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Study of morphology, physicochemical properties and antibacterial effect of gallium oxide coated implantable substrates submitted to thermal oxidation treatment

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MARIA YASEEN

“STUDY OF MORPHOLOGY, PHYSICOCHEMICAL PROPERTIES AND ANTIBACTERIAL EFFECT OF GALLIUM OXIDE COATED IMPLANTABLE SUBSTRATES SUBMITTED TO THERMAL OXIDATION TREATMENT”

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ABSTRACT

In the production of medical devices, surface modification of bioimplant is a key process to link with the biointerface for each other. Many strategies, however, implement a multi-functional approach that incorporates with the suitable deposition thin film growth and antibacterial coating mechanisms. Surface coating approach not only projected as protection layer but also justify with the less use of bulk material and expense. In biomedical arena, it's assisted as to prevent bacterial adhesion and reduce biofilm formation due to the increasing prevalence of antibiotic resistant bacterial strains. Present work focused on the use of a metal oxide (Gallium oxide) on different substrates to investigate the appropriate condition to prevent bacteria adhesion with and without subtract effect. Two thin film deposition techniques MOCVD and PLD choosed to deals with the certain parameters. For this, deposition technique applied to adhere the thin film of biomaterial on the substrates. After deposition major concern was to improve the surface properties. The interactions of Gallium Oxide (Ga_2O_3) were investigated using multiple characterization techniques. In results, surface features explored by morphology, XRD, surface roughness and wettability characterization. The diffraction peaks are indexed as with the crystalline structure which is confirmed by X-ray diffraction pattern. Further, interface behaviour study under different substrates effects coated with gallium oxide. It must be concluded that as surface treatment temperature approached to deposition temperature RMS values became equal or greater and thermal treatment under vacuum condition support good treated surface. From adhesion point of view, all surfaces uniquely hydrophilic with the gallium oxide thin film metal oxide. Through, antibacterial assessment of gallium oxide revealed more effectives on the E. coli bacterial cells the S. aureus. It was perceived that the coated surfaces possess strong different electrical properties and thus illustrate more efficient electron transfer mechanism with the Ti alloy when compared with the other surfaces counterpart. The death kinetics of bacteria with multiple coating surfaces was provided the surface potential in the design of medical devices. By the manipulation of band theory with charge transportation phenomena support to in the selection of suitable implant surface. Moreover, metal substrate surface with the metal oxide thin film provide promising results to modify the biomedical implant for as antibacterial coating.

Keywords: Biomedical implant. Biomaterial. Gallium Oxide. Thin film. Thermal Oxidation. Antibacterial.

RESUMO

Na produção de dispositivos médicos, a modificação da superfície do bioimplante é um processo essencial para vincular a biointerface entre si. Muitas estratégias, no entanto, implementam uma abordagem multifuncional que se incorpora aos mecanismos adequados de crescimento de filmes finos de deposição e de revestimento antibacteriano. A abordagem do revestimento de superfície não apenas é projetada como uma camada protetora, mas também justifica o menor uso de material e despesas a granel.z Na arena biomédica, é auxiliado a prevenir a adesão bacteriana e reduzir a formação de biofilme devido à crescente prevalência de cepas bacterianas resistentes a antibióticos. O presente trabalho enfocou o uso de óxido metálico (óxido de gálio) em diferentes substratos para investigar as condições apropriadas para evitar a adesão de bactérias com e sem efeito de subtração. Duas técnicas de deposição de filme fino MOCVD e PLD optam por lidar com determinados parâmetros. Para isso, a técnica de deposição aplicada para aderir ao filme fino de biomaterial nos substratos. Após a deposição, a principal preocupação foi melhorar as propriedades da superfície. As interações do óxido de gálio (Ga2O3) foram investigadas utilizando-se múltiplas técnicas de caracterização. Nos resultados, características da superfície exploradas pela morfologia, DRX, caracterização da rugosidade da superfície e molhabilidade. Os picos de difração são indexados como na estrutura cristalina que é confirmada pelo padrão de difração de raios-X. Além disso, estudo do comportamento da interface sob diferentes efeitos de substratos revestidos com óxido de gálio. Deve-se concluir que, à medida que a temperatura do tratamento de superfície se aproximava da temperatura de deposição, os valores de RMS se tornavam iguais ou maiores e o tratamento térmico sob condição de vácuo suporta uma boa superfície tratada. Do ponto de vista da adesão, todas as superfícies são hidrofílicas com o óxido metálico de filme fino de óxido de gálio. Por meio disso, a avaliação antibacteriana do óxido de gálio revelou-se mais eficaz nas células bacterianas de E. coli do S. aureus. Percebeu-se que as superfícies revestidas possuem fortes propriedades elétricas diferentes e, portanto, ilustram um mecanismo de transferência de elétrons mais eficiente com a liga Ti quando comparado com as outras superfícies. A cinética de morte de bactérias com múltiplas superfícies de revestimento proporcionou o potencial de superfície no design de dispositivos médicos. Através da manipulação da teoria das bandas com suporte a fenômenos de transporte de carga na seleção da superfície adequada do implante. Além disso, a superfície do substrato metálico com o filme fino de óxido de metal fornece resultados promissores para modificar o implante biomédico como revestimento antibacteriano.

Palavras-chave: Implante biomédico. Biomaterial. Óxido de gálio. Filme fino. Oxidação térmica. Antibacteriano.

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LIST OF ABBREVIATIONS

Average arithmetic means	S_{av}
Vertical tube furnace	F
Metal Organic Chemical Vapor Deposition	MOCVD
Pulsed Laser Deposition	PLD
Titanium alloy	Ti6Al4V
Silicon	Si
X-ray diffraction	XRD
Gallium Oxide	Ga_2O_3
Trimetallic gallium	TMGa
Chemical Vapour Deposition	CVD
Radio frequency	RF
Femtosecond Laser	Fs
Ultra-High Vacuum	UHV
Krypton Fluoride	KrF
Escherichia coli	E.Coli
Reactive Oxygen Species	ROS

CONTENTS

1 INTRODUCTION

This chapter deals with the general and specific objective need to achieve and explanation to introduce the area in which this project is designed. For this motivation and objectives are specified to achieve the target of the work. This chapter will justify the choice and explain the motivations for growing thin films.

1.1 Bioimplant in health care

Bio implants have covered enormous area of health by covering life expectancy and enriched the implant technology dramatically during the past decades.(1) Presently all over the world annually health disorder is spreading as orthopedic, maxillofacial, fractures that demands solution to regain high quality of life. In this way annual cost of bioimplant has exceeds billions in Euro worldwide. (2) Another accountability of consumption of bioimplant large in number is rapidly increase in elderly people growth rate to replace their body tissue or organ to compromised health condition prolonged.(3) The shape of human body fundamentally structured by hard tissue that not only protect but also support mechanically to perform moments of body like knee and joints function. Consequently, replacement as artificial implant concerned with the mechanical performance with existing organ and healing process.(4) With the extensive use of medical devices in health sectors its marketing is spreading worldwide day by day. From marketing point of view medical devices are majorly categorized by functionality including drug delivery devices, monitoring devices, surgical devices, diagnostic devices, bioimplant and neurostimulation etc.(5)

1.2 Bioimplant material

Many descriptions have been proposed for the term ‘biomaterials’. Among many others one definition is endorsed by the experts in the field is: “A biomaterial is a nonviable materials used in a medical devices, intended to interact with biological system (Williams,1987)”. To understand the goal of biomaterial science another term is accomplished as “biocompatibility”. “Biocompatibility is the ability of a material to perform with an appropriate host response in a specific application (Williams, 1987).” Biomaterials are such material need to replace a tissue or organ function in vivo. The reliability is concerned with the non-inflammatory, non-toxic and non-carcinogenic.[6] To estimate the biocompatibility there are two ways to distinguished in which process biomaterial is fabricated into devices and host environment. All these information's are prior to recognize to any medical application, but also term of change with time must be encountered in selection of biomaterial and design of device. Frequently, information related to the basic properties is available through international standers but in case of synthetic material that have different behaviour with host environment need to explore via research. Somehow, tissue response is tremendously different with specific area e.g., cardiovascular (flow of blood through streams), orthopedic (load bearing applications) and dental (filling, surface texture). The physiological interaction of biomaterial with the certain environment should be inert. Every material has its limitation in application like metals are being used from centuries, but their efficacy is limited due to it adsorption and infection property. On the other side steel corroded rapidly and produced inflammation. Nobel metals are mostly preferred in load bearing application due to low strength also considered to cost effective.[7]

Whereas, biomaterials are classified as three generations by its functional. First generation of biomaterial was established during 1960s to 1970s as the primary goal was intended to be inert and to find a suitable physiological environment to replace the organ or tissue with minimal hazards. As a consequences these material were designed for aerospace and construction industries not for medical purpose. For clinical evaluation millions of implantations were applied by assessing multiple materials. To extend the features of biomaterial the 2nd generation was arrived in 1980s. The particular interest was to deal with the tissue reaction these including bioactive glasses, biodegradable materials and many other materials contain bioactive assets. The material designing was key supportive for strong bonding with bone tissues to enhance the osteoblast proliferation and reduce the implant losing effect.[8] The 3rd generation of biomaterial is mainly support the cellular response at molecular level. It is deals with the specific cellular activity of bioactive and restorable material that enhanced the cell proliferation, differentiation and cellular matrix production and arrangement. There are two alternative ways to repair the tissue with biomaterial. *Firstly*, cells are seeds to develop the scaffold like properties on the outside of the body tissue that apparently grow mimic with the naturally occuring tissues. So it can help to replace the diseased or damaged part of body. With the passage of time material adopt the physiological environment to repair the fragment long lasting. *Secondly*, materials are doped in the form of solution and powder to stimulate the cell activity. The ions are released by diffusion network breakdown that activate the cell in contact with the stimuli at controlled rate. At the surface both osteoconduction and osteoproduction reaction happens with the cellular environment.[9] Several surface modification approaches are employed for all classes of biomaterials to engineer biofunctionality at the material-tissue interface in order to modulate biological responses without altering material bulk properties. Surface modifications are classified into two general categories that include physicochemical modifications involving alterations to the atoms, compounds, or molecules on the surface, and surface coatings consisting of a different material from the underlying support. Physicochemical modifications include chemical reactions such as oxidation, reduction, salinization, and acetylation and also involve etching and mechanical roughening/polishing and patterning. Methods for generating surface texture could be grouped into approaches for engineering either roughness or topography. Surface roughness indicates a random or complex pattern of features of varying amplitude and spacing, typically on a scale smaller than a cell, and surface topography refers to patterns of well-defined, controlled features on the surface. Overcoating alterations comprise grafting (including tethering of biomolecules), noncovalent and covalent coatings, and thin film deposition. Common noncovalent coating methods are solvent-casting, and vapor deposition of metals, parylene, and carbons. Covalent coating methodologies rely on direct tethering of overcoats onto the base material to improve film stability and adherence. Radiation grafting, both with ionizing radiation and high-energy electron beams, and photografting are extensively employed to modify polymer substrates in order to introduce chemically reactable groups into inert hydrophobic polymers and polymerize overcoats onto the base support. [10]

1.3 Surface features of bioimplant

Bioimplant deals with the surface performance of interface that can influence the tissue response also the implant life span. The ideal or permanent surface is considered to be less toxicity, ideal corrosion and non-inflammation that decrease the life time of bioimplant by producing the stress -corrosion and cracks. All these factors are related to the physiochemical process materialization on the surface. Another aspect of surface feature is wettability or hydrophlicity that play critical role in the cell adherence and protein. Success of implant is based on the osseointergation process in which implant body directly linked with the living system. Few topographical parameters have accountability in this case. Basic implant topographies are designed as smooth, rough and porous.

If surface with average arithmetic means S_{av} >1 um supposed to be smooth. If surface with average Sa<1 um supposed to be rough. Surface texturing mainly enhance the cellular activity, it evaluated that rough surface anchored better than smooth in the bone. [11]

1.4 Proposed research

> Main motivation of this work is one accidental happening in experimental lab of Department of Physics of the UFPE in 2013 with the leak of organometallic gallium (TMGa) from the tubing in a MOCVD system, on the hand and arm of a person. TMGa takes fire in contact with air, burning deeply the skin. In this case, after the skin burning (bubbles like), it was observed that no infection found there and no Scar (cicatriz) was present. After looking toward the consequence of this incident the project designed to study the effect of gallium to kill infection in the area of orthopedic or dentistry bioimplant surface. (Prof. Marco Sacilloti , DF, UFPE)

The proposed work deals with the exploration of biomaterial for orthopedic application. Presently, many types of biomaterials are availed that fulfill the basic need of implantation. Traditionally, bioimplant such as Ti/Ti alloy have good integration and load bearing property with the surrounding tissue. Currently, it is important to find such material that can react against infection. Additionally, surface can further modified by coating /depositing the biomaterial and cell adhesion can be increased by surface energy via suitable treatment.

Objective 1: - Determine the suitable parameters to deposition the metal oxide material with suitable technique on different substrates

In the project gallium oxide metallic biomaterials is under study to deposition and evaluate the antibacterial effect. Gallium belongs from IIIa metal, its shares its chemical properties with iron III. Iron helps in the formation of DNA but gallium don't have such applications in the body. Gallium compounds have potential application in therapeutic activities to killing infections.

The mechanism of action of gallium (III) complexes in anticancer chemotherapy has been briefly studied. Ga^{3+} ions usually compete with Fe^{3+} for binding to transferrin to reach the intracellular medium. In this way, a cellular uptake of large amounts of gallium is achieved. Analyzing all the biochemical pathways of the gallium (III) ion, it seems clear that the enzyme ribonucleotide reeducates is its biological target. Like iron, gallium doesn't deals with redox process. That's the reason gallium is to study in this project to modify and to kill the infection. Among many surface coating methods pulsed laser deposition and metallic organic chemical vapor deposition are choose to compare and achieved the homogeneity. Because each method deals with creation of vacuum that provide the less contaminated environment for deposition.

Objective 2: - Surface modification by thermal oxidation treatment to enhance the cell adhesion

Here in this study thermal treatment intending to apply in two different environments with and without vacuum to improve the interface. Further need to explore that which treatment provides more significant results. Surface oxidation in the form of thermal treatment help to modify surface. Impact of this treatment can increase the oxygen content for biocompatibity and surface roughness by changing the morphology of the deposited thin film.

Objective 3: - Evaluation of physical and chemical characterization of surface to control the stability and potential bonding

Response of the tissues to the implant is largely controlled by the nature and texture of the surface of the implant. Compared to smooth surfaces, textured implants surfaces exhibit more surface area for integrating with bone via osseointergation process. Textured surface also allows ingrowth of the tissues. The role of surface topography has been the interesting area of investigation in implant dentistry for several years. Some of these have the ability to enhance and direct the growth of bone and achieve osseointergation when implanted in osseous sites. Endosseous dental implants available commercially with many different surface configurations. Most implant systems of this category are based on the fact that bone tissue can adapt to surface irregularities in the 1 – 100 micron range, and that altering the surface topography of an implant can greatly improve its stability.

Objective 4: - In vitro analysis to test antibacterial effectiveness of biomaterial

All kind of surface interaction deals with the phenomena of cells osteoconductivity. There are many other methods to test the cell death in the biological testing but this work is mainly designed to explore the biointerface and how different substrates surfaces contributes in the charge transportation phenomena on the base of band theory in the solid state physics. Here need to explore the antibacterial properties of material in vitro analysis that can help to obtain the appropriate constraints to suggest for a good bioimplant.

2 LITERATURE REVIEW

2.1 Orthopedic implant

Mainly functions of orthopedic implant are bone fixation and joints replacement. Some fixation devices are temporarily inserted inside of the body for healing to support the affected part than removed. Joint replacement eliminates the pain by providing the alternative to improve the function of the system. These parts kept inside the body until its relief the pain. Consumption of the total hip replacement increased in USA more than two fold from between 1990 to 2007. Foreign implants are highly subjected to the bacterial and fungal infection that's why surface protection required for defense. Another latest global bioimplant market report is expected to garner $115.8 billion by 2020, registering a CAGR of 10.3% during the forecast period 2014 - 2020. Bio-implants have emerged as a promising solution for the variety of conditions such as cardiovascular, dental, orthopedic, ophthalmology, neurological disorders and others. (12,136)

2.2 Interaction of implant with bone

When implant exposed in the body the blood make first interaction, customarily its contains red cell, platelets and plasma protein. The bonding starts establishing with the round space and form clotting with the adhesion of protein like fibronectine, fibrinogen. The surface topography activates the degree of cell to provide the link between implant and bone. The cell start migrating and potentially healing process inaugurate between each other. Links start following the specific pattern to forming the structure with matrix of blood protein. Tension between matrixes creates the superficial forces to construct the network. During migration, cells began to differentiate and arrive at the surface and stop over it. Moreover, with certain situations sometime cell model itself to adheres on the surface. Typically, all cells don't resize in one direction and shape. The orientations of cell are always dynamic in the form of clusters with different protein cells. The links among the cells serve as transmitter to regulate the signals. (13)

In the first attachment osteoblast cell calcified collagen free bone cement on the prosthetic implant that create the bone matrix which turn the osteocyte matrix form. The staffing and transport the osteogenic cell defined the osteoconduction process. In the beginning, intimation of the bone growth with implants deals with critical parameters known as osseointergation. The contact of the bone with implant demands a reasonable gap that is mechanically strengths the bone grip. Certain unfavorable conditions can disturb the cell growth such as hydrophlicity, surface roughness and topography. (14)

The healing process with the wound tissue is not simple process that can be summarized by the four phases; hemostasis, inflammatory, cell proliferation and remodeling. Hemostasis promotes the protein adsorption on the surface by aggregation of platelets and thrombus. In flammatory phase initiate after 10 minutes of surgery and super cleaning surgical environment can avoid this problem. In the third stage, cell proliferation began within few days to few weeks. Remodeling of cell originates with the appearances of osteoclast and start replacing with the newly formed load oriented bone cells. Releasing of ions create significant important feature to differentiate the cell interference. (15)

2.3 Prosthetic associated infection

Infections are caused by the microorganism with the host body tissue. The infections that are associated with the surgery create most of the complicated issues. The drugs are administrated to the body to target the infections by relatively low concentration to avoid the effect of toxicity. In most of the cases infections are appeared in the early stage. The growth of biofilm decreases the susceptibility of antimicrobial agent. The presence of biofilms which protects adherence of bacteria in the company of poor vascularization of the bone/implant interface, make prosthetic joint infections extremely difficult to treat. So implant surface modifications are required in the pre-implant stage. The infection may appear in the form of inflammation or clotting the blood. (16)

2.4 Osseointergation of metal implant

Success of joint replacement and fracture fixation depends upon the mechanical and chemical biocompatibility. Currently, titanium, cobalt, zirconia, stainless steel and their alloy expected to provide good results clinically. These are considered to be inert for the interval part of the body. A fibrous layer is deposited on the implant that must be proportional to the biocompatibility of implant. Titanium is a reactive metal, the surface of which quickly oxidizes on exposure to air, creating a stable oxide surface which is incorrodible after implantation. This provides a surface onto which bone can grow and adhere. Titanium has advantages over cobalt alloys such as lower modulus of elasticity and higher biocompatibility. Jinno *et al.* showed that both titanium and cobalt alloys demonstrate good biocompatibility but less osseointergation was observed on cobalt alloy surfaces. However in general all metal implants are suffering from low osteoconductivity.[17] The coating on the surface implant composition of deposited material, adherence significantly plays an important role. The surface roughness is another important parameter that alternates the osteoblast cell activity. Surface wettability with water molecules help to adsorb the protein and subsequently cell adhesion. Among many other surfaces hydrophilic surface intimate the faster osseointergation and reduction of free surface energy. [18]

2.5 Why thin film?

History of thin films have not been newly performed mechanism but available to mankind as early as 4000 B.C. and were used by the Egyptians to decorate King Tutankhamen's tomb with films as thin as 0.5 μm. Electroplating has been widely used since its invention by Luigi Brugnatelli in 1805 to deposit thin films of metals on conducting surfaces, usually to add a protective layer or improve the aesthetics of a surface. Richard Feynman later made it possible to deposit metals on non-conducting plastic surfaces. [19] By pre-treating the plastics with sodium hydroxide and stannous chloride to ensure the metal film sticks to the plastic before placing it in an electroplating bath. The first evaporated thin films were those of Faraday [20], who exploded metal wires in an inert atmosphere in 1857.Depositing thin films by joule heating of platinum wires was discovered by Nahrwold [21] in 1887 and the following year adapted by Kundt [22] to measure the refractive index of metals. For the following couple of decades, interest in thin film deposition remained a rather niche area of academic interest until vacuum technology had developed to make it applicable to industrial applications. It could be asked why thin films are of interest for scientific and industrial applications. The principal reasons are cost of material, weight of material and the need for low-dimensional structures. In the first case, a good example would be the use of gold as a heat reflector. Gold is the best heat reflector known, but it is an expensive metal so depositing a thin film is often sufficient to take advantage of its reflecting properties while minimizing both expense and weight. Being able to control the film thickness to such a short length scale obviously implies such thin films are achievable. Another example, which takes us more towards the area of this research, is the deposition of thin semiconductor layers to form quantum wells, whose light-emitting layers differ in emission wavelength from the bulk material by virtue of quantum effects that become appreciable at such a small scale. In the architecture of nanoscale electronics, thin films and other nanosized structures become a natural consequence of the need to miniaturize electronic circuits to allow signal processing and data storage to increase while attempting to maintain device sizes at practical levels.

Thin film technology serving through different ways in biomedical applications. The uncountable material are presently its benefits and progress made in the last two decades in chemical vapour deposition technology has enabled the production of inexpensive, high-quality coatings made from diamond to become a scientific and commercial reality. Two properties of diamond make it a highly desirable candidate material for biomedical applications: first, it is bioinert, meaning that there is minimal immune response when diamond is implanted into the body, and second, its electrical conductivity can be altered in a controlled manner, from insulating to near-metallic. *In vitro,* diamond can be used as a substrate upon which a range of biological cells can be cultured. *In vivo*, diamond thin films have been proposed as coatings for implants and prostheses. Here, we review a large body of data regarding the use of diamond substrates for *in vitro* cell culture. We also detail more recent work exploring diamond-coated implants with the main targets being bone and neural tissue. We conclude that diamond emerges as one of the major new biomaterials of the twenty-first century that could shape the way medical treatment will be performed, especially when invasive procedures are required. [23, 24, 25, 26, 27, 28]

2.6 Thin film deposition techniques

The feature of surface implant provides the good interaction to the tissue and biocompatibility. It is observed that by increasing the surface roughness improve the cell migration and attachment of implant.[29] The surface can be modified in various ways either by adding or subtraction procedure. The modifying techniques can be mechanical, chemical and physical methods. The surface treatment deals with the topography, surface energy that ultimately helps to improve wettability.[30] With all other advantages modify surface can also assist in killing the infection that maintain the healing process for long term body implant.[31] Thin film coating is useful to protect large sophisticated surface not only for industrial applications but also for medical. Various methods are available but generally it has been divided into two sections; physical deposition techniques and chemical vapor deposition techniques although both are different in dealing but similar in many ways also.

2.7 Physical process coating

Almost all physical coating depositions occurred by forming the vaporing solid of the precursor changing through physical state. In this process any kind of chemical reaction does not required to forming the deposition.

2.7.1 Physical Vapor Deposition

PVD superficially a vaporization coating, that concern with the transportation of material at atomic level. In practically it is similar to chemical vapor deposition with the difference that PVD use raw solid material or precursors rather than in CVD precursors are introduced with gaseous state in reaction chamber. All process integrates such spraying form. PVD concerned with the four stages such as evaporation, transportation, reaction, and deposition. The whole process carried out under the vacuum condition in a closed chamber. Evaporation first stage is to sits the target precursor material into vaporized form using suitable beam of electrons or ions. Than the target start heating above its boiling point and transportation occurred by dislocation. The particles start transforming on the substrate to form a layer of thin film. Sometimes it appears in the form of coloured layers or in gray surface. PVD coating provides numerous benefits. It's improved the surface efficiency, hardness and wears resistance. With all reduced the friction by improving the oxidation resistance. This technique deals with all inorganic material as few organic materials also. Some technical process is as always on demand to control the inside machine factors at low or high vacuum.

2.7.2 Sputtering

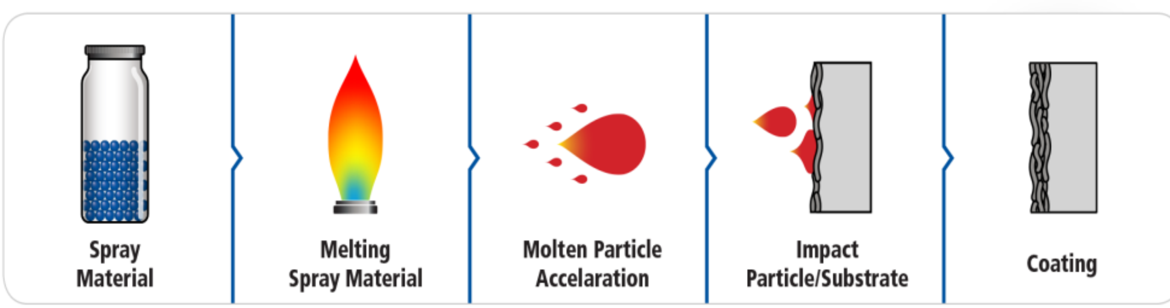
Sputtering is a physical vapor deposition technique in which atoms are physically ejected by the momentum transfer though electrical discharge in a closed vacuum chamber at low pressure. This momentum comes from inert gas (Ar^+) that is integral part of glow discharge. The target material is projectile to hit the substrate and appears in the form of deposition.(32) The material vaporized so it can be deposited in any direction. Whole process is performed at the low pressure so sputtering atoms experience many collision as before targeting , this is known as thermalization process, it play vital role to slow down the high energy particle to produce less damage that anchored as uniform thick layer.(33) Another advantage of this process is that atom composed on the substrate without requiring the high temperature. But in this technique thickness is difficult to control. [34] Sputter coating can be performed with two operation DC (diode & triode) and AC (radiofrequency) the frequency is always below the 50 KHz. Because above this value atoms unable to get mobility. The sputter yield of ion at constant energy related to the position of elements with position. [35]

2.7.3 Thermal spraying

Thermal spray process involved with solid precursor material. When the material is heated by applying electrical current the resulting particles are accelerated towards substrate. In this process the material is deposited by mechanical adhesion. It's covered a large area of surface as compared to the other physical techniques. Here in this thermal spraying thickness can never be controlled only valid to cover the surface by appropriate material. In this way, such depositions have limitation. The whole process is performed in the atmospheric pressure.

Plasma spraying, there is relatively similar method called plasma spraying. In this process precursor are more heated and convert in the form of ions/high energy particle. Plasma spray provide good adherence as compared to the thermal spray because of high temperature production. The particles are accelerated towards target with the high energy. However, this process demand lots of precise parameters to control otherwise particles will detracted from optimal condition and surface will not modified with high accuracy. This technique does not required vacuum conditions. [36]

Figure 1 - Schamatic process of Thermal spray coating on the substrtae surface to adhere the particles

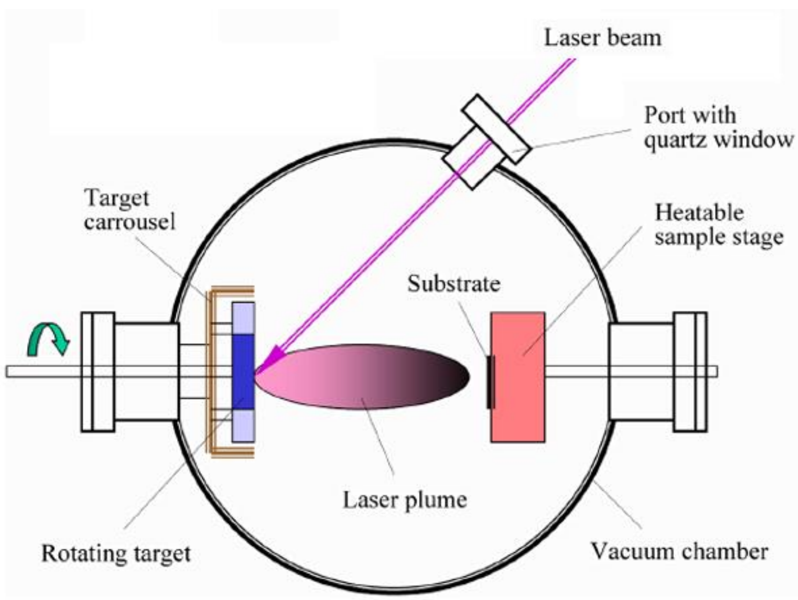


Taken from the Ref. [36]

2.7.4 Pulsed Laser Deposition (PLD)

One of the best versatile solid state and analytical technique inwhich material vaporized and condensed using photons. A very short range high power beam focused onto the sample by which finite volume convert into vapor phase. As a result, material absorbs the photo energy; significant amount of material is removed from the targeted surface. As threshold level is achieved plasma creation appeared in chromed form. Afterward; particles are accelerated with high velocity to grow in the form of film. Generally, PLD process is controlled by the ablation, temperature, plume formation, laser wavelength, and repetition rate. Different kinds of Laser are used in different frequency range.

Figure 2 - Schematic working process of physical depsition techniques by Plused Laser Deposition (PLD) with different components.



Taken from the Ref. [40]

In the initial stage when photon interact with the material by coupling the photon energy to the lattice over the electronic transitions. Over the optical depth, absorption of the photon transfer its energy in metal to the electronic system and in nonmetallic usually to the lattice. Moreover, the lifetime of electronic relaxation also altered in composition of matter. In case of metal, electronics transion is about *1.5ps* is considered to be very fast as compare to the nonmetal that is of varies among 10^{-12} to 10^{-3} s. The detailed description of this phenomenon strongly relies on the thermal conductivity, heat transfer and photon coupling with matter. The formation of plasma certainly deals with the time scale. Therefore, mechanism of plasma expected to be different at nanosecond and femtosecond.[37]

Selection of Laser: Different factors are considered in the selection of Laser for ablation. The ablation / plume can be created with different lasers. Ruby is among the first laser that was applied in the creation of plasma. After that Nd: YAG and excimer lasers can also produce ablation. The solid state laser has many advantages because required short maintenance, less divergence in beam and lest cost effective. In addition, beam profile is close to the Gaussian distribution. Other type is eximer laser filled with the halogen gas filled cavity that’s why its demand more care and maintainess having greater power output and definite beam profile. Generally, it is admitted that production of ablation get high with decreasing the laser wavelength. The reason could be behind of this phenomenon that shorter wavelength offered higher photo energy that is suitable to vaporize and ionized the target. At certain wavelengths like 266, 213 and 157nm equivalent photons have energy 4.66, 5.83 and 7.90 eV. Another additional factor of concern with the ablation also depends on the absorption of wavelength in the material. For getting high efficiency in the laser ablation UV wavelength because reflectivity in the metals quite lower that support to increase the ablation efficiency.

Femtosecond Laser: The *fs* pulse in the ablation process provides the successful benefit especially in the nanomaterial synthesis as compared to longer pules. However, with the *fs* laser can establish high temperature and pressure comparatively ns ablation because energy is processed as far as thermal condition happens.

On the otherside, no *secondary interaction* appeared among the laser beam and ablated process. Due to high density of photon means greater the kinetic energy of the plume creation of the thin film growth.[38]

Nanosecond Laser: Among many other lasers *ns* pulse consider to be the good for ablating the bulk material because having enough time for thermal excitation for melting and evaporation. In the plume absorption, deals with the two mechanism inverse i.e., Bremsstrahlung and photoionization. Both phenomena’s induce excite the atom through collision process.

Influence of background radiation: The background radiation reflects the high impact in the surface formation on the substrate. Two kinds of effects can be expected in the presence of background radiation: a) reduction of the kinetic energy of vapor flux, b) in the presence of background oxygen provides the high flux and additionally high flux can increase the oxygen content during the deposition of film. Beside the oxygen as background radiations inside the vacuum chamber can change composition of film on the surface growth such an NH3 to provide reactive N2 and CH4 form Si-C or C-N. (39)

Mechanism of plume expansion in vacuum: Plume expansion deals with the adiabatic process, in which no mass and energy transfer hence this expansion is collision free. Geometrically, this expansion appeared as an ellipsoid known as Anisimov model.(40) High particles densities are formed at the target surface as the ablation rate exceeds from 0.1monolayer p/s and multiple scattering formed between the plumes to establish the thermal plasma into equilibrium. Collision of particles start with one another until thermal equilibrium approach at the distance $\approx$ 50 μm from the target, called Knudsen layer. (41)

The plasma contains free ionic cluster that emitted the light caused by the fluoresce and recombination process. Meanwhile atomic transition having very short life time's usually of the order few nanosecond. Finally, particles are deposited in different mode like in layer, cluster and film and it can be characterized by microscopy techniques.

There are number of reasons to utilizing this technique. Almost all type of material can be ablating due to flexible wavelength. In the experimental setup laser is externally separate from the reaction chamber. That's why considerable degree of freedom in the ablation geometry possible. The growth of film can be controlled by kinetic energy of vaporized particles. Due to complex setup and high energy particle formation this technique has some defects. If particles deals with large kinetic energy of plume causes the defects in the form of inhomogeneous growing film. The inhomogenty indicate that distribution of energy is not symmetrical.

However, lighter and heavy elements also have its impact in plume creation with distribution as film because of different velocities, angular momentum. Therefore, some additional supplements in the form of gas required to obtain the desired surface belongings.

2.8 Chemical coating process

Chemical Vapor deposition comprise with the developing of thin layer on the substrate using chemical precursors. This technique can be eminent by others due to its specific methodology involve with the adsorption of atomic layer. Reaction can be initiated through thermal process, high frequency, photo assisted and plasma process. There are different ways to deals with the chemical vapor depositions having slightly difference in deposition are given as below:

In MOCVD, their systems operate with the metal organic precursor compound surrounded directly with the metal carbon-bond (metal, alkyls and metal carbonyls). However, MOCVD can be originated as Metal Organic vapor phase epitaxy (MOVPE) and Organic Metallic Vapor Phase epitaxy (OMVPE) and produced single crystal.

One of other techniques is Plasma enhance chemical vapor deposition (PECVD) inwhich electrical discharge initiate the homogenous reaction for activate the ions and radical to indulge the heterogeneous reaction that leads to formation of layer on substrate. All these reaction happens on the at very low temperature close to ambient. Atomic Layer Deposition (ALD) also known as Atomic layer epitaxy (ALE) or Atomic layer Chemical Vapor Deposition (ALCVD) , its deals with the surface modification process by which precursor are acquaint with the gaseous state and sequentially deposit to the substrate and reactor chamber attached with the inert gas. The chemical reaction assists to form thin layer on the substrate at the temperature below the thermal decomposition and gas phase at this stage are unimportant.

2.9 Surface modification treatments

The purpose of surface modification is to preserve the key bulk properties of the material while transforming the surface to advance biocompatibility. Usually, modifications can either alter the atoms, compounds, or molecules on the existing surface chemically or physically, or coating the existing surface with a different material. Some of the important surface modifications are discussed, with a great deal of highlighting on coating and texturing operations.

2.9.1 Chemical treatment

Chemical reactions can be categorized as specific and nonspecific types. If the result of a chemical reaction is a distribution of different functional groups on the surface, then it is a nonspecific reaction. The oxidation of a polyethylene surface by chromic acid and radio frequency glow discharge (RFGD) in different atmospheres are common examples of nonspecific reactions. When a surface reaction changes one functional group into another with few side reactions and high yield, it is called a specific chemical reaction. Alkylations and conversion to siloxane come under this category. [42]

2.9.2 Ion Beam Implantation

In this process, accelerated ions are injected into the surface of a material and the surface properties of the material are modified. Largely used for metallic biomaterials, the process involves the implantation of the desired ion at high kinetic energies on a localized surface zone. Applications of such a process include improvement in hardness, wear, corrosion, toughness, and bioreactivity. The corrosion resistance of Ti–6Al–4V alloy has been shown to improve after implantation of irridium ions. Similarly, silver ions implanted into polystyrene allow cell attachment on the surface.

2.9.3 Texturing

Texturing generally refers to the physical modification of the functional properties of the surface of an object by surface engineering. The properties and the reasons for changing them may be many and varied. There may be a requirement to reduce (or even increase) the friction between mating parts, to improve the formability of a metal sheet, to increase the surface area or to enhance its decorative appeal and reduce its sensitivity to surface scratches. Textured surfaces are found in everyday products, from steel body panels and bearings to book covers. (43) A range of biomaterial surface properties are involved tribological, mechanical, and chemical ones, influence the biocompatibility and functionality of the implants. As control of the surface morphology is of primordial an importance. It has been well established that physical structure plays a key role in determining the cellular responses and hence the range of biomaterial applications. The interactions of cells to surfaces occur at various length scales from microscale to nanoscale. (44) The protein molecule interactions associated with cell signaling occur at the nanoscale. This regulates cell adhesion, proliferation, and differentiation. The combined effects of configurationally hydrophobic/hydrophilic or capillary forces might be responsible for self-organization of protein molecules and cell attachment. The interactions between microsize features (e.g., grooves) with cells take place at the microscale. Finally, the interaction of tissues with porosity occurs at the microscale. This knowledge has driven research at the micrometer and the nanometer scale features of the implant surfaces. Materials textured at the microscale or the nanoscale have all showed improved performance. But so far the ideal surface textured has not been established. Most of these studies were concentrated on characterization of cell or protein adhesion to surfaces from a biological perspective. A great deal of interest was given to surface energy and charge, and hydrophilic/hydrophobic features. [45, 46, 47] Different features like grooves, ridges, cliffs, dots, spikes, pits, and mesh, were experimented. [48] The phenomenon that substrate topography influences the cellular behavior is referred to as 'contact guidance. [49] The phenomenon by which cells adapt and orient to the surface micro topography is influenced by contact guidance. On the other hand, chemistry of the surface influences cell signaling and the way cell surface receptors respond. Once cell surface signaling is established, the cells try to orient by achieving a biomechanical equilibrium alignment provided by contact guidance of the topography.

2.9.4 Surface modification by LASER

Some of the important laser processing is operations with respect to their applicability to surface. Laser surface engineering (LSE) is a material processing method, which utilizes the high power density available from focused and localized laser sources to melt, heat, or modify the material on and near the surface. Depending upon the particular material system and process parameters, it may involve only modification of microstructure, grain refinement, phase transformations, alloying and mixing of multiple materials, and mixing and formation of composite system on the surface without actually affecting the bulk material itself. The use of a laser for texturing surfaces presents many advantages; for instance, it is rapid and extremely clean. Lasers are useful for the selective modification of the surfaces. They have the ability to generate extremely complex microstructures/features with high resolution. If a laser beam is coupled with a noncontact mask and optical systems, it is possible to produce sub micrometer features. Laser ablation can be used for varied surfaces.[50] Furthermore, the common disadvantage among other conventional techniques like anodizing, chemical etching, and mechanical scratching is that they are incapable of local texturing, and they lack good control over the resulting roughness. Lasers are useful in the generation of short pulses of light of single wavelength, which allow them to focus a great amount of energy in one spot.

2.10 Gallium oxide use in biomedical application

Different literature has been reported the dual mechanism of killing bacteria of gallium oxide with the synthesis of other metals. Gallium oxide itself doesn't have any well-known physiological function in the body. Gallium oxide has displayed its highly transparent quality. Moreover, antimicrobial mechanism of gallium is related to the Fe^{3+} mimic but gallium doesn't participate in a redox reaction. Gallium is able to compare with the iron due to the similar ionic radius; interface absorption Iman A. Hassan investigated that composite of an amorphous thin film by Ga_2O_3-Cu_2O growth. Further its applications in antibacterial killing effect via a dual mechanism involving Ga^{3+} ions which disrupt Fe3+ metabolism pathway and Fenton type reaction though reactive oxygen-mediated by Cu^{1+}. By combining both of these chemical agents the productivity of killing mechanism enhanced. The action of resistivity gets stronger due to dual effect. Ga_2O_3-Cu_2O thin film applied by aerosol assisted chemical vapor deposition on quartz substrate annealed at 1000^0C. By characterization, spectra were deals with the amorphous phase coated with the surface area. The important factor of the coating is that it helps to increase the surface contact with other interfaces through the topographical factor which consists of micro or nanostructure. So this kind of coating has a huge impact to treat the wide variety of surface for the biomedical purpose. (132)

Another factor that is associated with the biofilm formation appeared as infection. Such infection destroys the living organ and produces poison. Andrea Cochise tried to address kill the biofilm formation by grafting gallium and silver using anodic spark deposition. The sample preparation mainly concerns with the combination of the chelating and antibacterial agent to produce the biomimetic coating by anodizing spark deposition on the titanium surface. The antibacterial agent shows supportive features of biomimetic coating for homogenous microspores morphology that helps to cell attachments, cell proliferation, and differentiation. The advantage of the coating was return by the effectiveness killing of gallium about 40-48 % as compared to control. (133)

The biomaterial can be synthesized in different form depends upon the adopted method and purpose. K.Girija proposed the single crystalline b-Ga_2O_3 nanorods for testing antibacterial effectiveness of the material. The pure nonclinical phase was synthesized by the reflex condensation process. Two species of gram-positive and negative arranged to investigate the efficacy against *E.Coli* and *S.areus*. The significant difference observed by the disk diffusion method which provides to see the zone of inhibition at various concentrations. The estimated zone was different for both of bacterial species. Clearly, observed results indicated that monocrystalline beta gallium oxide is more effective for gram-negative than gram-positive. This observation can be explained on the bases of cell structure and its composition because gram-negative has less thin cell wall than positive in which penetration more facilitated as compared to thicker. Another effect could be illuminated as shape dependency of material that interacts with the out cell wall. A final conclusion can be estimated through positive utilization of β-Ga_2O_3 nanorods as an antibacterial agent and selective in killing effect. (134)

Sriyutha investigates the inhibition of biofilm formation using functional group carboxyl and amine with gallium oxide nanoparticles for surface modification. The reduction of biofilm was interfering differently on gram positive and gram negative. It was found that the susceptibility of gallium oxide with functional groups was more effective for gram-positive than negative. This phenomenon can be described on the bases of cell wall adherence, charged surface, toxicity and molecular matrix coagulation to extract their liquid removal from the cell. It is concluded that gallium oxide is still beneficent with other functional groups. (135)

3 MATERIAL AND METHODS

This chapter outlines the selection of material used in this study with the selection of different substrate surfaces. For thin film deposition pulsed laser deposition chooses among many other techniques. Surface cleaning procedure explain before implemented the thin film. To improve the surface modification thermal oxidation methods addressed as surface treatment. Target preparation by sintering method explained in each step. Details of the equipment used to characterize the structural, elemental analysis and surface roughness properties of the samples are also presented.

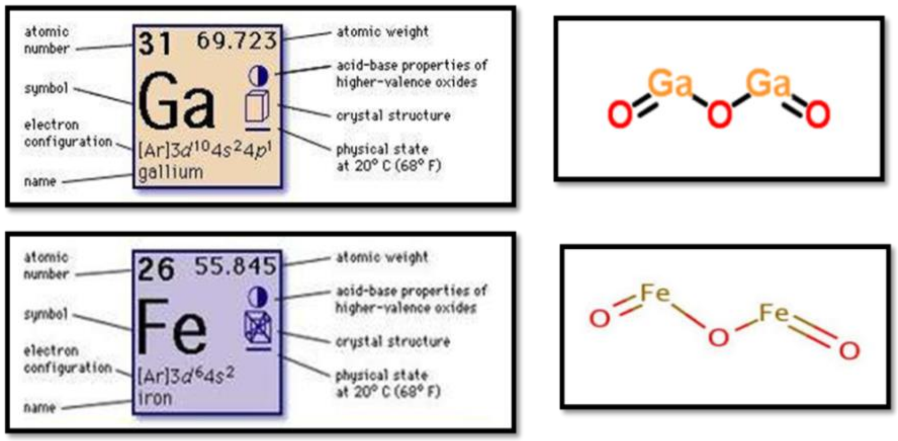
3.1 Material selection

The present study concerns with the development of metal oxide deposition using PLD technique on three different surfaces. Different surface are covered with gallium oxide just to make sure and compare the validity of results with eachother. For this gallium oxide is choose due to its antibacterial effectiveness. Different studies were reported regarding deposition of gallium oxide but study with hydrothermal oxidation treatment after deposition and its impact to explore surface roughness to improve osseointergation process did not acknowledge more. Therefore, this project is mainly designed to make a small contribution to acknowledge novel aspect of gallium oxide in the area of biomedical.

The implant surface is immediately colonized by the microorganism. So, initially, antibacterial coating essentially required to avoid any kind of failure. The keys factors such as surface roughness, texturing and wettability have a significant correlation with the accumulation of charges on implant. (51, 52) It has already been reported that metal and its oxide form contains an antibacterial effect. The model of action is complex and expected goes through the Fenton type reactions that produce reactive oxygen species which damage to the antibacterial cell membrane by oxidative stress. (53) Gallium formed in different combination with nitride and oxides. Gallium (Ga^{3+}) with trivalent charge correspond its physical and somehow chemical properties with iron with Fe^{3+}. The pharmacological mechanism of gallium contains chemical mimicry. However, iron deals with redox active but gallium is not. Conventionally, gallium doesn't have any physiological function in the body but the iron is important for cell division with formation of

DNA. Therefore, if it's exceeding from a certain level then break down the continuity of DNA replication, ultimately leads to its death. [54, 55]

Figure 3 - Gallium Vs Iron chemical formation and in the form of compound Gallium Oxide & Iron oxide



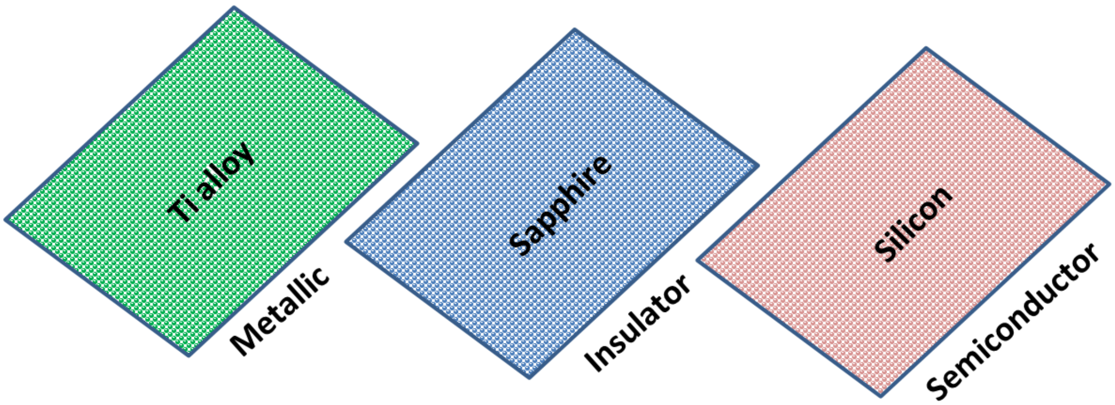
Taken from the Ref. [57]

On the other hand gallium oxide has various physical, chemical properties and its application in the area of electronic. The gallium oxide contains wide gap 4.9eV. Wide band gap semiconductors are highly recommended for high power application in optoelectronic. Because these are high saturated, conductive, stable and balanced electron drift velocity. Moreover, it is transparent in visible and IR region and that's why it is considered to be good candidate for high power electronic devices. [56, 57] Recently, gallium oxide semiconductor have gain the attention for high power devices fabrication due to its large band gap, excellent chemical stability, large breakdown voltage phenomena. Previously, reported that gallium oxide has five crystal structures and among all of that beta-gallium oxide is more stable and more convenient in production.[58] For blue light emitted diode hexagonal semiconductor material developed at advanced level. In high energy applications, it has huge impact in creation of solar blind photo detector, high temperature gas sensor, and ultraviolet photo diode. [59]

3.2 Substrate selection

In this study three different kinds of substrates were selected. Main study was dedicated to explore the properties of prosthetic implant surface. In this project Titanium (Ti-6Al-4V) alloy grade IV choosed. On the other hand silicon (100) and sapphire substrate (1102) utilize to compare with the results. After thin film deposition, thermal oxidation treatment applied to examine the surface roughness, particle size and phase variation before and after treatment.

Figure 4 - Classification of substrate



Source: The Author, 2019.

Note: The metallic substrate deals with Ti alloy, insulator as Sapphire (1102) and semiconductor as Silicon (100) wafer.

3.2.1 Cleaning of substrate

Thin films find applications in modifying the reflectivity of the substrate's surface, altering its rigidity, or modifying its surface chemistry. To achieve uniform surface, defect-free deposition, the substrate must be free of dust and other particles.

Most commonly acetone and ethanol are used for cleaning the samples in the laboratory; these solvents are easily available and easy to handle as compare to acid. [(60)] In case of metal, substrates the alumina powder with the grain size 0.3um were used as polishing. In the first step, alumina powder wet by the DI water and substrate insert on the surface of SiC 1200 grade paper with wet alumina. The cleaning process stay continues until it looks mirror like surface. Afterward, removing the inorganic particles using a mixture of acetone and ethanol diluted with 60% of pure water was prepared and inserted all samples for 15 mints and stirred ultrasonically. After this cleaning step, the sample was rinsed twice with ultrapure water followed by an ultrasonic bath for 15 min. In the end dry in the presence of N2 gas with suitable pressure.

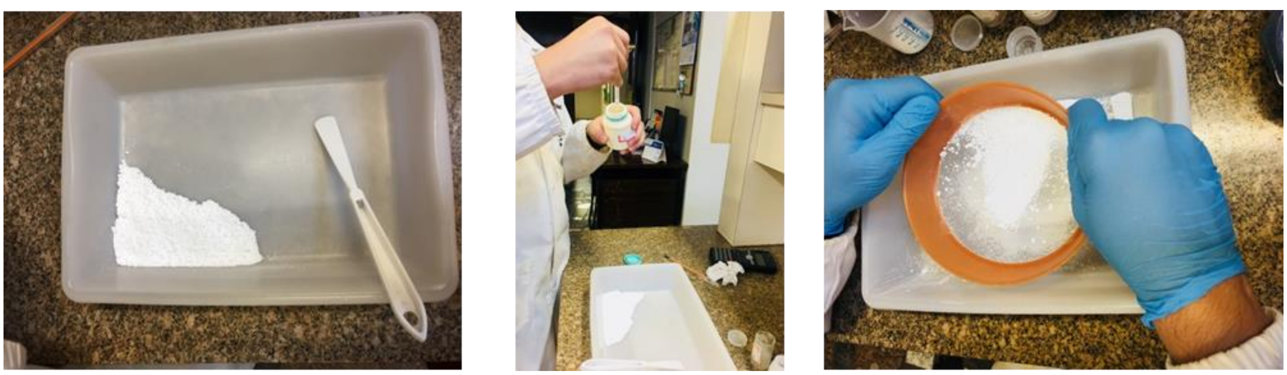
3.3 Gallium oxide (Ga2O3) target preparation for PLD

Target preparation for pulsed laser deposition required in compact solid shape. The porous powder passes through different stages to reduce in proper form. [61] The powder is sintering under with two parameters pressure and temperature which plays an important role to defined an appropriate volume. The process is as followed:

3.3.1 Sintering process

In the target formation, porous powder spread to discriminate the grain and mixed with binder like alcohol (1mL) (Fig. 5). After strainer the powder was inserted in the space holder and lid holder placed to form the certain shape (Fig. 6). The porous structure partially densified by limiting pressure. Until space holder was removed after forming the shape. Sintering of hollow particles or spheres allows obtaining porous materials with a closed-cell structure. After the removal of the space holder, the porous compact may require further sintering at a higher pressure and temperature.

Figure 5 - Gallium oxide powder spreading with addition of binder (alcohol) and straining



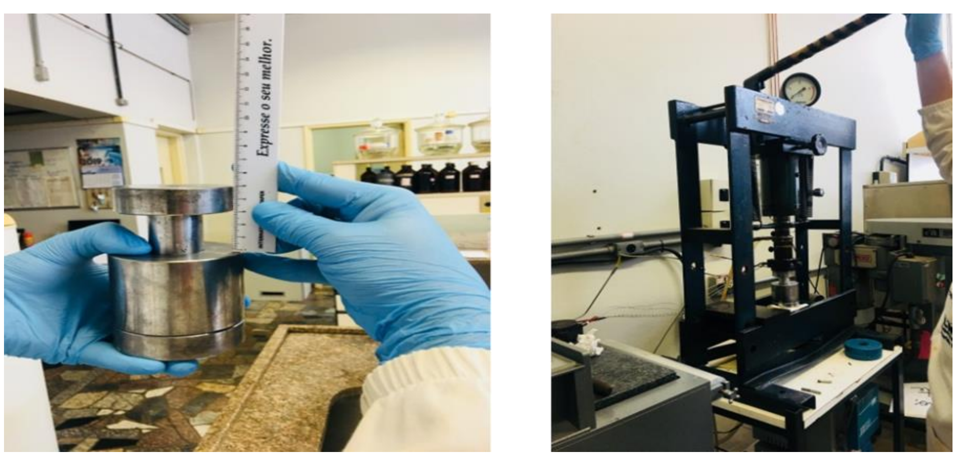
Source: The Author, 2019.

Figure 6 - Space holder to form the pellet and pouring the powder with certain shape

Source: The Author, 2019.

During the target preparation sintering temperature was the main concern to make it more compact. The sintering process deals with the two parameters heat and pressure. To achieve this, pellet was shaped in circular mold. The pressure was applied on the pellet/target in two steps; in the first step mechanical pressure insert about 10 Tone for few mints just to reduce the volume (Fig. 7) and in the 2^{nd} step hydrostatic pressure 150 Tone kept for 5mints to reduce its remaining spaces in a certain volume (Fig. 8).

Figure 7 - Mechanical pressure for forming the target more compact by sintering process



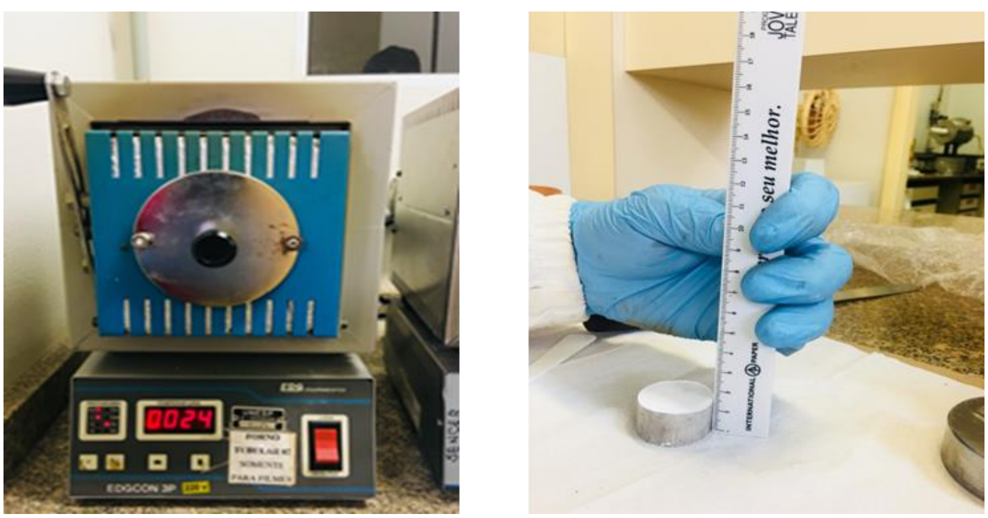
Source: The Author, 2019.

Figure 8: Hydrostatic pressure for reducing the spacing in pellet sintering process

Source: The Author, 2019.

The last step followed as sinterization in which temperature further practiced in two stages. The first stage 400^{0}C temperature stabilized for 3h by the rate change 30^{0}C/min that helps to remove all unnecessary residual present in the material and 2nd stage 1200^{0}C temperature puts for 3h by the rate change 30^{0}C/min to compact the target. Finally, pellet/target of gallium oxide (Ga_2O_3) ends up and placed as target with the dimensions diameter 2.7cm, 1.2 cm thickness (Fig. 9).

Figure 9 - Furnas heating process for pellet sintering process



Source: The Author, 2019.

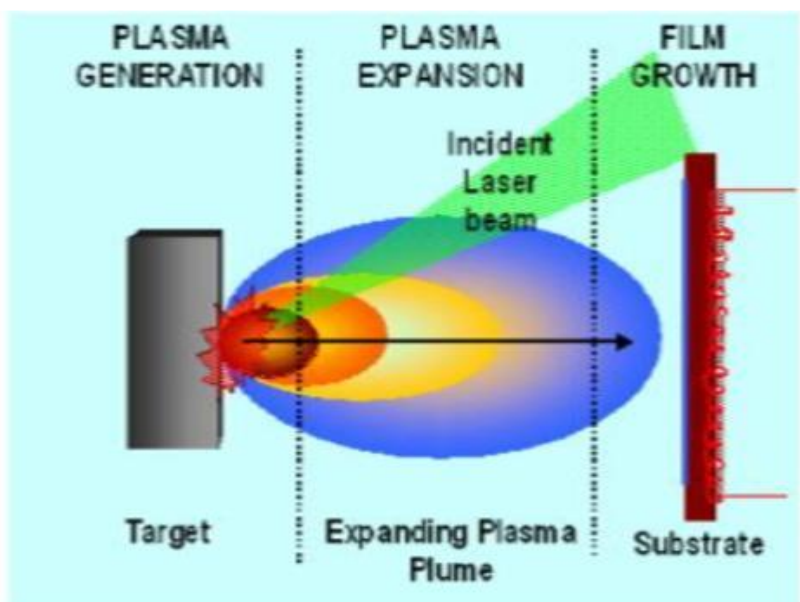
3.4 Deposition system

The present work deals with the deposition of thin film by Pulsed Laser Deposition (PLD). Idea behind this deposition was to modify the bioimplant surface and thin film to fight against the bacterial effect.

3.4.1 Experimental setup of PLD

The PLD technique was firstly practiced by [62] in 1965 to fabricate semiconductors and dielectric thin films using a ruby laser, which is measured as a very adaptable thin film growth process. Since the laser source is positioned outside the deposition chamber, PLD deposition can be accomplished either in ultra-high vacuum or in ambient gas. [63] It is possible to deposit all kinds of carbon-based materials, including fullerenes, [64] carbon nanotubes [65] graphite, and diamond-like carbon. [66, 67] With the PLD technique, the laser ablated species have high kinetic energy up to a few keV, allowing depositing adherent thin films at moderately low temperatures compared to other techniques.

Figure 10 - Schematic setup of Pulsed Laser Deposition and its components.



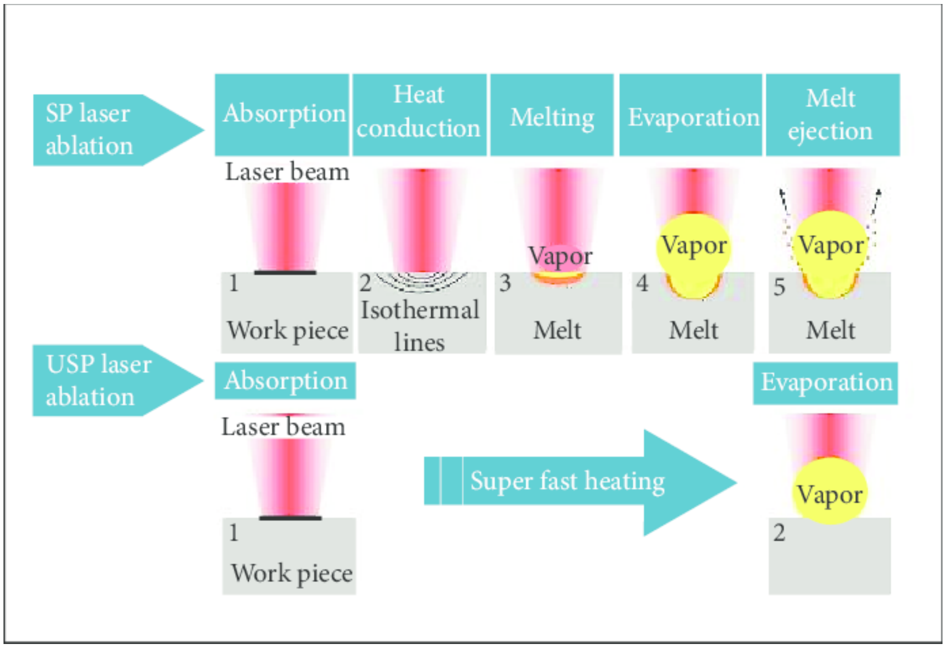
Taken from the Ref. [70]

Inside the vacuum chamber (ultra-high vacuum, UHV), targets of elementary or alloy elements are struck at an angle of 45° by a high energy focused pulsed laser beam. The species ablated from the target(s) are deposited directly onto the substrate. The principle behind the PLD mechanism can be concisely described as follows. The focused laser beam, striking the surface of a solid target during a short time, induces an energetic plasma plume containing ions and atoms, impacting the substrate in front of the target. Depending on various process parameters, including the substrate temperature, a single-crystal, polycrystalline or amorphous film can be attained. [68, 69] The quality of the deposit can be controlled by adjusting the experimental parameters, mainly the laser parameters (fluence, wavelength, pulse-duration, and repetition rate), and the deposition conditions (target-to-substrate distance, temperature, nature and pressure of the environment, etc.). [70, 71, 72]

Compared to CVD, the PLD method is conceptually simple, versatile, rapid, and cost effective, enables good control of thickness and morphology, requires only a low temperature for growth, and can be used with temperature sensitive materials, especially those with an active chemical surface. In addition, composite thin films with complex composition can be deposited by using several targets to perform co-ablation in a controlled and reproducible way. Another difference between the CVD and PLD is the carbon source. In CVD, the carbon source is a gaseous gas mixture, whereas PLD requires a solid carbon target, thus limiting the carbon source during segregation to that supplied during target ablation. Lastly, the PLD energetic source of carbon allows ablated species to penetrate deep into the substrate surface, rather than remaining on the film surface. [73] Eclipse PLD is one of the techniques used to reduce the number of large particulates reaching the substrate. The fact that the ablating source is a laser means that it is decoupled from the chamber and hence deposition can also be carried out in UHV. [74]

When focused on the surface of a solid target, pulsed-laser radiation can be absorbed through various energy transfer mechanisms, leading to thermal and non-thermal heating, melting, and finally ablation of the target. Laser ablation has shown itself as one of the most efficient physical methods for micro, and more recently, nanofabrication, due to the high resolution capability, low heat deposition in the target and high level of flexibility. On the other hand, the ablation of the target yields to an ejection of its constituents and to the formation of nanoclusters and nanostructures on the PLD process. When the target is ablated in vacuum or in a residual gas, the nanoclusters can be deposited on a substrate, placed at some distance from the target, leading to the formation of a thin nanostructured film. The properties of synthesized nanostructures can be efficiently controlled by parameters of laser ablation (fluence, pulse duration, wavelength) and properties of the environment.

Figure 11: Schematic process of laser ablation plume creation with superfast and ultrafast laser.



Taken from the Ref. [76]

Femtosecond (fs) laser ablation is opening up new perspectives in the ultra-precise processing of organic materials and in the PLD synthesis of thin films with tailored structure and composition. Focusing fs laser pulses into a target leads to optical breakdown and generation of a micro-plasma. Because of the nonlinear nature of plasma formation, very fine and highly localized laser effects can be induced while minimizing thermal and mechanical damage to the surrounding material. For fs pulses, the pulse energy threshold for material modification is reduced by some orders of magnitude compared with ns or ps pulses. (75)

In the first case, macroscopic particles (a few micrometers in diameter) are generally present on the surface of the films. The origin of these particles is related to liquid droplets that are generally assumed to be the consequence of thermal effects during laser-matter interaction. This problem can be possibly solved by using femtosecond laser deposition. With ultra-short (fs) pulsed laser, energy deposited does not have enough time to move into the bulk, which neglects thermal effects minimizing the molten droplets present in ns-PLD. (76)

3.4.2 Artifact to successful thin film growth by Pulse Laser Deposition (PLD):

The main issues which contribute to particulates formation are the morphology of the target surface, target density, laser wavelength and fluence, ambient gas nature and pressure, and/or the target-collector separation distance. According to literature, the physical processes which lead to particulates formation are: (i) explosive dislocation of the substance caused by subsurface over boiling of target, (ii) gas phase condensation (clustering) of the vaporized material, (iii) liquid phase expulsion under recoil pressure of ablated substance, (iv) blast-wave explosion at liquid (melt) – solid interface and/or (v) hydrodynamic instabilities evolving on target surface. The optimization of process parameters (use of smooth target surface, combined rotation and scanning the target with respect to the laser beam, proper choice of distance target-collector surface, tilting of substrate orientation against the direction of the plasma-plume expansion, off-axis deposition, appropriate choice of the laser wavelength and fluence, as well as ambient gas nature and pressure) led to the reduction of the particulates density. According to material deposition, parameters are variant and suitable conditions of smooth thin film deposition need to be estimated by testing. Before starting a study was made of the parameters that would be used for the deposition of all films on Ti alloy, Silicon and Sapphire (Al_2O_3). For this the tests were made by varying the pressure, spot size and distance between the target and the substrate. The laser used in this process is **KrF Excimer**, wavelength **248nm**. The pressure ranged from 10^{-3} to 10^{-1} (mbar) and the distance between the target and substrate ranged from **3 to 5.5** cm.

Phase I:

Number of laser pulsed shots for all thin film was 7200
Deposition time 30 minutes
P_{base} - Base pressure before deposition
P_{dep} - Pressure during deposition
T_{dep} - Deposition temperature
D_{T-S} - Distance between target and substrate
f - Deposition frequency

Table 1 - Selected thin film growth parameters by PLD phase I

P_{base} (mbar)	P_{dep} (mbar)	T_{dep} (°C)	D_{T-S} (cm)	f (Hz)	Flux (J/cm2)
1.2X10-4	6.5X10-1	650	4.1	4	1.6

Source: The Author, 2019.

Observation: These parameters did not produce a good film as show in the fig. 12 (a, aa) so it was necessary to modify the deposition pressure to a smaller order of magnitude to adjust the plume, in addition to increasing the distance between target and substrate.

Phase II:

Number of shots for all movies was 7200.
Deposition time 30 minutes.

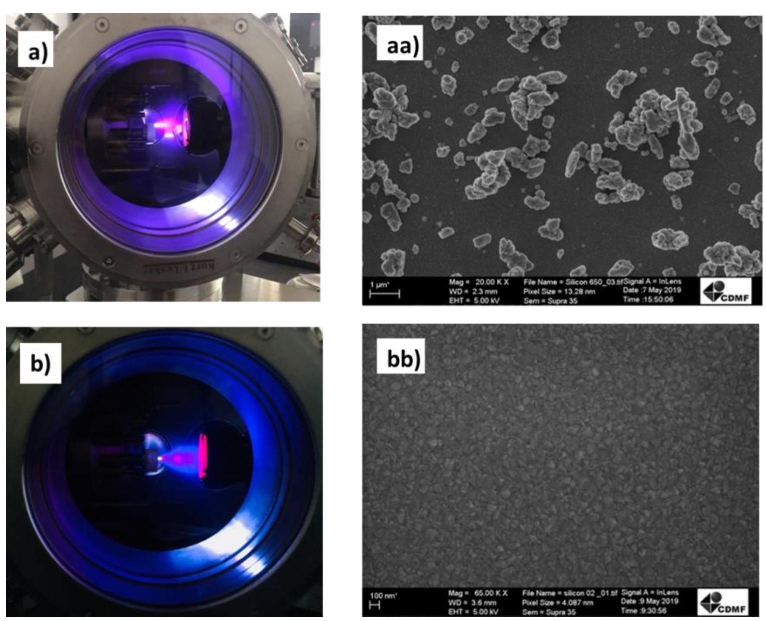
Table 2 - Selected thin film growth parameters by PLD phase II

P_{base} (mbar)	P_{dep} (mbar)	T_{dep} (°C)	D_{T-S} (cm)	f (Hz)	Flux (J/cm2)
1.1X10-4	3.4 X10-1	650	5	4	1.4

Source: The Author, 2019.

Observation**:** These parameters produced a good thin film as show in the fig. 12 (b, bb) and all films were deposited with the following parameters in phase II.

Figure 12 - Thin film growth of gallium oxide by Pulsed Laser Deposition with the impact of plume creation geometry for deposition of Gallium oxide on the substrate surface



Source: The Author, 2019.

Note: a & aa Phase I , b & bb Phase II.

Besides, several other techniques have been developed to decrease the density of particulates, if possible till their complete exemption. These are mainly shadow masks, magnetic filters, gas jet directed into the plume during film growth, post deposition annealing, or dual-laser beam ablation from one target. A significant reduction of particulates can be produced by choosing appropriate parameters. One special reason mention is related to the fragmentation of particulates by means of an additional laser beam propagating parallel to target surface. It was demonstrated that this technique allows for the reduction of particulates density. Nevertheless, the particulates complete elimination was not reached, possibly because of the improper choice of the delay time between ablating and reheating laser pulses surface. [(77)] *As show in the figure 12 morphology of the plume creation with geometry and distance between target and substrate having enormous impact.*

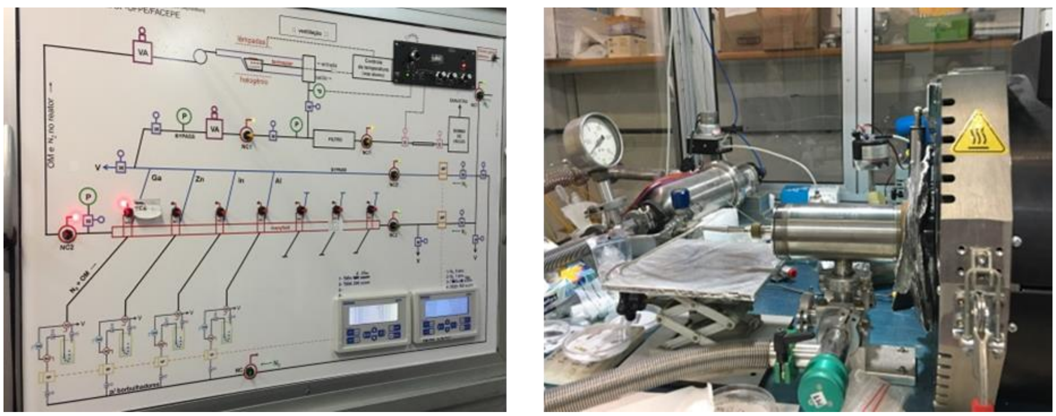
3.5 Deposition system of MOCVD:

In this system the precursor are injected in the form of gas after that it's injected to deposit a thin layer of atoms. There are certain organic or metal organic compounds for chemical reactions that required creating the conditions for crystalline growth. The basic working of deposition started with to bring the precursor in to the reaction chamber where actual event happened to distribute the layer on the substrate. Generally, source of nitrogen for the III-nitrides in the liquid form has sufficient high equilibrium vapor pressure at the room temperature directly delivered to the chamber as gas. For instance this situation is much more complicated for the metallicorganic species. Most frequently metallicorganic are trimethylaluminum (TMA), Trimethylgallium (TMG), and Trimethylindium (TMI) and are found in the liquid form only TMI in solid form. Three of these substances are formed in sealed containers known as bubblers because of pyrophoric nature. Due to this nature ultimate care required to avoid the leakage between the bubblers and reactor. This gas flow as a carrier where it's entre into the dip tube and gets saturated with the vapor phase. The delivered amount depends upon the equilibrium vapour pressure that is usually in the order of micromoles.

3.5.1 Reaction chamber:

For ultra-thin film deposition, specific arrangement in chamber prerequisite to be done the whole process in a closed system. In MOCVD reactor body comprise with the three main parts: top and bottom plate with the side walls chamber. In this process, the top plate helps to enter gases and disperses across the chamber side wall and bottom plate. Along with this side bottom plate deals with electrical heater linked with the rotational shaft motor outside the reaction chamber to provide with the exhausted pump.

Figure 13 - Overview of control system, control panel with electronic system, horizontal reaction chamber reaction chamber



Source: The Author, 2019.

Side by side there is cooling system to prevent from high temperature during deposition. Inside chamber there is substrate sample support typically known as susceptor made of SiC coated by graphite. Graphite is easily machinable material with high thermal conductivity and SiC makes a chemically inert surface to avoid any kind of contamination during deposition. To deal with temperature measurements thermocouple works with the overall side and substrate temperature monitored by the pyrometer. Among many other parameters pressure inside the chamber impact the flow dynamic depending on the design of the chamber reactor normally 50-760 torr. Final major component in MOCVD reactor is loading that consist with the load lock separated by the gate valve with the nitrogen to remove existing oxygen or reactant before running the deposition.

3.5.2 Process of thin film formation of Gallium oxide by MOCVD

For thin film deposition three different surfaces (Titanium, Silicon and Ceramic) substrates are selected to understand the surface composition and morphological phenomena. Commercially pure titanium Ti6Al4V alloy (Bioconect, Medical equipment supplier, Itapira, sao paulo) in the form rectangular bar , 3mm*1.5mm, were cut in 2 mm thick samples by means of an auto-matic cutter provided with an alumina blade. The samples were mechanically polished with liquid alumina of grain size 0.3 and 0.5um on abrasive SiC papers (upto 800 grit) and finally washed in acetone and double distilled water, in an ultrasonic bath, in order to remove surface contamination and oxidized layer.

MOCVD is sophisticated technique to grow the particles on the surface. Deposited layer assisted in different ways to interact with the other surfaces. The main concern in any type of deposition is adherence of particles with substrate. Among many other parameters temperature, time, pressure and precursor play wide role in layering the film on substrate. The mechanical and chemical bonding is primary parameter after controlling the physical parameters. Variations in physical parameters make lots of possible changes in this way. In the present work, after experiencing different issue regarding adherence of deposited material with substrates has got attention to resolve and find such parameters that satisfied the growth conditions.

For thin film deposition metalorganic chemical vapor depositions (MOCVD) deals with the deposit on three different oriented substrates like titanium (Ti–6Al–4V), silicon (0 0 1) and sapphire (1 1 0 2) are desired to deposit gallium 99.99 % under certain physical controlled conditions. The Titanium substrates cleaned with SiC 1200 grad paper with 0.5um alumina to get the mirror like surface. After that substrates washed with organic solvent, dried and preserve. The schematic **MOCVD** chamber deals with the geometry as horizontal reactor manufactured as stainless steel and surrounded by cooled water. For certain growth temperature lamp heated at the graphite subsector to achieve stander growth for deposition at 650^0C for 10 mints. The base pressure was 1.0×10^{-3} Torr maintained and working pressure at 760 Torr. The liquid nitrogen used as carrier gas flowed through **TMGa** bubbler to maintain the negative temperature with the flow rate maintained at 5 sccm. The TMGa (Trimethylgallium) 99.99% was used as precursor medium for suitable growth. After growth, reactor chamber turns off and before taking out the substrates firstly temperature reduce at atmospheric.

3.6 Thermal heat treatment

Thermal heat treatment support in different ways likes to improve the surface adherence, hardness of the material, deposit the oxide layer to make surface more active. The heating furnace with different model and shapes helps to performer this activity. The designs of furnace are choosed according to the desire properties to achieve. It can also work under atmospheric temperature or with ultra-high vacuum condition. Surface oxidation thermal heat treatment (HT) and plasma immersion is commonly used to increase the crystallinity of the material/thin film. This treatment produces a surface roughness on the nanometer scale with high specific surface area and has been found to release no ions *in* vivo. (78) And the role of the oxide layer on the chemical and biological properties biomaterials has been demonstrated in several studies. This oxide layer also has an important role in the adsorption of proteins and calcium and phosphate ions on the bioimplant surface. Oxide thickness and porosity have been shown to have a strong effect on the *in vivo* performance. (79, 80, 81, 82)

Selected parameters for thermal treatment inside the PLD chamber as referred below:

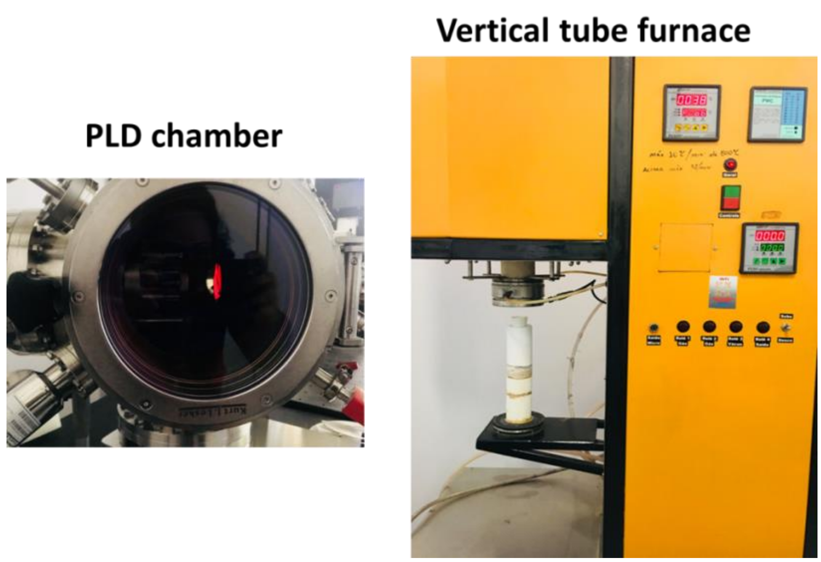
T_{oxy} - Temperature during oxygenation (heat treatment).
P_{oxy} - Pressure during oxygenation.
t_{oxy} - Oxygenation time

Table 3 - Thermal oxidation treatment parameters inside the PLD chamber

T_{oxy} (°C)	P_{oxy} (mbar)	t_{oxy} (min)
600	$4.9X10^2$	60

Source: The Author, 2019.

Figure 14 - Thermal heating electrical furnace and PLD heating treatment chamber



Source: The Author, 2019.

3.7 Microorganism, sample preparation and Culture method Microorganism:

Almost all the groups outside the animal and plant are considering being fall in the microorganism. By classification microorganisms are characterized into *five* types. Bacteria, Virus, Algae, Fungi, Protozoa. Among all of these Bacteria consist of unicell with the body range of about 3-5um. Its contain cytoplasm with cell of protein *mucopeptide* also missing nucleus inside the cell. Diseases causing bacteria having *gelatinous* cell wall that cause harmful effects in the form of pores and binary fusion through their reproductive system. [108]

Identification of the bacteria involved in an infection can help with the rational selection of antibacterial agents. The most commonly used classification of bacteria provides identification on the basis of criteria such as cell wall composition (Gram-positive, Gram-negative), morphology, differential staining, oxygen requirements and biochemical testing. The cell wall of Gram positive bacterial predominantly 40-80% made up of peptidoglycan which is a polymer of N-acetylglucosamine and Nacetylmuramic acid, containing mainly carboxyl, amide, and hydroxyl functional groups. The two other important constituents of Gram-positive cell walls are teichoic acid, a polymer of glycopyranosyl glycerol phosphate, and teichuronic acid, which is similar to teichoic acid, but replaces the phosphate functional groups with carboxyls. The cell walls of Gram-negative bacteria are more complex due to the presence of an outer membrane in addition to a thin peptidoglycan layer, but do not contain teichoic or teichuronic acids. Instead, the outer membrane contains phospholipids, lipoproteins, lipopolysaccharides, and proteins. Several recent investigations examined the surface chemistry of intact bacterial cells and their cell walls using both macroscopic (e.g., potentiometric titration, ion adsorption) and molecular tools (microscopy and spectroscopy). [109,110, 111]

Among many others two species are choosed to test the antimicrobial effect. One related to gram-positive *(S.aureus)* and other gram negative *(E.Coli)*.

The *Staphylococcus* is found in the form of group that can harvest number of infections over the body. Shortly, *Staphylococcus* may know as staph (pronounced "staff).The word *Staphylococcus* derived from the greek *staphyle* significance of bunch of grapes and *kokko* as berry, the reason could be pretend as because its looks like bunch of grapes or little round berries. Its belong from the class of gram positive, facultative anaerobic, usually un encapsulated cocci. Various type of staphylococci can cause infection but mostly are found in the form of *Staphylococcus aureus* that can found over the skin or respiration in the nose. Usually bacteria don’t cause any disease however, wound, injury or skin damage could be lead infection in these conditions. *Staphylococcus aureus* is weakly negatively charged, and therefore attracted to positively charged areas on the surface of orthopedic implants. (112)

Escherichia coli is one of the first causes of Gram-negative orthopedic implant infections, but for pathogenicity of this species is not known as much details. Through experimental conditions only a small amount of E.Coli found to be the biofilm formation on inert surfaces. It is found that E.coli interacts with the bone cell and majorly with the osteoblasts and osteoclasts. Major cause of the infection come from the studies of bone cells stimulated with the bacterial cell wall component LPS (lipopolysaccharide). (113)

Sample preparation: The experimental setup was essentially designed to deposit thin film on Titanium (Ti-6Al-4V), Silicon (100) and Sapphire (1102) substrates by Pulsed Laser Deposition (PLD). The samples were cut with the dimensions (1cm × 1cm ×1cm). The substrates were carefully cleaned with the ethanol and ultra-pure DI water to remove all the extra unnecessary residual on the surface. Moreover, substrates were dried with the N2 gas supply. To follow the deposition procedure already published articles estimated to achieve the uniform thin film. (114, 115) For ablation process Krypton Fluoride (KrF) laser choosed that works in the deep ultraviolet region also known as Excimer laser (λ = 248nm) fixed as a source and Gallium oxide 99.99% prepared as a target. During the film growth, substrate temperature was chosen 650 ^{o}C to attain the crystalline uniform deposition. The deposition chamber was evacuated at the base pressure 1.1×10^{-4} mbar and the working deposition pressure maintained at 3.4×10^{-2} mbarr. The distance was maintained 5cm between target and substrate respectively. The oxygen gas rushed inside the chamber during the film growth to sustain the concentration of oxide on the substrate surface. The Excimer laser sets for the ablation source irradiated with the repetition rate 4 Hz and flux of energy density 1.4J/cm^2 respectively.

Culture method: The bacteria were cultivated by using the agar diffusion method. The antimicrobial activity of the three different substrate surfaces deposited with gallium oxide were tested against the Gram-negative bacteria *Escherichia coli (ATCC 8099, Rockville MD, USA)* and Gram-positive *Staphylococcus aureus* (S.aureus) *(ATCC 6538, Rockville MD, USA)*. The concentration of *Staphylococcus aureus* and *E.coli* was adjusted to 1×10^6 colony-forming units (CFUs)/mL and was introduced to the substrate surfaces of the density about 60uL/cm^2 before all this substrates were sterilized about 120 ^{o}C for 15 minutes [116, 117]. This concentration was hosted to the substrates by drop casting method and incubated at 37 ^{o}C for 24h. In the next step agar plates were prepared by the Luria-Bertain medium further plates were also incubated for 37 ^{o}C for 24h. In the next day, culture plates with active bacteria were spread over it. The bacterial solution dissociated from the substrates and spread over the agar plates to test the effect of antibacterial susceptibility. Moreover, these prepared plates incubated for a day and finally photographed to display.

3.8 Growth characterization techniques

Characterization: The Scanning Electron Microscopy (SEM) was used to study the morphology of the liquid metallic Ga deposition on different substrate. The images were obtained using the commercial Oxford instrument system, operating with 15 keV electron beam and being the secondary electrons collected and analyzed with Silicon Drift Detectors.

The crystal structure of the samples has been evaluated by x-ray diffraction (XRD-7000_maxima.X). The incident beam is formed in a Cu x-ray tube, focused on mirror and finally collimated with a monochromatic source. The incident angle was fixed and the 2θ angle was varied from 10^{o} up to 90^{o}.

The contact angle was measured with the sessile drop model. It is important factor to know for compatibility of surface with biological samples. The sample was examined with DI water in the form of static image at the room temperature.

Three-dimensional images of rough and smooth implant surfaces were obtained by means of atomic force microscopy (AFM) using the tapping mode scan of an AFM. The topography images were obtained using a commercial AFM (MultiMode Nanoscope V, Bruker). The system was equipped with a probe (NCH, Nanoworld, 42 N/m). This system was also used to determine the RMS values. We scanned several randomly selected areas measuring inwhich $10\ \mu m \times 10\ \mu m$ was finding to be with more compatible resolution.

4 RESULTS AND DISCUSSION

This chapter is mainly divided into two parts. In the first part results and discussion explained on the base of surface characterization and the part second deals with the interface assessment by microorganism.

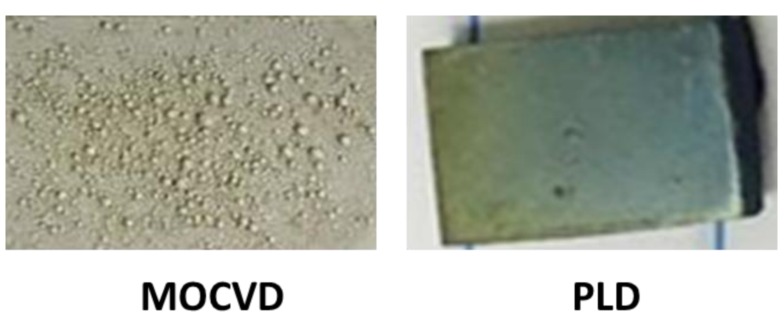
RESULTS AND DISCUSSION: PART I

In this chapter, results are mainly concerned to explain the thin film growth on different substrates. The different deposition techniques are applied to explore the differences and similarities in the following results. The samples are grown in the MOCVD and PLD system by substrate temperature 650^{o}C for 30 mints under appropriate conditions. The among both of these it is expected to explore which technique provided appropriate results in case of uniform film deposition and lower surface roughness. To modify the surface after deposition heat treatment applied under oxygen atmosphere. Two methods are choosed for thermal oxidation treatment, first method applied inside the vertical tube furnace and 2nd inside the PLD chamber. Both treatments are practiced in the presence of oxygen. The discussions are as followed:

4.1 Thin film growth at 650^{o}C by PLD and MOCVD

After optimizing the deposition growth regarding both of these technique MOCVD growths appeared as 3D liquid metallic gallium oxide sphere but weekly attached with the substrates. With the deposition temperature at 650 ^{o}C certain deposited particles separations were found by SEM image that projected the increase in the surface roughness. Likewise deposited thin film by PLD anticipated the uniform growth on three of the substrates. It must be concluded that physical

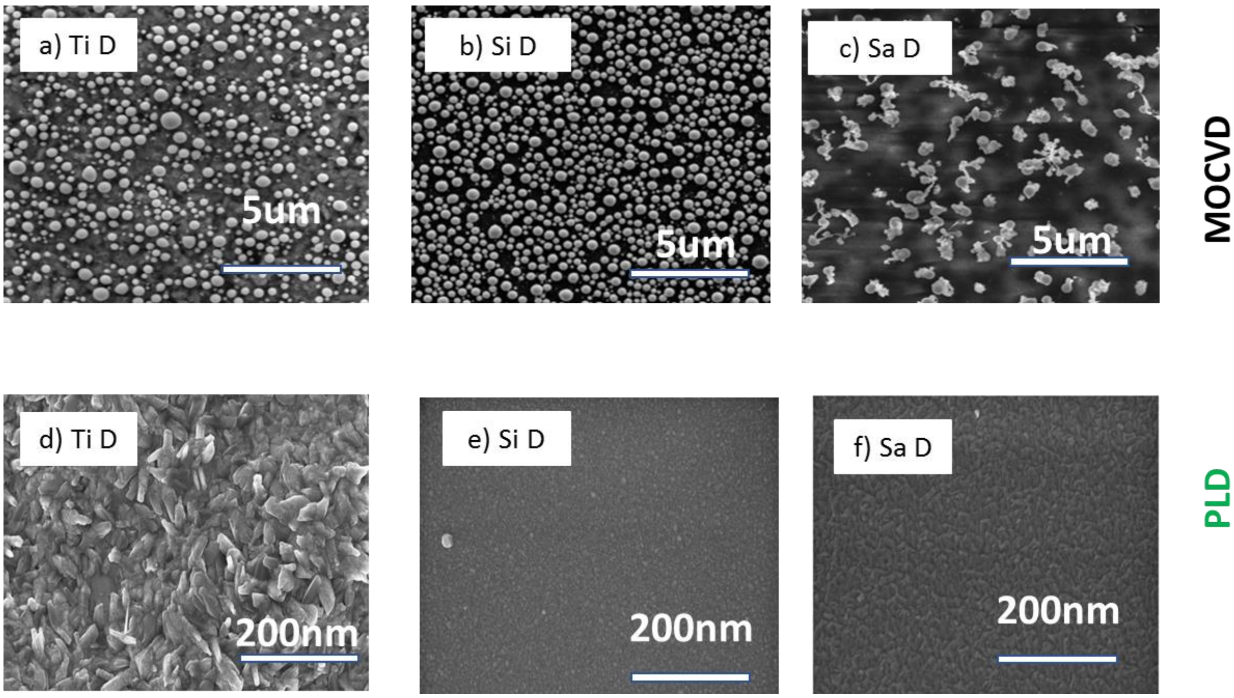
Figure 15 - Physical appearance of thin film deposition of Gallium oxide by PLD and MOCVD



Source: The Author, 2019.

ddeposition supporting the parameters that are required to improve the surface texture for osseointegration phenomena at low temperature and suitable time.

Figure 16 - SEM images presenting the morphology of deposition of gallium oxide by MOCVD on Ti alloy substrate



Source: The Author, 2019.

Note : (a), Silicon substrate (b), Sapphire substrate (c) and deposition of gallium oxide by PLD on Ti alloy substrate (d), Silicon substrate (e), Sapphire substrate (f), also comparison between thin films of both at the temperature 650 ^oC

4.2 Field Effect Scanning Electron Microscopy (FE-SEM) analysis

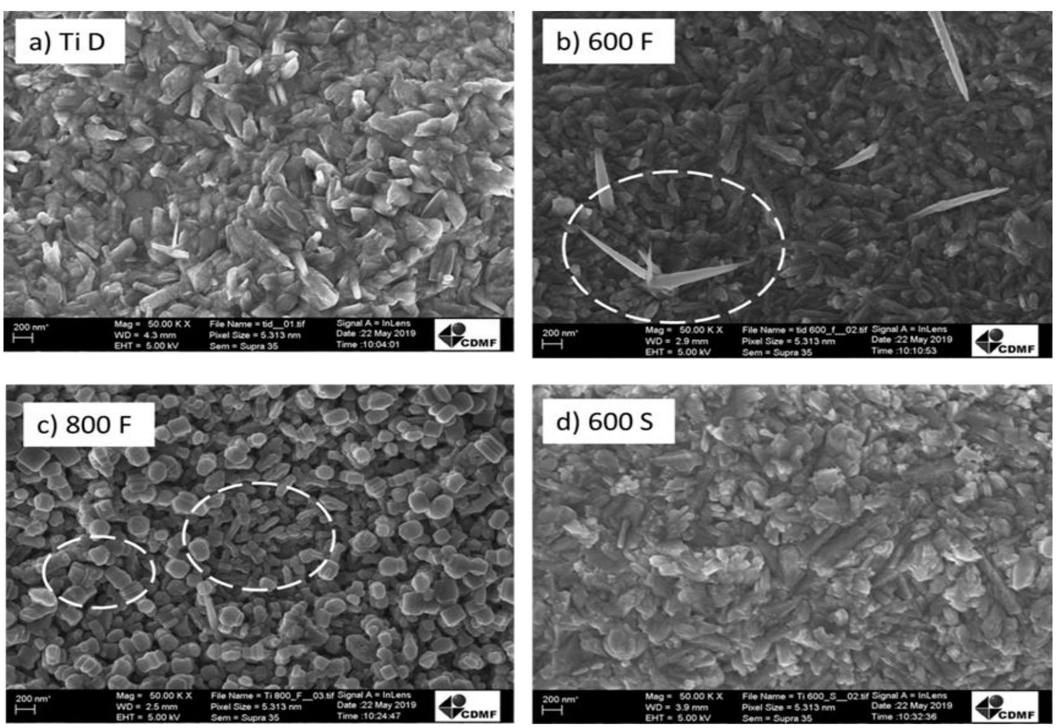
The morphology of the substrates surface with deposition explained by SEM as follow:

4.2.1 Thin film growth of Ga2O3: Ti alloy substrate

The surface morphology of Gallium oxide (Ga_2O_3) this film deposition on Ti alloy substrates showing in the Fig.17. The deposition was carried out by PLD with the substrate temperature 650 ^oC under high vacuum condition. FE-SEM images captured with the resolution 200nm at 50k magnification. Fig.17; (a)**TiD** significance for the deposition on the Ti alloy substrate, it must be observed that the shape of the particle after deposition looks like short length nanorods and these rods are randomly oriented in all directions. These nanorods are uniformly distributed and covered all the surface of Ti alloy substrate. Moreover, tails of the nanorods are embedded with each other and which supporting the adherence on the surface of substrate. However, thickness of nanorods heads certainly not with sharp tips instead of having uniform thickness.

Fig.17; **(b) 600F** indicates the gallium oxide deposition and treatment inside the vertical tube furnace at atmospheric pressure with 600 ^{o}C. Clearly observed that at 600 ^{o}C treatment few nanorods produced with extra sharp edges (showing with dash circle). The reason could be explained on the base of heat diffusion more at certain location on the substrate or interaction of oxide with the Ti or Al present on the surface of substrate. No other difference experienced in the morphology of nanorodes at this heat treatment. Due to heat treatment in oxygen enviroment oxide layer form on each part of rod.

Figure 17 - FE-SEM images of gallium oxide deposition on Ti alloy substrates,



Source: The Author, 2019.

Note: (a) TiD deals with depostion of gallium oxide,(b) 600F indicate that 600 ^{o}C heat tretament in the furnace, (c) 800F deals with 800 ^{o}C heat tretament in the furnace and (d) 600S elaborate heat tretament inside the PLD chamber

Fig. 17; **(c) 800F** is deal with the heat tretament at 800 ^{o}C inside the tube furnace. As the heat tretament increased it was expected that morphlogy will be changed by its shapes.Many studies already has been reported that effect by the heat treatment [84,85,86,87,88].The most notiable change was observed in the **800F**. In this treatment two changed are visualized that can be described as uper and lower part of the surface. For instance on the lower side the morphology is same as nanorods with specific length but at the uper side the morphology is governed as rectangluar and sphere form. Due to heat treatment in oxygen enviroment oxide layer form on each part of rod. This transformation could be due to production of chagres from the target surface or possibly due influence of pressure when the particle accelerate from target to substrate as mentioned in the dash circle form (c). fig. 17; **(d) 600S** point towards the heat treatment isde the PLD chamber in the presence of high vacuum. From the **(d)** FE-SEM analysis it is observed that after heat treatment morphology appeared as nanorods but in the end tips branched into aparts and unable to maintained the shape. In this heat treatment transferred as by the conduction mechanism in which substrtae placed on the heater plate.

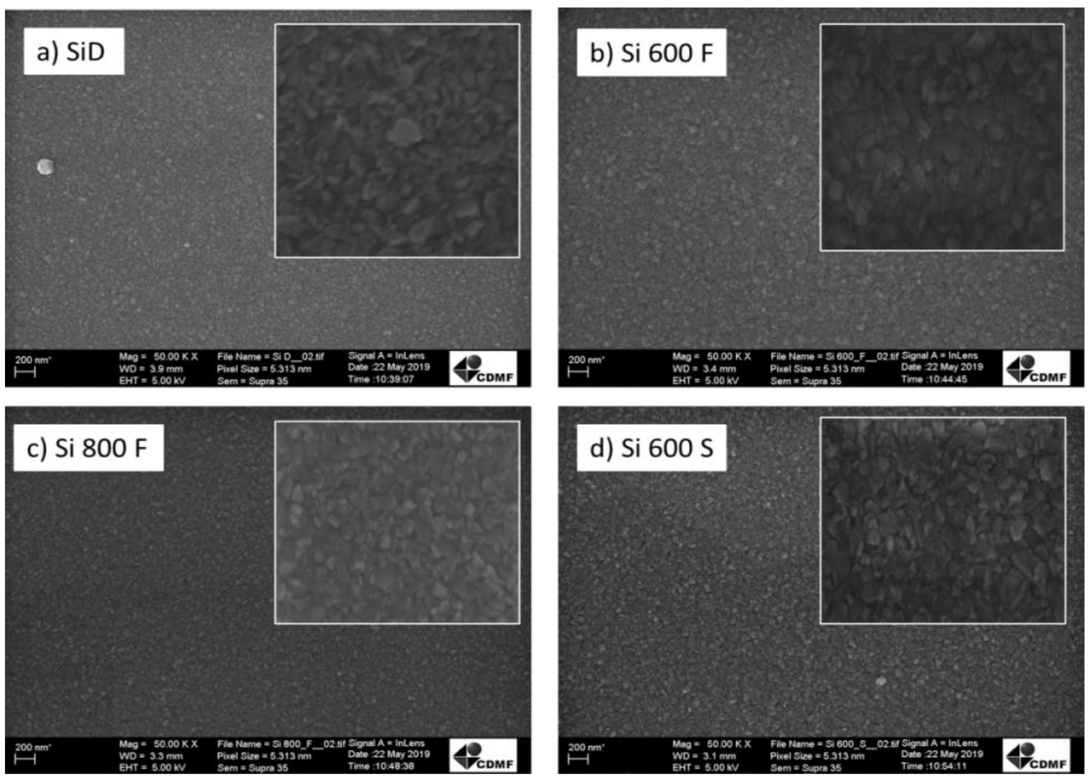
By comparing the two thermal oxidation treatments **(b) 600F** and **(d) 600S** both at the sample temperture 600 ^{o}C but at different pressures. In 600F change is observed in the form of extended sharp edges and at 600S with the branched edges. If we looks towards the 600F heat treatment is under atmospheric pressure further in 600S high vacuum environment. So here its must be conclded that inside the chamber the physical parameters and quality can be assured but under atmospheric pressure treatment cant be approved due to presence of gases in the surrounding.

4.2.2 Thin film growth of Ga2O3: Si substrate

The surface morphology of gallium oxide deposition on Si substrate as thin film produces successfully with less and less defects or residuals. As the deposition method is PLD by heating the substrate at 650 ^{o}C. Before deposition surface of substrate was having the structure as Si (100). Fig. 18; **(a) SiD** shows the deposition growth on the Si substrate. By clearly observed the deposited film grain size appeared in the with random boundary although the size of the grain is too small all appeared as smooth film. After thermal oxidation at **(b) Si600F** inside the furnace no modification in the grain size witnessed. Further inducing the more temperature up till **(c) Si800F** in the furnace at atmospheric pressure no alternation in the grain size perceived. By using the 2nd

heat treatment method inside the PLD chamber **(d) Si600S** the deposition expected to be with the same results as **Si600F**. So in this situation it can be concluded that if the gain size is less and less in the nm range thermal effect unable to influence the thin film particle size ultimately its experienced smooth. On the other side, it can be assumed that at this stage might be chemical alternation possible rather than morphological. Moreover, all the surfaces contain the oxide layer on the surface of gallium oxide deposition because treatment was done under oxygen presence to avoid the loss of oxygen more due to heating effect.

Figure 18 - FE-SEM images of gallium oxide deposition on Si substrates



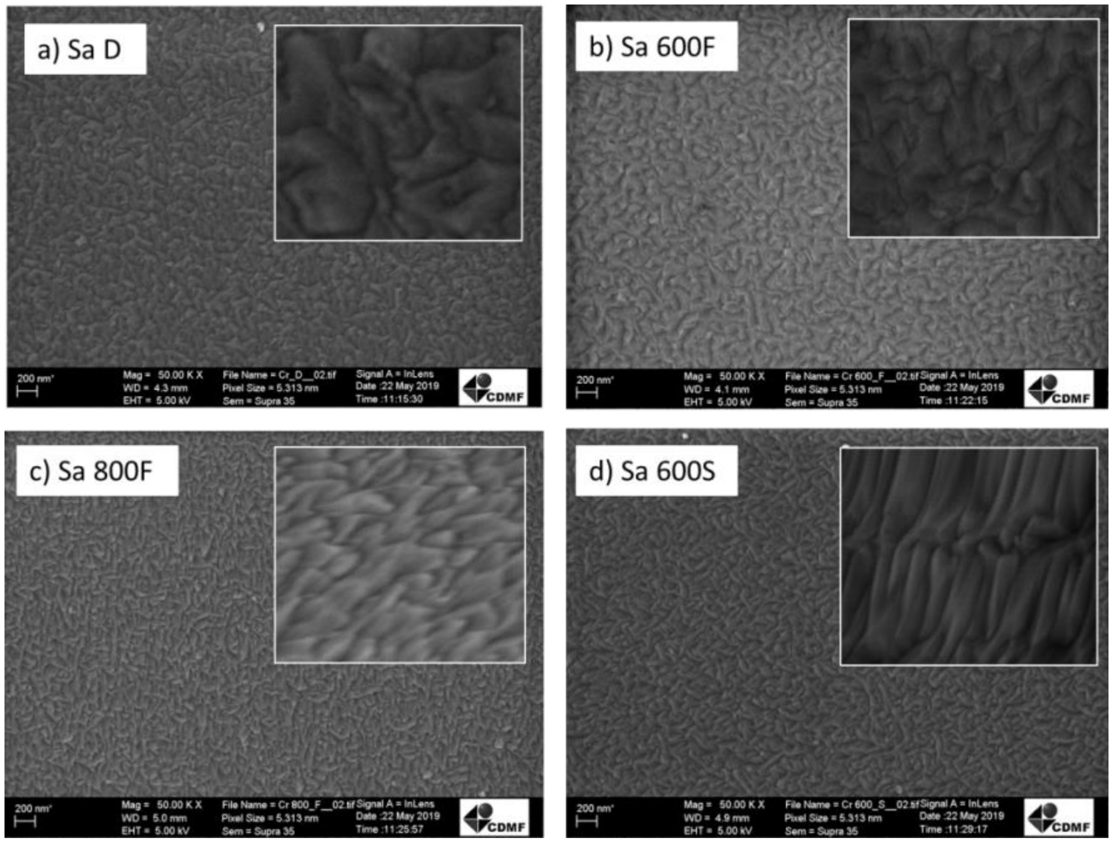
Source: The Author, 2019.

Note: (a) SiD deals with deposition of gallium oxide,(b) 600F indicate that 600 ^{o}C heat treatment in the furnace, (c) 800F deals with 800 ^{o}C heat treatment in the furnace and (d) 600S elaborate heat treatment inside the PLD chamber

4.2.3 Thin film growth of Ga2O3: Sapphire substrate

The topography of gallium oxide deposition on Sapphire substrate as thin film yields effectively. Without deposition the sapphire substrate is surface having the orientation as (1102). Thin film

Figure 19 - FE-SEM images of gallium oxide deposition on Sapphire (Sa) substrates



Source: The Author, 2019.

Note: (a) SaD deals with deposition of gallium oxide, After deposition (b) 600F indicate that 600 ^oC heat treatment in the furnace, (c) 800F deals with 800 ^oC heat treatment in the furnace and (d) 600S elaborate heat treatment inside the PLD chamber

method adopted by PLD through heating the substrate at 650^{0}C. Fig. 19; **(a) SaD** shows the deposition growth on the Sapphire substrate. By clearly observed the deposited film oriented in specific direction and reason could be estimated that as substrate surface already oriented. Thin film appeared continuous as layer by layer. The topographical view seems to be smooth and layers are unidirectional, overlapping on each other continuously. After thermal oxidation, at **(b) Sa600F** inside the furnace minuet dislocation in layer observed due to heating effect. Further, these dislocations assumed to be layer by layer assembled with the adjacent eachother. Further, persuading the more temperature until **(c) Sa800F** in the furnace at atmospheric pressure dislodgment in the layer are found centered merging and oriented in a specific direction. By using the 2nd heat treatment method inside the PLD chamber **(d) Sa600S** the deposition expected to be having consequence of treatment effect. So in this situation it is experienced that interruption classified as longitudinal extended as elastic band with the adjacent central part. Moreover, all the surfaces contain the oxide layer on the surface of gallium oxide deposition because treatment was done under oxygen presence to form the active layer of oxide and to avoid the loss of oxygen due to heating effect.

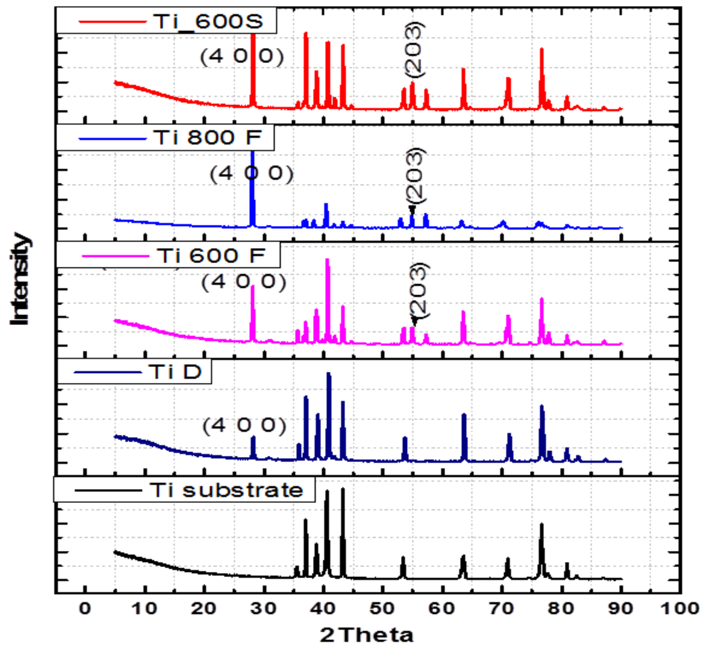
4.3 X-ray diffraction analysis

Fig. 20 displays the XRD patterns of the gallium oxide films grown at Ti alloy substrate. When the substrate temperature was heated to 650°C and thin film was deposited as shown in the Fig. 20,**TiD** only a Ga_2O_3 (400) diffraction peak can be detected in the film, which was attributed to the crystalline nature of the successfully deposited gallium oxide as already repeated [89,90]. The diffraction peak indexed was very narrow and rests of the peaks were matched with the substrate surface.

Further increasing the substrate temperature to 600 and 800°C respectively as **Ti600F, Ti800F** treatment inside the furnace, the XRD patterns presented single peaks indexed to (400) and (203) planes both within these two gallium oxide films. The peak fitting to the plane family was associated to the β-Ga_2O_3 phase. However, the XRD patterns also implicit that the 600 °C- and 800 °C-grown gallium oxide films both possessed single crystalline structure. With growing thermal treatment temperature from 600 to 800 °C, the intensities of these diffraction peaks all modified longer and sharp. This singularity can be explained by the thermal energy supplied from the substrate temperature or environment inside furnace. In the last, **Ti600S** substrate with surface deposition and heat treatment implies inside the furnace present the gallium oxide crystalline structure with the peak indexed at (400) & (203).

Compared with the 600 °C-grown film, **Ti600F and Ti600S** two different heat treatment methods found that peaks indexed are same but with different intensities. So from these results it can be concluded that **Ti600S** provided the more good results because this treatment was under clean high vacuum conditions other was under atmospheric pressure. If more thermal energy can be provided to the ad-atoms on the substrate while the substrate temperature was enlarged to 800 °C, leading to enhancements in the surface mobility and the intensity of plane family positioning. Another key point was originated that after heat treatment one more peak detected that witness of the crystalline structure modification.

Figure 20 - XRD patterns of the gallium oxide films grown at Ti alloy substrate temperature 650^{o}C by PLD



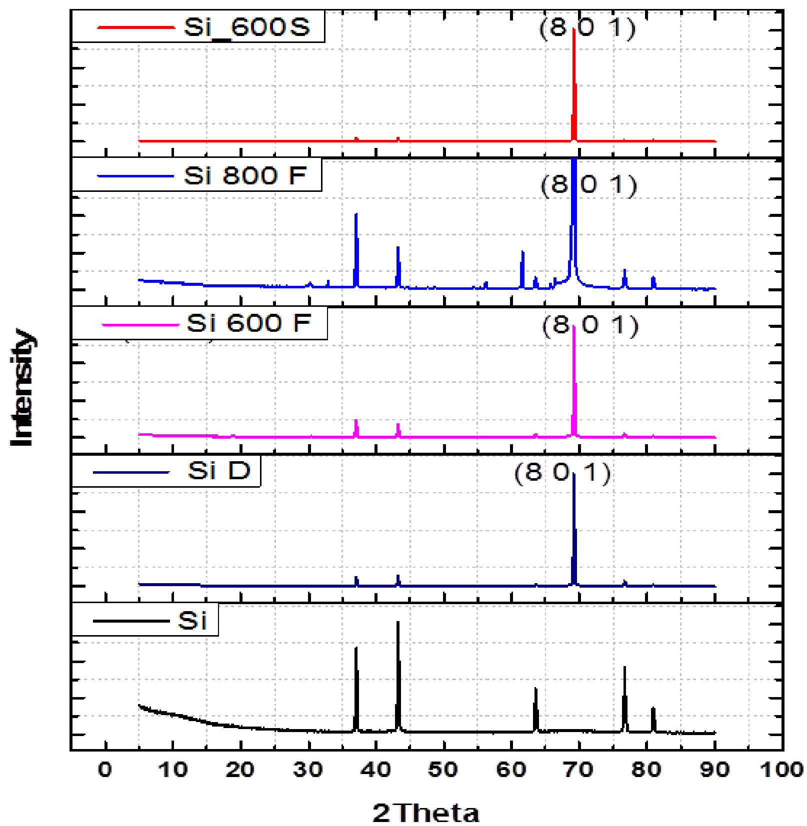
Source: The Author, 2019.

Note: (a) TiD significance for deposition, (b) Ti 600F heat treatment inside the furnace at 600 ^{0}C after deposition, (c) Ti 800F heat treatment inside the furnace at 800 ^{o}C after deposition, (d) Ti 600S heat treatment inside the PLD chamber at 600 ^{o}C after deposition.

Fig. 21; showing the gallium oxide thin film deposition diffraction pattern by using Pulsed Laser Deposition (PLD) with the substrate temperature 650 ^{o}C. In the Fig.21; the displays the XRD patterns of the gallium oxide films grown at Si substrate. When the substrate temperature was heated to 650°C and thin film was deposited as shown, **SiD** only a Ga_2O_3 (801) diffraction peak can be detected in the film, which was attributed to the crystalline nature of the successfully deposited gallium oxide [91]. The diffraction peak indexed was very narrow and rests of the peaks were matched with the substrate surface.

Further increasing the substrate temperature to 600 and 800°C respectively as **Si600F, Si800F** treatment inside the furnace, the XRD patterns presented single peaks indexed to (801) plane in **Si600F**. The peak fitting to the plane family was associated to the β-Ga_2O_3 phase. However, the XRD patterns also implicit that the 600 °C grown gallium oxide film possessed single crystalline structure but at 800 °C gallium oxide peak was detected as in **Si600F** but change in intensity counts increased as indication of the modification in the crystalline structure. The reason could be explained on the base of high temperature treatment and all deposition lose it adherence and evaporate in the air. The singularity of **SiD, Si600F** can be explained by the thermal energy supplied from the substrate temperature or environment inside furnace. In the last, **Si600S** substrate with surface deposition and heat treatment implies inside the furnace present the gallium oxide crystalline structure with the peak indexed at (801).

Figure 21 - XRD patterns of the gallium oxide films grown at Silicon substrate temperature 650 °C by PLD.



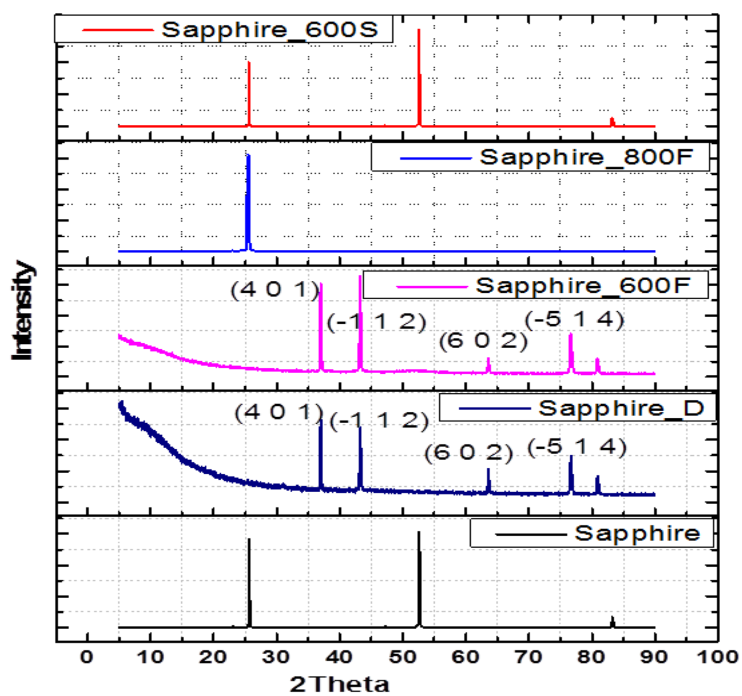
Source: The Author, 2019.

Note: (a) SiD significance for deposition, (b) Si 600F heat treatment inside the furnace at 600 ^{0}C after deposition, (c) Si 800F heat treatment inside the furnace at 800 °C after deposition, (d) Si 600S heat treatment inside the PLD chamber at 600 °C after deposition.

Compared with the 600 °C-grown film, **Si600F and Si600S** two different heat treatment methods found that peaks indexed was same but with different intensities. So from these results it can be concluded that **Si600S** provided the more good results because this treatment was under clean high vacuum conditions other was under atmospheric pressure. If more thermal energy can be provided to the ad-atoms on the substrate while the substrate temperature was enlarged to 800 °C, leading to removal of deposition film from the silicon substrate. So temperature must be less than 800^0C to safe the film. Another point was noticed that **SiD, Si600F** and **Si600S** were appeared with the same diffraction index peak no alternation in the plane angle.

Fig. 22; showing the gallium oxide thin film deposition diffraction pattern by using Pulsed Laser Deposition (PLD) with the substrate temperature 650 ^{o}C. In the Fig. 22; the displays the XRD patterns of the gallium oxide films grown at Sapphire substrate.

Figure 22 - XRD patterns of the gallium oxide films grown at Sapphire substrate temperature650 ^{o}C by PLD.



Source: The Author, 2019.

Note: (a) SaD significance for deposition, (b) Sa 600F heat treatment inside the furnace at 600 ^{o}C after deposition, (c) Sa 800F heat treatment inside the furnace at 800 ^{o}C after deposition, (d) Sa 600S heat treatment inside the PLD chamber at 600 ^{o}C after deposition

When the substrate temperature was heated to 650°C and thin film was deposited as shown, **SaD** polycrystalline structure grown of Ga_2O_3 with (401),(-112),(602) and (-514) diffraction peaks can be detected in the film, which was attributed to the crystalline nature of the successfully deposited gallium oxide . The diffraction peak indexed was very narrow and rests of the peaks were matched with the substrate surface.

Further increasing the heat treatment to 600 and 800°C respectively as **Sa600F, Sa800F** treatment inside the furnace, the XRD patterns presented multiple peaks indexed to plane in **Sa600F**. The peak fitting to the plane family was associated to the β-Ga_2O_3 phase. However, the XRD patterns also implicit that the 600 °C grown gallium oxide film possessed polycrystalline e structure but at 800^{o}C no gallium oxide peak was detected. The reason could be explained on the base of high temperature treatment and all deposition lose it adherence and evaporate in the air. On the other hand, it could be estimated that peak intensity increased as compare to sapphire substrate which support that growth of film orientation resembled with the substrate orientation surface. The singularity of **SaD, Sa600F** can be explained by the thermal energy supplied from the substrate temperature or environment inside furnace. In the last, **Sa600S** substrate with surface deposition and heat treatment implies inside the furnace present the gallium oxide crystalline structure with the peaks indexed (-5 1 4).

Table 4 - XRD different peaks indexed of Gallium Oxide on different substrates

Substrates	**2Ө (Degree)**	**Ga2O3 peak detection (hkl)**	**Intensity (CPR)**	**Ref. Article**
TiD	28.2	(400)	1000	Ref. [89,90]
Ti600F	28.2, 54.92	(400), (203)	2555, 777	Ref. [89,90]
Ti800F	28.2, 54.92	(400), (203)	10264,1093	Ref. [89,90]
Ti600S	28.2, 54.92	(400), (203)	3016,1093	Ref. [89,90]
SiD	69.14	(801)	102480	Ref. [91]
Si600F	69.14	(801)	50882	Ref. [91]
Si800F	69.14	(801)	17895	Ref. [91]
Si800S	69.14	(801)	167635	Ref. [91]
Sapphire D	36.98,43.22, 63.56,76.68	(401), (-112), (602), (-514)	1148,1098, 493, 687	Ref. [89,90,91]
Sapphire 600F	36.98,43.22, 63.56,76.68	(401), (-112), (602), (-514)	3355,3645, 685, 1565	Ref. [89,90,91]
Sapphire 800F	No peak	None	None	None
Sapphire 600S	No peak	None	None	None

Source: The Author, 2019.

Compared with the 600 °C-grown film, **Sa600F and Sa600S** two different heat treatment methods found that peaks indexed was not similar. So from these results it can be concluded that **Sa600S** diffraction peak grown with the same dimension as the substrate surface. If more thermal energy can be provided to the ad-atoms on the substrate while the substrate temperature was enlarged to 800 °C, leading to removal of deposition film from the substrate. So temperature must be less than 800 ^{o}C to safe the film. Another point was noticed that **SaD, Sa600F** were appeared with the same diffraction index peak no alternation in the plane angle but with the more counts rate.

4.4 Energy Disperse Spectroscopy (EDS):

Table 05; show the chemical composition of gallium oxide thin film deposition on different substrate surfaces. The present results contained the information of elemental composition before and after treatment. The substrates are thermally oxides with the temperature 600 and 800 ^{o}C. The composition values are varied by different percentage. Although the EDS results of the chemical composition investigation vary slightly, it can be concluded that they are consistent and indicate that the oxide layer is formed mainly of gallium oxide. The atomic percentage significant only the estimated values not exact.

Table 5 - Energy Disperse Spectroscopy (EDS) calculated results of elemental composition with atomic and weight % with the presence of Gallium, oxygen and substrates elements

Composition	**Gallium**		**Oxygen**	
Substrate	**At%**	**Wt%**	**At %**	**Wt%**
Ti D	5.61	13.85	65.44	37.09
Ti 600_F	18.17	39.77	63.11	32.03
Ti 800_F	5.75	13.83	63.42	35.12
Ti 600_S	2.83	7.23	66.80	39.37
Si D	12.21	29.18	33.09	18.15
Si 600_F	20.75	45.51	40.88	20.58
Si 800_F	11.56	27.65	30.93	16.97
Si 600_S	11.87	28.22	30.66	16.73
Sa D	23.30	52.41	54.08	27.73
Sa 600_F	27.23	58.18	54.54	26.74
Sa 800_F	19.44	47.12	59.47	33.09
Sa 600_S	15.39	38.81	53.87	31.19

Source: The Author, 2019.

4.5 Surface roughness

For surface topographical investigation at the micrometer or nanometer level two major standards exist, contact and non-contact methods. With a contact stylus is among the most widely used industrial method. Basic principle for contact stylus instruments designed with the diamond tip that is traversed over the surface at a contact velocity inwhich load applied to make assure tip never loss contact. The moment is controlled by the electrical signal which is amplified before being converted into digital information. As far as non-contact method deals with the optical profilometers provide the same possibilities for surface roughness parameter calculation but with image production. Compared to the mechanical stylus, optical techniques are relatively new, but have reached an increasing popularity, in part since the non-contact technique has an advantage when measuring soft materials. Other important advantages are that optical methods, in general, are faster than contact methods, and that they often have better resolution in the horizontal direction. Vertical measuring range is up to 1 mm. Examples of different principles used in commercially available devices are: interferometry, auto focus detection and confocal laser scanning microscopy.(92) Measurement of the roughness parameter of the orthopedic implant surface morphology is important because its value influences the adhesion, adsorption and differentiation of the cells. The most regularly used dental implant roughness parameter is Ra, the arithmetic medium value of the deviations of the roughness profile in relation to a medium line. Other important roughness parameters are the quadratic medium value of the roughness (Rq or Sq). (93) An important parameter for the clinical success of dental implants is the formation of direct contact between the implant and surrounding bone, whose quality is directly influenced by the implant surface roughness. (94)

Table 6 - Surface roughness (RMS) values with the SD on different substrates

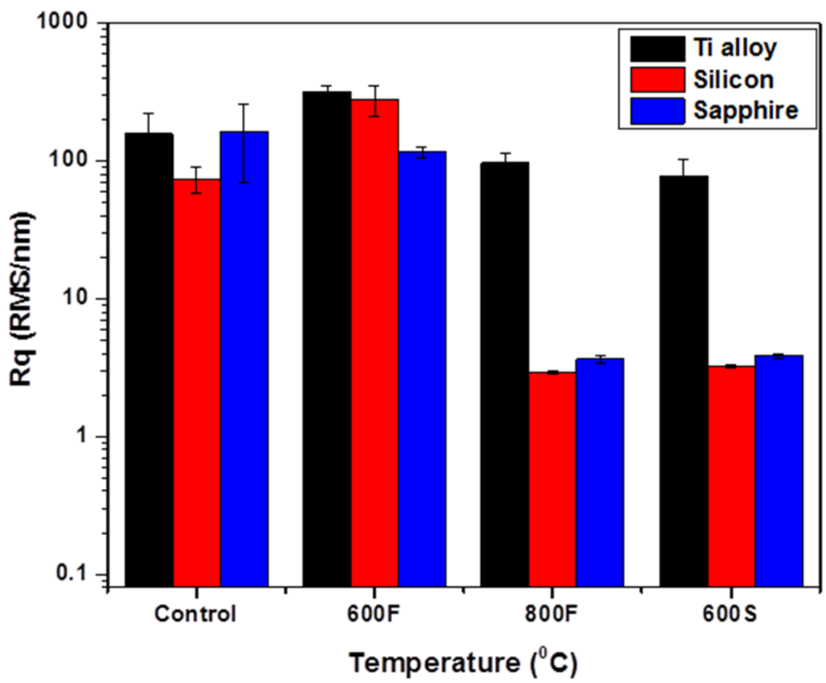
Substrates	**Ti alloy**	**Silicon**	**Sapphire**
Temperature	**Mean ±SD**	**Mean ± SD**	**Mean ± SD**
Control	157.53333± 65.95797	74.7± 16.17436	164.26667± 94.81779
600F	318.33333± 318.33333	281.66667± 70.23057	116.33333± 10.21437
800F	97.63333± 18.20449	2.9± 0.08185	3.64333 ± 0.24194
600S	77.56667± 26.05002	3.24± 0.0755	3.83667 ± 0.15535

Source: The Author, 2019.

From the graphical analysis fig. 23 thin film deposited on three different substrates by PLD with the substrtae temperture 650^0C for all. The data is divided in four groups"Control" group deals with only surface deposition at 650^0C with no thermal tretament. Next 600F and 800F deals with the thermal oxidation treatment under atmospheric pressure in vertical tube furnace. However, group 600S indicate the surface treatment in the PLD chamber with the high vacuum.

Different surface roughness values are observed with varing the temperature range. It is oberved that when the deposition temperture (Control 650^0C) reached with the (600F) the surface roughess slighly matched with eachother with no big difference. As the thermal temperature increased the roughness value decreased at 800F as compare to 600F because of nano structure formation on the substrate. Moreover, as compare to the 600F and 600S, the surface roughness has the less value incase of tretament inside the PLD chamber. Its reason could be explained on the base of quiality of environment and influence of temperature on the morphlogy of the structure of the deposited particles.

Figure 23 - Surface roughness calculated on different substrates surfaces with and without thermal oxidation treatment.



Source: The Author, 2019.

Note: Here "F" deals with the heat treatment under atmospheric pressure inside vertical tube furnace. Moreover, "S" significance for heat treatment inside PLD chamber/system.

Surface roughness, as a component of the surface texture, gives an indication about the quality of the surface. The average roughness of Ti alloy, silicon and sapphire substrates films annealed at temperature 600, 800 ^{o}C respectively. The thermal oxidation treatments are tested under two kinds to environments. First is among atmospheric pressure and other one under high vacuum condition. This shows that as the temperature of films increases, average roughness increases and then decreases. The decrease in roughness leads to good homogeneity of films. It indicates the good interaction of gallium oxide particles in different layers of films. As a result, good film has been formed. The increase in roughness is due to nucleation of the particles with increment of temperature. [95] It must be noted that at 800F and 600S the surface roughness deals with almost same results. From this it can be concluded that 600S consider as good because for this it consume less temperature to approach the same results as in the 800F. Moreover, 600S texture formation under quality controlled environment.

There are several approaches to improve the blood compatibility with the bioimplant surface. This includes alteration of the surface free energy, introducing of positive or negative charges or more complex functional groups. These studies indicate that simple morphological or chemical characteristics have less effect than functional groups. [96, 97, 98]

From surface texture point of view the surface roughness must be less and less to found more compatible with the biological adherence. Materials in contact with blood frequently show different behavior concerning the activation of blood platelets or the clotting cascade. Surface roughness below 50 nm or crystal structure have only minor effects on the blood compatibility.[99]

From the present study it must be concluded that if the substrate surface roughness is less before the depositing than thin layer low surface roughness value can be achieved.

4.6 Contact angle/ surface wettability

The phenomenon of wettability mainly deals with interaction of solid surface with liquid known as contact angle (CA). The concept of contact angle is not new which was first described in 1890 by Thomas Young. After this during the following years many theoretical and experimental studies have been done. By these studies many relationships established from different aspects among surface roughness, surface energy and hydrophlicity. It has been reported that wettability and surface energy play vital role in the bioimplant surface to interact with the biological environment. If the surface is treated in a suitable way than absorption reaction materializes more easily on the surface. From surface modification point of view surface energy and wettability has good reciprocal relationship with each other. By increasing the surface energy wettability angle decreased which established the more compatible surface as hydrophilic. [100, 101] In the article it is mention that heating the substrate alter the morphology of the sample that relatively vary the roughness. It is expected that contact angle is diminished in the range of 90^0-15^0 degree when the surface is treated. [102]

Figure 24 show that static contact angle and surface wettability was measured by sessile drop casting method using DI water at room temperature (22 ^{o}C). The water droplet was captured 30 second after delivered on the surface. In this work, it was tried to explore the wetting behaviour dynamics on three different substrates surfaces before and after thermal treatment. The surfaces are dividing as metal, insulator and semiconductor with the deposition of gallium oxide at 650 ^{o}C for 30 mints. During the investigation of contact angle performance there is significant difference in the wettability angle after treating the surface thermally.

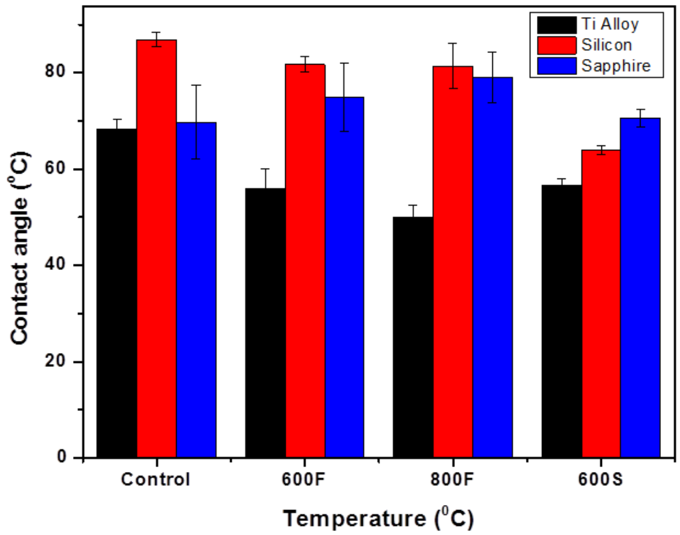
Table 7 - Average behaviour of Contact angle variation with the Mean±Stander deviation values of different substrates

Substrates	**Ti alloy**	**Silicon**	**Sapphire**
Temperature	**Mean ±SD**	**Mean ± SD**	**Mean ± SD**
Control	68.26667 ± 1.98578	86.9 ± 1.51327	69.73333 ± 7.6068
600F	55.83333 ± 4.26654	81.73333 ± 1.60104	74.9 ± 7.04486
800F	49.93333 ± 2.55408	81.36667 ± 4.73849	79.13333 ± 5.24817
600S	56.66667 ± 1.45029	63.83333 ± 0.90185	70.6 ± 1.73494

Source: The Author, 2019.

From the graphical analysis when the surfaces are not treated the contact angle is less than 90^o but after treatment the surfaces gets completely hydrophilic. As increasing the heating temperature in the presence of oxygen the contact angle decreased and the surface behaved hydrophilic. This hydrophilic property helps to enhance the surface to adhere the biological substance. It must be concluded that as the surface energy increased the contact angle decreased.

Figure 24 - Contact angle calculated on different substrates surfaces with and without thermal oxidation treatment.



Source: The Author, 2019.
Note: Here "F" deals with the heat treatment under atmospheric pressure inside vertical tube furnace. Moreover, "S" indicates for heat treatment inside PLD chamber/system

The healing process around the bioimplant occurs through a gradual mineralization process from the bone towards the implant. The biological cells in contact with the turned surface allow the bone mineralization. Besides this, the healing process takes several days, and the remodeling process takes weeks or years. The healing time for dental implants without surface treatment is higher than that for implants with treated surfaces. With smooth surfaces, the biological processes at the bone implant interface are slower. To minimize the mineralization time, titanium surface treatment is carried out. This procedure accelerates the adhesion micro mechanisms between the implant and the bone. [103, 104, 105] With surface treatment, it is possible to change the surface features of the implant, such as chemical composition, energy level, morphology, topography and roughness. The morphology and roughness can be controlled by treatment with acid solution or oxidation. [106, 107]

RESULTS AND DISCUSSION: PART II

This part II enclosed with the investigation of antibacterial assessment of gallium oxide thin film coated with the Pulsed Laser Deposition (PLD) on the three different substrates. The antibacterial efficiency is already reposted in many articles but after deposition of gallium oxide on the substrate did not stated any study. In this study, substrates are classified as metal, semiconductor and insulator after that gallium oxide thin layer embedded with the physical deposition technique. Two microorganism species tested to support the antibacterial effect and compare the differences. The investigated results were described on the bases of charge transportation due to the fact of band theory and different surfaces consequence. For further explanation the I-V electric characteristic curves explored to support the overall mechanism of thin film effectiveness.

Aim and objectives:

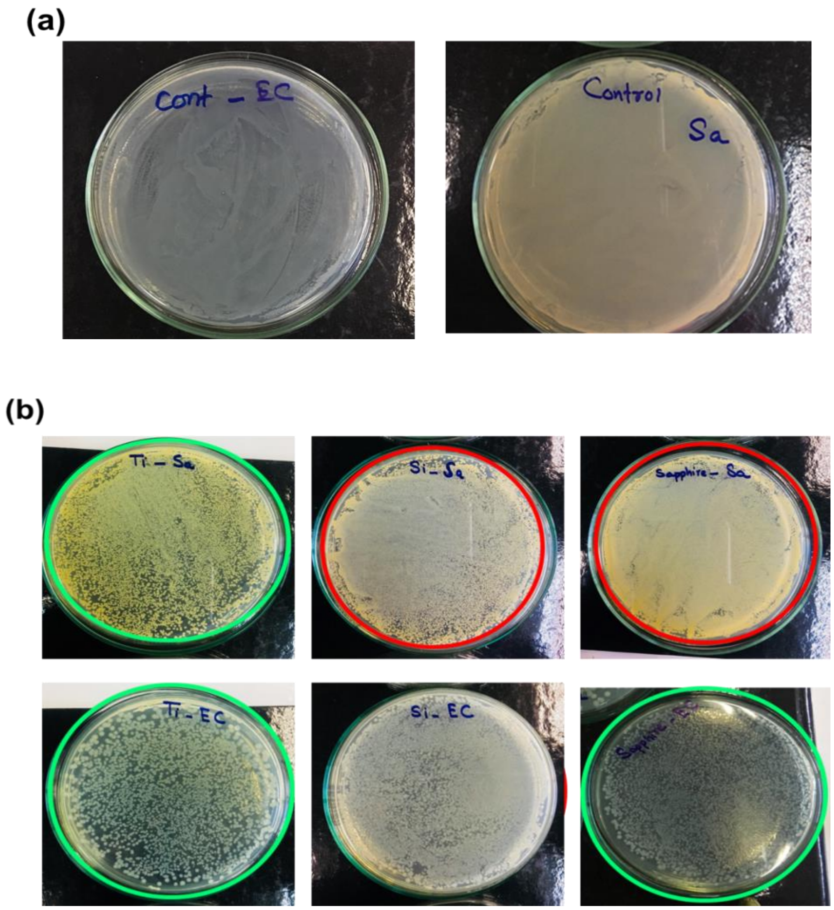
The purpose of this investigation is to understand the antimicrobial mechanism of cell death and charge transportation phenomena with and without substrate effect. To study this certain steps need to follow as:

- To evaluate efficacy of Gallium Oxide on gram positive and negative bacteria
- Comparison of cell death morphology between S. aureus and E.Coli biofilms using SEM image
- Interface assessment by I-V electrical characteristic of the thin film with charge transfer phenomena from the substrates

4.7 Bacterial response to Gallium Oxide thin film on cell culture media

From the fig. 25 (a), was presenting the control cultivation of bacterial growth S.aureus and E.coli to compare with the other results. In the fig. 25 (b) response of the gram positive and gram negative bacterial were presented to evaluate which specie provide the more effective outcome after the bacterial-material interaction. In order to inspect the responses of both Gram-positive *Staphylococcus aureus (S. aureus)* and Gram-negative *Escherichia coli (E. coli)* cells to the gallium oxide thin films on different substrates, for example Gallium oxide @Ti alloy, Gallium oxide@Si and Gallium oxide@sapphire, the adhered bacteria were dissociated from the surfaces, re-cultivated on agar, and evaluated by using the bacteria counting method.

Figure 25 - Typical analysis of antibacterial-material assessment of E.Coli and S.aureus colonies



Source: The Author, 2019.

Note: (a) Control colonies; (b) on agar culture plates, with the seeded concentrations of bacteria onto gallium oxide film being 10^6 CFU

Fig. 25 (b) offers the distinctive photographs of *E. coli* and *S. aureus* bacteria colonies number on three types of gallium oxide films the $1{\times}10^6$ CFU concentrations of bacteria. It must be scrutinize that Gallium oxide thin film more effective on the E.coli as compare to S.aureus. On the other hand results can be evaluated on the bases of substrates surfaces as: E.coli was not able to survive on the Gallium oxide@Ti alloy and Gallium oxide@sapphire surface respectively. As far as when culturing S.aureus bacteria's only Gallium oxide@Ti alloy was not able to persist on the surface and rest of the surfaces unable to confirmation the results.

It must be concluded that gallium oxide with the substrate having different effect on the bacteria because substrates were also having its impact. Metal surface are highly charged, high energy surfaces and hydrophilic as compare to insulator which govern the less susceptibility of charge. Despite of the fact many others factor influence like physical adhesion, charge surface of bacterial, chemical transportation between two interfaces pointed in antimicrobial.

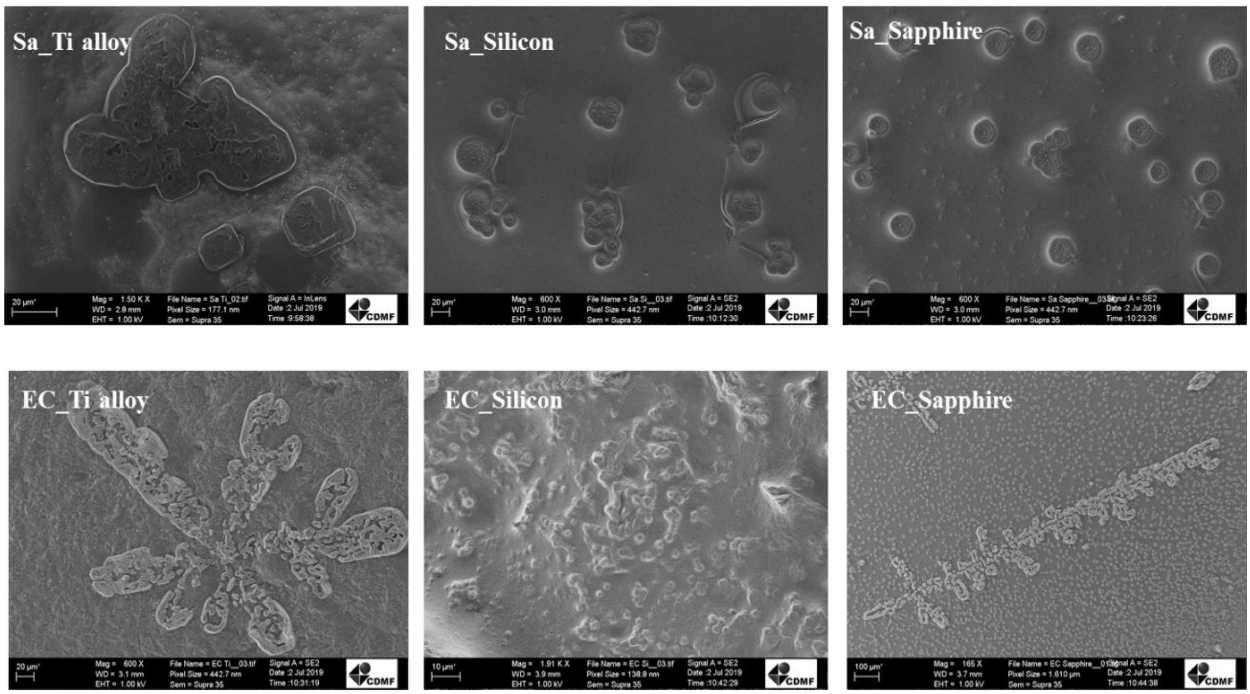
4.8 Efficacy of Gallium Oxide thin film on the bacterial cell membrane morphology analysis

Biofilm formation is a multifaceted developmental process comprising attachment and immobilization on a surface, cell-to-cell interaction, and micro colony establishment, formation of a confluent biofilm and development of a three-dimensional biofilm structure. Bacterial biofilms are incapable to form on either biotic or abiotic surfaces. The deduction of biofilms from the infected site poses a great challenge. With time bacteria gets more resistance of biofilms to antibiotics, new strategies to increase the sensitivity of pathogens in biofilms or new bacteria-killing agents are obligatory. The replacement of Fe^{3+} with Ga^{3+} interferes with bacterial DNA and protein synthesis, and blocks the redox reactions that depend on Fe electron acquisition. The replacement of Fe has been demonstrated to inhibit *S.aureus and E.coli* growth and biofilm formation and kill planktonic and biofilm bacteria *in vitro*. In the present study, we further evaluated the effects of a Gallium oxide coating on different substrate surfaces often used for medical implants. The Ga is already FDA-approved for the treatment of hyperkalemia of malignancy. (118)

As shown in the fig. 26, for the cultivation of the *S.aureus* and *E.coli* on the gallium oxide film with the substrate surface impact. Whenever, bacteria cell interact with the metals or metals oxide than it's suffered from the membrane disruption, reduction of oxygen and leakage of fluid due to ions exchange phenomena from the cell result into death.

On the other side it must be subjected that Gram-negative bacterium cell has a complementary outer membrane composed of phospholipids and lipopolysaccharides (LPS) which governed with the net negative charge. This net negative surface charge of the bacterial cells helps them to adhere and proliferate as biofilms on positively charged abiotic surfaces. Thus, the assistance of an electron transfer interaction between the negatively charged bacterial membranes to an electron-withdrawing or electropositive surface may disrupt the surface potential of the bacterial cell membrane, thereby resulting in cell death. (137)

Figure 26 - SEM images morphology of bacterial membrane killing effect that were seeded on the gallium oxide film with the concentration of 10^6 CFU/mL



Source: The Author, 2019.

It has been reported that eventually deactivating the function of bacterial cell happen by interacting the thiol, hydroxyl and carboxyl groups of the biomolecules existing in the system by releasing of the ions substitutes. The creation of ion through redox mechanism and donating the ions with thiol and phosphate groups inhibiting the cell replica. It is clear that metals nanoparticles bind with the sulfur and oxygen electron donors who ultimately arrest the vital function of the organ leads to the death of cell. (119, 120, 121)

The Gallium attributed as ferro mimicking metals as reported which reduce the biofilm formation to the corresponding availability of planktonic cells.(122) The antibacterial study demonstrated the significant promising results against E.coli and S.aureus. (123, 124, 125) The gallium participate with other composite to attack the bacterial with the dual mechanism as disruption of the metabolism and Fenton type reactions that produce ROS unlikely the bacteria resistance arise importantly where multidrug resistance bacteria are major concern. (126)

In the first sight SEM topographical results can be explained on the bases of thin film with the substrates influence. The morphology of the bacteria species used in the experiment characterized as S.aureus found in the form of grapes cluster and E.coli appeared as in the shape of rods. It should be noted that when S.aureus bacteria membrane an interaction with the gallium oxide that caused the death as clearly observed in Gallium oxide@Ti alloy disrupted the cell membrane. It reveals that death of cell were in the unit/colony not a single bacteria. Infact, it can be reported that PLD deposited film act as an efficient charge the particles the deformation starts occuring. In the next two images Gallium oxide@Si, Gallium oxide@sapphire it was showing the gallium oxide absorb in the cell wall and amount of dose seems to be insufficient to disrupt the cell wall or can be explained on the base of insufficient charge supply/transportation from the substrate to invade bacteria cell. So it can be estimated that bacterial S.aureus was able to sustain on the silicon and sapphire substrate. With the regard to the gallium oxide film interaction with the E.coli found to be more affective on the Gallium oxide@Ti alloy and Gallium oxide@sapphire. The intracellular communication found to be very interactive with the E.coli to damage the cell wall of bacterial. The gallium oxide film sufficient to stop surviving the bacteria on the surface which may provide the leakage of cell fluid or reduce the oxygen of the bacterial cell to pointed into death. And with the silicon substrate E.coli was unable to destroy the cell and colony of bacterial remains same on the substrate. The SEM results were in fig. 26 quite consistence with the fig. 25 to conform the overall situation.

4.9 Manipulation of antibacterial effect on the base of band theory concept

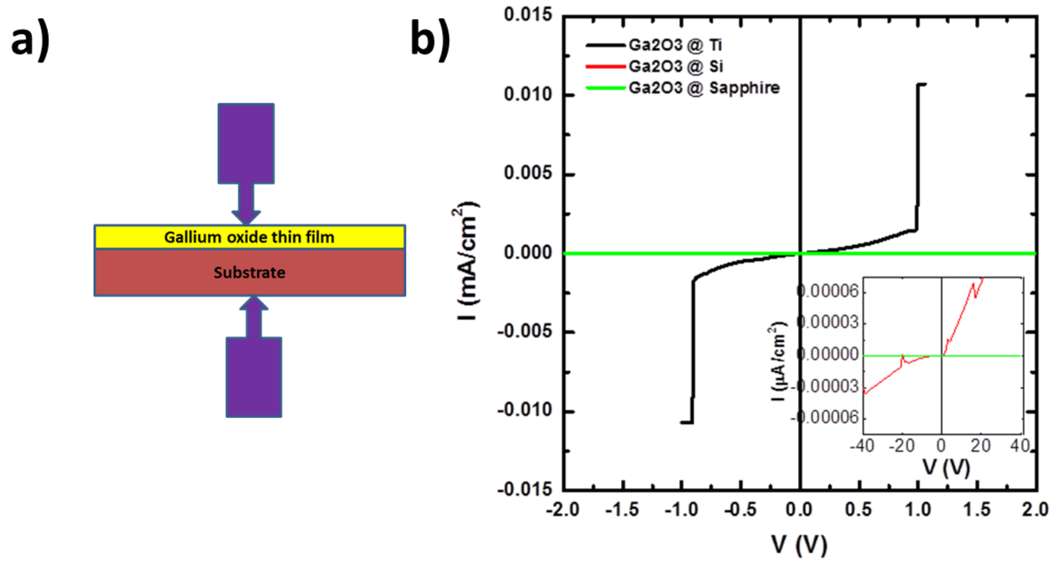
Like many other ways to describe the antibacterial killing effect here it can also be interpret on the bases of band theory due to avaibility of electron transportation from the microbial cell membrane and thin film. It is known that bacterial cell walls are composed of many ions and bacteria carry out respiration to produce energy. During this process, extra electron transportation required to realize the electron transport. So schematically electrons are conduct on the base of respiratory protein and extracellular environment to produce energy. [127, 128,129] So by electronic configuration this respiratory protein possesses n-type semiconductivity with the band gap 2.6 to 3.1 eV. [130]

The metal transport phenomena provided another advantage through this metals based nanoparticles having potential to differentiate prokaryotic (bacterial cells) from eukaryotic (mammalian cells). However, metal based nanoparticles physically interact with the antimicrobial activity through three different major pathways. Firstly, metal ions can possibly interact with the **phospholipid bilayer** further disruption of cell membrane integrity apart by which ions release. This communications is majorly conceivable by electrostatic interaction of ions between metals and membrane. As a result oxidative stress that damage the bacterial protein. Overall, this disproportion of ions and membrane solidity results in impaired respiration, interruption of energy transduction, and eventually cell death. Secondly, another ways to induce antimicrobial response is to binding cytosolic proteins as enzymes and DNA which lead to reduce the respiration or metabolic pathway. This results in diminished membrane integrity and a buildup of ROS within the cytosol of the cell. Thirdly, alternative pathway by which bacterial death can be possible by reactive oxygen species (ROS) or free radical. The ROS lead to severe oxidative stress and damage to the cell's macromolecules which overall alter of protein. This reactive oxygen causes holes or pits in the bacterial membrane. [(131)]

In this present study, physical contact of micro bacterial and gallium oxide (metal oxide) produces the diode conductive ohmic behaviour with linear and nonlinear curve. Here, results are presented on the base of band theory concept which deals with the valance to conduction band by electron transport phenomena to distinguish the conductor, semiconductor and insulator by electrical behaviour. To demonstrate the perceived sensations of microbial response to the thin film of gallium oxide on the metallic Ti alloy, semiconductor Silicon wafer and insulator as Sapphire substrates. In the figure 27 electrical behaviour outcome of Ti alloy substrate (Ga_2O_3@Ti alloy) with gallium oxide showing that conductive non-ohmic trend. It can be assumed that substrate junction built up a circuit for electron transportation and bacterial cell membrane lose electron due to negative charge. From the calculated graphical data, it can be seen that electrons are easily accessible to the gallium oxide film with the underlying conductor substrate which form a circuit for electron transfer until the bacterial cell loss its viability. So in this way survival of bacteria's are impossible by interacting with this kind of interface.

On the other surface, electron carrying ability under the Silicon substrate (Ga_2O_3@Si) with the gallium oxide film kill the bacterial but behaviour of ions viability is less strong than Ti alloy surface as shown in the graph ohmic linear behaviour. That's why; there are still some bacterial entities that were able to maintain their integrity in the poor living state. However, it has been found that with the sapphire surface ($Ga_2O_3@Al_2O_3$) electro transfer ability is negligible that don't help to form a circuit and in graph it predicted as straight line with the reference to the zero. As a result, bacteria's are able to survive.

Figure 27 - Schematic illustration for the electrical based response to obtain the current voltage (I–V)



Source: The Author, 2019.

Note : a) electric contact with substrate and film; b) characteristics of Ga_2O_3@ Ti alloy, Ga_2O_3@ Silicon, and Ga_2O_3@ Sapphire at room temperature respectively, indicating three different contacts of Gallium oxide films deposition by PLD with the underlying substrates

From the figure 25and 26 antibacterial assessments can be observed as agar diffusion method and SEM morphology destruction. Moreover, gallium oxide found to be more effective on the *E.coli* for killing with the Ti alloy surface. As the microbial membrane with the negative charge potential and substrate junction act charge to jump as more possible which create the ROS stress to destruct the membrane eventually bacterial died. So it can be concluded that substrate surface may play an important role and can be interpret on the base of band structure theory to design the bioimplant surface.

5 CONCLUSIONS AND PERSPECTIVES

The dealing between the tissue and the implant surface is a dynamic process. The ions exchange phenomena start appearing in surrounding the implant surface during the few seconds after implantation. These ions may contain water molecules, free biomolecules and dissolved ions. All these cells start adsorbing by creating the layers with cells in convinced patterns. As the time passes, layers anchored with the other tissues and this process called osseointegration which refer the direct contact between a bone and implant. During all of this process surface texture in term of physical and chemical play an important role. The physical textural features at atomic, molecular, and higher levels act as contact areas for biological units and the different types of bonding associated with each of these biological units influence the hierarchical integration of a surface. A material experience different chemical reactions at the surface depending on the environment and thus complicates the understanding of the exact nature of the interactions.

The present work deals with the deposition of gallium oxide thin film on different substrate surfaces to explore the better condition to grow and modify the surface by thermal oxidation treatment. Surface roughness is important parameter in this work to find the compatible texture and temperature constrains to reduce the biofilm on the bioimplant. In this study two deposition techniques applied to support two parameters surface roughness and temperature effect.

Optimum growth conditions have been established by interpretation of thin film through substrates temperature 650 ^{o}C using PLD technique. The gallium oxide distribution is uniform and particles size, shape alters due to substrate surface effect in nano range. It must be concluded that surface roughness deals with the promising results ≤ 50um in this work with 1 hour thermal treatment. As the surface treatment increased roughness change by some values. The chemical composition elements changes by thermal effect also. Further, gallium oxide conformation found by EDS peaks that reside on the substrates. Moreover, wettability investigated as hydrophilic with the all substrates. The diffraction pattern indexed with the crystalline phase conformation. Both thermal treatments methods provide the compatible results but because of clean environment PLD system consider among good one. It must me found that surface roughness under vacuum condition at 600 ^{o}C provide the same results as 800 ^{o}C without vacuum that observation calling the attention for vacuum as suitable treatment. So, low temperature treatment under vacuum environment can be supportive treatment to consume less consumption of temperature.

The inhibition growth of bacteria on the thin film provided the impression that it's influenced by the gallium oxide and substrate. In case of metal surface avaibility of charge are more to kill the bacterium that's why Ti alloy surface with gallium oxide having more antibacterial effect than others. Additionally, SEM results further conformed the deformation of bacterial structural wall because of gallium oxide killing effect. On the other side band theory provided the more accurate vision to understand the bacterial killing effect on the base of charge transportation between the cell wall and substrates. From the above observed results it can be determined that substrate surface may play an important role and can be interpret on the base of band structure theory to design the bioimplant surface on the base of charge transportation. Further, in future this study may be responsible for new vision for better understanding to fabricate the the biomedical devices.

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