material. If the resultant vibrations are enough to break inter molecular bonds, the vaporization of material surface occurs without passing through heating and melting processes. In case of photo chemical ablation, photon energy of incident laser beam is equal to bonding energy of constituent molecules of substrate. So, the absorbed energy is enough to break inter molecular bonds without heat affected zone [94].

Photo chemical ablation can be achieved by using short wavelengths, well defined ablated area with minimum heat affected zone can be obtained [93].

1.8. Literature Review

Author and year	Type of laser	Laser parameters	Test	Results
Noda et al 2010 [111]	Nd:YAG laser 1064nm	pulse duration(5 ms)	SEM	Surface damage due to cracks
Arami et al 2014 [112]	Nd:YAG laser 1064nm	Power(1.5,2,2.5)W Pulse duration(10)µs	SEM	Surface cracks
Omer et al 2014[113]	Nd:YAG laser 1064nm	Energy (100)mJ Frequency(10)Hz	SEM	Surface cracks
Kakura et al 2014[114]	Nd:YAG laser 1064nm	Energy(0.15)mJ Power(3)W	SEM	Improved bone implant contact
Liu et al 2015[115]	Nd:YAG laser 1064nm	Power(1,2,3)W Pulse duration(150)µs	SEM	Surface microcracks
Guilitsyi et al 2016[116]		Femtosecond laser	Cell adhesion And prolifration	Higher cellular attachment and prolifration
Roiteroa et al 2017[117]	Nd:YAG laser 355nm	Energy density(0.15 and7.15)J/cm² Frequency (1)Hz	SEM	Surface microcracks
Soltaninjad et al2017 [118]	Nd:YAG laser 1064nm	Energy density(5 and9)J/cm²	SEM	Surface microcracks
Saygen et al2017[119]	Nd:YAG laser 1064nm	Power(1.5,2)W Frequency(10)Hz Exposure time (20)sec	SEM AFM	Improved surface roughness without surface microcracks
Abdulsatar A.M.2018[107]	Nd:YAG laser 1064nm	Energy density(35)J/cm² Frequency (10)Hz	SEM AFM	Improved surface roughness without surface microcracks.

Aims of study

1. Modification of zirconia implant surface roughness by irradiation with Nd-YAG laser of (1064 and 532) nm wavelength.
2. Assessment of osteoblast cells activity to the modified surface roughness regarding attachment and cellular proliferation (in vitro study).
3. Assessment of Alkaline Phosphatase level during different incubation periods.

Introduction

In this chapter, the steps of present study will be explained in details. The work of present study had been done through two main steps including, laser irradiation and in vitro biologic work.

The first step includes, zirconia samples preparation, sintering and laser irradiation. This step was done in The Institute of Laser For Post Graduate Studies (University of Baghdad).

Surface topography and surface roughness assessment had been done and their results will be displayed next in this chapter. Scanning Electron Microscope Imaging was made in University of Technology/ Department of Mechanical Engineering.

Atomic Force Microscope Imaging was done in Central Service Laboratory/ College of Education, Ibn Al Haitham. Contact angle measurement also was done in University of Technology.

The second step of present study was done in ASCO learning center, during one month December 2019.

List of Equipments

A. for laser irradiation work.

1. Q- switched Nd :YAG laser system (DAESHEN ENTERPRISE/Korea)
2. CAD\CAM (inlab 16 /Sirona /Germany).
3. CNC. Costumized in the Institute of laser.
4. Scanning Electron Microscope (TESCAN / VEGA3/ Czetch Republic/ Germany)
5. Atomic Force Microscope. (NANO PHYWE–Germany)
6. Contact Angle Measurement System. Meter Series-Creating Nano Technology Inc.(China)
7. Grinding and Polishing Machine. (HERGON MP 200V, Italy).
8. Optical Microscope. (BX51/OLYMPUS/Korea).
9. Ultrasonic cleaner (NEW TREND/China).

10. Sintering furnace. (InfireHTC speed/Sirona/Germany).
11. UV Spectrophotometer (Shimadzu/ India).

B. for biologic work

1. Inverted Microscope(AmScope/China)
2. Incubator (Lab teach /Korea).
3. Light Microscope(AmScope/China)
4. Class II Biological Safty Cabinet (Thermo fisher scientific /USA).
5. Centrifuge (Quality Lab System/UK).
6. Micropipette (Human/Germany).
7. 24 and 96 micro well plates.
8. Petri dish.
9. 50 cm^3 Flask.
10. Test tubes.
11. Heamocytometer (China).
12. Spectroscopic plate reader(Glomax Discover/Promega /USA)
13. Tweezers, surgical knives.

List of Materials

A. for Laser Irradiation work

1. Zirconia block (Incoris TZI C / Sirona / Germany / lot no. 2016310139).
2. Diamond paste and Lubricant.

B. for the Biologic Work

1. D-MEM High Glucose (*Euro*- lone, Italy, expiry date ,June 2020).
2. Collagenase type II (Sigma /Germany, expiry date, November 2020).
3. Hank Buffer Solution (Euro- lone, Italy, expiry date May 2020).
4. Phosphate Buffer Solution (Euro- lone, Italy, expiry date July 2021).
5. Fetal Calf Serum(biowest. USA,expiry date, November 2020)
6. Trypsin 1x with EDTA (biowest. USA, expiry date, June 2021)
7. Trypan Blue dye
8. Alkaline Phosphatase kit (ALP – DEA. BioSystems, Spain, expiry date,may2020).

2.1.Sample preparation

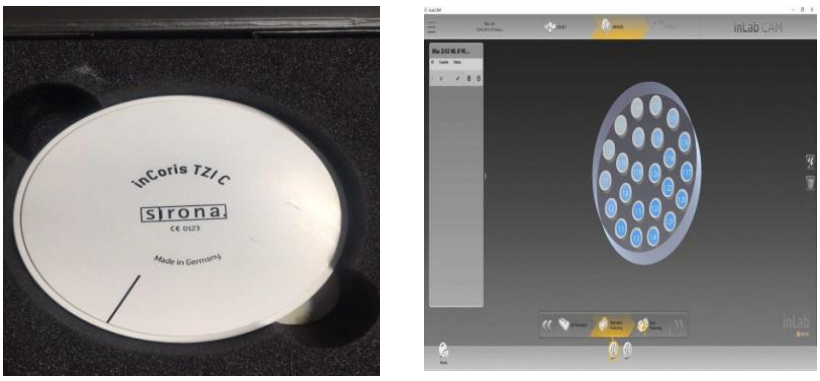
Zirconia blocks were used in samples preparation for this study. Table (2.1) shows the percentage constituent of each compound incorporated in the block.

Table (2.1), the chemical composition of zirconia implant

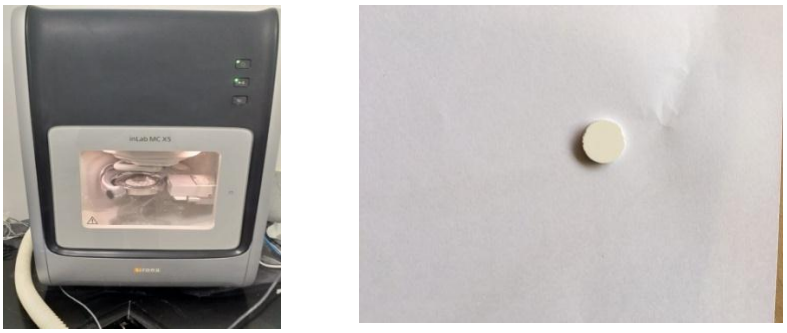
Component	inCoris TZI
$ZrO_2+HfO_2+Y_2O_3$	≥ 99.0%
Y_2O_3	> 4.5 - ≤ 6.0%
HfO_2	≤ 5%
Al_2O_3	≤ 0.5%
Other oxides	≤ 0.5%

The block was sectioned by CAD/CAM technique into discs of (7,2)mm diameter and thickness respectively as shown in figure(2.1). The selection of these dimensions was based on suitability with those of micro well plates. These pre sintered discs were polished by using diamond paste and grinding machine (figure2.2) and by using maximum system speed to get standardized smooth surface for all samples. Then, they were cleaned using ultrasonic cleaner.

All polished samples were sintered in a sintering furnace according to manufacturer instructions to be ready for using in next step of this study. The temperature was elevated from (25to750)Cº with average speed of heating (99Cº/minute). Then the temperature raised to (1510)Cº with (50Cº/minute) heating rate, then the samples were hold at this temperature for (30)minutes. Later, samples retained at (800)Cº for (5)minutes. The temperature reduced with (15Cº/minute) cooling rate.(figure 2.3).



A B



C D

Figure (2.1) steps of sample preparation. (A) zirconia block. (B) computer designing.(C) computer aid manufacturing. (D) obtained disc.

A B

Figure (2.2) polishing of working samples.(A) diamond paste and lubricant.(B) grinding and polishing machine.

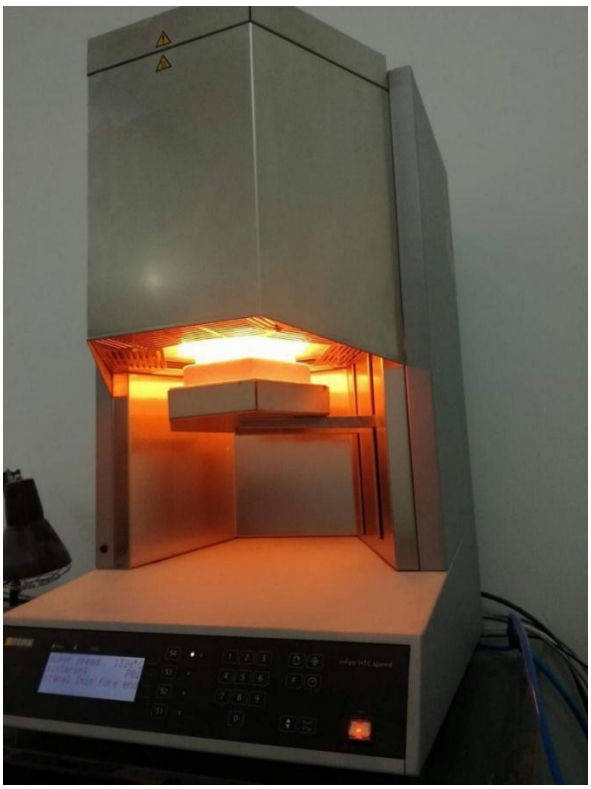


Figure (2.3), sintering furnace

2.2. Absorption spectrum assessment

Sintered zirconia sample used for this test in (UV., visible) region to determine the absorptivity of zirconia to Nd:YAG laser of (1064 and 532)nm.

2.3. Pilot study

The aim of pilot study is to determine the most appropriate laser parameters which can enhance surface roughness of zirconia without thermal damage like, cracks. Q-switched Nd:YAG laser system was used to enhance surface roughness of the prepared samples in this study Figure (2.4).

A B

Figure (2.4), (A) Q-switched Nd: YAG laser system (B), sample handling.

2.3.1. Nd: YAG laser of 1064nm wavelength

Since zirconia ceramics characterized by hardness and high melting temperature, the pilot study was started with maximum allowable energy density for this system which is 38.22 J/cm². Table (2.2) shows the parameters which were used in this step.

Table (2.2), Set parameters used in pilot study

Energy density(J/cm²)	Hand piece grade	Frequency(Hz)	Exposure time(sec)
38.22	15	1	1
38.22	12	1	1
38.22	10	1	1
38.22	7	1	1
38.22	5	1	1

No effect on irradiated surfaces was noted under optical microscope.

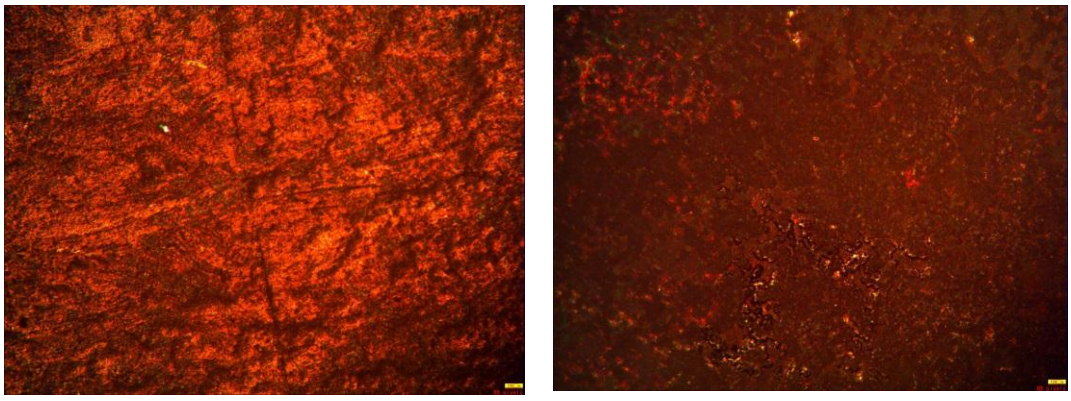
The trial was repeated by using same previous parameters except frequency (5 Hz) then (10 Hz) instead of (1 Hz). Also no effect was detected under optical microscope.

Then the exposure time was prolonged to (2 sec) with same previous parameters. No observable effect was detected under optical microscope.

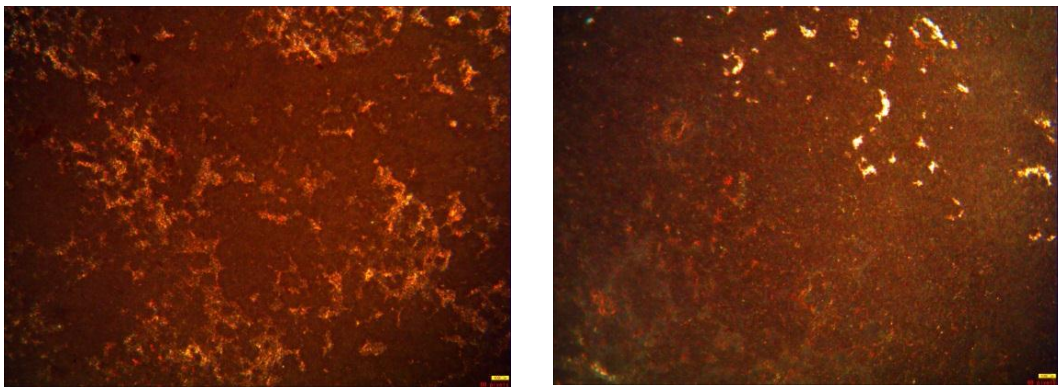
Another trial was done with (3 sec) exposure time. Surface changes appeared but on (10, 7, 5) handpeice grades. Grade (10) was selected to complete the experiment to facilitate larger coverage area for surface treatment. Three samples were selected randomly and exposed to Nd: YAG laser (1064)nm of following parameters (table 2.3). Figure (2.5) shows zirconia surface changes.

Table 2.3 selected parameters for SEM and AFM

Sample number	**Energy density(J/cm²)**	**Frequency (Hz)**	**Exposure time(sec)**	**Hand piece grade**
1	38.22	10	3	10
2	38.22	10	5	10
3	38.22	10	7	10



A B



C D

Figure (2.5) optical microscope images of zirconia surface with different exposure times of (1064) nm irradiation. (A), control (B), 3sec(C), 5sec (D), 7sec.

2.3.2. The second harmonic generation of wavelength 532 nm

Sintered zirconia samples of dimensions (7,2) mm diameter and thickness respectively were used in this step of present study. The used laser system in this study has the same parameters of (1064 nm) including frequency and pulse duration except maximum power density which is (19.22) J\cm².

Four samples were selected randomly and irradiated by different parameters. Details have shown table (2.4) ….

Table (2.4), parameters used in first trial for pilot study of (532)nm Nd:YAG laser

Sample no.	**Hand piece grade**	**Energy density J\cm²**	**Frequency Hz**	**Exposure time sec.**
1	10	19.11	10	1
2	7	19.11	10	1
3	5	19.11	10	1
4	3	19.11	10	1

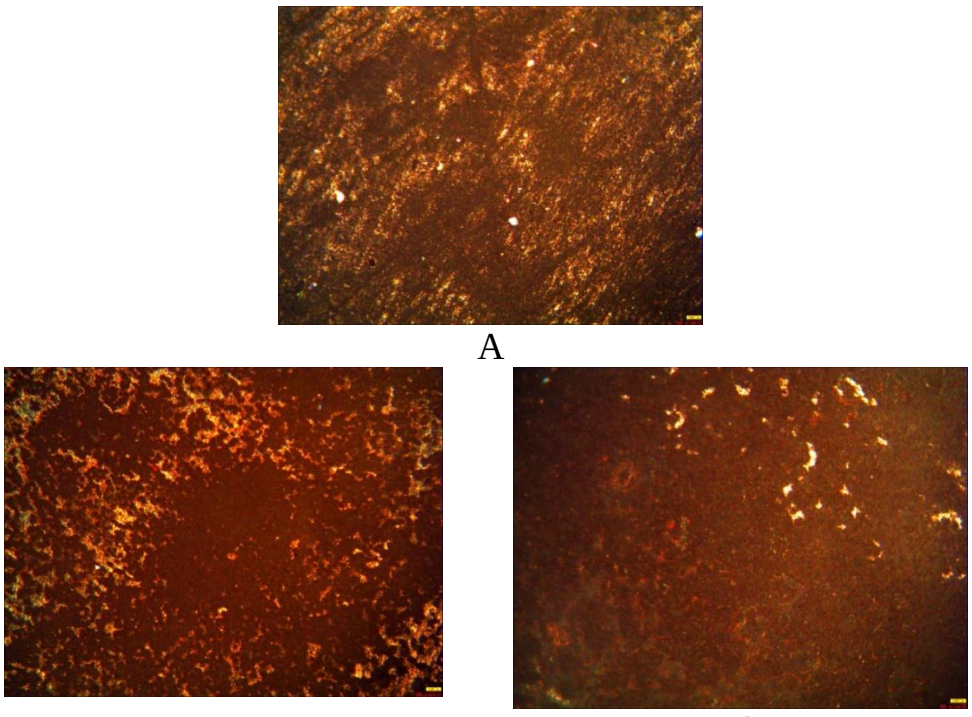
No surface changes were observed under optical microscope using such parameters.

Another trial was done by using the same parameters except exposure time, which was prolonged to (2 sec). The same result of first trial obtained, also no observable surface changes under optical microscope. The third trial was made by using the same parameters with (3 sec) exposure time. Surface changes were observed under optical microscope in the sample no. 3, which was irradiated by using hand piece grade (5). Figure (2.6) shows zirconia surface changes.

The following step was irradiation of another three samples with same parameters except the exposure time, as explained in table (2.5)

Table (2.5), selected parameters of Nd: YAG laser of (532)nm for samples irradiation for AFM and SEM assessment

Sample no.	**Hand piece grade**	**Energy density(J\cm²)**	**Frequency (Hz)**	**Exposure time(sec.)**
1	5	19.11	10	3
2	5	19.11	10	5
3	5	19.11	10	7



A B C

Figure (2.6) optical microscope images of zirconia surface irradiated by 532 nm Nd:YAG laser with different exposure times (A) 3sc. (B)5sec. (C) 7sec.

2.4. Surface roughness

The surface roughness of all irradiated samples were assessed by AFM. For the samples which irradiated by Nd:YAG laser 1064nm, the surface roughness value increased with prolonged exposure time. As in table (2.6), and figure (2.7).

Table (2.6), shows the surface roughness values of zirconia samples irradiated by Nd:YAG laser 1064nm at different exposure times.

Sample no.	Exposure time (sec)	Surface roughness(nm)
1	Control	98.56
2	3	401.35
3	5	447.26
4	7	497.5

For the samples which were irradiated by second harmonic Nd-YAG laser 532nm, surface roughness increased with prolonged exposure time. Table (2.7) explains that, and figure (2.8).

Table (2.7), surface roughness values of irradiated zirconia by second harmonic generation Nd-YAG laser 532nm

Sample no.	Exposure time(sec)	Surface roughness(nm)
1	3	190.17
2	5	214.67
3	7	444.63

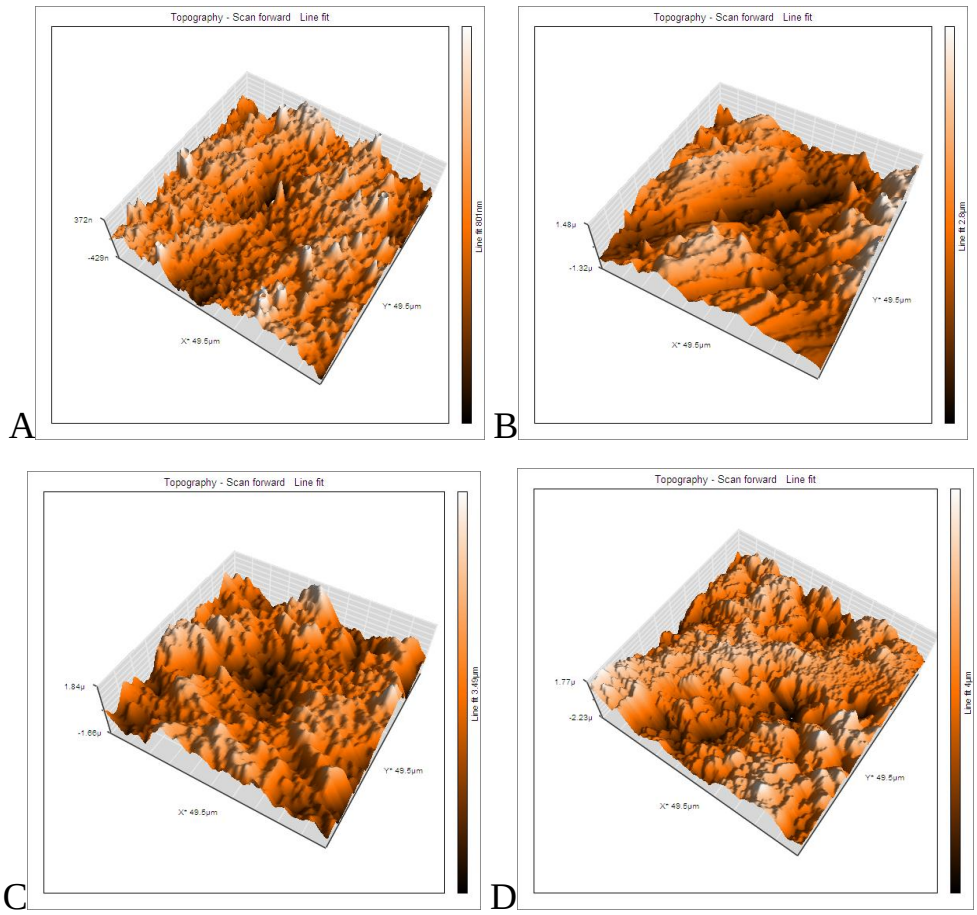
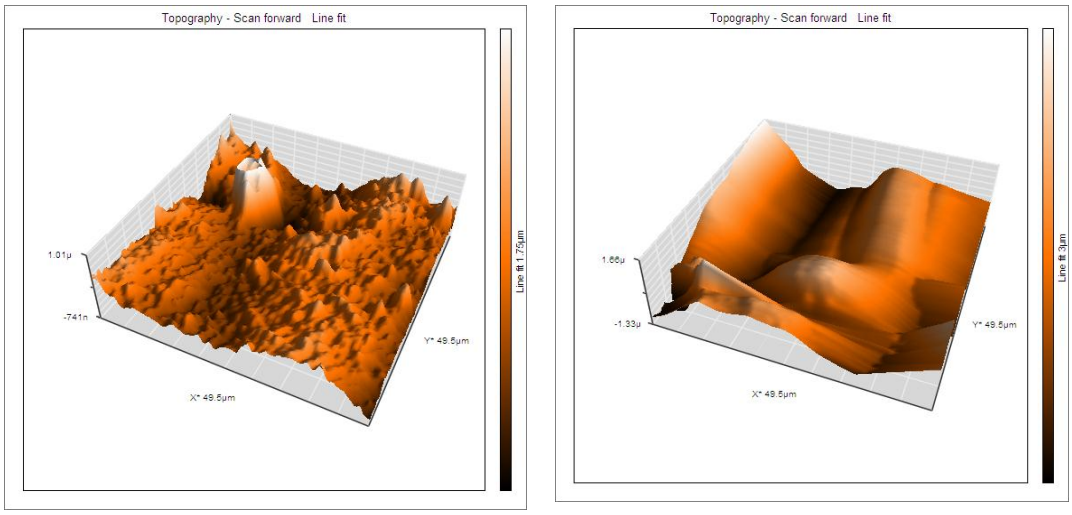
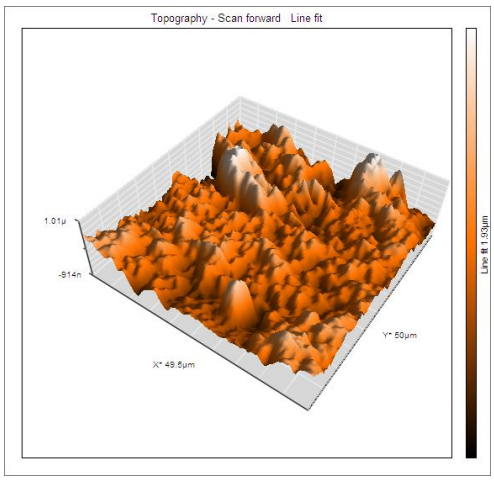


Figure (2.7), 3D images of AFM of samples irradiated by Nd-YAG laser 1064nm for different exposure time (A).control (B). 3 sec (C). 5 sec(D). 7 sec.



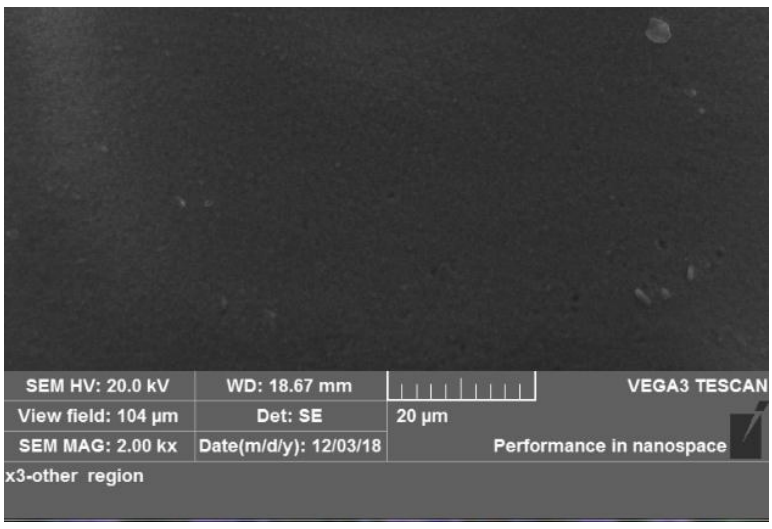


C

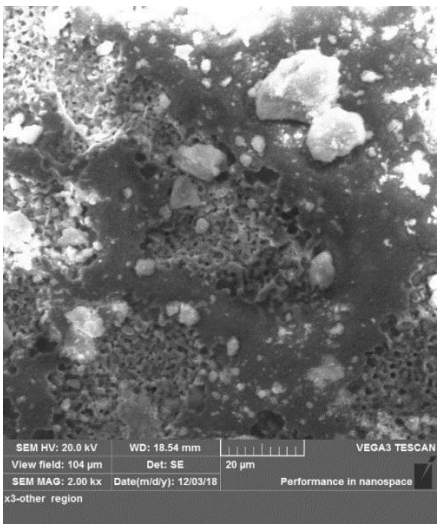
Figure (2.8), 3D images of AFM. of samples irradiated by Nd-YAG laser (532)nm for different exposure time (A). 3 sec (B). 5 sec (C). 7 sec.

2.5. Surface topography assessment

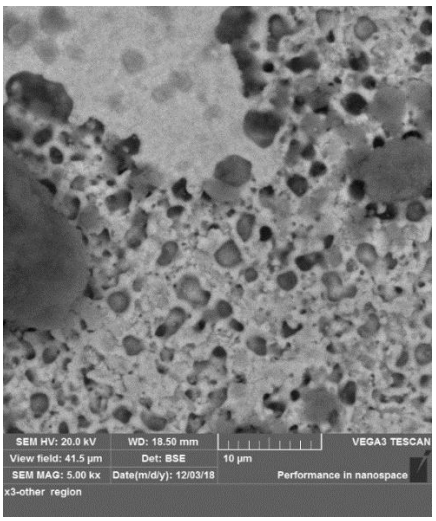
The samples which were irradiated by three different exposure time of Nd:YAG laser of 1064 nm wave length were assessed by SEM for surface topographic changes determination. The same assessment was applied for the group which was irradiated by second harmonic Nd:YAG laser 532 nm and different exposure times. Figure (2.9) and (2.10).



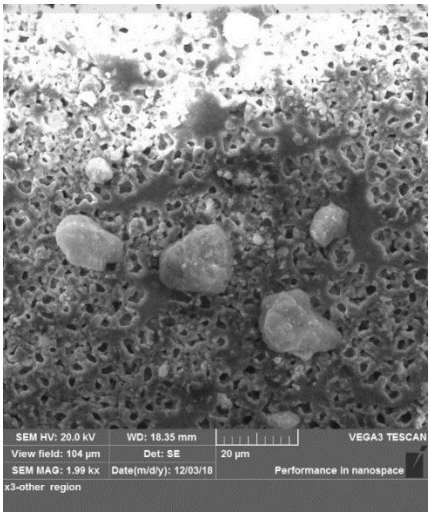
A



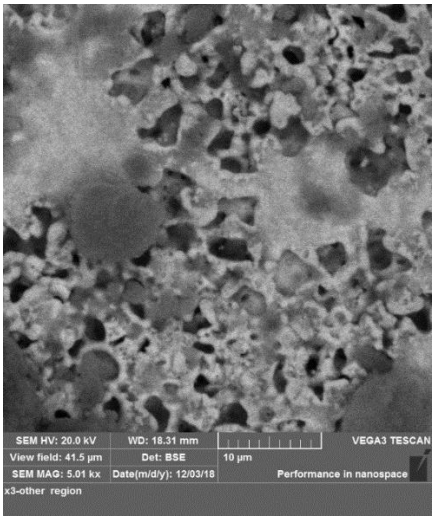
B



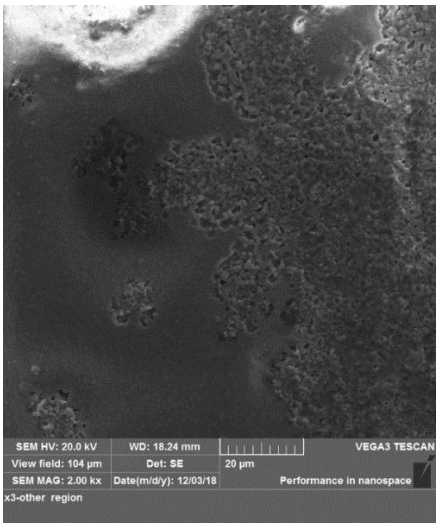
C



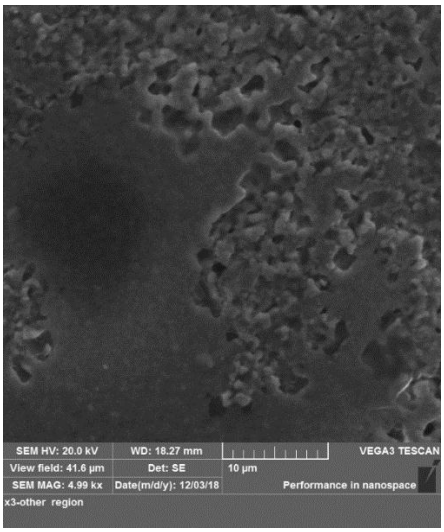
D



E



F



G

Figure (2.9), SEM images for samples irradiated by Nd:YAG (1064)nm at different exposure times in 2000 and 5000 magnification. A control (B and C) 3sec. (D and E) 5sec. (F and G) 7sec.

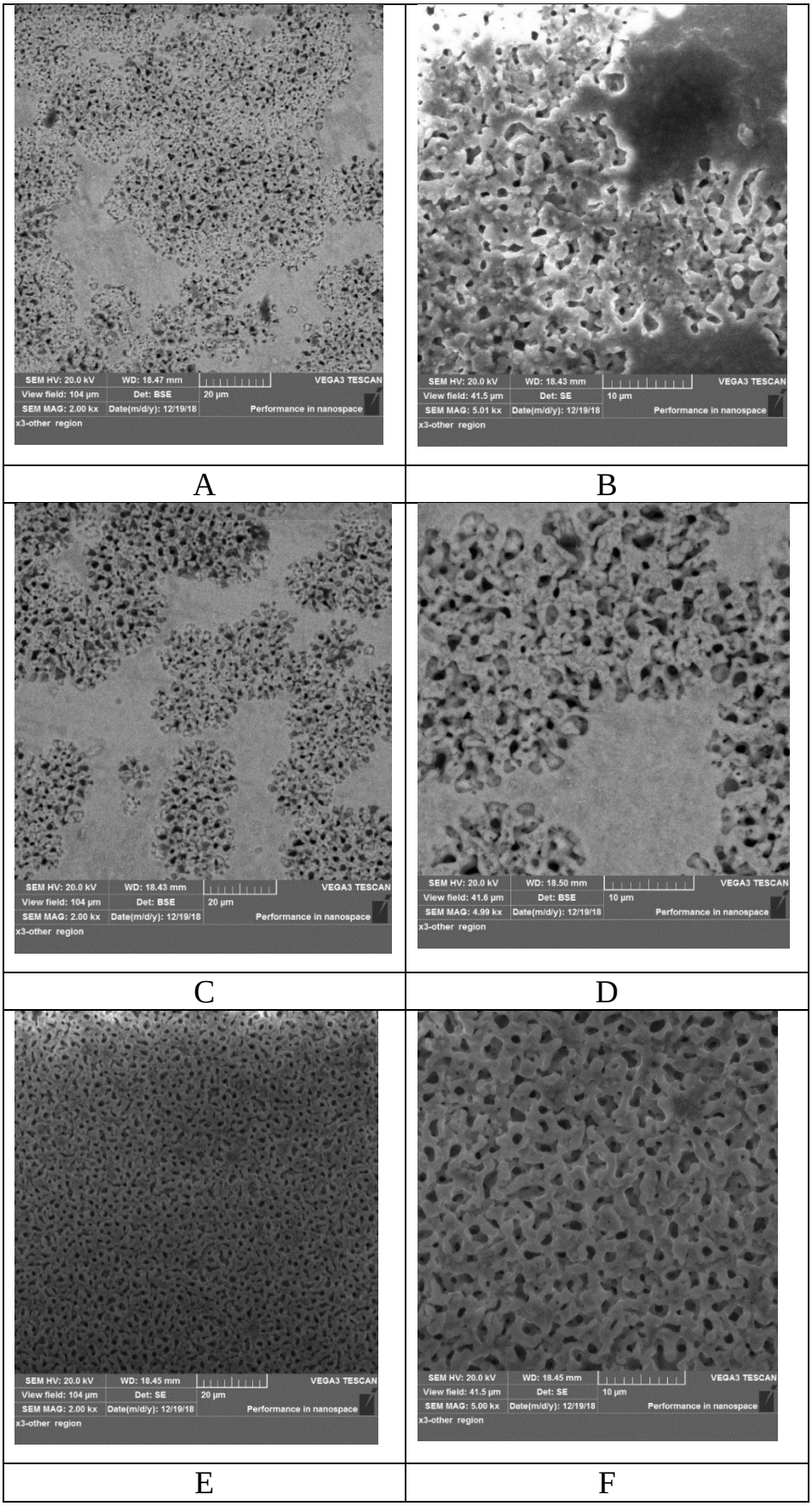


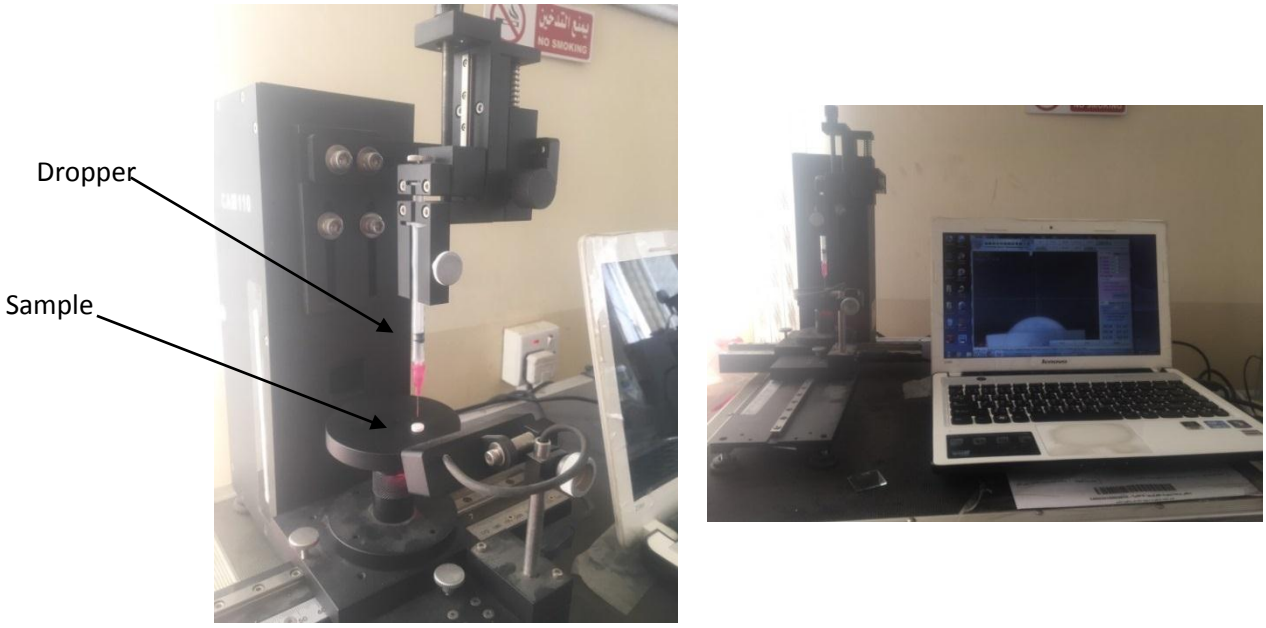
Figure (2.10), SEM images for samples irradiated by (532)nm Nd: YAG laser with different exposure times(A and B)3 sec. (C and D)5 sec (E and F) 7sec.

2.6. Cross sectional SEM assessment

Samples after irradiation by Nd:YAG laser of (1064 and 532)nm at parameters of Energy Density (38.22 and 19.11) J\cm² respectively and frequency (10)Hz for both lasers and (3,5,7)sec exposure time for both wavelengths, prepared for cross section. imaging. The samples were fixed in a mold made from cold cure acrylic. Then, after perfect material setting, the molds were sectioned by diamond disc. The samples then released from surrounding mold ruminant to be ready for cross cestion SEM assessment.

2.7. Contact Angle measurement

The sample was placed on a removable tray, drop of water was released on. The water filled the syringe which was connected to the machine in vertical syringe holder. The syringe was released carefully to drop the water on the sample. The drop volume was measured using micropipette to be 5 µl. The contact angle was measured from both sides and the average contact angle was obtained..(Figure 2.11) shows the system.



A B

Figure (2.11), contact angle measurement system (A), sample position. (B), software measurement system.

2.8. In vitro study

2.8.1. Preparation of culture media

Dulbecco`s Minimal Essential Minerals media (D-MEM) (readymade) was used in this work. Complete media was prepared by adding 1% antibiotic, 0.5 % antifungal and 10% fetal calf serum (figure 2.12)[120].

Figure (2.12) used materials, D-MEM media, Phosphate Buffer solution, penicillin, fetal calf serum, Trypsine.

2.8.2. Sterilization

The used tools and flasks and plates were sterilized by (UV) light before using. Zirconia discs were sterilized using Auto clave, on 121ºC, 15 psi pressure and 15 minutes time.

2.8.3. Preparation of collagenase type ll solution

(100) mg of powder collagenase was mixed in a test tube with (49) ml of HBSS and (1) ml distilled water. Preparation of collagenase solution was done immediately before using [121].

2.8.4. Isolation of osteoblast cells

New born rat of (2-3) days age was used in this procedure. The animal was slaughtered after sedation with chloroform. Skin and soft tissue were removed gently from clavarial bones. The obtained bone was

washed three times by phosphate buffer solution to be ready for enzymatic digestion procedure.

The calvarias bone was sectioned into three pieces to facilitate placement in the test tube. 4ml of trypsin were added to these bony pieces and incubated for 10 minutes in an incubator in 37ºC with 5% CO2 supply.

Later on, the trypsin solution was washed by adding 4ml of D-MEM media and centrifuged at 1500g for 10 minutes. The supernatant liquid was discarded and 5ml of collagenase ll solution were added to the test tube. The test tube was incubated for 10 minutes at 37ºC and 5% CO^2 supply. Then centrifuging at 1500g for 10 minutes was done.

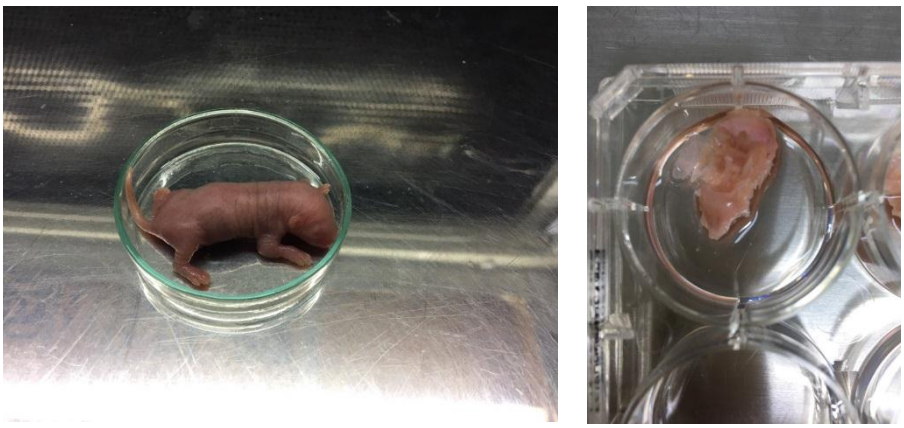
The supernatant was discarded and further fresh collagenase type ll solution was added and incubated for 60 minutes in 37ºC with 5% CO2 supply.

The content was filtered by using filter allows passing cells and prevents larger bony remnants. The collected solution was washed by adding 5ml of D-MEM media then centrifuged at 1500 g for 10 minutes.

The supernatant was removed and remaining cell were incubated into $50cm^3$ flasks in 37ºC with 5% CO2 supply.

Small cellular population were stained by Trypan blue stain and examined under optical microscope to ensure vitality of isolated cells, darker blue stained cells represent the vital cells.

The flasks were under observation and the media was changed every (2-3) days.[120]. Figure (2.13) and (2.14) and (2.15) show these steps.



A B

Figure (2.13), (A) the new born rat (B), obtained calvarias bone

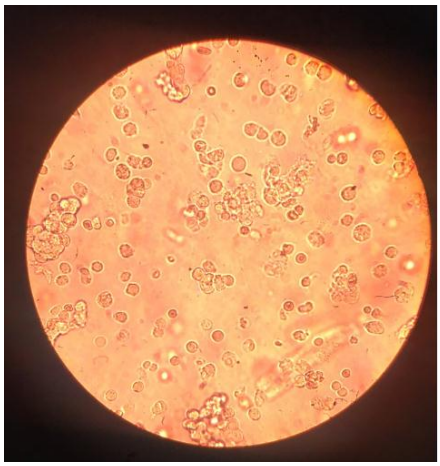
A

B C

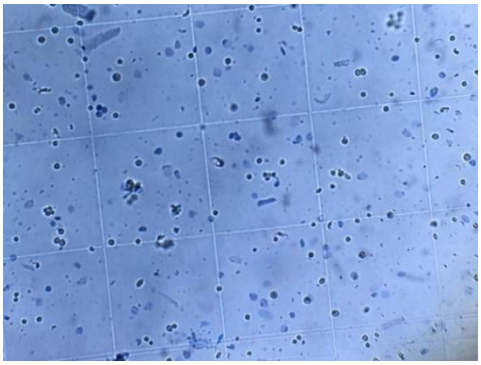
D

E

Figure (2.14), steps of osteoblast isolation. (A) Freshly prepared collagenase solution. (B)the same solution after 60 minutes incubation of bone. (C) Filtering (D), centrifuging. (E), cellular precipitation.

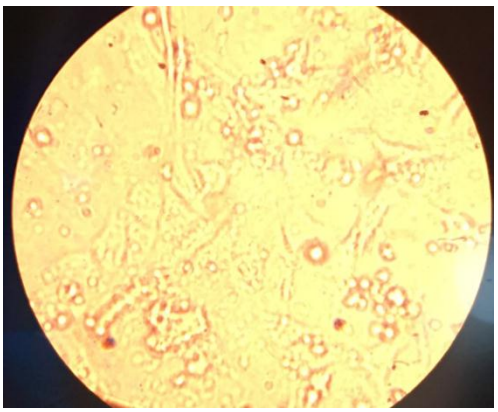


A

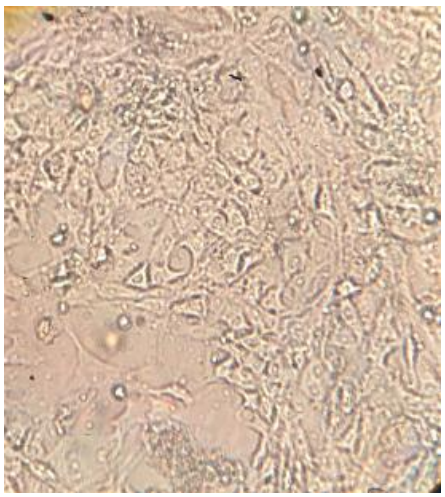


B

C



D



E

Figure (2.15). osteoblast cells, (A), immediately after enzymatic digestion. (B), with trypan blue stain. (C), flasks insde incubator. (D), cells after two days incubation. (E) cells after one week incubation.

2.8.5.Cell culture

The sterilized zirconia discs were placed in the 24 wells microtiter plates, one plate for each group. The media of each flask was collected and removed, each flask was washed with the PBS three times. Two ml of trypsine were added to each flask followed by (10) minutes incubation in 37ºC and 5% CO2 supply.

The trypsine solution then washed with 10ml complete media solution and centrifuged for (10) min. on 1500g.

Cells free solution was discraed, and 1ml of media was added for cell counting by heamocytometer. On each disc (10^3) were seeded then 1ml of complete media was added, the plates were covered and incubated in 37ºC with 5% CO2 supply.[122]. Figure (2.16) shows the discs embeded in the culturing media.

2.8.6.Attachment assay

After 24 hours incubation, the number of attached cells was counted. The followed method was trypsination method. In which, each disc was removed form well and washed three times with PBS in a graduated test tube separatly. Then (1)ml of trpsine was added to each test tube, the test tubes were incubated for (10) minutes in 37ºC with 5%CO2. Later on, 2ml of media were added to stop trpsin activity. The solution centrifuged for (5) minutes in 1500g, cells free liquid discraded and precepetated cells were counted by using heamocytometer[37].

2.8.7.Prolifration assay

The rate of cellular prolifration was assessed during three different incubation periods, (3, 6, 9) days of incubation period. The same trypsination method was followed for cellular releasing and counting from each disc[37].

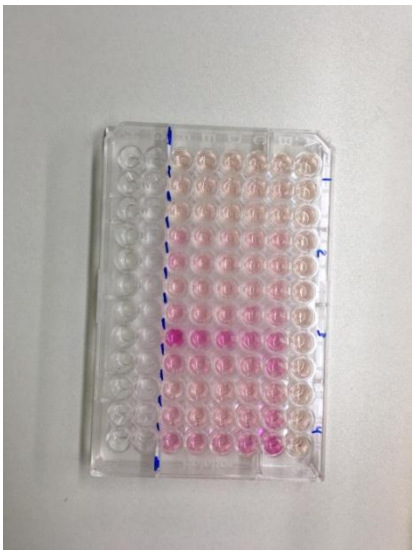
A B

Figure (2.16), (A) seeding of culturd cells on discs. (B) Heamocytometer.

2.2.8.Alkaline Phosphatase (ALP) level measurement

ALP secretion was measured after four different intervals including, 24 hours, 3, 6 and 9 days incubation. Measurement was achieved by using ALP kit and spectrometric plate reader system. According to manufacturer instructions, each (10)ml of working reagent mixed with (20)µl of working media. The plate placed inside the plate reader system and two readings obtained for calculation, the first one after one minute and the second is three minutes later, the difference between two values was calculated and inserted in special formula for calculation the real value of ALP. Figure(2.17)

A



C

B

Figure (2.17), ALP assessment, (A) plate reader system. (B), ALP kit. (C), plate contains mixture of media and ALP reagent ready for assessment.

2.9 Statistical Analysis

Analyzing and assessing of the result were done using “Statistical Package for the Social Sciences (SPSS/21/9i)” methods which includes:-

I) Descriptive statistics:

1. Arithmetic mean.
2. Standard deviation “SD”.
3. Statistical tables.

II) Inferential statistics:

1. (Analysis of variance) ANOVA test was carried out to exam the significant difference between the means of groups.

2. LSD-test to detect the significantydifferences between every two groups.

Where, Probability value (P) was determined as:

A. Significant at $P \leq 0.05$.

B. Non-significant at $P > 0.05$.

C. Highly significant at $P \leq 0.01$.

Introduction

In this chapter, different results will be displayed and compared with the results of previous studies.

This chapter includes, the result of zirconia absorption spectrum, cross sectional SEM and contact angle measurement.

The statistical results of the biologic work will be displayed and discussed in this chapter.

Results

3.1. Absorption spectrum

The zirconia absorptivity spectrum in (U.V., visible) spectral region revealed that, the Nd: YAG laser of (532 and 1064)nm wavelengths can be absorbed by zirconia. As shown in figure (3.1)

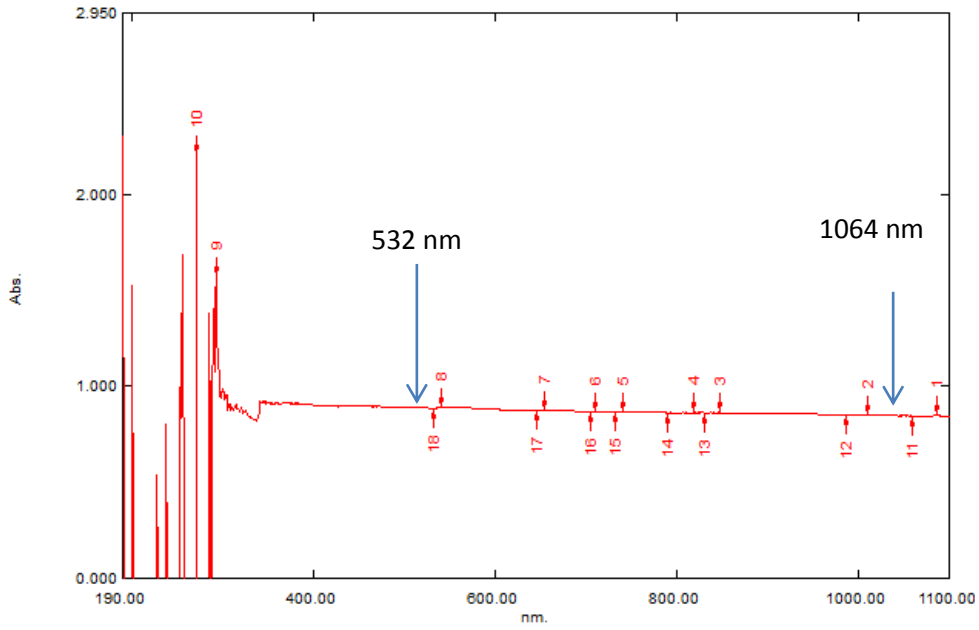
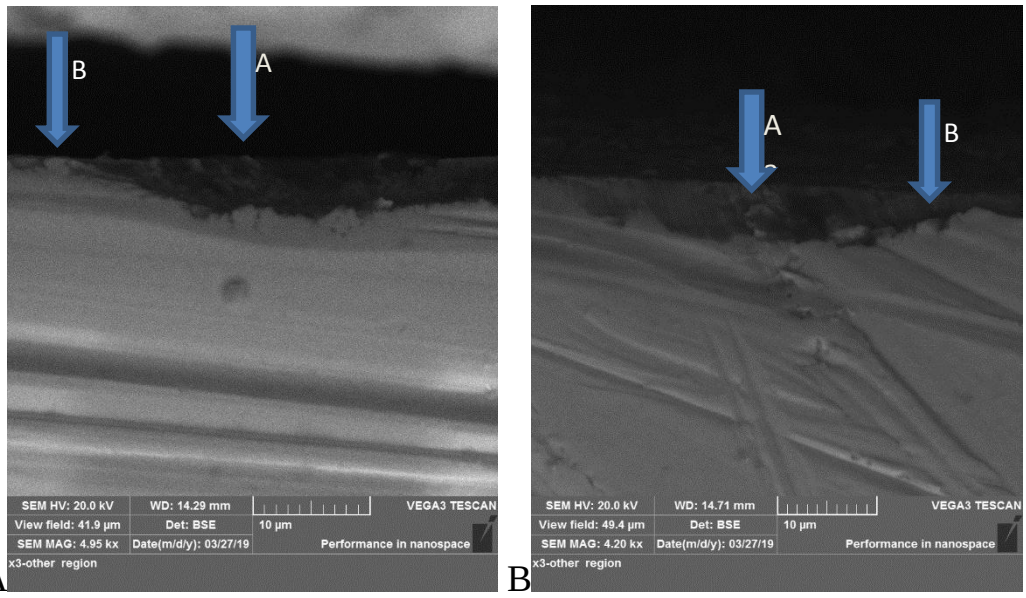


Figure (3.1), Absorption spectrum of zirconia in (U.V, visible region).

3.2. Cross section SEM

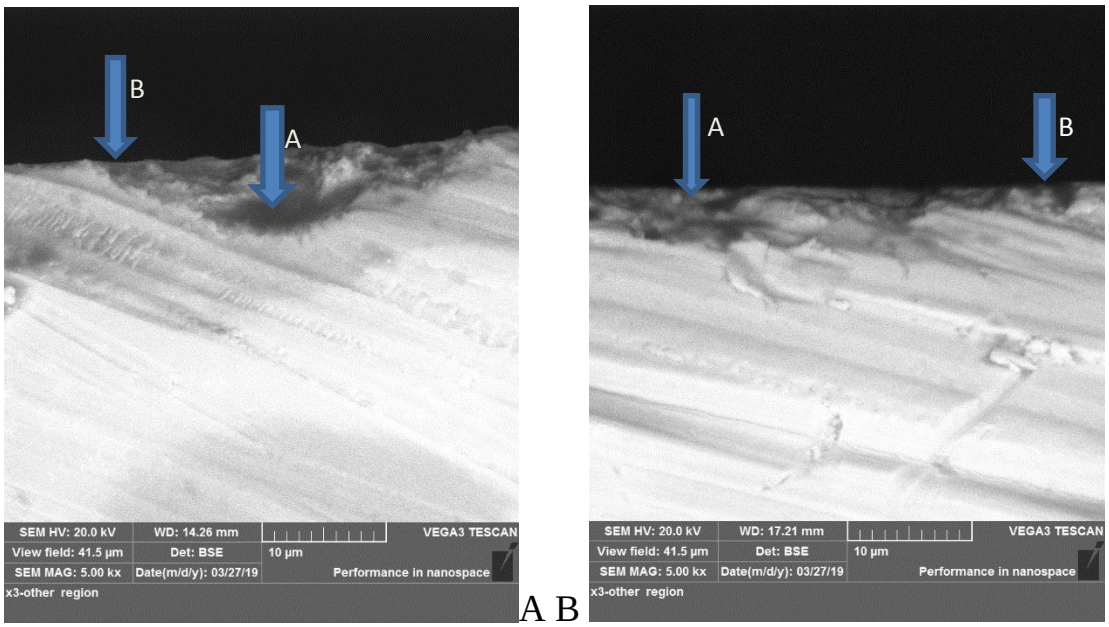
Images of cross sectional SEM explained that, unequal energy distribution on irradiated zone during each irradiation period. The maximum depth is located in the center of each irradiation zone, and

gradually decreased toward peripheries. Figure (3.2) and (3.3) show that.



A B

Figure (3.2), Cross sectional SEM for zirconia samples irradiated by Nd:YAG laser(532)nm (A).5 sec. (B).3 sec, Arrow (A) represents deep centeral zone, Arrow (B) represente perefries.



A B

Figure(3.3), Cross sectional SEM images for samples irradiated by Nd:YAG laser (1064) nm. (A).5 sec.(B) 3 sec., Arrow (A) represents deep centeral zone, Arrow (B) represente perefries

3.3. Cellular attachment after 24 hours

Mean, SD and P-value of ANOVA and LSD tests used to compare between groups. ANOVA test revealed a high significant difference in the number of attached cells after 24 hours,P-value <(0.05), table(3.1).

Table (3.1), ANOVA test for the mean number of attached cells after 24 hours between study groups

Study group	**Mean number of attached cells after 24 hrs. Mean ± SD**	**p- value**
Control	325.0 ± 192.0	0.01
532 nm	600.0 ± 158.1	
1064 nm	700.0 ± 141.4	

Further LSD test was done to assess the difference between every two groups, the results indicate a significant difference in the number of attached cells on the experimental group irradiated by Nd:YAG laser of (532)nm wavelength in comparison with the control group, non-significant difference between two experimental groups. Table (3.2) shows details.

Table (3.2): (LSD) test to confirm the differences between study groups in mean number of attached cells after 24 hours.

Control Mean ± SD	**532nm Mean ± SD**	**1064nm Mean ± SD**	**p- value**
325.0 ± 192.0	600.0 ± 158.1		0.022
325.0 ± 192.0		700.0 ± 141.4	0.004
	600.0 ± 158.1	700.0 ± 141.4	0.357

3.4.Proliferation rate after three days

A high significant increase in the proliferated cells after three days when the three groups were tested using ANOVA. Table (3.3) shows detailes.

Table (3.3): Comparison between study groups in mean proliferation number after three days.(ANOVA test)

Study group	**Mean proliferation rate after 3 Days. Mean ± SD**	**p- value**
Control	220.0 ± 130.38	0.001
532nm	500.0 ± 70.71	
1064nm	720.0 ± 83.66	

Using of LSD test was done to assist the difference between every two groups, the results indicate a high significant difference in the number of proliferated cells on the experimental groups irradiated by Nd:YAG laser of (532 and 1064)nm wavelength in comparison with the control group and a high significant difference between two experimental groups. Table (3.4) shows details.

Table (3.4): LSD test to confirm the differences between study groups in mean proliferation rate after three days

Control Mean ± SD	532 Mean ± SD	1064 Mean ± SD	p- value
220.0 ± 130.38	500.0 ± 70.71		0.001
220.0 ± 130.38		720.0 ± 83.66	0.001
	500.0 ± 70.71	720.0 ± 83.66	0.004

3.5. Proliferation rate after six days

ANOVA Statistical test showed a significant difference between the mean values of proliferated cells counted for the three study groups. As shown in table (3.5)

Table (3.5): Comparison between study groups in mean proliferation rate after six days.

Study group	**Mean proliferation rate after six days. Mean ± SD**	**p- value**
Control	780±83.66	0.02
532nm	960±54.77	
1064nm	960.0 ± 151.65	

LSD test was used to show the difference between every two groups, the results indicate a high significant difference in the number of proliferated cells between both irradiated groups and control group, while no significant difference between two experimental groups. Table (3.6) shows detailes.

Table (3.6), LSD test between three groups after six days incubation

Control Mean ± SD	532nm Mean ± SD	1064 nm Mean ± SD	P-value
780±83.66	960±54.77		0.019
780±83.66		960.0 ± 151.65	0.019
	960±54.77	960.0 ± 151.65	1

3.6. Proliferation rate after nine days

A significant difference in the number of proliferated cells between two irradiated groups and control one after nine days when ANOVA test was used. Table (3.7) shows results.

Table (3.7): Comparison between study groups in mean proliferation rate after nine days.

Study group	Mean proliferation rate after nine days. Mean ± SD	p- value
Control	840.0 ± 89.44	0.032
532nm	900.0 ± 223.6	
1064nm	1120.0 ± 109.54	

LSD test was done for this period, the results indicate a high significant difference in the number of proliferated cells between the experimental group irradiated by (1064)nm Nd:YAG laser and control group. Also, a significant difference was seen between two irradiated groups. No significant difference resulted between the group irradiated by (532)nm Nd:YAG laser and control group. Detailes showed in next table.

Table (3.8): (LSD) test to confirm the differences between study groups in mean proliferation rate after nine days

Control Mean ± SD	532 Mean ± SD	1064 Mean ± SD	p-value
840.0 ± 89.44	900.0 ± 223.6		0.546
840.0 ± 89.44		1120.0±109.54	0.013
	900.0 ± 223.6	1120.0±109.54	0.042

3.7.Alkaline phosphatase level assessment

Assessment of ALP level during the same intervals of cells counting revealed, there is a significant increase in ALP level after 24 hours incubation period. Table (3.9), (3.10), (3.11), (3.12) and (3.13) show mean and SD and p- value for three experimental groups.

Table (3.9): Comparison between study groups in mean of alkaline phosphatase after 24 hours.

Study group	**Mean alkaline phosphatase after 24 hrs. Mean ± SD**	**p-value**
Control	13±7.78	0.04
532nm	25.34±6.9	
1064nm	17.28±5.67	

LSD test was done, the results indicate a high significant difference in ALP level between the experimental group of (532)nm and control group, also significant difference between the group irradiated by (1064)nm Nd:YAG laser on control group. While, no significant difference between two irradiated groups. Table (3.10) shows details.

Table (3.10), LSD test for ALP level after 24 hours incubation.

Control Mean ± SD	**532nm Mean ± SD**	**1064nm Mean ± SD**	**P- value**
13±7.78	25.34±6.9		0.015
13±7.78		17.28±5.67	0.35
	25.34±6.9	17.28±5.67	0.08

Table (3.11): Comparison between study groups in the mean value of alkaline phosphatase after three days incubation period.

Study group	**Mean alkaline phosphatase after three days. Mean ± SD**	**p- value**
Control	12.79 ± 11.4	0.702
532nm	13.15 ± 11.5	
1064nm	18.57 ± 13.0	

Table (3.12): Comparison between study groups in mean of alkaline phosphatase after six days.

Study group	**Mean alkaline phosphatase after six days. Mean ± SD**	**p-value**
Control	14.18 ± 12.0	0.936
532nm	14.95 ± 9.6	
1064nm	16.49 ± 8.8	

Table (3.13): Comparison between study groups in mean of alkaline phosphatase after nine days.

Study group	**Mean alkaline phosphatase after nine days. Mean ± SD**	**P value**
Control	18.47 ± 8.7	0.9
532nm	21.35 ± 13.3	
1064nm	20.85 ± 9.1	

3.8.Contact angle measurement

The results of ANOVA test revealed a high significant increase in contact angle mean value, as in table (3.14). Figure (3.4).

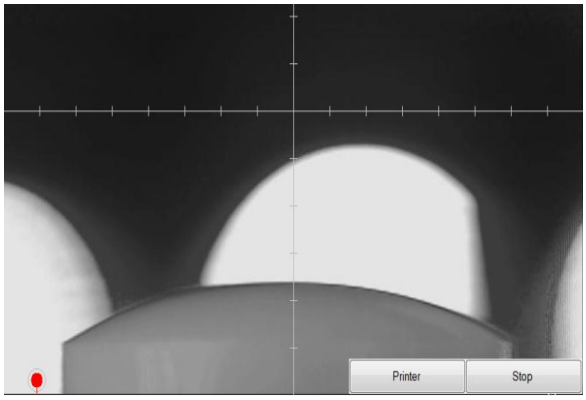
Table (3.14): Comparison between study groups in mean of contact angle, ANOVA test

Study group	Mean of contact angle Mean ± SD	p- value
Control	16.17 ± 0.54	0.003
532nm	45.78 ± 8.9	
1064nm	37.4 ± 17.03	

LSD test was done and the results revealed a high significant difference in contact angle measurement of the control wit both irradiated groups and no significant difference between two experimental groups. Detailes in table(3.15).

Table (3.15): (LSD) to confirm the differences between study groups in mean of contact angle.

Control Mean ± SD	532 Mean ± SD	1064 Mean ± SD	P value
16.17 ± 0.54	45.78 ± 8.9		0.001
16.17 ± 0.54		37.4 ± 17.03	0.011
	45.78 ± 8.9	37.4 ± 17.03	0.256



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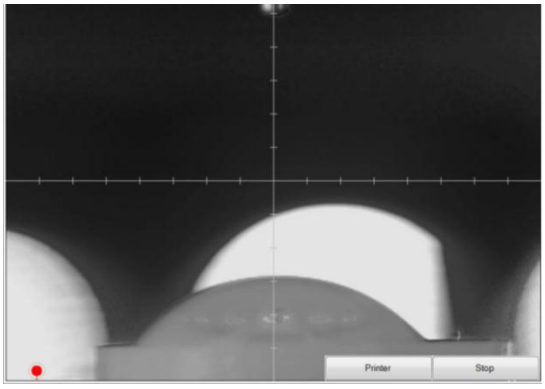

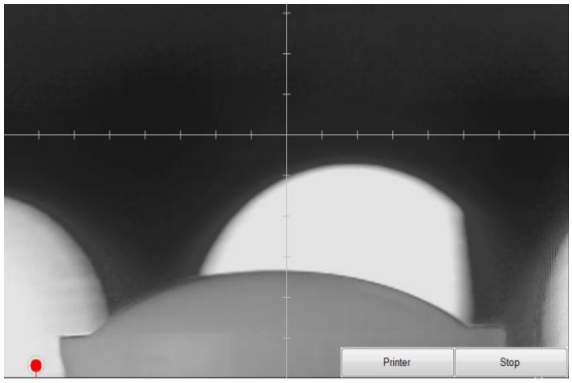

B C

Figure (3.4), Contact angle measurement.(A), control.(B), 532nm. (C), 1064nm.

3.9.Effect of time on present vitro study

The effect of different incubation periods in this study was assessed statistically for control and other two experimental groups separately.

ANOVA and LSD tests were used to determine the statistical difference in the cellular proliferation rate during different period for each group. For control group, ANOVA test revealed a high significant difference in the proliferation rate of control group during different three intervals. Table (3.16) explains details.

Table (3.16), show the result of ANOVA test between the mean value of cellular proliferation of control group after three incubation period

Incubation period	Mean and SD	p-value
3 days	200±130.3	0.0001
6 days	780±83.66	
9 days	840±89.44	

Further LSD test was done, the results indicate a high significant difference in the proliferation rate after six days incubation period compared with three days one. Also, high significant difference obtained after nine days incubation period. While, no significant difference between six and nine days incubation. More detailes in table (3.17).

Table (3.17), LSD test between the mean value of cellular proliferation of control group after three incubation period

Mean and SD of 3 days	Mean and SD Of 6 days	Mean and SD Of 9 days	p- value
220±130.3	780±83.66		0.0001
220±130.3		840±89.44	0.0001
	780±83.66	840±89.44	0.37

For the experimental group that was irradiated by Nd-YAG laser of (532)nm wavelength, ANOVA test showed a high significant difference in the proliferation rate during three different incubation period. Table (3.18) shows detailes

Table (3.18) the result of ANOVA test between the mean value of cellular proliferation of (532)nm group after three incubation period

Incubation period	Mean and SD	P- value
3 days	500±70.7	0.0001
6 days	960±54.7	
9 days	900±223.6	

LSD test was done and the results revealed that, there is a high significant difference in the cellular proliferation rate after(6 and9) days incubation period compared with three days incubation. Detailes in table (3.19).

Table (3.19) LSD test between the mean value of cellular proliferation of (532)nm group after three incubation period

Mean and SD of 3 days	Mean and SD Of 6 days	Mean and SD Of 9 days	P-value
500±70.7	960±54.7		0.0001
500±70.7		900±223.6	0.001
	960±54.7	900±223.6	0.5

ANOVA test was done for the group which irradiated by (1064)nm wavelength. The results indicate a high significant difference in the proliferation rate after three incubation period. Table (3.20) explains details.

Table (3.20) the result of ANOVA test between the mean value of cellular proliferation of (1064)nm group after three incubation period

Incubation period	Mean and SD	P- value
3 days	720±83.66	0.001
6 days	960±151.6	
9 days	1120±109.5	

Further LSD test was done, the results indicate a high significant difference in the proliferation rate after both (6 and 9) days incubation period compared with three days one. A significant difference obtained between (6 and 9) days incubation period. Table (3.21) shows detailes.

Table (3.21) LSD test between the mean value of cellular proliferation of (532)nm group after three incubation period

Mean and SD Of 3 days	Mean and SD Of 6 days	Mean and SD Of 9 days	P- value
720±83.66	960±151.6		0.008
720±83.66		1120±109.5	0.0001
	960±151.6	1120±109.5	0.05

Discussion

Laser material interaction is mainly depending on conversion of an optical energy to thermal energy following the absorption of an incident laser beam[123]. The generated thermal energy represents the emitted energy after a certain period of time after excitation process[124]. In this study, maximum obtainable Energy Density from the laser system was the starting energy selected in the pilot study for both wavelengths. Since, zirconia processing requires a high thermal energy to achieve surface roughness(vaporization). The irradiation time has an impact role

in material processing. Longer irradiation time related to further heat generation on substrate surface, so the frequency used was the maximum of the system then the exposure time was prolonged to obtain the required surface modifications.

3.10.Scanning Electron Microscope Images

In this study, the scanning electron microscope imaging revealed that, zirconia surfaces which were irradiated by both (1064 and 532)nm wavelength Nd:YAG laser had enhanced surface roughness without surface micro cracks. This results match with Abdulsattar[107]and Saygen et al, [119] and not match with Roiteroa E. et al and Soltaninjad et al,[117],[118].

During laser material interaction period, energy accumulation is focused onto a small area of substrate surface. This results in thermalization of the irradiated area to the limit which causes melting or vaporization of such zone. Elevation of surface temperature in such process is mainly governed by several parameters, some are related to laser beam characteristics including, wavelength, pulse duration, frequency and fluence. While, others are related to substrate properties including, thermal and optical properties[125].

Zirconia as a ceramic material differs from metallic in case of laser surface processing. Since, it requires higher excitation energy to achieve the electronic transition to higher energy levels from their stable valance band. For this reason, shorter pulses are recommended to be used in zirconia surface treatment by laser[126]. Therefor the laser irradiation system used in this study was of pulse duration range of Nano – second.

In case of pulsed laser processing, the pulse width has an impact role in determination of the final result of laser material interaction. Since, the pulse duration and the thermal affected zone are proportional. In other words, long pulses are related to heat dissipation deeply into core material resulting in more molten material than vaporized[127].

For ceramic material, it is preferable to use lasers of pulse durations within the range of nanosecond, picosecond, femtosecond instead of long pulses to obtain the required surface modification with less thermal damage[128,129], as explained in following equqtion[130]….

Peak power (W) = Laser pulse energy (J) / Pulse duration (s)

From this equation, higher peak power can be obtained by using lasers of short pulses. Also, the role of short pulses can be explained in the following equation….

$$l_{\mathrm{d}} = \sqrt{D\tau_{\mathrm{l}}}$$

Where, ld is the thickness of heat diffusion layer.

D is diffusion coefficient factor.

tl is pulse duration.

This equation indicates that, using of short pulses is related to less heat dissipation deeply into core material and more heat accumulation on the substrate surface.[110]

According to SEM imaging, the resulted surface roughness of irradiated surfaces indicate the process of material vaporization. Since, laser material processing using nanosecond pulsed laser is achieved by photo-thermal interaction including, melting and vaporization.[101]

As shown previously in SEM images of irradiated samples, there were multiple scattered zones of molten material. This finding represents that the local temperature didn't reach to the threshold of vaporization process[131]. The same findings also were documented in previous studies Daniel et al and Heiroth et al [132,133] in which zirconia also was treated with nanosecond laser.

The cross sectional SEM imaging of irradiated samples explains un equal energy distribution, in which the maximum energy located in the center of the irradiated area which had the highest depth compared with the depth at peripheries which was less.

SEM of group irradiated by Nd:YAG laser of (1064) nm wavelength show presence of scattered surface droplets which considered due to vapor condensation, this matched with Heiroth et al [133].

3.11.Atomic Force Microscope Assessment

AFM results reveal increase surface roughness with prolonged exposure time for both laser wavelengths. This can be explained by, case of vaporization process initiation at the surface of the material with continued laser irradiation, the liquid–vapor interface down movement inside the material whould result. This is assotiated with the removal of material from the irradiation zone above the liquid–vapor interface [96]. That`s why, the surface roughness increased with prolonged exposure time in both irradiated groups.

As mentioned previously, the surface roughness of the zirconia samples irradiated by Nd:YAG laser of a wavelength (532)nm increased inspite of lower energy density used compared with that of (1064)nm.

Absorption coefficient of the material plays an important role in determination of the depth of heat generation inside material [110].

$$l_s = \frac{1}{\alpha}$$

Where l_s is the thickness of the layer absorbed energy initially penetrates and α is the absorption coefficient[110].

This equation and absorption spectrum test of zirconia explained the interaction of Nd:YAG laser of (532)nm wavelength with zirconia and its role in modification of surface roughness. Since, absorption of incident laser energy and heat dissipation into core material are inversely proportioned (as shown in equation above). This results in heat accumulation in superficialy and reaching vaporization threshold.

3.12.Contact angle measurement

Implant surface properties including wettability have been received a special attention in dental implant studies[134]. Surface wettability has an impact role on the process of osseo integration. Since, it affects on the initial contact between the biological fluids and implant surface following the insertion procedure. This includes interaction with the water molecules and formation of protein layer ending with cellular interaction [135].

In the present study, water contact angle measurement for three experimental groups was done to determine the degree of surface wettability for machined zirconia implant, moreover the effect of laser irradiation on this surface property.

ANOVA test results revealed that, there is a high significant difference (increase) in the mean value of contact angle measurement for three experimental groups depending on p value(0.003). This result matches with Jemat A. et al,[136]

In spite of increased contact angle measurement in both irradiated groups, its value still within accepted range of hydrophilicity ($<90^{o}$)[5].

Increased surface roughness closely related to higher surface free energy [137] consequently wettability. Since, increased surface roughness results in larger surface area that becomes in contact with the surrounding biologic fluids[138]. In case of extreme hydrophilic implant surface, cellular attachment may be affected negatively[139].

3.13.Cellular attachment assessment

An intimate contact between the implant surface and surrounding bone considered as a main parameter that determines the success rate of the implantation procedure. Implant surface properties directly influence the bone response to implant unit insertion procedure. Osteoblast cells considered as a main element in such healing process[140].

In this study, the impact of modified zirconia implant surface (roughness) was assessed in vitro by seeding of osteoblast cells isolated from calvarias bones of new born rats. The effect of obtained roughness on osteoblast cells response was determined by counting the number of attached cells after 24 hours culturing and number of proliferated cells during three different interval periods.

The surface roughness can directly affect on the surrounding cells or on reactions that occur on it. In other words, it may affect on protein adsorption which considered as a main mediator for cellular attachment on implant surface. Clinically, improved surface roughness leads to higher protein adsorption consequently, higher cellular attachment results.[37].

According to the result of ANOVA test, the result of mean comparison of three experimental groups (p value of 0.01), this value indicates a high significant difference, consequently, increase in the number of attached cells after 24 hours culturing. This result matched with Markhoff J. et al, Renan D et al, Naji S.S., [141,71, 121]. Not matched with Strickstrock et al, in their study, the implant sutface was roughened by sand blast technique with particle size (1.7-3)μm which produce wider surface pores compared with that of laser surface processing [142].

Improved surface roughness considered a useful surface property that enhances cellular attachment [143,144,145, 146].

The contact area between the implant surface and surrounding fluids has an impact role on cellular attachment and proliferation.[38].

This fact explains the increase in cellular attachment population on both roughed zirconia surfaces. Enhancement of surface roughness results in larger surface area becomes in contact with surrounding fluids. Consequently, further positions for protein adsorption and cellular attachment.

3.14.Cellular proliferation

Statistical comparison was done between the mean value of cellular proliferation for three experimental groups using ANOVA test. The obtained result showed a high significant difference consequently, increase in the number of proliferated cells on both irradiated groups compared with the machined (control) group after three days incubation period, p value is 0.001.

This result matches with Song Y. et al, Gnilitskyi et al and Naji S.S., [38,116, 121]. And not matched with Strickstrock et al [143], that probably due to using of different technique in surface modification.

This result due to the higher cellular population attached on both roughened surfaces.

3.15.Alkaline Phosphatase Level

Alkaline phosphatase level measurement considered as an indicator for osteoblast cells differentiation. It plays an important role in mineralization of newly formed bone[38].

In the present study, the ALP level had a significant difference between control group and other two irradiated groups.

This is matching with Rosa A.L. and Song et al, [37,38]. During further three intervals, ALP level had no significant difference between control and two irradiated groups. Since, ALP secretion considered an indicator for early osteoblastic differentiation, where as matrix mineralization associated to final differentiation phase [144]. ALP level start to decrease once other markers start to increase like (osteocalcin)[147]

In both previous studies, ALP activity was measured after 2 weeks and three weeks incubation period. both are longer than measurement period of present study.

3.16. Conclusions

1. Q-switched Nd:YAG laser of 532 and 1064nm wavelength can be used in modification of zirconia implant surface properties. Both wavelengths from Nd:YAG laser have an almost similar effect.
2. Proper selection of laser parameters for zirconia surface treatment affects clearly on the surface properties. Comparable effect was seen in regard to surface roughness and surface topography.
3. Improved surface roughness of zirconia dental affects positively on the biologic response of osteoblast cells, but more favorable for 1064 nm.

3.17. Suggestions for future work

1. Study the effect of longer exposure time of Q-switched Nd:YAG laser of (532 and 1064)nm wavelength.
2. Assessment different types of growth factors associated with healing process including, Inerlukine-1, TGF-b.
3. Assessment ALP level during longer incubation period.
4. Application present study in vivo.
5. Modification of zirconia surface roughness by using femtosecond laser.

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