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Influence of Surface Modification on Corrosion and Biocompatibility of Titanium Alloys

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INFLUENCE OF SURFACE MODIFICATION ON CORROSION AND BIOCOMPATIBILITY OF TITANIUM ALLOYS

A Thesis

by

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ABSTRACT

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Titanium alloys enhance the quality and longevity of human life by replacing or treating various parts of the body. However, the aggressive body fluids lead to corrosion and metal ions dissolution. These ions leach to the adjacent tissues and causes adverse reactions. Surface modifications improve corrosion resistance and biological activity. In this investigation, electropolishing, magnetoelectropolishing, titanium coating and hydroxyapatite coating were carried out on commercially pure titanium (CPTi), Ti6Al4V and Ti6Al4V-ELI (Extra Low Interstitials). These surface modifications are known to affect surface chemistry, morphology, wettability, corrosion resistance and biocompatibility of these materials. *In vitro* cyclic potentiodynamic polarization tests were conducted in phosphate buffer saline in compliance with ASTM standard. The surface morphology, roughness and wettability of these alloys were studied using scanning electron microscope, atomic force microscope and contact angle meter, respectively. Moreover, biocompatibility of titanium alloys was assessed by growing MC3T3 pre-osteoblast cells on the surfaces.

DEDICATION

The completion of my Masters studies and this dissertation is dedicated to my mother Jehan Ara. This would not have been possible without her love and support. Thank you for your love and patience.

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CHAPTER I

INTRODUCTION

Biomaterials

In the last few decades, biomaterials have been used in wide range of medical implants devices such that, in present era, it constitutes an important area of the medical industry [1]. The purpose of the biomaterial implantation is to improve body function, replace the damaged tissue to recover the structure of the organs [2]. According to D. F. Williams et al. the term biomaterial is defined as "a material intended to interface with biological systems to evaluate, treat, augment or replace any tissue, organ or function of the body; further defined the term biocompatibility as "the ability of a material to perform with an appropriate host response in a specific application"[3]. In 1986 the European society of Biomaterials define the biomaterial as "A biomaterial is a nonviable material used in a medical device, intended to interact with biological systems"[4]. Natural or artificial materials are used in a variety of medical implant devices, including artificial organs, rehabilitation devices and implant materials [5], [6]. Metals, ceramics, polymers have found the applications to the extant that many are now the routine armamentarium of medical profession [7]. Biomaterials are used in different parts of human bodies as artificial heart valves, stents in blood vessels, replacement implants for spinal disks, hip, knees, shoulders, elbows ears and orthodontic structures. The number of implants used for spinal, shoulder, hip and knee replacement are extremely high, which are complex structures capable of functioning

under critical conditions [8]. Hip, knee and shoulder joints are also called synovial joints, as these joints are combination of articular cartilage, a load bearing connective tissue, which covers the bone while the synovial fluid within these joints, covers the outer surfaces of bones to reduce fraction between them [9].

Several degenerative and inflammatory diseases may result in pain and joint stiffness. Primary and secondary osteoarthritis, rheumatoid arthritis (inflammation of synovial membrane), and chondromalacia (softening of cartilage) are the most common diseases of degeneration of synovial joints [10] These diseases generally lead to orthopedic implants. To achieve pain relief and improve mobility, the degenerated natural surfaces are replaced with advanced orthopedic biomaterials [11]. Surgical implantation is one of the solution for restoring the function of the impaired tissues [12].

History of Biomaterials

The history of the field of biomaterials date back 4000 years age. Egyptians and Romans used linen for sutures, gold and iron for dental implant, and wood for toe replacement. The Bronze and copper has been practice since the pre Christian era and until the mid 19^{th} century [13]. Nylon, Teflon, silicone, stainless steel and titanium became widely used in the medical field after World War II.

Serious attempts have already been made to repair the body with foreign material by the mid 19^{th} century. In 1880, Gluck, used ivory prosthesis as implant in the body. The participation of biomaterials earlier was unsuccessful due to the occurrence of infection and toxicity but later on in 1860 Dr. J. Lister develop an antiseptic surgical techniques [14]. In 1902 gold was use as the interphase between the articulate head of the implant. This experiment proved to be

successful, which lead to further studies on chemically inert and stable materials. Currently, the knowledge of materials and the availability of advanced diagnostic tools for implantology have great significance in the field of biomaterials.

Future Trends and Demands

In recent years, the demand for biomaterials has been increasing tremendously with relative proportion of senior citizens in society. An 8% of annual growth is anticipated in the orthopedic industry from $6 billion in 2007 to $13 billion in 2017 [15] . It is projected that by 2015, there will be 133 million Americans over 45 years of age, at this age the incidence of heart diseases is documented. Many will require heart stents (small metal mesh sleeves implanted in unclogged arteries by angioplasty). On the other hand the number of revision surgeries increases simultaneously with the increase of replacement surgeries and at this very moment, there is an "accelerated rate of approximately 60%" revision surgical procedures and still growing in just the United States.

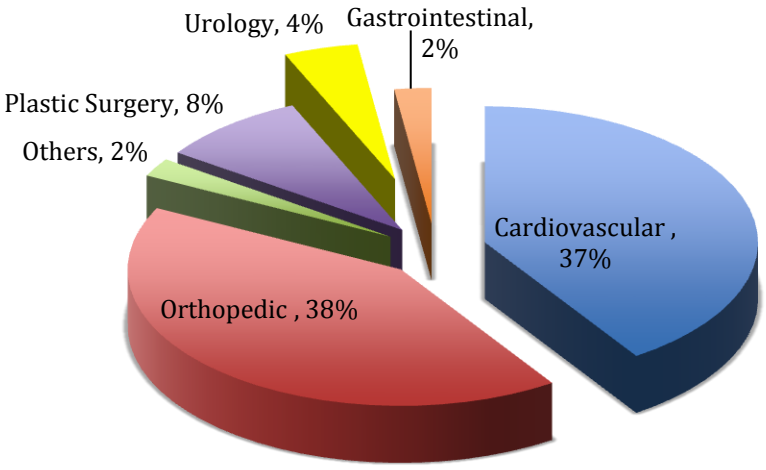


Figure 1: World market of Biomaterials **[16]**

In the year 2000, about 152,000 total hip replacements were performed in which 12.8% were involved the revisions of the previous hip replacement while in 2006 the number of primary hip replacement and knee arthoplasty has been estimated about 800,000 in United States alone [17]. It is projected that approximately 272,000 total hip replacements will annually be performed by 2030 [20]. According to statistics, there will be an increase of 174% (572,000 procedures) in total hip arthoplasties and 673% (3.48 million procedures) of total knee arthoplasties by the end of 2030 [18]. It is projected that approximately 272,000 total hip replacements will annually be performed by 2030 [20]. According to statistics, there will be an increase of 174% (572,000 procedures) in total hip arthoplasties and 673% (3.48 million procedures) of total knee arthoplasties by the end of 2030 [18]. Figure 1 shows the world market of bioimplants where, the largest share is for orthopedics (38%) and cardiovascular applications (37%).

Classification of Biomaterials

On the basis of the nature of the installation, the biomaterials have different classes. Some materials have no direct body contact are called class I materials. The common example of the class I biomaterials is the dialysis machine. The machine doesn't have direct contact with the body but the hoses in which the blood circulates and the material in which the blood contact takes place in the dialysis machine must be biocompatible [19]. The materials having intermittent contact are class II biomaterials while class III has direct contact. Class III materials are used to install inside the body, mostly for permanent basis. These materials start leeching ions that affects the adjacent tissues and sometimes causes complication [20]. The approved Class III materials comprise of titanium, cobalt-chromium and stainless steel alloys [21]. Metallic implants mostly covers the class III section and are commonly used in orthopaedics in the form

of plates, pins, fixing screws for bone fractures and as devices as parts for total hip prostheses or as femoral and tibia components in total knee orthoplasty [22]. A list of commonly used biomedical applications of metals is given in Table 1.

Table 1: Classification of Biomaterials [1,23].

Types of Material	Examples	Applications
Polymers	Nylon, silicones, Teflon, polyester fibers, high strength acrylics, polyurethane, hydrogels, polycarbonate, polypropylene	Contact lenses, Vascular grafts, wound dressings, Maxillo facial operations, absorbable sutures, Drug-release system, Renal dialysis cartilages, trocars, extra cellular matrices.
Metals	Nitinol, titanium alloys, cobalt-chromium alloys, 316L stainless steel, platinum alloys, silver, magnesium alloys, iron alloys	Joint replacements, dental root implants, bone plates, bone grafts, cardiac stents, electrodes, anti-bacterial material
Ceramics	Alumina, Zirconia, Hydroxyapatite, bio-glass	Joint replacements, bone spacers, tooth implants, bone bonding applications, bone cement fillers, cardiac stents
Composites	Carbon-carbon, calcium phosphate cement	Joint implants, heart valves

On the basis on the nature of the materials the biomaterials are classified into bioinert, bioactive and bioresorbable. The bioinert refers to a material that retains its structure in the body after implantation and does not induce any immunologic host reactions [24]. The bioactive are those biomaterials that form direct chemical bonds with bone or even with soft tissue of a living organism and materials that degrade in the body while they are being replaced by regenerating

natural tissue; the chemical by-products of the degrading materials that are absorbed and released via metabolic process of the body are bioresorbable materials [25]. The following are the main requirements for the implant for long term and implantation without rejection [26].

Major Requirements of Implant Materials

The choice of the appropriate material for the living organism depends on its application and the intended place to install. Mechanical properties are the key component for bone implants while surface properties determine the success of blood contacting devices The following are the main requirements for the implant for long term and implantation without rejection. Some of the other requirements are mentioned in figure 2.

Biocompatibility

The first foremost requirement for the choice of implant is biocompatibility, which means that the material is acceptable to human body and the material should not cause any adverse effect. These adverse effects come in the form of allergy, infection, inflammation and cytotoxicity [27]. The biocompatibility can be measured from the body reaction or the adverse reaction of the tissues [28]. The biocompatibility is defined as "The ability of a material to perform with an appropriate host response in a specific application"[29]. Thus the two main factors for biocompatibility are the host response and the behavior of the implant material in the environment. Some implants have adverse reaction to tissues depending on the targeted area, in the form of allergy and inflammation and thrombosis. Thrombosis is the blood coagulation and the adhesion of the blood platelets to biomaterial surface and the loosening of implants by fibrous tissue encapsulation of biomaterials, are the common adverse body response [30].

Mechanical Properties

The joints are working in continuous cyclic loads [31,32]. The response of the material to the cyclic loads is determined by its fatigue strength, modulus of elasticity and ductility. The successful implantation may be achieved when the modulus of elasticity of implant is closer to the modulus of elasticity of bone. The modulus of elasticity of the bone varies from 10-30 GPa [4]. Higher modulus of elasticity of implant material has a risk of stress shielding effect. This effect leads us to the loosening of the prosthetic device and ultimately the death of the bone cells [33].

Corrosion Resistance and Wear Resistance

The corrosion of metallic implant is critical because it can affect the biocompatibility and mechanical integrity [34]. The physiological environment in the human body consists of various elements namely, physical and chemical environments, cells and cellular matrices and tissue fluids and blood circulatory systems [35]. The physical and chemical environment in the body is interrelated and changes in physical environment affects the chemical environment. The chemical environment within the body is controlled by the body fluids [36]. These fluids are buffered saline having a temperature of 37 °C and a pH of 7.4 under normal conditions [37]. The chemical reactions and biological environment are responsible for corrosion and the dissolution of the surface oxide layer. Extensive release of ions from prosthesis can result in adverse biological reactions which can lead to the mechanical failure of the device [38]. Metal ions release to the adjacent tissues may cause long-term complications. This reduces service life of the implant and increases the chances of the revision surgery [36]. Figure 2 shows the brief and major requirements of the implant materials.

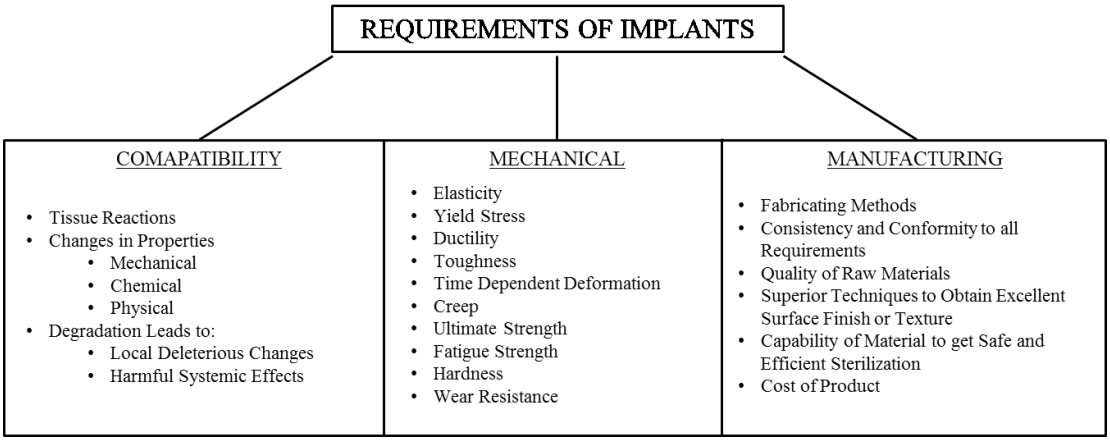


Figure 2: Major requirements for implants [36]

Currently used Metallic Biomaterials

Cobalt-chromium, titanium and 316L stainless steel have proven to be biocompatible to the human body and are used for implants applications, however titanium alloys are considered to be far more superior comparatively to the others [39]. The metallic materials are mostly used as load-bearing implants because of high mechanical resistance, this guarantee the good load transmission over a long time as well as mechanical stiffness [40]. The corrosion resistance of metals in the living organism is one of the major prerequisites to avoid impairment of the materials properties due to degradation. The release of ions from these metallic implants to the adjacent tissues has been observed. Different animal studies have proven that nickel ions causes the skin related diseases while cobalt shows carcinogenicity [41]. In addition the stainless steel and cobalt chromium alloys possesses higher elastic modulus than the bone, which leads to stress shielding and implant loosening after some years of implantation [42].

Titanium and its alloys are famous for its low density and high strength. Ti6Al4V alloy is known for its excellent tensile strength and pitting corrosion resistance and light weight [30], [43]. Nitinol or nickel titanium creates devices having shape memory effects that can be applied

to dental restoration wiring and cardiovascular applications [44]. A list of commonly biomedical applications of metallic implants is given in Table 2.

Table 2. Metallic Materials and Applications [45]

Metallic Materials	Common Applications
Stainless Steel alloys	Joint replacements (hip, knee), bone plate for fracture fixation, dental implant for tooth fixation, heart valve, spinal instruments, surgical instruments, screws, dental root implants, pacer, fracture plates, hip nails, shoulder prosthesis
Cobalt-chromium alloys	Bone plate for fracture fixation, screws, dental root implant, pacer, and suture, dentistry, orthopedic prosthesis, mini plates, surgical tools, bone and joint replacements, dental implants
Titanium alloys	Cochlear replacement, bone and joint replacements, dental implants for tooth fixation, screws, sutures, parts for orthodontic surgery, bone fixation devices like nails, screws and plates, artificial heart valves and surgical instruments heart pacemakers, artificial heart valves

The AISI 316L, a single phase austenitic stainless steel, is one of the most popular type of steel for implant applications [46], [47]. Its composition contains about 17-19% Cr, 12-14% Ni, and 2-3% Mo. The percent compositions of the metals in alloys have great influence on the corrosion resistance and mechanical properties of metallic implant. Research demonstrates that Mo has improved 316L stainless steel corrosion resistance, and a carbon content of 0.03 wt%, improves its corrosion resistance to chloride solution. In the mechanical properties, the 316L elastic modulus is about 200 GPa, which makes it an excellent choice for load-bearing bone applications are to be considered [48], [49]. The strength of the titanium is very close to the 316L and Co-Cr but its density is 55% less than the steel, when compared by specific strength (strength per density) the titanium alloy outperform any other implant material. Some of the

important alloys employed in the field of biomaterial along with their mechanical properties are listed in the figure 3.

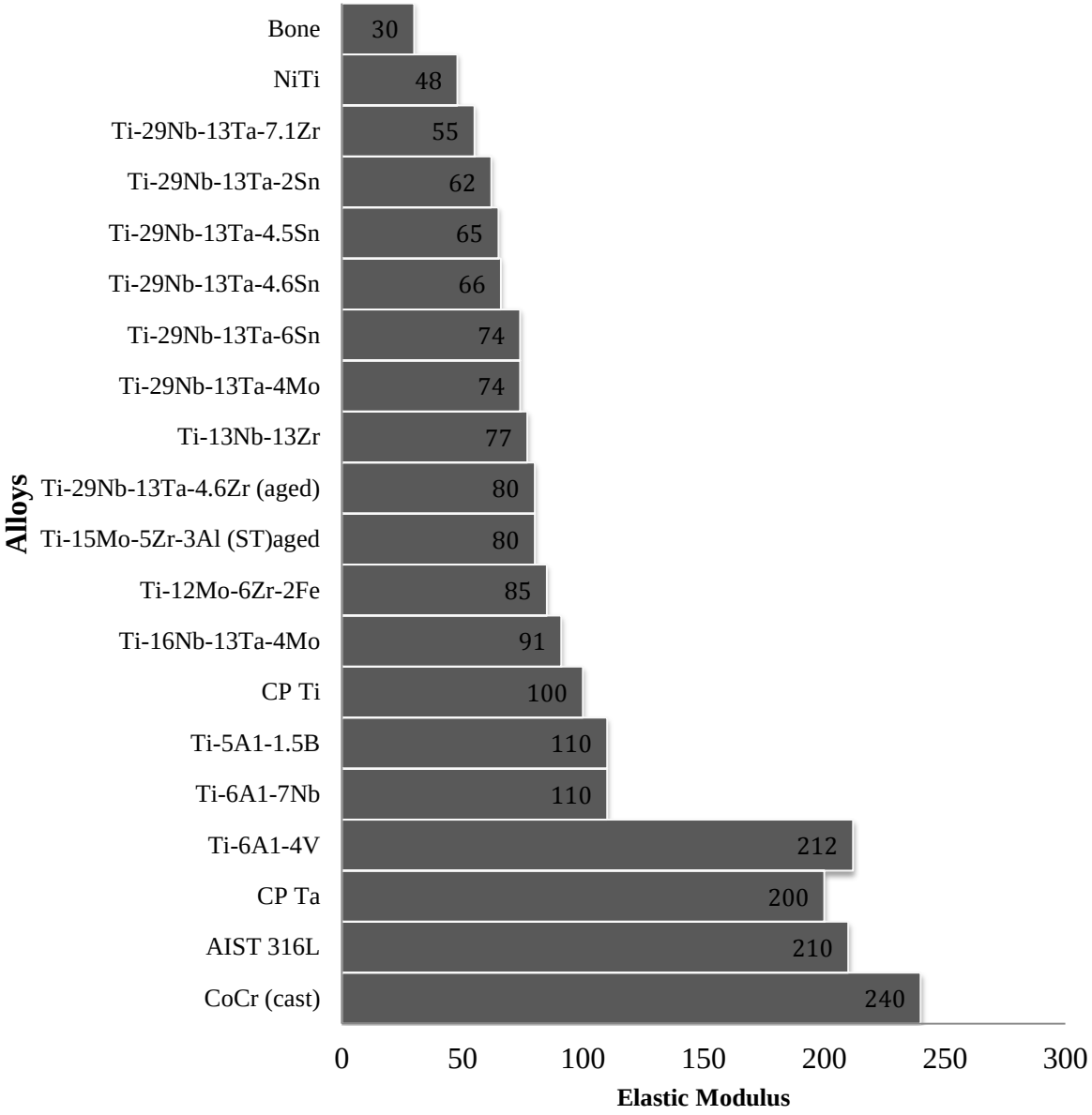


Figure 3: Elastic modulus of various alloys

Titanium as Biomaterial

Titanium alloys further increased the use of titanium as a biomaterial; the titanium alloy, Ti-6Al-4V ELI, is commonly used for total joint prostheses. The Ti-6Al-4V alloy offered many advantages, such as high strength, a low elastic modulus, high corrosion resistance, and high tissue tolerance. The high strength and low elastic modulus rendered this alloy suitable for many orthopedic applications, including the following: "hip and knee prostheses, trauma/fixation devices (nails, plates, screws, and wires), instruments, and dental implants"[50]. The Ti-3Al-2.5V alloy was soon found to have cold shaping properties in combination with high mechanical properties and good corrosion resistance. Ti-3Al-2.5V alloy became used for the femoral and tibia rod in total joint replacement. In the 1980's, drawbacks of titanium were soon discovered as "black debris" were found in patients with total knee and total hip replacements, both of which exhibit high wear. The concern for toxicity from these alloys was increased, although titanium alloys were still used as biomaterials [50]. The use of surface coating soon became prominent important in titanium alloys in order to prevent toxicity. Today, the most commonly used titanium alloys include Ti-6Al-4V ELI and Ti-6Al-4V [51]. The development of new titanium alloys and new processing methods has become important. Due to its high biocompatibility, corrosion resistance and "specific" strength as compared to other biomaterials, titanium and its alloys are finding continuing use [50].

Titanium Alloys and its Metallurgy

Today there are more than 100 different types of titanium alloys, however, only 20 to 30 have reached the commercial status. Of these, the classic alloy Ti-6Al-4V covers more than 50% of commercial usage while another 20 to 30% is unalloyed, pure titanium [52]. The chemical

composition and the microstructure are the two factors, which determine the properties of titanium alloys. Pure titanium is a transition element and undergoes allotropic transformations of from hexagonal closed pack to body cubic center. At low temperatures it has a closed packed hexagonal crystal structure (HCP), which is commonly known as α, whereas above 883°C it has a body centered cubic structure (bcc) termed as β [53]. The alloying elements in most Ti alloys are added to control the constitution of the alloy, to alter and/or control the transformation kinetics, and to solid-solution-strengthen one or more of the microstructural constituents. The alloying behavior of titanium is indicated in terms of the effect of different solutes on the allotropic transformation temperature of the pure metal and is given in figure 4 [54].

Titanium alloys are classified as a, near-α, α + β, metastable β, or stable β depending upon the room temperature microstructure. In this respect, alloying elements for titanium fall into three categories: (1) α-stabilizers, such as Al, O_2, N_2, C ; (2) β-stabilizers, such as Mo, V, Nb, Ta (Isomorphous), Fe, W, Cr, Si, Co, Mn, H (eutectoid); (3) neutrals, such as Zr [55].

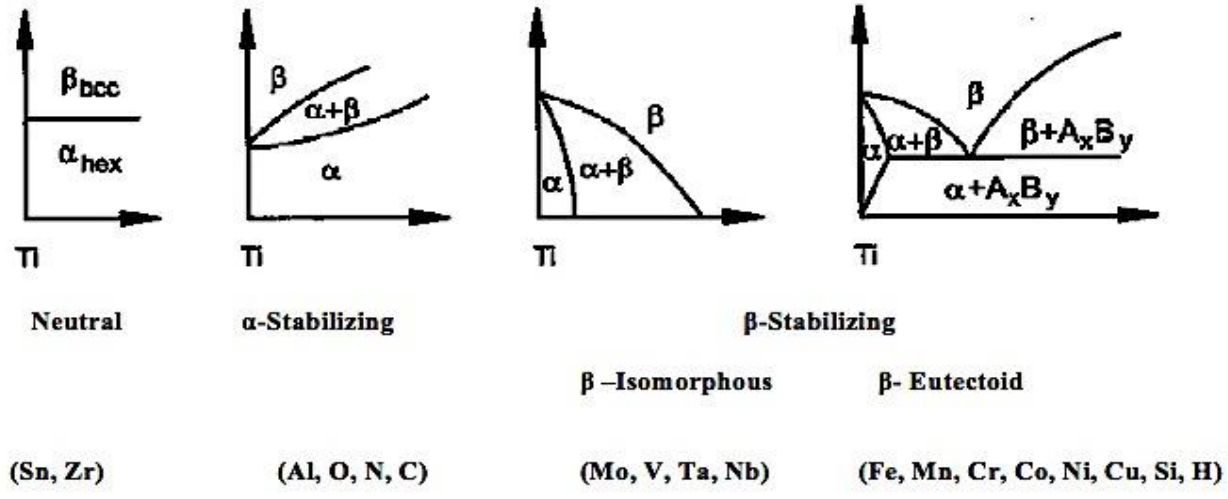


Figure 4: Influence of alloying element on phase diagram of titanium [52]

Oxygen concentrations in α-stabilizer have great influence on the strengthening of alloys while N_2 and C are both soluble enough as solid-solution strengtheners and have significant effects on the nucleation of the α-phase. The β-isomorphous elements are significant due to their much higher solubility in titanium. On the other hand, even very low volume fractions of β-eutectic elements, e.g. Fe, Mn, Cr, Co, Ni, Cu, Si, and H_2, can lead to the formation of intermetallic compounds and they decrease the transformation temperature [56]. Si atoms tend to segregate to dislocations and thus effectively prevent dislocation climb, which improves creep behavior. The solubility of H_2 is very high in the β-phase and relatively low in the α-phase, because the Ti-H_2 system forms a eutectoid and the solubility of H in the α -phase in equilibrium with titanium hydride is small. In addition to the transition metals, the noble metals Au, Cu and Ag as well as the heavy transition metals Pt and Pd are eutectoid formers. Sn, Hf and Zr are considered neutral elements since they have (nearly) no influence on the α/β-phase boundary. However as far as the strength is concerned, they are not neutral since they primarily strengthen the α phase. Zr tends to homogenize fine silicide precipitates.

The α and near-α titanium alloys exhibit superior corrosion resistance but have limited low temperature strength. In contrast, the α + β alloys exhibit higher strength due to the presence of both the α and β phases. The properties of the materials depend on the composition, relative proportions of the α and β phases, thermal treatment, and thermo–mechanical processing conditions. The β alloys also offer the unique characteristic of low elastic modulus and superior corrosion resistance[54], [57]. The figure 5 and figure 6 shows the influence of thermo-mechanical effects on the microstructure of Ti alloys.

The most commonly used titanium alloys are commercially pure (CPTi), Ti-6Al-4V, and Ti-6Al-4V-ELI [58]. Nickel titanium, also known as Nitinol, has recently been added as another

important alloy. Although these alloys are commonly used for orthopedic applications, there are still more titanium alloys being developed and tested. In the next few paragraphs, there will be brief descriptions of the alloys that were previously mentioned.

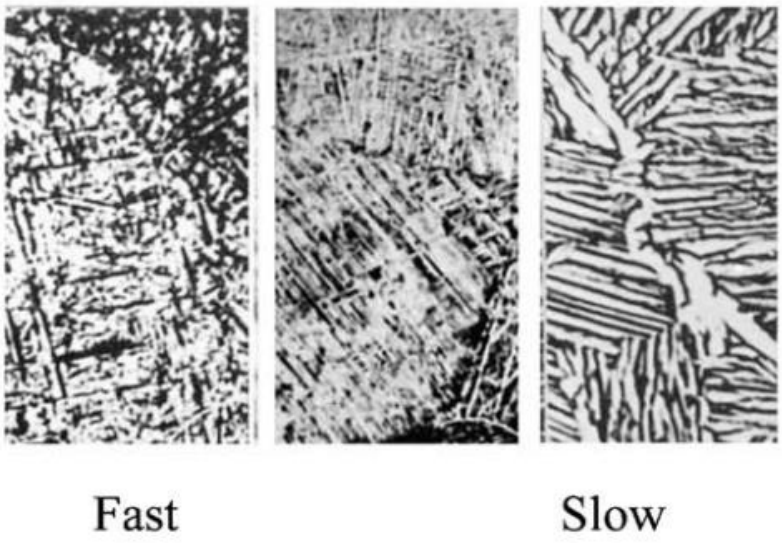


Figure 5: β-titanium microstructure from fast to slow thermo-mechanical process

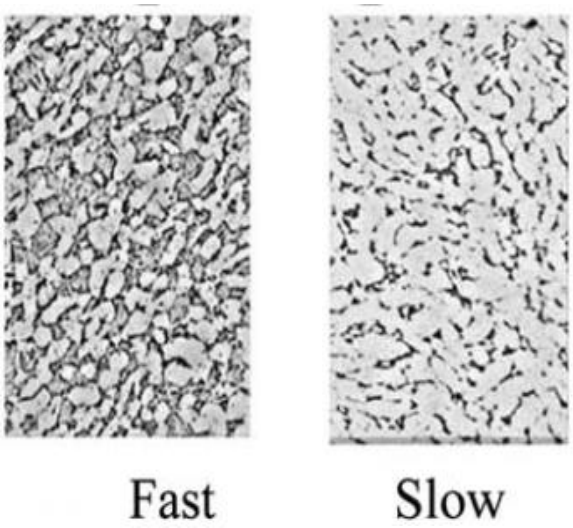


Figure 6: α-titanium microstructure from fast to slow thermo-mechanical process

Commercially Pure Titanium (CPTi)

CP-Ti is the most common type of titanium base implant and is classified as biologically inert. This material does not promote any adverse reaction to the tissue and it is tolerated well by tissues when implanted. This alloy is made up of 98.9-99.6% of titanium and it comes in four grades, Grade 1 has maximum percentage of 0.18% oxygen, Grade 2 has 0.25% oxygen. Grade 3 has 0.35% maximum and oxygen, and Grade 4 has 0.40% oxygen. Compared to stainless steel CPTi has better corrosion resistance and tissue tolerance [59].

Ti6Al4V–ELI (Extra Low Interstitial) Alloy

Ti-6Al-4V-ELI has highly used in the aerospace industry; however, it's high corrosion resistance and being well agreeable to the human body made it very useful in the biomedical field [60]. It even has a lower elastic modulus than stainless steel and cobalt chromium alloys. Ti6AL4V-ELI alloy has a few disadvantages, which was part of the reason for the development of new alloys. Aluminum and vanadium ions are released in the body, and these metals are toxic enough to be fatal [60].

Shape Memory Alloys (SMAs)

Shape Memory Alloys (SMAs) are a group of metallic materials that demonstrate the ability to return to some previously defined shape or size when subjected to the appropriate thermal procedure. A. Ölander discovered the pseudoelastic behavior of the Au-Cd alloy for the first time in 1932. In 1938, Greninger & Mooradian observed the formation and disappearance of a martensitic phase by decreasing and increasing the temperature of a Cu-Zn alloy. In the early 1960s, Buehler and his co-workers at the U.S. Naval Ordnance Laboratory discovered the shape

memory effect in an equiatomic alloy of nickel and titanium (NiTi), which is considered as a breakthrough in the field of shape memory materials. Other SMAs includes Cu-Al-Ni, Cu-Zn-Al, Fe-Mn-Si. However, NiTi is the most common shape memory alloy [61]. This alloy has quickly made itself popular due to its properties. Nitinol is able to have a shape memory effect, biocompatibility, super plasticity, and is closer to bone when it is porous. High nickel release from nitinol can be toxic.

CHAPTER II

LITERATURE REVIEW

Titanium was initially made commercially available in the 1940's during World War II. Soon after, titanium was explored and used as a surgical implant material. Early in vivo experimental analysis by Bothe et al and Leventhal showed that titanium had outstanding tissue compatibility [62]. Few internal fixation devices were used in the United States as early as the 1950's and 1960's. In the 1960's a British orthopedic surgeon, Sir John Charnley pioneered modern total hip arthroplasty. Further research in the late 1960's, by P.I. Branemark et al. introduced titanium shape memory alloys to medicine. At a meeting held at Clemson University in South Carolina, Nitinol (NiTi) was officially recognized as biomaterial [63].

In 1973, it was found that the pigmentation was caused by commercially pure titanium, and the traces of metal ions were observed in the tissue, which had adverse reactions. In 1982 Williams introduced the Ti6Al4V in the field of biomaterials [64]. Furthermore, in era of 1980s, black debris was found in the tissue surrounding the Ti6Al4V under condition of high wear [50]. Breen Stoker in 1993 named above mentioned black debris as metallosis and titanium cyst. Lalor et al in 1993 analyzed the concept of titanium immunogenicity [65]. Haynes et al. in 1993 found that titanium induces release of inflammatory mediators [64]. Owing the poor wear resistance and its effects on the biocompatibility of the titanium alloys, it was soon realized that implants leaches the metal ions in the tissues and their high concentration has adverse effects. In this view

surface modification of these alloys were considered complimentary for the titanium implants [66]. Meanwhile, 316L Stainless Steel and cobalt-chromium were introduced as biomaterials. Due to their high elastic modulus, loosening of implant and stress shielding may take place. The high modulus material also leads the healing bone to the bone absorption. Mitsuo Niinomi in 2008, implanted three different alloys, Ti-29Nb-13Ta-4.6Zr (referred to as TNTZ), Ti6Al4V-ELI and 316L, in the tibia. Figure 7 shows the X-ray photographs of the healing states of experimental tibial fractures at 24 weeks after the implantation of intramedullary rods made of low modulus TNTZ, Ti-6Al-4V ELI and 316L stainless steel. The posterior tibial bone becomes very thin 24 weeks after the implantation of the intramedullary rods made of 316L stainless steel. Therefore, the bone absorption occurred in the case of the 316L intramedullary rod, but did not occur in the cases of the other two types of titanium alloys. Further, the remodeling is much better in the case of the TNTZ [67].

The titanium alloys have usually form a thin oxide layer of 10nm approximately, which act as a protective barrier between the alloy surface and the surrounding environment. The protectiveness of this passive film is determined by the rate of ion transfer through the film and by the stability of the film against dissolution (S. Virtanen 2008) [66]. In 2008, J.V.S Shabalovskaya found that 100 nm thick oxide layer appears to be more reliable. These variations in the surface film thickness are known to affect surface charge, chemistry, morphology, wettability, corrosion resistance and biocompatibility of these materials. These are also influence the bioactivity of the implant. Thus different type of coatings were introduced, e.g. hydroxyapatite, polymers, calcium phosphate and other techniques of surface modification were applied to surface to make Ti more bioactive [68]. However, these available surface coating techniques are not reliable for long tenure and tends to fail by fracture and delamination [69].

The plasma sprayed hydroxyapatite is the common coating used on metallic implants but with a high degree of porosity, poor bonding strength, and non uniformity make it unfit for long tenure use [70]. Dip coating, ion sputtering, and sol-gel coating are the other techniques that can be used as surface treatments [71], [72].

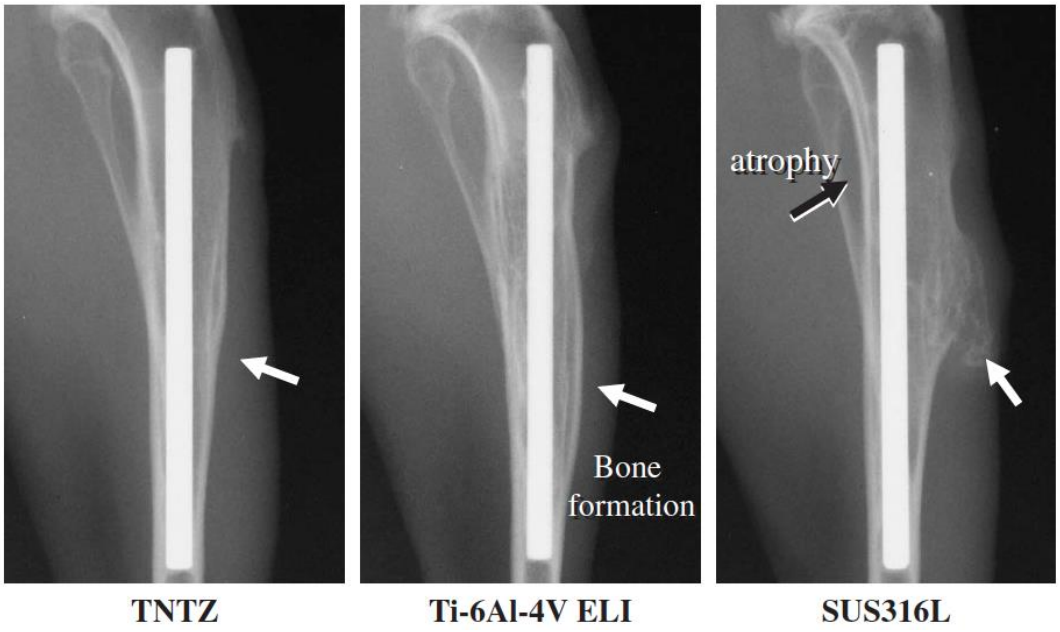


Figure 7: X-ray photographs of tibia at 24 weeks after implantation [67]**.**

Cyto-compatibility of Titanium Alloys

Titanium and titanium alloys are relatively inert, biocompatible and have good corrosion resistance because of the passive thin surface oxide layer and typically do not undergo significant corrosion in a biological environment. Also, titanium readily adsorbs proteins from biological fluids [73]. Example of some specific proteins including albumin, laminin, fibronectin, glycosaminoglycans and collagenase (carbohydrates), complement proteins, and fibrinogen has been found to adsorbed onto titanium surfaces. Titanium surfaces can also support cell growth and differentiation. Much work has been devoted to the investigation of cell interactions with

titanium surfaces. After the materials are implanted into the body, neutrophils and macrophages are first noted on the implants, followed by the formation of foreign body giant cells from activated macrophages. It is generally accepted that osteoprogenitor cells migrate to the implant site and differentiate into osteoblasts that aid in formation of bone. After the materials have been implanted into the body, the first stage of the reaction (after interaction with water and ions) is a non-specific protein adsorption. Afterwards, neutrophils and macrophages "interrogate" the implant. The macrophage interaction and cytokines released by the macrophages are believed to attract fibroblasts and drive the foreign body encapsulation process [2].

Toxicity Affects of Elements in Titanium Alloys

Most of titanium implants are in alloyed form with other elements. The physiological environment in the human body consists of various elements namely; physical and chemical environments, cells and cellular matrices, tissue fluids and blood circulatory systems [35]. The physical and chemical environment in the body is interrelated and changes in physical environment affects the chemical environment. The chemical environment within the body is controlled by the body fluids [36]. These fluids are similar to buffered saline having a temperature of 37 °C and pH of 7.4 under normal conditions [37]. Extensive release of ions from prosthesis can result in adverse biological reactions which can lead to the mechanical failure of the device [38]. In the some times, metal ions release to the adjacent tissues may cause long-term complications. This reduces service life of the implant and increases the chances of needed revision surgery. The released ions leach to the adjacent tissues and having toxic effects in the form of different types of diseases. The Nickel ions can act as cofactors or inhibitors in enzymatic processes involved in protein synthesis and cell replication, modifying cell

morphology, destroying the cell organelles and even decreasing cell numbers. Ni ions in solution have been reported to cause expression of inflammatory mediators, such as interleukin-1β (IL-1β), tumor necrosis factor-α (TNF- α) [74]. Skin related diseases like dermatitis can also be caused excess nickel ions.

It is observed that the corrosion of cobalt in the wet and a salty surrounding in human body results in the releasing of toxins into the body, which in turns leads to the formation of cancerous tumors. List of other elements that have cytotoxic effects of the tissue are shown in table 3.

Table 3: Effects of ions released during implant corrosion in human body [39].

Biomaterials Metals	Effect of ions on tissue
Nickel	Affect skin causing dermatitis and Necrosis
Cobalt	Anemia B inhibition iron from being absorbed into the blood stream
Aluminum	Epileptic effects and Alzheimer's diseases
Vanadium	Toxic in elementary state
Chromium	Ulcers and central nervous system

Surface Treatments of Titanium Alloys

The susceptibility of the corrosion of implants and the release of ions compelled the minds of the researchers towards the surface modification and coatings in the field of biomaterials. Presently a lot of interest has been generated in the fabrication of films and coatings for the inhibition of ions released from titanium implants. The surface treatment plays a critical role in enhancing its corrosion resistance and biocompatibility. Several surface treatments have been used among which are; mechanical and electrochemical treatments, chemical etching, heat treatments, conventional and plasma ion immersion implantation, laser and electron-beam irradiation and application of bioactive surfaces [75]. The main intent of most of these techniques is to produce a uniform, stable and highly adherent TiO_2 layer.

Generally titanium alloys have a strong oxide passive layer. This protectiveness of the passive film is determined by the rate of ion transfer through the film and by the stability of the film against dissolution [35]. Furthermore, surface properties such as heterogeneity chemical composition, crystallinity, roughness, and wettability, are also of great importance to the stability of the TiO_2 layer and thus to the life of the biomedical implant [76].

Anodization

Electrochemical anodization is the surface modification method mainly used for titanium based biomedical alloys. In this approach, the implant material is exposed to an anodic voltage in an acidic solution. This treatment is commonly used for increasing the thickness of oxide layer for corrosion protection applications. Particularly, soluble selective ions releases can be suppressed in anodization of specific alloys by the altering the parameters of anodic potential, electrolyte composition, temperature, and galvanostatic conditions. The thickness of the oxide layer is bounded to the applied voltage. At voltage of 100V or above the formation of the oxide layer stops and the alloys reaches to dielectric or avalanche breakdown limit.

Yang et al. investigate the anodization of titanium at 90V to 180V using different concentrations of H_2SO_4. They indicated that with the increase in the applied voltage, the structural feature at the micrometer level could be obtained as shown in the figure 8. On the other hand the crystalline nature of the formed oxide layer changed to the applied potential from anatase to rutile [77]. Yang et al. concluded that the microporous structure of the titanium may have apatite. The other investigation showed that this type of anodized surface represent strong segmentation in bone and good osteointegration.

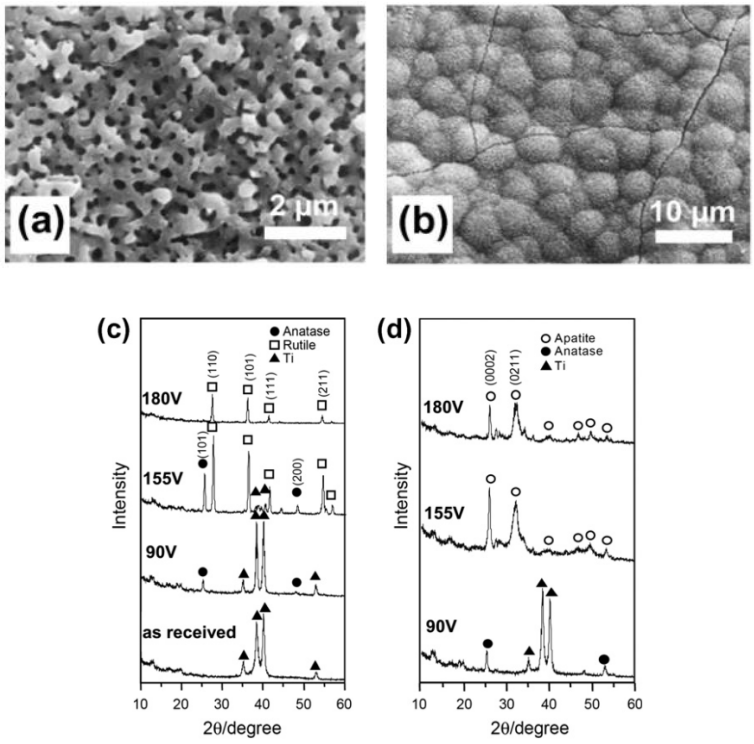


Figure 8: (a) SEM image of anodically oxidized titanium surface at 155V in 1M H2SO4 (b) after soaking for 6 days in SBF (c) XRD patterns taken from titanium surface anodized at 90V, 155V and 180V in 1M H2SO4 for 1 minute. (d) After soaking these samples for 6 days [77]**.**

Plasma Spraying

During this process, the coating is produced at high temperatures by using plasma jet, that project particle to the targeted area. The thickness of the layer varies from micrometers to nanometers. Bioactive and bioinert ceramic with excellent mechanical properties are used, such that, titania, zirconia and alumina. Most of the titanium-based materials are using this technique for the surface roughness and at the same time to modify the surface chemistry. Alumina and zirconia coatings are mostly used due to their high wear resistance. Plasma sprayed titania has been used to produce rough implant surface of 20 μm. The plasma praying in also commonly used for spraying Hydroxyapatite followed by other modifications of the surface for example

etching or blasting; moreover the surface modifications of with hydroxyapatite shows ver good structural influences in terms of bioactivity [78].

Lee et al. investigated the mechanical stability and in vivo behavior of implants of three surface designs: smooth surface (SS), rough titanium surface with a plasma-sprayed coating (PSC),and alkali- and heat-treated titanium (AHT) surface after plasma spraying. Implants have been grafted onto the dog bone, and the measured pull out forces of the SS, PSC, and AHT implants are 235, 34.25, 710, 142.25, and 823, 152.22 N, respectively. They observed that the AHT implant have good bone-bonding strength after 4 weeks of healing because of the mechanical interlocking in the micrometer-sized rough surface and the large bonding area between the bone and implant.

Alkali Treatment

Kim et al. first introduced alkali to improve the bioactivity. The method enables the formation of a biologically active bone-like apatite layer on the surface of bioactive ceramics, such as bioglass hydroxyapatite and glass–ceramic [79]. The materials are first immersed in a 5–10 M NaOH or KOH solution for 24 h, followed by rinsing with distilled water and ultrasonic cleaning for 5 min. The specimens are then dried in an oven at 408 °C for 24 h and finally heated to around 600–800 °C for 1 h. Because of the strong tendency of titanium to oxidize, the heat treatment is performed at a pressure of 10^{-4} to 10^{-5} Torr. After the treatment, a porous surface forms on the surface of titanium as shown in figure 9. The structural change of the titanium surface during alkali and heat treatments and mechanism of apatite formation on the treated surface in simulated body fluids are described as follows. During the alkali treatment, the TiO2 layer partially dissolves in the alkaline solution because of the attack by hydroxyl groups.

$TiO_2 + NaOH \Rightarrow HTiO^-_3 + Na^+$ (1)

This reaction is assumed to proceed simultaneously with hydration of titanium.

$Ti + 3OH^- \Rightarrow Ti(OH)_3 + 4e$ (2)

$Ti(OH)^+_3 + e^- \Rightarrow TiO_2 . H_2O + 1/2H_2$ (3)

$Ti(OH)_3 + OH^- \Leftrightarrow Ti + OH_4$ (4)

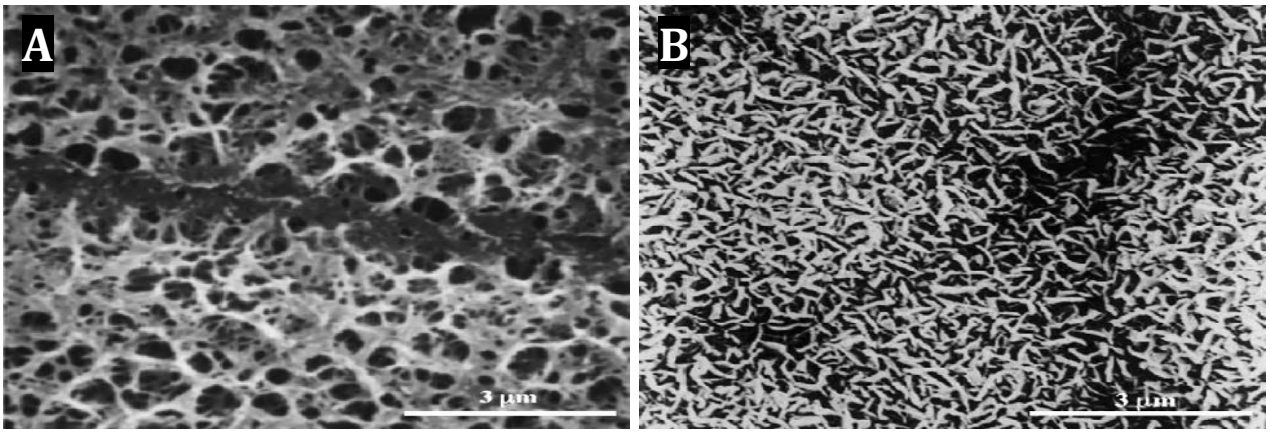


Figure 9: (A) Surface of alkali and heat treatment of titanium; (B) after soaked in SBF for 4 weeks [80]

Nishio et al. investigated the osteoblastic differentiationof bone marrow cells on the alkali- and heat-treated titanium. This examination revealed that apatite formation played an important role in osteoblastic differentiation. Bone-like apatite-formed by titanium after alkali- and heat treatment were observed to provide the most favorable conditions for bone marrow cell differentiation. Alkali and heat treated titanium bonds to bones directly; however but titanium that is only alkali-treated does not [80]. The detaching failure loads of untreated, alkali-treated, and alkali- and heat-treated titanium were investigated for 16 weeks after implantation and observed the failure of alkali and heat treated titanium alloys [81].

Porous Surfaces of Titanium

The scientists are now introducing new techniques in the field of orthopedic biomaterials in order to improve the bone implant bond. Bones, which appear to be a solid, are actually porous materials. Because of this, material must mimic the natural structure of bone for many different biomaterials applications. Many different methods to create porous biomaterials were explored [82]. One of the methods that have been used to create porous biomaterials was to solidify metals in the presence of a gas atmosphere, such as hydrogen, nitrogen, or oxygen. During metal solidification insoluble gases form pores within the metals. Techniques such as mold casting, continuous zone casting, and continuous casting each rely on the principle to form pores within the metal. Each technique utilizes slightly different mechanisms in which the pores are formed. Different techniques thus allow for the formation of various pore sizes, pore diameters, pore aspect ratios, pore orientation, and porosity [83]. Further techniques developed by Ryan et al. involved metal slurry and a wax template to be used to form porous materials. Rapid prototyping utilizes computer-assisted drawing and computer assisted manufacturing in order to form a wax template. The use of rapid prototyping allows for high level of details and also offers high levels of reproducibility. Through this method, porous titanium metals can be easily fabricated, and the porosity and the pore size can be controlled. Different porous titanium metals are represented in figure 10, and further on tested to determine their use as orthopedic biomaterials. The porous materials fabricated exhibit high mechanical properties and anisotropy. The mechanical properties reported were higher than that of bone, although it is expected that b reducing the porosity of the material will result in mechanical properties similar to the bone. The anisotropic nature of the material is similar to that of bone. Overall, the development of rapid prototyping offers many advantages to form porous titanium as orthopedic biomaterials [84].

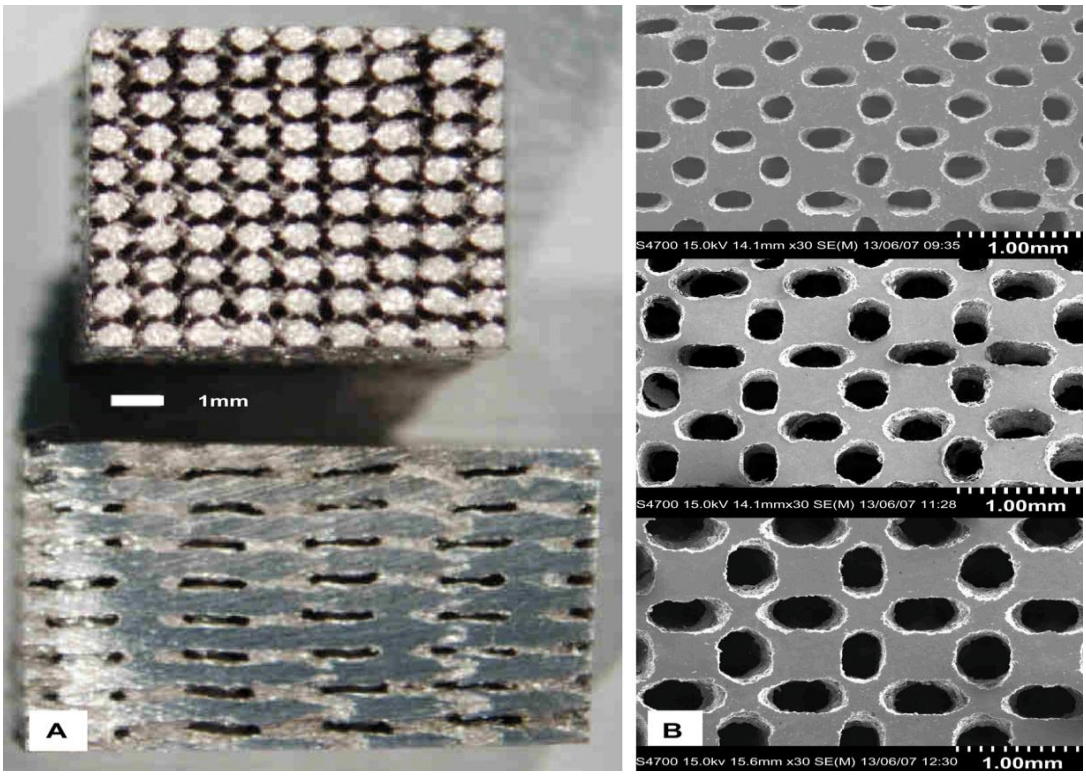


Figure 10: Porous titanium-based metals formed from rapid prototyping [84]

A second study conducted by Li, et al. utilizes a similar rapid prototyping technique in a method known as three-dimensional fiber deposition. This method utilizes a 3-dimensional bioplotter to eject metal slurry in the form of fibers onto specific locations, specified by the bioplotter. As the metal slurry is ejected from the bioplotter, the metal solidifies quickly forming the desired three-dimensional structure. This method was used to make porous Ti-6Al-4V. The titanium alloy was then studied as a scaffold for the lumbar spine in goats. From this study it was found that this scaffold was found to have high potential as an orthopedic biomaterial due to its biocompatibility, however further studies are necessary to determine the optimal porosity and pore size for orthopedic biomaterials [85]. There are many current studies being conducted in order to find new methods of fabrication for porous materials, and an important aspect for porous orthopedic materials is determining the effect of porosity of bone ingrowth and the resulting

mechanical properties. In a study conducted by Shen et al., the bone ingrowth and the resulting mechanical properties for various levels of porosity were determined. Bone ingrowth, also known as osseointegration, is influenced by many factors and is important in the success of the biomaterial.

Research Objective

Corrosion reduces the life span of metallic implants. The debris and the ions leaching from these implants have adverse reaction to the tissue, which leads to the generating great pain and short term and long-term complications. In order to overcome the problem different surface modifications were carried out without changing the bulk properties of titanium. These surface modifications are known to effect surface charge, chemistry, morphology; wettability, corrosion resistance and biocompatibility of these materials. Surface treatment should have the following effects on implants:

- Improve the corrosion resistance
- Reduces the leaching of ions and debris
- Develop stable oxide film
- Reduce cytotoxicity
- Promote good osseointegration and cell implant adhesion

Research Methodology

The research plane is shown in the figure 11. In order to plane the research methodology, the there titanium alloys were selected i.e. CPTi, Ti6Al4V and Ti6AL4V-ELI. For surface characterization the scanning electron microscopy (SEM) is carried out and Energy dispersive X-ray spectroscopy (EDS) is used for analysis of surface chemistry. Atomic force microscopy (AFM) is conducted to study the average roughness changes at nano scale. For surface wettability studies Kyowa contact angle meter is used. Electrochemical behavior of the alloys was studied before and after the surface modifications. Cyclicpoalzation scans were carried out to understand the corrosion susceptibility of each alloy. For Biocompatibility studies, cytoscane cytotoxic assay were conducted.

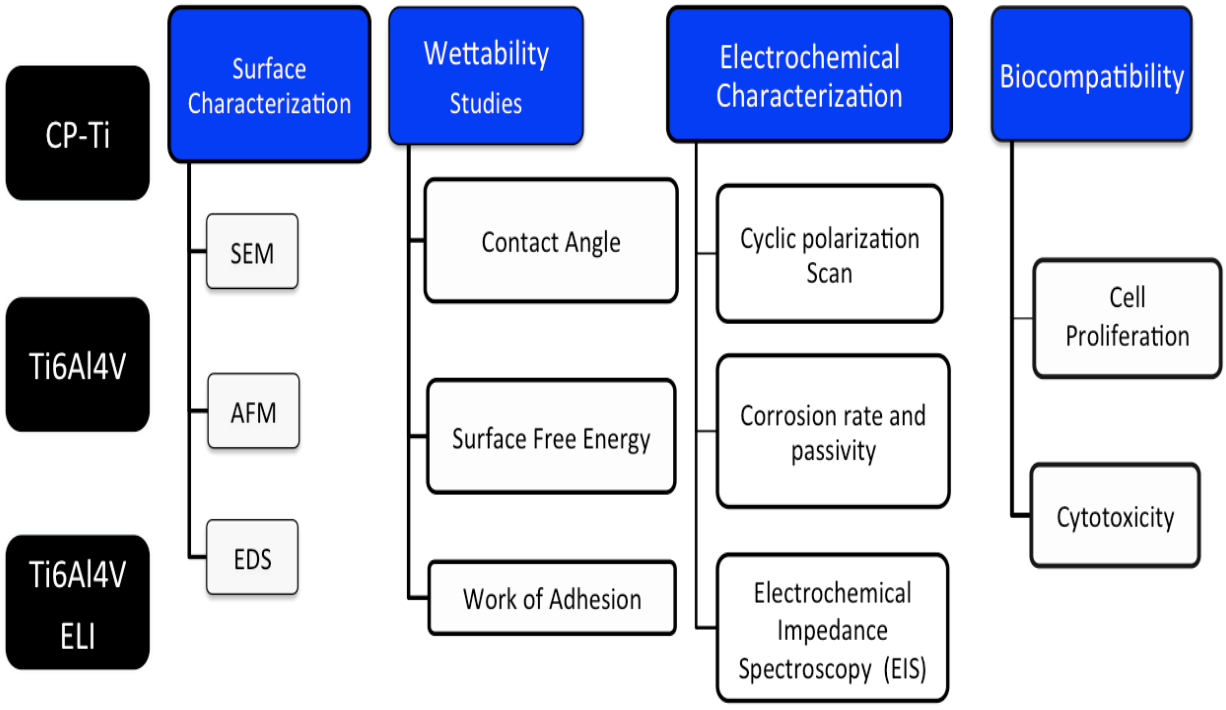


Figure 11: Research Methodology

CHAPTER III

MATERIAL SELECTION & METHOD

The commercially pure titanium (CPTi), Ti6Al4V and Ti6Al4V-ELI were obtained in the form of rods. The chemical composition of these alloys is given in the table 4, table 5 and table 6. The Ti6Al4V and Ti6Al4V-ELI have same composition but the Ti6Al4V-ELI have low interstitial atoms.

Table 4: Chemical composition of Commercially pure titanium

C	O	N	Fe	H	Others	Ti
0.006	0.16	0.006	0.08	0.0009	0.10	bal

Table 5: Chemical Composition of Ti6Al4V

C	Si	Mn	Mo	Al	V	Fe	Cu	Ti
0.006	<0.01	<0.01	0.01	6.06	3.94	0.13	<0.01	bal

Table 6: Chemical Composition of Ti6Al4V-ELI

C	Si	Mn	Mo	Al	V	Fe	Cu	Ti
0.006	<0.01	<0.01	0.01	6.06	3.94	0.13	<0.01	bal

Sample Preparation

CPTi, Ti6Al4V and Ti6Al4V-ELI rods of medical grade were cut with high-speed saw into circular disks samples having 0.2 inches of thickness and 0.625 inches diameter.
The samples were grinded using silicon carbide grit papers on Buehler® abrasive belt grinder. Grit paper sizes: 180, 240, 320, 400, 600, 800, 1000 and 1200 were used simultaneously in order to achieve smooth and mirror like surface finish. Each sample is first cleaned with deionized water and then ultrasonically cleaned with acetone for 15 minutes.

Surface Treatments

The mechanically polished samples were sent to different companies in order to modify their surface. For this purpose the help was taken from the Electrobright® (Macungie, PA, USA). This company performed the Electropolishing (EP) and Magnetoelectropolishing (MEP). The Hydroxyapatite plasma spraying and titanium coatings was accomplished by APS Materials®, which is an Ohio based company.

Mechanical Polishing

The circular disks of the grinded alloys of CPTi, Ti6Al4V and Ti6Al4V-ELI were cleaned with DI water soon after grinding. These untreated or mechanically polished samples were ultrasonically cleaned with acetone for 15 minutes and carefully packed and sealed in plastic bags to avoid contamination.

Electropolishing (EP)

EP is the standard method of surface treatment employed as a final finishing of the substrate of selected titanium alloy. The general procedure for electropolishing is accomplished by applying an electrical potential, which result in the ionic dissolution of the surface. In this process, the surface chemistry and morphology of the material are altered. Resulting in the removal of the imperfections and inclusions by dissolving metal ions [66]. It is generally stated that during electropolishing the oxide layer of the substrate is completely dissolved and new, stable and homogeneous passive film is obtained [86]. The American Society of Testing materials (ASTM) recommends elctropolishing and nitric acid, for the surface treatment of the medical devices to remove the deformation in the native oxide layer.

Magnetoelectropolishing (MEP)

MEP is one of the effective methods of altering and modifying surface properties. This modification is obtained by applying a constant external magnetic field to the electropolishing system by using neodymium ring magnet. The acidic electrolyte is mostly used in this procedure. [87].

Hydroxyapatite (HA)

It is a complex phosphate of calcium $Ca_5(PO_4)_3OH$ is a mineral , main structural element of the vertebrate bone, and the main inorganic component in the mammal bones or teeth and has attracted attention as a surface-coating compound because of its high osteoconductivity. Many pyroprocessing methods of forming HA and other calcium phosphate coatings on metallic substrates have been reported (e.g. plasma spraying, sol-gel method, electron beam sputtering

method, and ion beam sputtering method). However, all have disadvantages related to coating with HA, on complex-shaped implants. Plasma spraying remains the most commonly used technique for HA coating on a Ti or Ti alloy substrate for the fabrication of artificial joint replacements and in endosseous dental implants. On the other hand, many hydrocoating techniques e.g. cathodic electrolysis method, electrophoretic method and thermal substrate method also have been proposed as approaches to forming thin-film coatings on metallic implants [88].

CHAPTER IV

ELECTROCHEMICAL ANALYSIS

Corrosion is the degradation or destruction of a metal by means of chemical or electrochemical reactions with its surrounding environment that deteriorates the material and its properties [89]. It is very important to determine the capacity of materials to resist deterioration without modifying their surface when exposed to aggressive media during service. In order to determine whether a material can be considered as resistant to corrosion, methods and characterization techniques are available which include the study of the chemical and metallurgical processes, the design and application of methods, respectively [2, 30]. The corrosion process involves two reactions: anodic and cathodic which are governed by the following equations:

$$M \Leftrightarrow M^{n+} + ne^{-} \quad (5)$$

$$O_2 + 2H_2O \Leftrightarrow 2e^{-} + 4OH^{-} \quad \text{for } pH \geq 7 \quad (6)$$

$$O_2 + 4H \Leftrightarrow 2e^{-} + 2H2O \quad \text{for } pH < 7 \quad (7)$$

The oxidation of the metal is shown in the equation (1) while the reduction process of hydrogen is shown in equation (2) and equation (3) is for reduction process in conditions of pH > 7 and pH < 7.

Corrosion is the first consideration for a material of any type that is to be used in the body because metal ion release takes place mainly due to corrosion of surgical implants [90]. Therefore, various in vitro and in vivo tests have to be carried out in order to identify appropriate materials for use as surgical implants. The environment of the body is extremely well buffered so that the pH is maintained at around 7.4 at 37 °C. Two factors control the severity of this environment. Firstly, the saline solution is an excellent electrolyte and facilitates the electrochemical mechanisms of corrosion and hydrolysis [91]. Secondly, there are many molecular and cellular species in the tissues that have the ability to catalyze certain chemical reactions or rapidly destroy certain components identified as foreign [92]. The primary aim of the surface treatments is to enhance the protective passive film by changing its composition, structure and thickness, and/or by reducing weak points such as non-metallic inclusion.

Passivation

Passivation is the formation of a thin, protective, hydrated oxide, corrosion product surface film that act as a resistive barrier between the solution and the metal, and leads to a marked reduction in the rate of the anodic reaction. Typical used metallic implants like titanium and their alloys have the ability to form a stable oxide layer spontaneously. This layer has the thickness of the few nanometers, which act as a protective barrier to corrosion in aggressive environment [93]. A variety of factors affecting the stability of passive layer are chemical composition, structural thickness, defects and inclusions [94].

When the potential exceeds the equilibrium potential, the oxygen evolution reaction starts to occur on the surface of the passive film. The rate of this reaction will depend on the electronic resistance of the passive film (since electrons must pass through the film to permit the oxygen

evolution reaction to occur) and on its electrochemical reactivity. If the metal oxide can be further oxidized to a more soluble state, transpassive (meaning 'above passive') corrosion can occur [95].

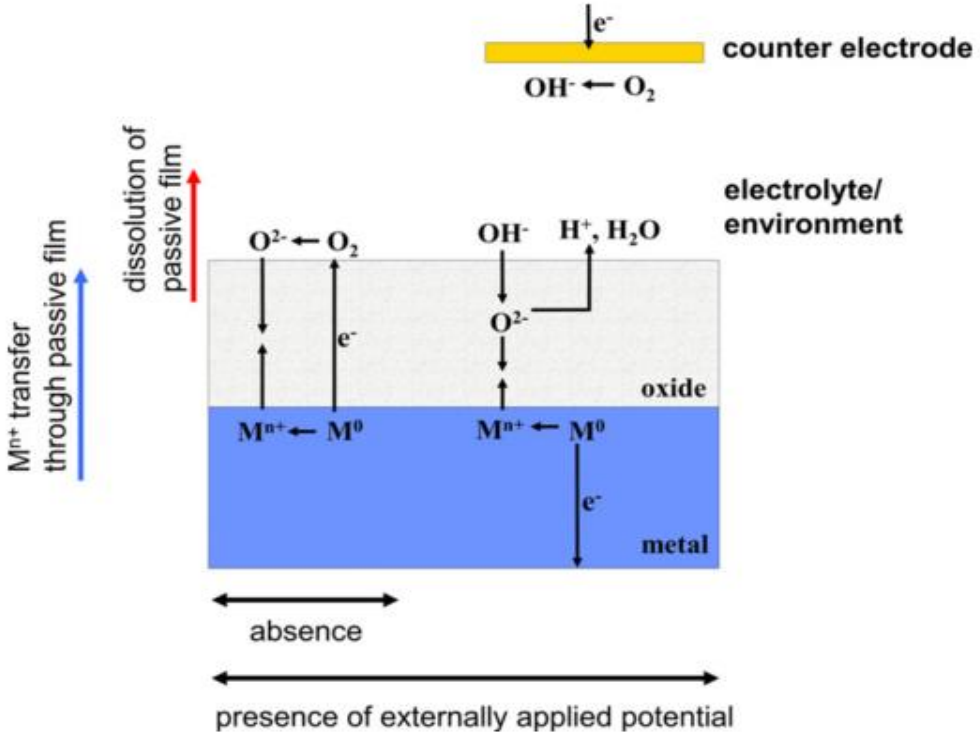


Figure 12: Surface dissolution and growth of oxide layer [4]

The passive state of the implants is prone to localized corrosion under some circumstances. If the metal is susceptible to localized corrosion, local breakdown of the passive film can occur, leading to pitting corrosion, crevice corrosion, and embrittlement by stress corrosion cracking. Which means the implant failure [94]. The titanium alloys has the sbility to spontaneously form a stable self-protecting oxide layer on its surface in the reaction with air or aqueous environments. In titanium alloys mostly the passive oxide film composed of TiO_2. During the oxide formation, one oxide compound is dissolved while other oxide compound

remain enriches on the surface. Which provides the barrier to aggressive biological fluids for Titanium metallic implants. The ions transfer through the film kinetically determines the quality and stability of the passive oxide film and the stability of the film against dissolution are shown in figure 12.

Common Corrosion Types in Metallic Implants

Pitting Corrosion

Pitting corrosion is a localized corrosion that is caused by the local dissolution of the surface passive film, which results in the formation of pits or cavities, surrounded by an intact passivized layer. Due to small size and distribution close to each other, the implant surface appear very rough. Pit is a cavity or hole with the surface diameter about the same or less than its depth [96]. In biomedical applications failure caused by pitting corrosion is often observed, especiall in 316L alloys. Pitting is an autocatalytic process i.e. corrosion processes within the pit produce conditions which are necessary for the sustained activity of the pit. In this process fast dissolution occurs within the pit and oxygen reduction takes place on adjacent surfaces. Pitting corrosion can cause an implant device to fail because of perforation with only a small percent weight loss of the entire implant. Pitting usually occurs in halide containing solutions. Rapid dissolution produces an excess of positive charge which attracts chloride ions to maintain the electro-neutrality. Both chloride and hydrogen ions stimulate the dissolution of metals. This process accelerates with time since there is a very low concentration of the oxygen available within the pit, no oxygen reduction occurs. Oxygen reduction on the adjacent surfaces tends to suppress corrosion and in that way pits cathodically protects the rest of the alloy surface [97].

Crevice Corrosion

Crevice corrosion is a similar localized form of corrosion like pitting corrosion but it is preferentially found in regions of the metal and alloys where mass transfer is limited such as narrow crevices, voids, gaps and under deposits. Metals that show the affinity to the pitting corrosion usually also suffer from crevice corrosion. Crevice corrosion leads to the depletion of oxygen and the enrichment of the aggressive species, which results in the acidification and the activation of surface in the crevice area. During crevice corrosion the metallic surface inside the crevice acts as anode and the bulk metallic surface acts as cathode. For the electrochemical reaction , the concentration of metal ions in the crevice increases due to anodic dissolution. These metal ions in the crevice attract the chloride ions from the bulk solution and forms unstable metallic chlorides, which then hydrolyze to produce H+ ions, and results in the decrease of the pH. The increase in acidity increases the dissolution rate of the metal, thus attracting more chloride ions and further on lowers the pH. This is an autocatalytic process and the solution in the crevice is becoming more and more aggressive as the concentration of Cl^- and H^+ ions are increasing [66]. Passive metallic materials are highly susceptible to crevice corrosion in chloride or hydrogen ions. Titanium critically shows the crevice corrosion at the temperature above 80°C in neutral chloride solution[98]. However the deplition of oxygen in the biomedical devices is critical and leads to localize corrosion [99].

Fretting Corrosion

The oscilations in the implantable joints lead to the fretting corrosion, commonly occuring at the interface. The mechanical wear in passive material, can lead to constant removal of the passive film, typically followed by a repassivation process. In detail, the damage is mostly

of a localized form and can be a defect at the surface that does not show repassivation, resulting in the formation of a pit, or a continuous cyclic process of activation and repassivation [100]. Moreover, fretting and crevice corrosion have been identified as one of the most important problem occuring in implant corrosion as the risk of metal ion release should be taken into account as a direct consequence of these continuous activation and repassivation events [101].

ASTM F 2129 Standard

The ASTM F 2129 is the standard method for the cyclic potentiodynamic polarization test for small implantable devices and was used in this study. The American Society of Testing and Materials was introduced this standard in 2001 and they updated it time by time. It is basically a test procedure for measuring the corrosion susceptibility of small metallic implants using cyclic potentiodynamic polarizations. The Food and Drug Administration (FDA) requires medical-device manufacturers to provide corrosion data to ensure that their device possess sufficient corrosion resistance and ASTM F 2129 is frequently used to satisfy this FDA requirement.

In general, three common methodologies exist as possibilities for an acceptance criterion. The first is that the E_b of an implantable device should have similar or better corrosion resistance than approved devices currently in the market with no known corrosion problems. The second methodology that has been proposed is that the E_b of the implantable device should be more than some threshold value. It is generally agreed that a Nitinol implantable device should have an E_b higher than 0.600 V with respect to the saturated calomel electrode (SCE). The third methodology is to use the difference between the breakdown potential (E_b) and the rest potential (E_r), (E_b- E_r), as a measure of corrosion resistance. The typical curves are mentioned in figure 13.

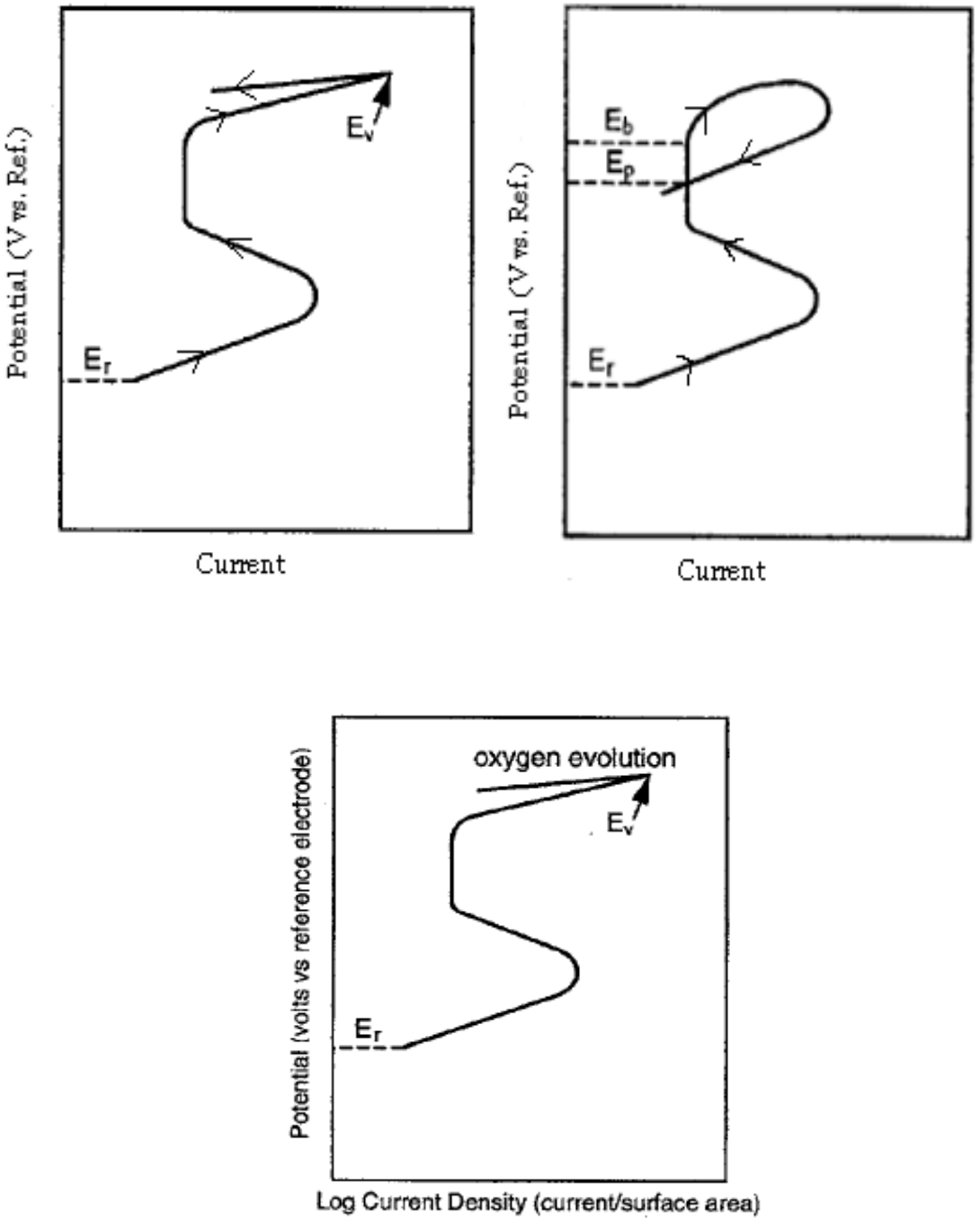


Figure 13 : Typical cyclic potentiodynamic curves illustrating different corrosion parameters

Breakdown potential (E_b) is the least noble potential at which pitting or crevice corrosion or both will initiate.

Protection potential (E_p) is the potential at which the reverse scan intersects the forward scan at a value that is less noble than E_b.

Rest potential (E_r) is the potential of the working electrode relative to the reference electrode measured under virtual open-circuit conditions.

Vertex potential (E_v) is the potential at which the scan is reversed.

Electrochemical Analysis of Titanium alloys

The conventional three-electrode cell was assembled using saturated calomel electrode (SCE) as reference electrode, carbon graphite as counter electrode and titanium sample as working electrode. The schematic diagram for the corrosion cell is shown in the figure 14. The cyclic potentiodynamic corrosion tests were conducted inside an incubator (temperature 37°C maintaining at 5% CO_2) at the scan rate of at 1mV/s and potential range of -500 to 1500 mV against SCE.

Reagent

The phosphate buffer saline tablets (Part # P4417-50TAB Sigma Aldrich® USA) are used to make 1x solution by dissolving one tablet in 200 ml of deionized water. This solution is used as an electrolyte and the chemical composition of the electrolyte solution in g/l is mentioned in the table 7. The PBS is also called the simulated body fluid as this solution has the same chemical composition as body fluids. In order to decrease the concentration of dissolved oxygen and to achieve the deaerated environment for the corrosion analysis, pure Nitrogen

(99.9%) gas was purged through the electrolyte for 15 minutes before each test. The pH and dissolved oxygen concentration was monitored before and after each test.

Table 7: Chemical Composition of PBS solution (g/L)

NaCl	Na2HPO4	NaHCO3	KCl	KH2PO4	MgSiO4	7H2O	CaCl2
8.0	0.06	0.35	0.4	0.06	0.2	bal	0.14

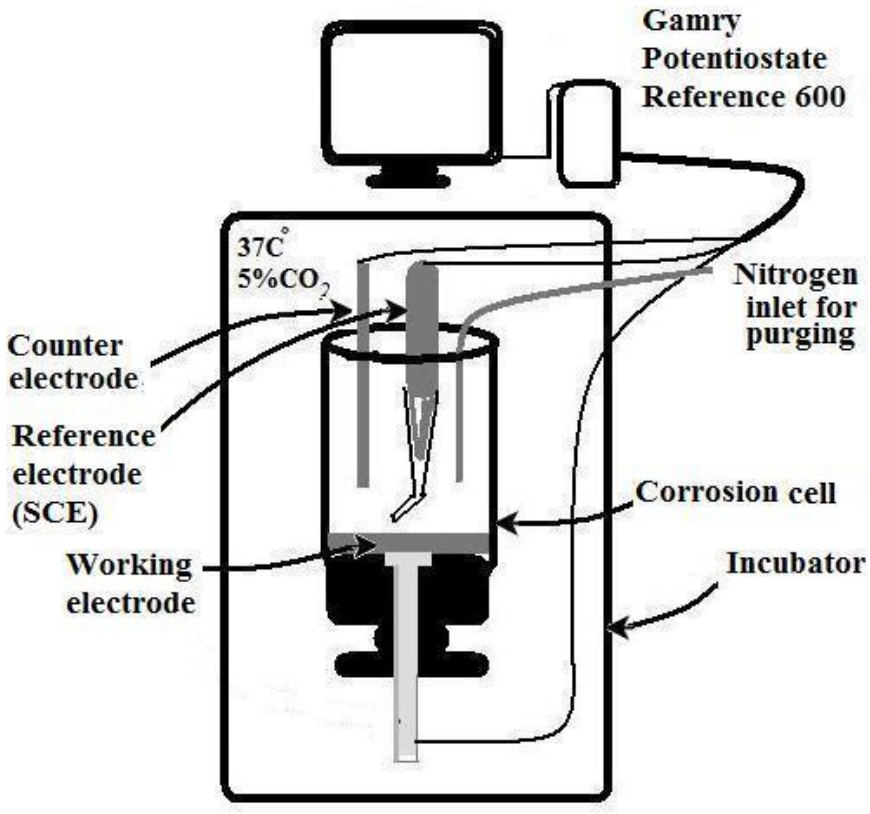


Figure 14: Schematic diagram of the three electrode cell with in incubator

Gamry potentiostate (reference 600) is used for the cyclic potentiodynamic polarization scans (shown in figure 15). For the corrosion data analysis, Gamry framework and Gamry Echem Analyst software are used. The current flowing between the electrodes and the graphite counter electrode is measured on high impedance. The data was plotted with the current density in A/cm^2 on the x-axis using logarithmic scale versus potential in V on the y-axis. Each test for the cyclic polarization scans was conducted in accordance with the ASTM standard F2129 and EIS test were performed in accordance with G-39 at 37°C. EIS tests were conducted in the same PBS environment under high purity nitrogen to determine the effect of alloying element on the charge transfer resistance and were conducted in the frequency range from 10^5 HZ to 0.01HZ with 10 points per decade.

Figure 15: Gamry Potentiostate

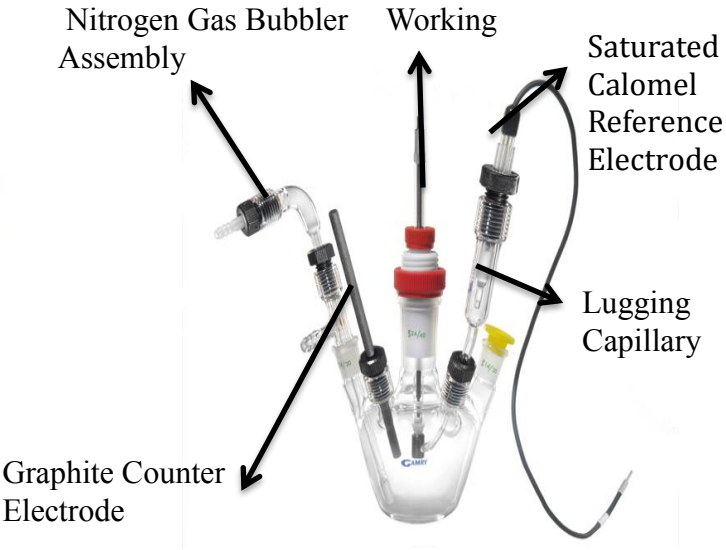


Figure 16: Typical Euro electrochemical glass cell

Cyclic Potentiodynamic Polarization Test

In order to understand the stability of metallic implants with body fluid, in a specific environment, the simple method is cyclic polarization technique (forward and reverse polarization). A technique in which the potential of the test specimen is controlled and the corrosion current is measured by a potentiostat. The potential is scanned in the positive or noble (forward) direction. The potential scan is continued until a predetermined potential or current density is reached. Typically, the scan is run until the transpassive region is reached, and the specimen no longer demonstrates passivity. The potential scan direction then is reversed until the specimen repassivates or the potential reaches a preset value. This is a highly useful method for determining the susceptibility of metals or alloys to pitting when placed in a specific corrosive environment. Potentiodynamic Cyclic polarization measurements were used to determine the active-passive characteristics of samples before and after surface treatment.

The Tafel fit and Tafel extrapolation method was employed to analyze the polarization curve where passivation control occurs by selecting two points' ±10mV of E_{corr} values. Each surface modification of electropolishing, magnetoelectropolishing, hydroxyapatite and titanium coating show improvement to crevice and pitting corrosion.

From the polarization scan of each sample no hysteresis loop was observed and all the scans have same general features of active, passive and transpassive regions. In general the pitting and crevice corrosion can be evaluated based on the formation of a loop and an evaluation of the sample can be made based on the area of loops that form in the cyclic polarization curves. The higher the loop area the greater is the tendency to pitting and crevice corrosion. In all cases the reverse scan takes an entirely different path, which is a clear indication of excellent pitting, and crevice corrosion resistance.

In case of CPTi, alloys the maximum corrosion rate was observed for untreated alloys. There is no significant difference in Icorr values therefore the difference in corrosion rate is smaller but the EP and MEP samples behave noble and more passivized to corrosion as compared to hydroxyapatite and Ti coated samples. The significant shift of the I_{corr} values of EP and MEP to more positive potentials make it more noble to crevice and pitting corrosion. The small shift in potential has been observed in Ti coated and HA coated of CPTi alloys are shown by using the scan after overlapping the graphs. The comparison shows that the E_{corr} values of CPTi EP are higher than MEP more the Ti and HA treatments while CPTi untreated Ecorr value is comparatively very low to Ti and HA. Which indicates that untreated CPTi is more susceptible to corrosion that is clearly shown in the figure 17. Each treatment indicates the reverse scan above the forward scan, which shows the passivation. The complete values of the E_{corr}, I_{corr}, I_{pass} and corrosion rates are mentioned in table 8.

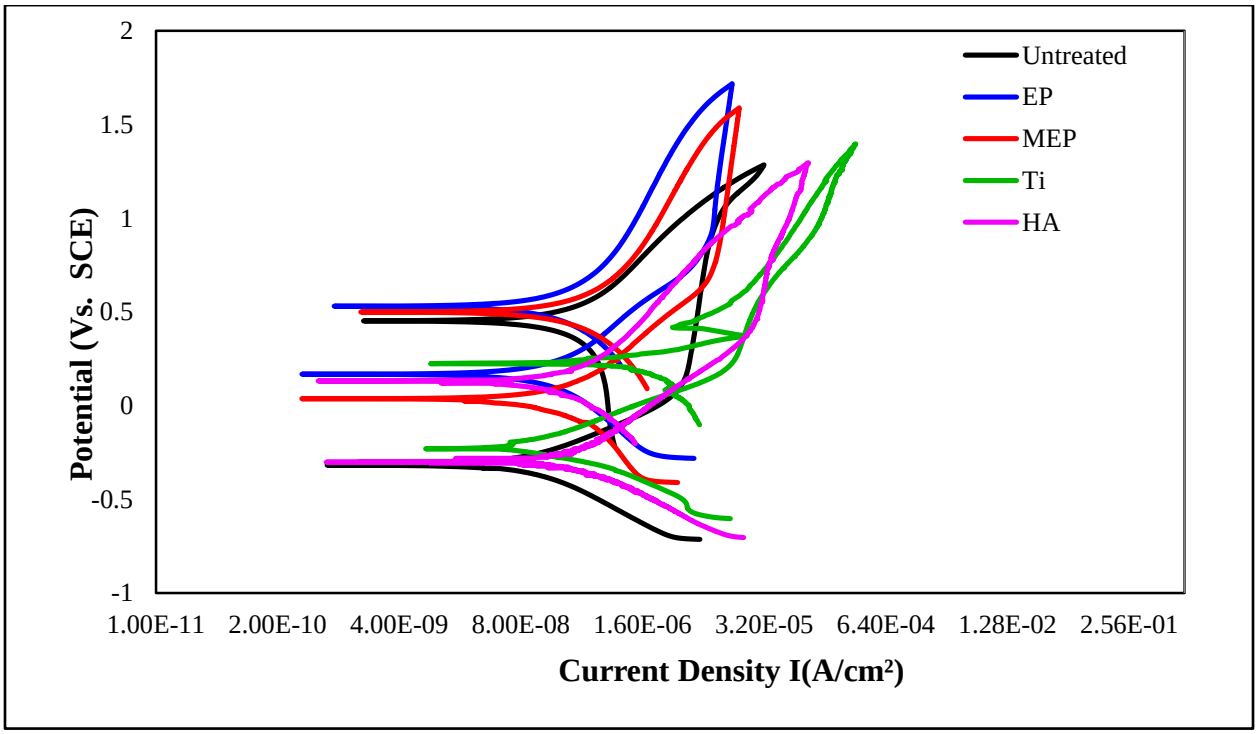


Figure 17: Cyclic potentiodynamic scans of CPTi

Table 8: Average values of CPTi cyclic polarization scan

CPTi	Area (Cm^2)	Dissolve Oxygen (mg/L)	pH before test	pH after test	E_{corr} (mV)	I_{corr} (A/cm^2)	Corrosion Rate (MPY)
Untreated	1.282	0.8	7.20	7.00	-317.0	64.40E-9	21.65E-3
EP	1.282	1.0	7.10	7.02	197.0	43E-9	14.63E-3
MEP	1.282	0.8	7.21	7.20	6.650	10E-9	3.41E-3
Ti Coated	1.282	1.3	7.22	7.28	-230.0	85.00e-9	46.22e-3
HA Coated	1.282	1.5	7.20	7.32	-296.0	2.28E-10	76.53e-2

In the case of Ti6Al4V, Ti coated alloy is shifted to more positive potential and is showing the smallest loop as compared to EP, MEP, and HA but its scan shows the hysteresis, meaning that the alloy is more susceptible to pitting corrosion. By comparing all the readings, the optimum corrosion resistant is EP. It has the smallest loop area and having no hysteresis as clearly shown in overlapped cyclic polarization scans in represented in figure 18. The table 9, included all the reading and corrosion rates measured by Tafel extrapolation method. Each surface indicates passivation after surface modification of electropolishing and magnetoelectropolishing, Ti and HA coating. EP and MEP shift the alloys to more noble potentials and make it more resistant to crevice and pitting corrosion.

Figure 19 depicts the overlapped scans of cyclic potentiodnamic polarizations of Ti6Al4V-ELI. There are variations and zigzag patterns in some of the places of HA and Ti coated scans. This is due to the porous and unsmoothed surfaces topography. The porous

topography always provides more surface area to the electrolyte for the interaction that's why sometimes its results are unpredictable. From the scans it is clear that the EP and MEP are more passivized and are showing more corrosion resistant behavior to HA and Ti Coated. The HA and Ti coated alloys are showing good shift to noble potential after the surface treatment. As represented in table 11, the corrosion rate of the untreated alloy is very high as compared to the surface modified alloys. Thus it can be concluded that each coating have positive impacts on the corrosion rate.

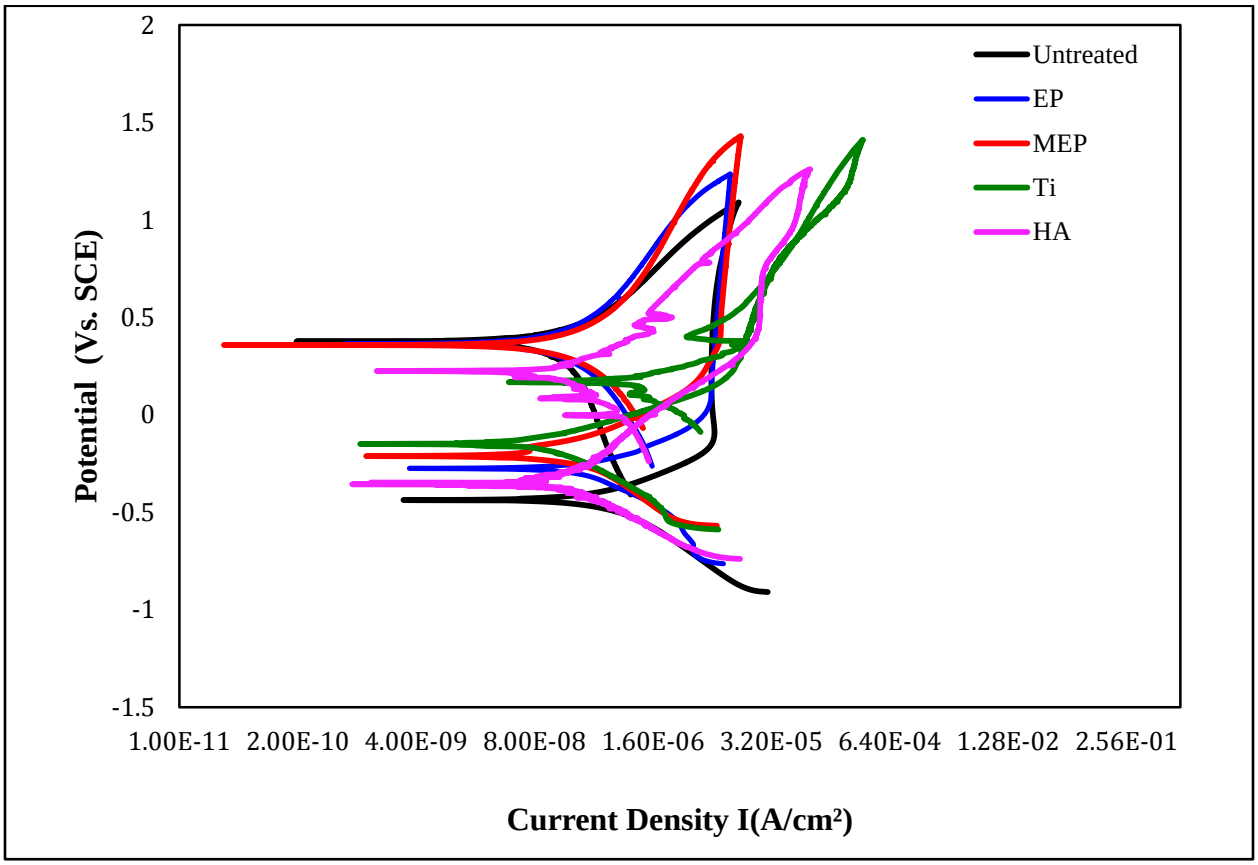


Figure 18: Cyclic Potentiodynamic Scans of Ti6Al4V

Table 9: Average values of Ti6Al4V cyclic polarization scan

Ti6Al4V	Area (Cm^2)	Dissolve Oxygen (mg/L)	pH before test	pH after test	E_{corr} (mV)	I_{corr} (A/cm^2)	Corrosion Rate (MPY)
Untreated	1.282	1.7	7.21	7.10	-437	159E-9	54.88E-3
EP	1.282	1.7	7.24	7.1	-228	115E-9	39.78E-3
MEP	1.282	1.6	7.28	7.13	-211	18.10E-9	6.164E-3
Ti coated	1.282	1.5	7.26	7.29	-150	9.62E-08	3.33E-02
HA coated	1.282	1.9	7.22	7.3	-355	9.62E-08	6.47E-02

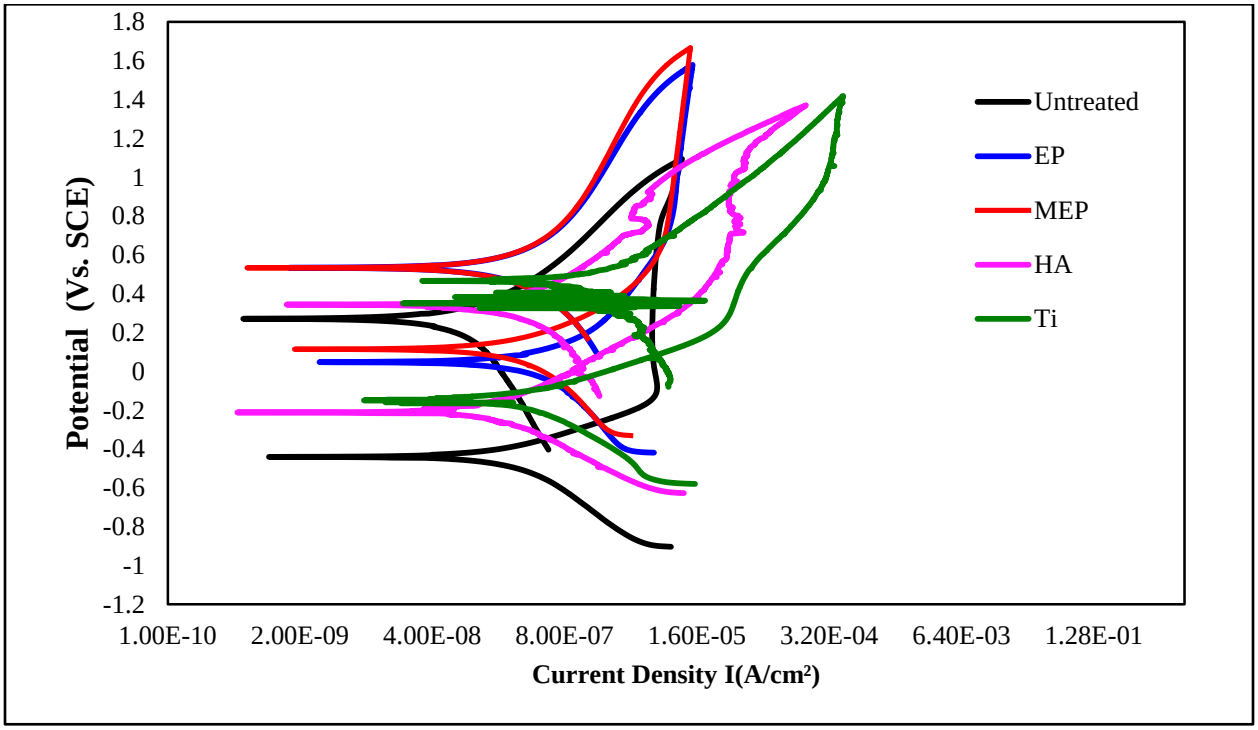


Figure 19: Cyclic potentiodynamic scans of Ti6Al4V-ELI

Table 10: Average values of Ti6Al4V-ELI cyclic polarization scan

Ti6Al4V-ELI	Area (Cm^2)	Dissolve Oxygen (mg/L)	pH before test	pH after test	E_{corr} (mV)	I_{corr} (A/cm^2)	Corrosion Rate (MPY)
Untreated	1.282	0.5	7.2	7.00	-44.7	48.20E-9	16.69E-3
EP	1.282	1.1	7.21	7.13	47.60	31.40E-9	10.87E-3
MEP	1.282	1.7	7.25	7.15	114.0	33.70E-9	11.65E-3
Ti coated	1.282	1.5	7.22	7.29	-408.0	3.71E-07	128.5e-3
HA coated	1.282	1.8	7.21	7.3	-212.0	7.36E-08	25.47e-3

CHAPTER V

MATERIAL CHARACTERIZATION

The surface morphology of the specimens was analyzed by scanning electron microscope (Carl Zeiss, Germany), the SEM micrographs were taken before and after each test of corrosion. The elemental distribution on the surface of each alloy was investigated by energy dispersive spectroscopy (EDS).

Surface roughness of the substrates was measured by using atomic force microscope (DI-Veeco, Dimension 3100 USA) and standard Si_3N_4 tips were used for imaging. One specimen of each alloy was analyzed by using three individual measurements were made on each specimen. Average roughness (R_a), maximum roughness (R_{max}), skewness (K) and the difference between the maximum and the average surface heights (R_q) were measured.

Kyowa contact angle meter (DM-CE1, Kyowa, Japan) was used for measuring contact angle, surface free energy and work of adhesion by sessile water drop method. The three chemicals approach (Di-water, Diiodomethane and Ethylene Glycol) was used in order to determine the surface free energy and work of adhesion.

Surface Morphology

Surface modifications are known to improve surface morphology and chemistry of titanium alloys. Figure 20 shows the SEM photomicrographs and EDS analysis of commercially

pure titanium alloys. SEM photomicrographs reflect that the surface morphologies are significantly changed after surface treatments. Untreated surface represented in the passage (A1) exhibits the grinding marks. The electropolished CPTi (A2) has less uniform morphology and the EDS data are showing alumina particles incrusted with in large pores. The CPTi electeropolished sample shows the porous structure on the surface. Magnetoelectropolised CPTi (A3) surface depicts the granular structure, having titanium oxides. The porous structures are known to effect cell proliferation and gives better osteointegration [102]. Ti coated (A4) and HA (A5) coated exhibited rough morphologies.

Figure 21 shows the SEM photomicrographs and EDS analysis of Ti6Al4V alloys. The SEM photomicrographs reflect that the surface morphologies are significantly changed after surface treatments. Magnetoelectropolised Ti6Al4V (B3) surface exhibited enhanced micro granular structure as indicated in the figure 21B. By careful observation, electropolished Ti6Al4V (B2) surfaces have different surface appearance (less porous) when compared with electropolished CPTi (more porous). HA surface is highly porous while titanium coatings have dendritic pattern. Magnetoelectropolised Ti6Al4V-ELI (C3) surface is very smooth when compared with untreated and electropolished Ti6Al4V-ELI (C1 and C2) is represented in figure 22.

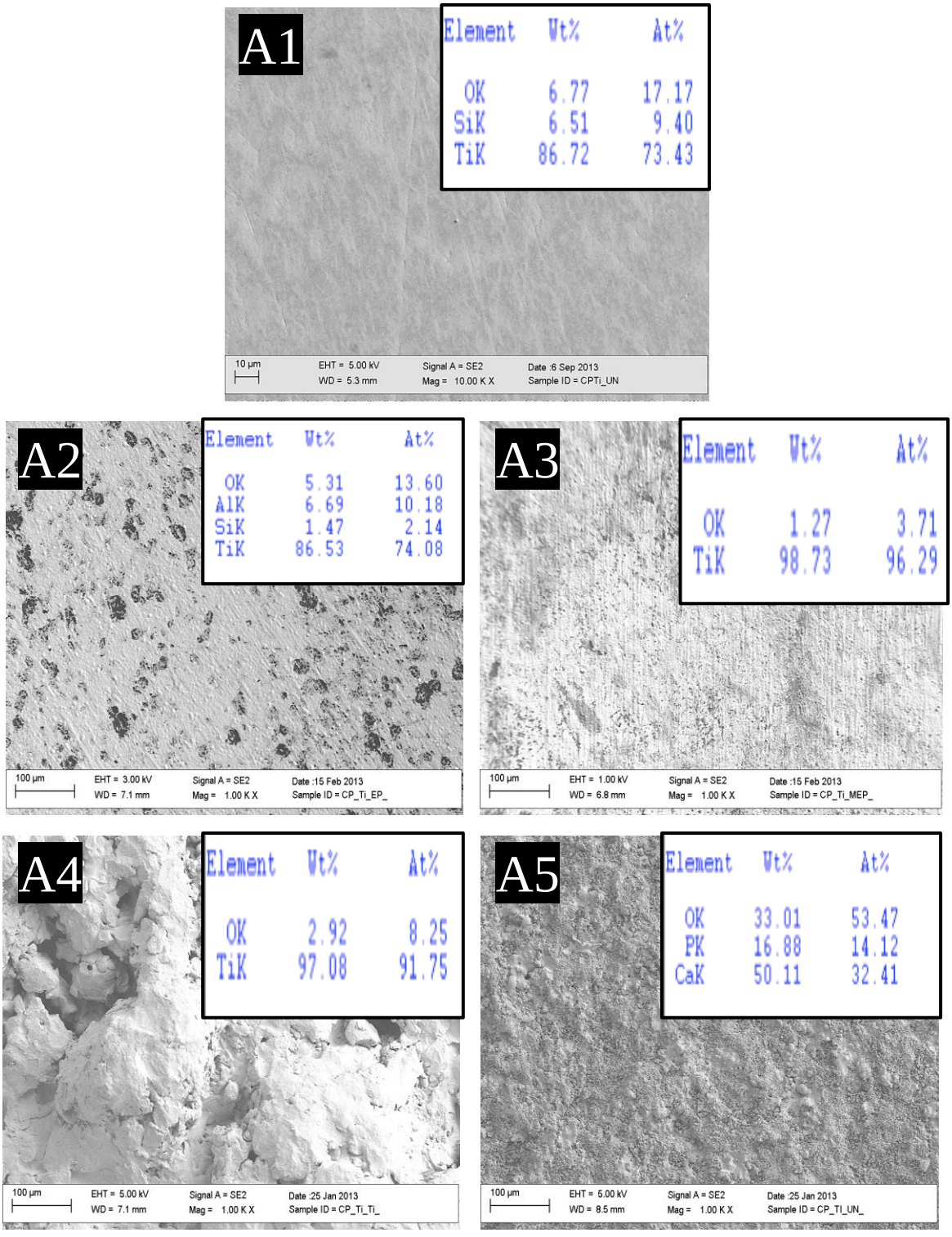


Figure 20: SEM surface morphology & surface chemistry of CPTi having different modified surfaces: (A1) Untreated; (A2) EP; (A3) MEP; (A4) Ti Coated; (A5) HA Coated

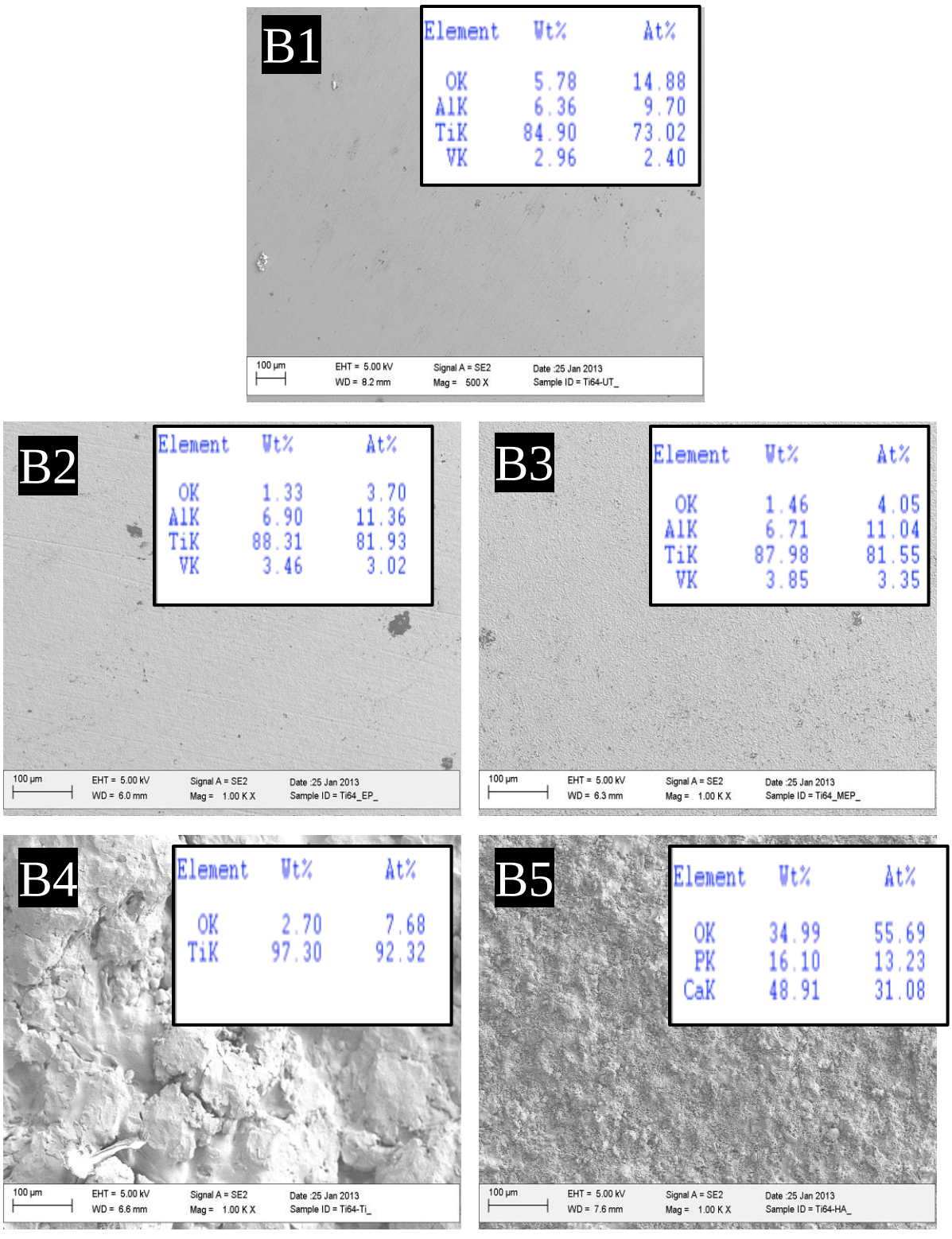


Figure 21: SEM surface morphology & surface chemistry of Ti6Al4V having different modified surfaces: (B1) Untreated; (B2) EP; (B3) MEP; (B4)Ti Coated; (B5) HA Coated

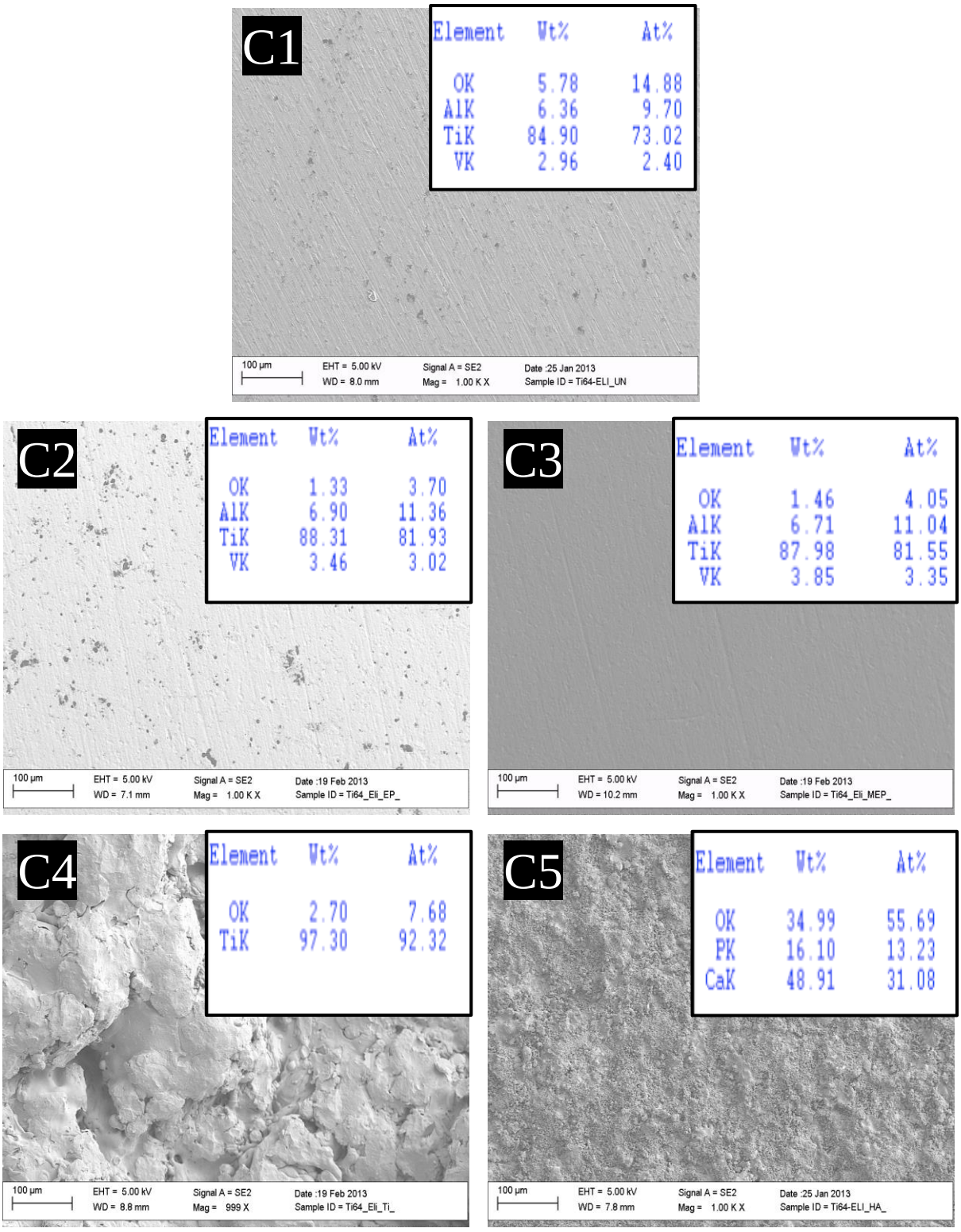


Figure 22: SEM surface morphology & surface chemistry of Ti6Al4V-ELI having different modified surfaces: (C1) untreated; (C2) EP; (C3) MEP; (C4) Ti Coated; (C5) HA Coated

Surface Roughness

The integration of the bone tissues to the surface of implant influences the performance of the biomaterial. The imperfect integration of the implant with the surrounding tissue results in the failure of the implant. From the biomaterial's point of view, the main factors contributing to the integration of an implant are the surface topography and surface free energy (SFE) [103]. Studies suggest that surface roughness influences cell adhesion. Ti surfaces with increased roughness and complex microstructures enhance bone-to-implant contact and result in better osseointegration [104]. The connection between implant and living remodeling bone without any soft tissue component at microscopic level is known as osseointegration [105].

Deligianni et al has found better cell proliferation and enhanced protein adhesion on rough hydroxyapatite (HA) surface when compared with smooth and polished surface. It was concluded that cell adhesion, proliferation and detachment strength were sensitive to surface roughness and increased as the roughness of HA increased [106]. The micron and submicron scale surface roughness is influential for permanent implants, which have long-term biomechanical integrity of bone-implant interface. The rougher surface have positive impact on the osseointegration and displaying exceptional cell adhesion which reduces the micro-motions and improve biomechanical interaction [107]. However, roughness makes the implants more susceptible to corrosion and initiates pitting in its oxide layer [108]. Optimal mechanical interlocking of implant and optimum surface roughness to the host tissue is required to achieve acceptable integration of implant and tissue. The AFM surface topography of CPTi, Ti6Al4V and Ti6Al4V-ELI is shown in figure 23. All untreated surfaces look very similar with prominent feature of retained titanium intermetallic inclusions. The results indicated that in each group of samples the MEP has the lowest roughness than EP and untreated alloys. Figure 23 shows the

AFM 3D simulation of roughness of untreated, EP, MEP and HA of CPTi, Ti6Al4V and Ti6Al4V-ELI Rp is root mean square of roughness, Ra shows arithmetic mean of roughness, RMax is maximum Peak height and K is skewness.

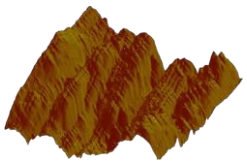
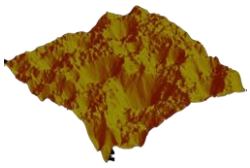
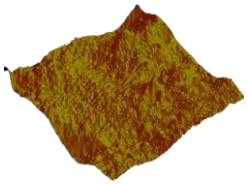
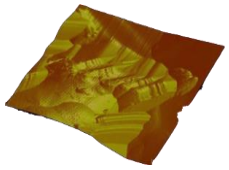
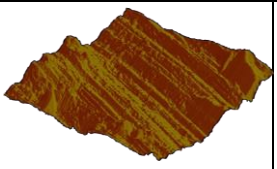
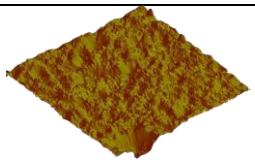
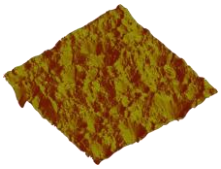
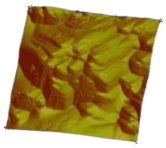
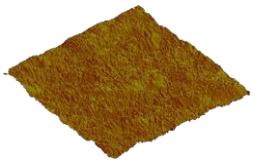
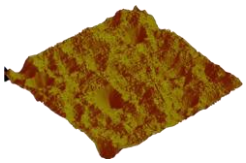
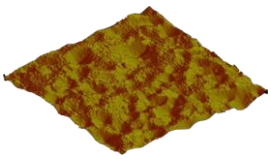
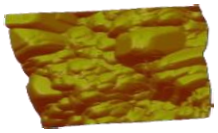
	Untreated	EP	MEP	HA
CPTi	 R_q=148.8±31nm R_{max}=402.8nm Ra=121.3±23nm K=-0.204nm	 R_q=158.37±24.6nm R_{max}=1.07±0.1nm Ra=121.53±18nm K=-1.15±0.1nm	 R_q=101.5±9.8nm R_{max}=147.21nm Ra=78.94±9.98nm K=-0.204nm	 R_q=898±50.8nm R_{max}=4.21µm Ra=718.4±19.9nm K=-0.020nm
Ti6Al4V	 R_q=75.12±15.3nm R_{max}=655.08nm Ra=53.825±126nm K=-0.347nm	 R_q=67.5±3.5nm R_{max}=615.7nm Ra=48±0.8nm K=-1.65nm	 R_q=57.8±3.8nm R_{max}=641.6nm Ra=40.9±2.5nm K=-1.0nm	 R_q=415.5±9.8nm R_{max}=47.21µm Ra=413.94±9.98nm K=-0.204nm
Ti6Al4V-ELI	 R_q=67±3.5nm R_{max}=662.72nm Ra=51.91±1.9nm K=0.83nm	 Rq=75.73±15.32nm Rmax=666.1nm Ra=52.81±8 nm K=-0.86nm	 Rq=73.9±0.4nm R_{max}=894.6nm Ra=49.5±1.2nm K=-0.642nm	 R_q=313.5±9.8nm R_{max}=147.21µm Ra=578.94±9.98nm K=-0.204nm

Figure 23: AFM 3D simulation of roughness of untreated, EP, MEP and HA of CPTi, Ti6Al4V and Ti6Al4V-ELI R_p

Surface Wettability

Wettability of materials is of significant interest in biomedical application because of its effects on the cell adhesion and cell proliferation. Relatively higher wettable surface with low contact angle and higher surface energy is termed hydrophilic while less wettable surface with low surface energy and high contact angle is hydrophobic surface [109]. Implants are always in contact with body fluids, and these body fluids have significant effects on the surface of the implant in the form of protein adsorption, platelet adhesion/activation, cell and bacterial adhesion and proliferation [110].

The sessile drop method was used by employing three solvents; DI-water (mild polar), Ethylene glycol (neutral) and Diiodomethane (highly polar). The test were performed per solvent on each specimen at a location separated by sufficient spacing in order to prevent the potential influence of previous tests. The Kyowa contact angle meter was is used for wettability studies as shown in the figure 24.

Figure 24: Kyowa contact angle meter

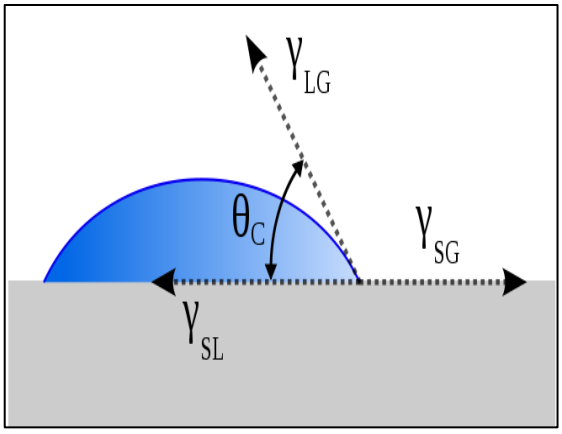


Figure 25: Contact angle by Young Dupree

According to Young-Dupree equation the contact angle θ, can be expressed as,

$$\gamma_{lg}^{cos\theta} = \gamma_{sg} + \gamma_{sl} \qquad (8)$$

where, γ_{sg} is the surface energy of the solid, γ_{sl} is the solid liquid interfacial energy, γ_{lg} is the surface energy of the liquid and θ is contact angle as shown in figure 25.
Famas analysis software were used to evaluate the surface free energy (SFE) . the Lifshitz-Van der Waals (LW) acid base interaction and Kitazaki Hata theory. The SFE was calculated by using the following equation:

$$\gamma^{total} = \gamma^{d} + \gamma^{p} + \gamma^{h} \qquad (9)$$

γ^{total} is the total surface free energy, γ^{d} is the SFE dispersion component , γ^{p} is the SFE polar component and γ^{h} is the SFE hydrogen bond.

In case of the CPTi alloys, the contact angle shows significant changes after surface treatment because the surface chemistry is changed and the content of oxygen was decreased as mentioned in the EDS data. This decrease in the oxygen content changes the dipole movement of the water drop on the surface of the substrate which affects the orientation of molecule and drop behavior with the solid surface. Ti6Al4V wettability show an increase in the contact angle for the electropolished surface, but it was observed a significant decrease in the contact angle of the magnetoelectropolished surface. However, in case of the Ti6Al4V-ELI magnetoelectropolished surface is more hydrophobic. Roughness also effects the contact angle orientation and has great influence on the wettability of the surface [110].

Surface free energy is inversely proportional to contact angle, guides the first events occurring at the biomaterial/biological interface, such as interaction of water and proteins with

biomaterial [111]. The surface energy estimation from contact angle measurement is a hard task because biomaterial surfaces are always rough and/or heterogeneous [104]. It has been observed that the advancing contact angle is influenced more by the microscopic inclusions and discontinuities than by the interfacial energetics that cause hysteresis if $R_a \geq 0.1$ μm [31]. The surface roughness, wettability and cell interaction properties are interrelated phenomenon, and could have counter effects on each other. Their relationship is a complex in nature and needs further research.

In this investigation it was observed that the surfaces of titanium alloys exhibited high electron donor (basic) and low electron accepter (acidic) characters , which are conductive for cell viability as shown in the table 11. Similar characteristics were reported by Ponsonet ae al.in 2003, who investigate good cell health [113]. The oxide layer on surface has great influence on the polar bonds and their interaction with water and aminoacids. Studies have also reveal that lower SFE values correspond to favorable cellular adhesion and cell activities [114]. Ponsonnet et al. investigated that 30-40 mJ/m^2 SFE corresponds to higher cell proliferation and he also indicated inflecting points in the mentioned range [113].

The average values of calculated surface free energies are shown in the figure 26. From the data it is obvious that hydroxyapatite has the highest SFE. In each case the Ep and MEP shows similar behavior, therefore the investigation shows that each surface modification increases the surface energetics for titanium alloys. The EP and MEP treated alloys of Ti6Al4V and Ti6Al4V-ELI indicates less than 40 mJ/m^2. Which is almost equal to that of the CPTi untreated sample. Which can be favorable for the cellular activity.

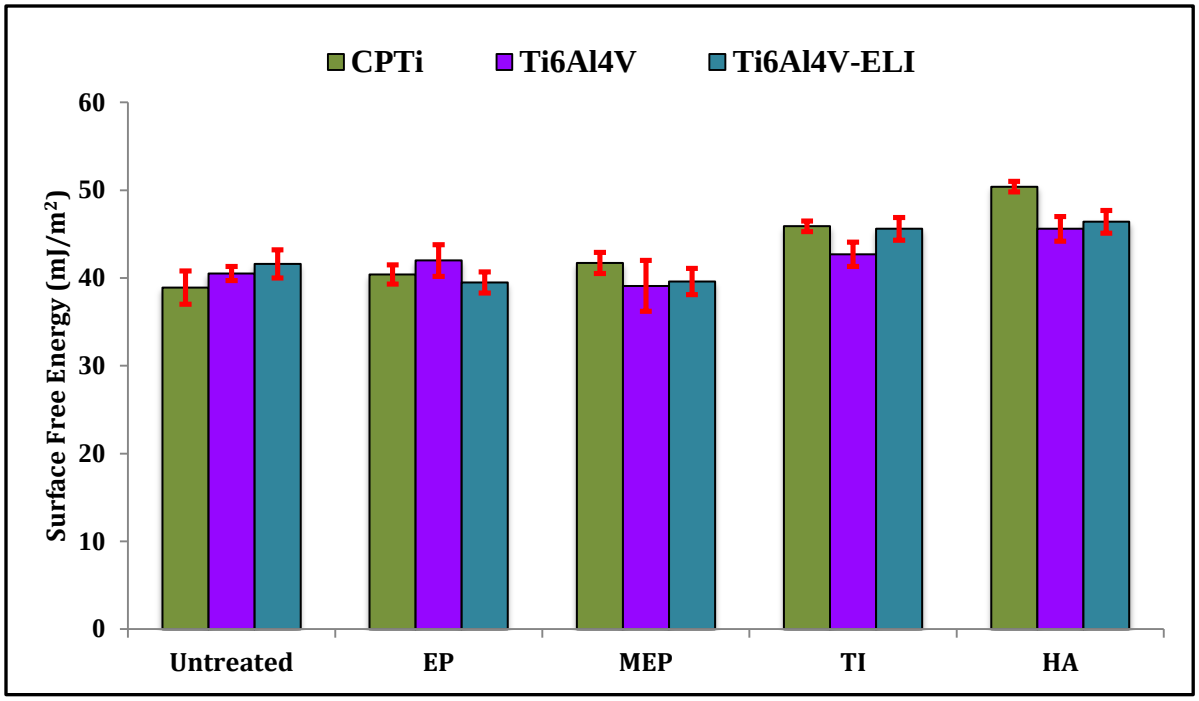


Figure 26: Acid-Base Surface Values of Titanium Alloys

Table 11: Acid –Base average surface free energy components (mJ/m^2) of titanium alloys

Alloy	Lifshitz-van der Waals	Acid (electron Acceptor) +	Basic (Electron Donor) -	Total SFE
CPTi Untreated	38.9	0	18	38.9
CPTi EP	40.4	0	28.9	40.4
CPTi MEP	41.7	0	20.7	41.7
CPTi Ti Coated	45.2	1.2	0.1	45.9
CPTi HA Coated	44.5	0.3	28.9	50.4
Ti6Al4V Untreated	40.5	0	15.2	40.5
Ti6Al4V EP	42	0	10.7	42
Ti6Al4V MEP	39.1	0	20.3	39.1
Ti6Al4V Ti Coated	42.7	0.1	0	42.7
Ti6Al4V HA Coated	45.6	0	49.1	45.6
Ti6Al4V-ELI Untreated	41.6	0	35.3	41.6
Ti6Al4V-ELI EP	39.5	0	25	39.5
Ti6Al4V-ELI MEP	39.6	0	14.8	39.6
Ti6Al4V-ELI Ti Coated	45.6	0.1	0	45.6
Ti6Al4V-ELI HA Coated	45.6	0	49.1	46.4

The contact angle, interfacial free energy and work of adhesion are shown in figure 27, figure 28 and figure 29. The Young's contact angle values are shows that the HA coated alloys have significant influence on each surface. From the data it is obvious that HA coated surfaces have the lowest contact angle, which leads to better cell proliferation, and adhesion as discussed in several previous studies [111]. Each HA coated sample shows better work of adhesion, which is the indication for a better osseointegration. Electropolished Ti6Al4V shows the highest contact angle and lowest SFE. Similarly, magnetoelectropolished Ti6Al4V-ELI has the lowest contact angle and highest SFE as depicted in figure 29. High SFE leads to cell proliferation and cell activity. The acid-base surface average values indicate the significant changes in surface wetabilities and adhesion properties. Cell adhesion is an important term for successful implantation, as better cell adhesion results in strong osseointegration. SFE and hydrophilicity of implant surfaces may be specially important during the initial conditioning by proteins and during initial stage of cell adhesion. Gopinath Mani et al found that the thrombogenicity of a material's surface increases with increasing surface energy [66]. In orthopedics and trauma surgery the success of implants surgery is based on the osseointegration. It is generally it noticed that on hydrophilic surface the cell adhesion is more stable when compared to hydrophobic surface. The hydrophilic surface permit higher osseointegration and more mechanically stable structure thus the chances of loosing implant becomes smaller [115].

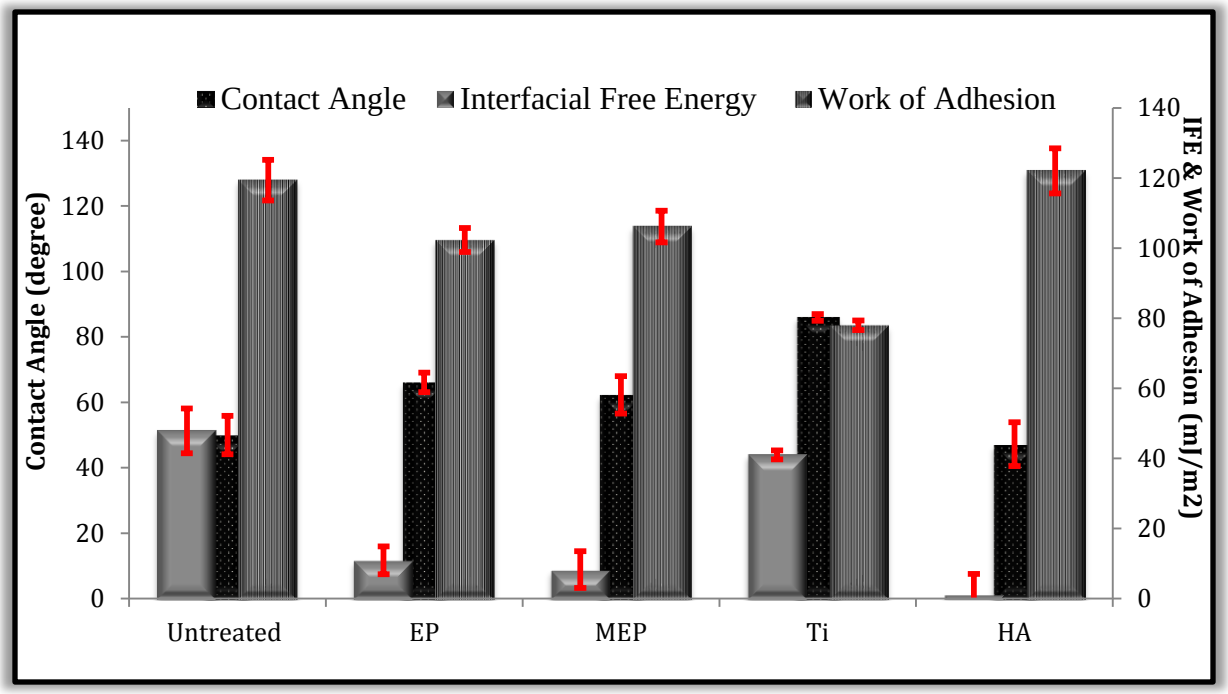


Figure 27: Acid base average values of CPTi

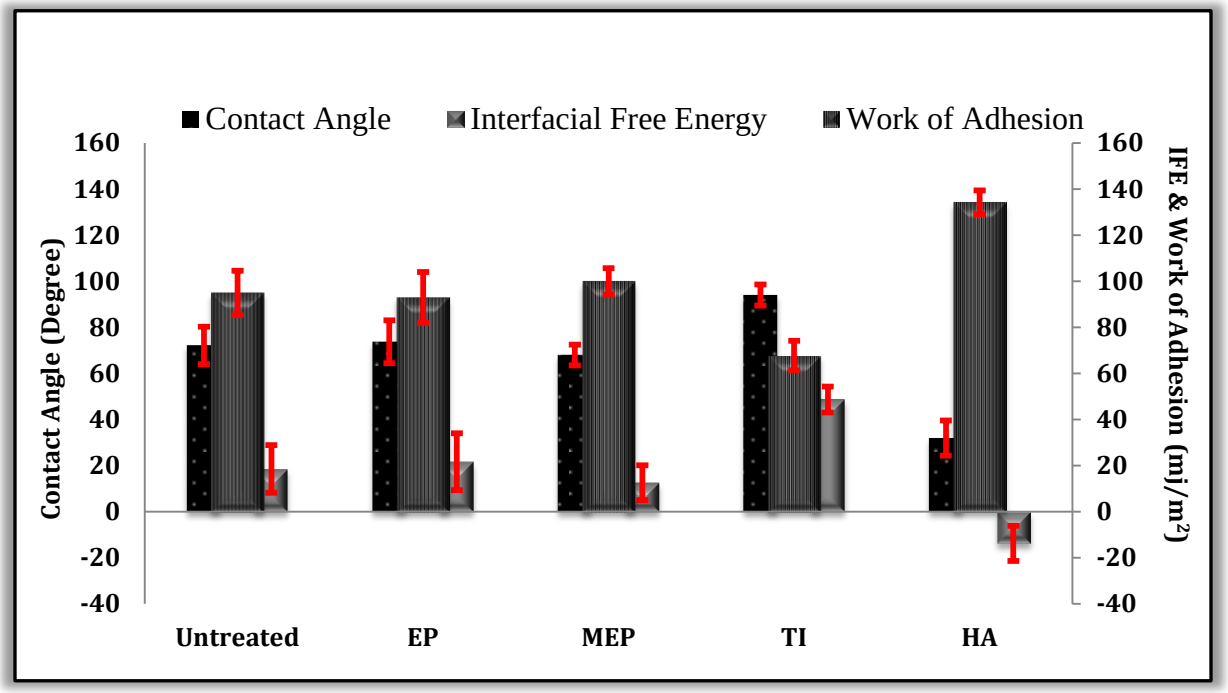


Figure 28: Acid base average values of Ti6Al4V

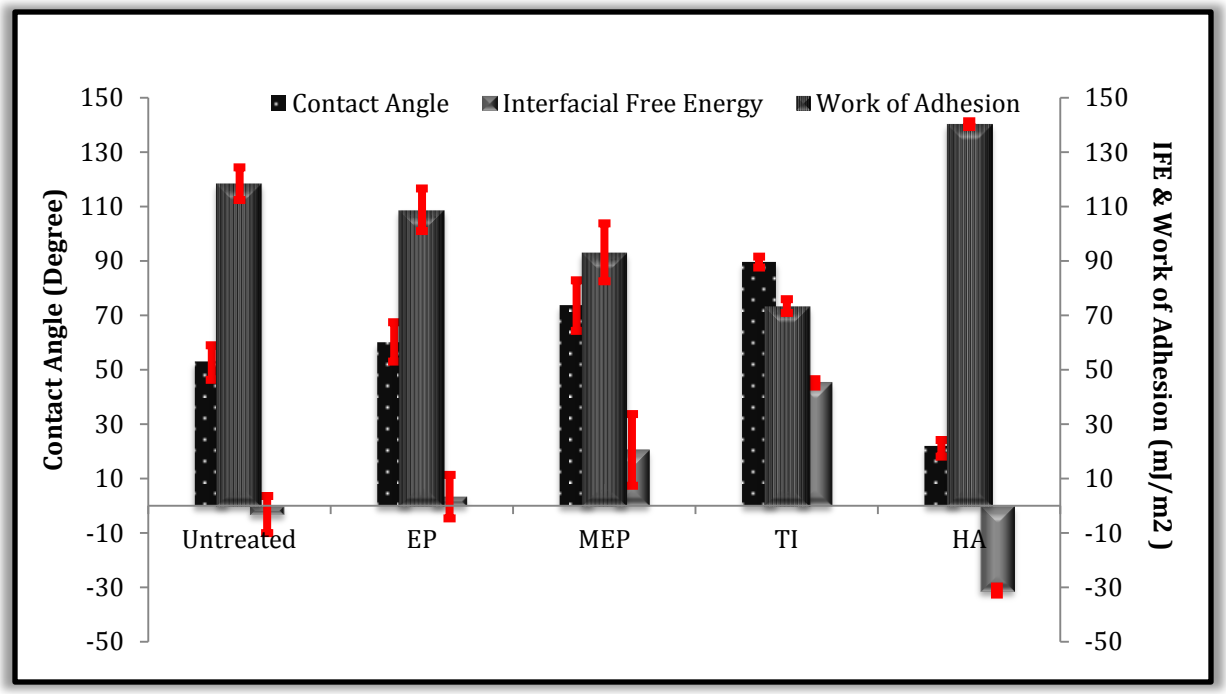


Figure 29: Acid base average values of Ti6Al4V-ELI

CHAPTER VI

BIOCOMAPTIBILITY ANALYSIS

MC3T3 Pre-Osteoblast Cell Culture

In order to investigate the effects of surface morphology on the MC3T3 pre-osteoblast cells, the surface of each untreated and treated alloys were exposed to these cells. For this determination the MC3T3-E1 Subclone 4 (ATCC® CRL-2593™) cells were grown in a culture flask. After the incubation, 90% confluency was achieved and the cells were trypsinized, centrifuged and suspended in culture media for further cell seeding.

The cell culture media was prepared by adding 10% fetal bovine serum (FBS) (Thermo Scientific™ HyClone™ SH3008803HI), 1% Penicillin Streptomycin (Sigma-Aldrich P4333) to MEM alpha from (part # SH4007-13, Thermo scientific, USA).

Immersion Test

The effect of metal ions released from Ti alloys was assessed by immersion tests. In this test, CPTi, Ti6Al4V and Ti6Al4V-ELI alloys were exposed to 5 ml culture media for 21 days. The culture media was collected from each immersed alloy after every three days and replaced with fresh culture media. The extracted culture media was labeled and stored in centrifuge tubes. The different concentration of ionized media was collected and stored after 3, 6, 9, 12, 15, 18,

and 21 days. The culture media was used to culture MC3T3 cells in order to observe their viability via MTS biological assay.

MTS Assay of Ionized Media

MTS assay (G3580, Celltiter 96® AQ_{ueous} One Solution Reagent, Promega Corporation) was used to determine the percentage of viable MC3T3-E1 Subclone 4 (ATCC® CRL-2593™) cells in extract solutions exposed to different titanium alloys. The cells were cultured in MEM alpha modification media (Thermo Scientific™ HyClone™ SH3026501), 10% fetal bovine serum (FBS) (Thermo Scientific™ HyClone™ SH3008803HI), and Penicillin-Streptomycin (Sigma-Alridch P4333) at 37 °C in a humidified atmosphere of 5% CO_2. The titanium alloys were immersed in MEM alpha modification media for 21 days and the media was changed and collected after periods of 3 days. Cells were counted and plated in 96-well plates at 20 x 10^3 cells with a working volume of 200 µl per well. The cells were incubated for 24 hours to allow attachment. After the 24-hour incubation, the media was replaced with 100% concentrations of the extract media. Pure culture media with cells was used for the control groups. The cells were incubated for 24 hours to allow cell interaction with the collected media. After the 24 hours, 100 µl of media were removed from the 96-well plates, and the remaining 100 µl media was treated with 20 µl/well with Celltiter 96® AQ_{ueous} One Solution Reagent. The 96-well plates were incubated at 37 °C in a humidified 5 % CO_2 atmosphere for 4 hours. Immediately after the incubation procedure, the optical density measurements were recorded using ELx800™ BioTek absorbance microplate reader controlled by Gen5 software with a 490 nm wavelength absorbance excitation filter.

The values of absorbance are converted to the line graph by using Microsoft Excel. For untreated alloys, the cell proliferation viability is decreasing day by day which indicates toxic effects of metal ions on cell viability.

Figure 30, 31, 32 exhibits cell viability versus time exposure of media graphs. It shows the sudden decrease in the cell viability on three day ionized culture media. This may be due to high release of leached metal ions which directly affect the cell viability and proliferation.

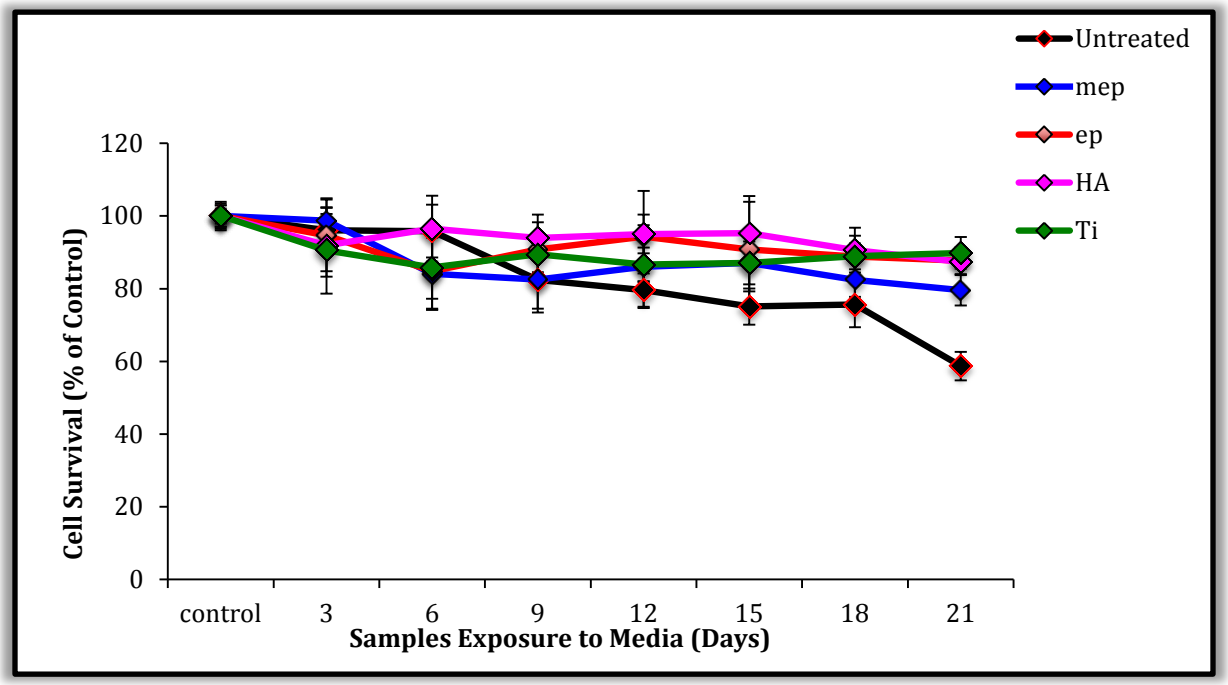


Figure 30: Effect of leached metal ions of CPTi on MC3T3 cells

In the case of CPTi, the untreated alloy showed low cell viability. HA coated showed the highest cell viability. Ti coated alloys also showed high cell viability after 21 days time period. However, EP and MEP exhibited somehow consistent linear behavior, which is the positive sign for cell viability as shown in figure 31.

Ti6Al4V alloys showed low cell viability after day three as indicated in figure 32. Ti coated samples also showed better cell viability after 12 days while EP and MEP have similar linear relationship as in case of CPTi. Ti6Al4V HA coated alloy showed low cell viability from day three to day 21. MTS results of Ti6Al4V-ELI are shown in figure 32. Untreated and HA coated alloys showed low cell viability while EP and MEP showed higher cell viability. The graph shows that the Hydroxiapatite has almost the same behavior as in Ti6Al4V. The EP and MEP shows better viability while titanium coated ionized media shows the variations in the cell viability.

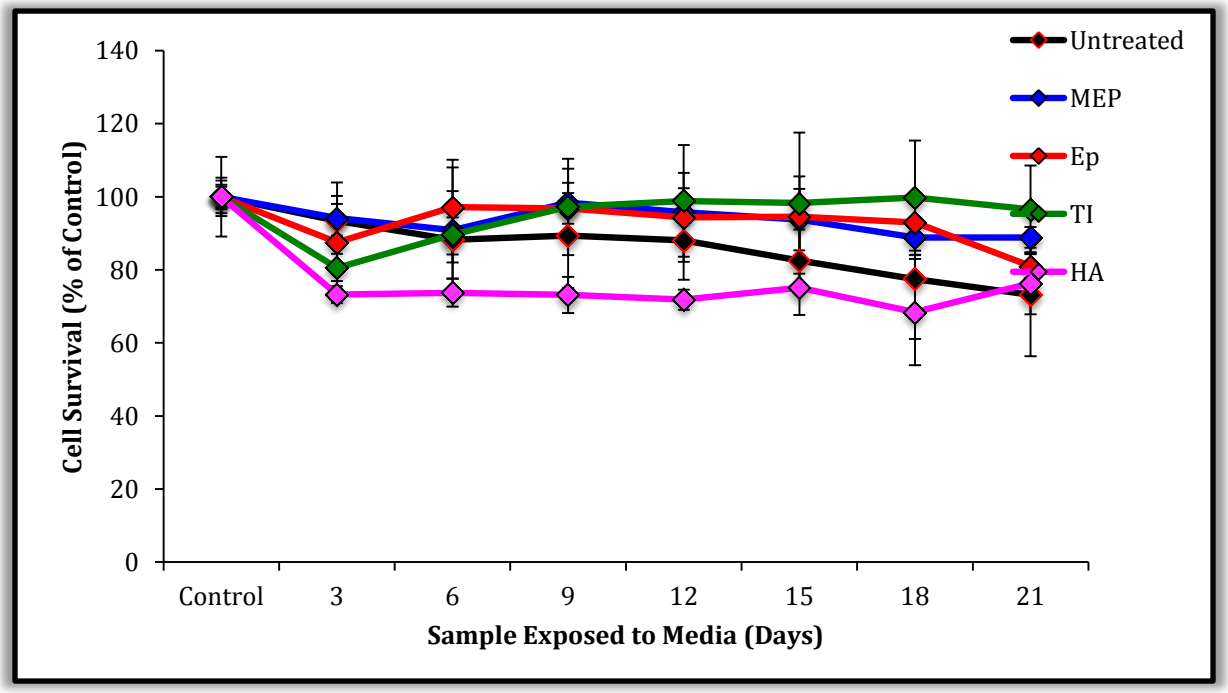


Figure 31: Effect of leached metal ions of Ti6Al4V on MC3T3 cells

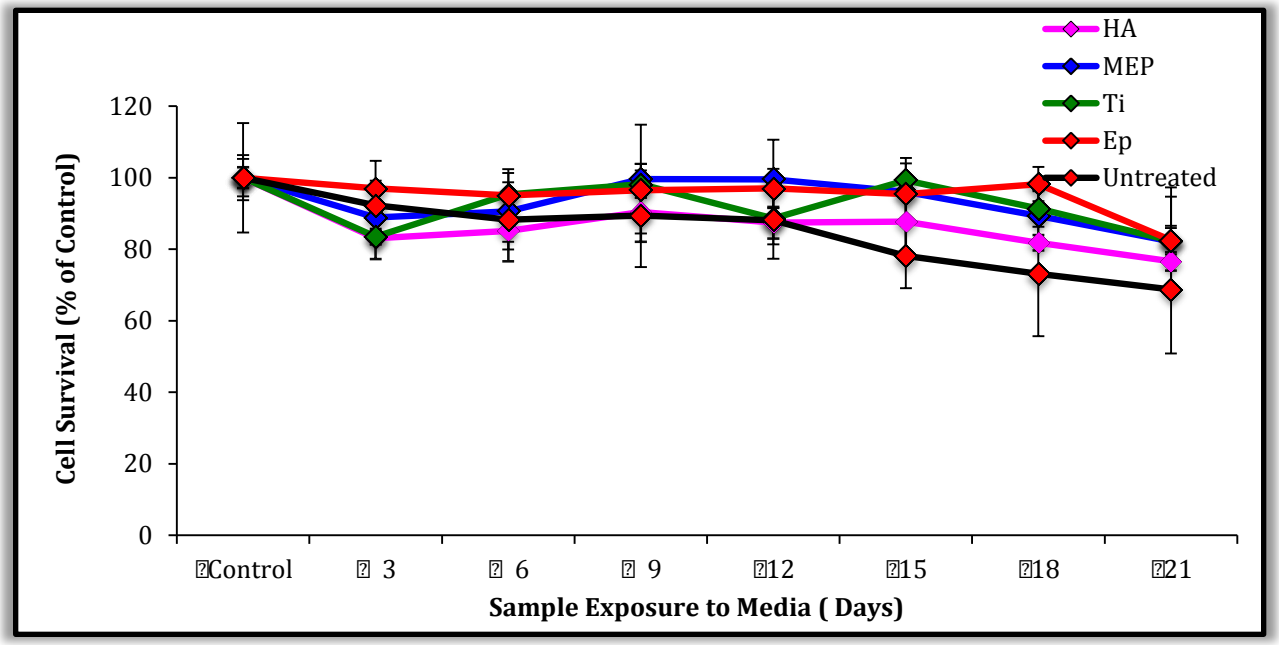


Figure 32: Effect of leached Metal ions of Ti6Al4V-ELI on MC3T3 cells

Cell-Surface Interaction

The cell culture media was prepared by adding 10% fetal bovine serum (FBS) (Thermo Scientific™ HyClone™ SH3008803HI), 1% Penicillin Streptomycin (Sigma-Aldrich P4333) to MEM alpha from (part # SH4007-13, Thermo scientific, USA). For cell proliferation on metal surface, the special glass cell was used as shown in figure 33. The sample was fit into the glass cylinder and 30,000 cells for each sample were counted by using the hemocytometer. These cells were allowed to proliferate on the surface of each sample in order to examine the direct effect of surface treatments on MC3T3 cells. After 48 hours incubation, the media were removed from the cells and cell staining was carried out. For cell staining the "NucBlue live ready probes reagent" (Hoechst reagent part #.33342) from Life Technologies® was used for nucleus while Mitrotraker red part #. M7512, molecular probe of Life Technologies was used for cell mitochondria staining.

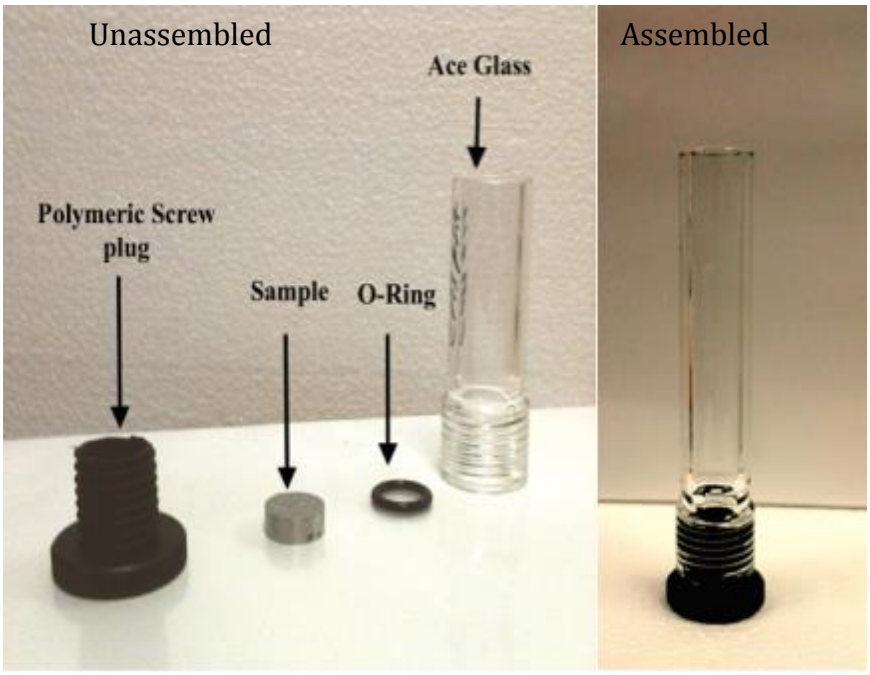


Figure 33: Unassembled and assembled glass cell

Cell adhesion is the initial interaction with an implant surface followed by spreading, which forms the basis for further proliferation. Initially cells do not communicate directly with the implant, but are guided to sites by biological interactive molecules. This interaction results in the formation of either a fibrous tissue or a stronger bond. The protein adhesion has great influence on the surface chemistry and surface energetics. Wettability studies and roughness data suggested that each alloy has different values and thus cell proliferation on these surfaces must be different. In order to understand the cell-biomaterial interaction, cells were allowed to proliferate. Figure 34 exhibits MC3T3 cells on titanium alloys after 3 days of incubation. Excellent cell proliferation was observed on Ti6Al4V MEP surface when compared with untreated and EP surfaces. The poor cell proliferation was observed on all untreated alloys when compared to EP and MEP. This was due to high concentration of leached metals ions from the bare surface.

The Ti6Al4V shows that on third day each sample reduces the cell viability as indicated in the figure 29 but days later on the cell and ions interaction was good. Ti shows better results while EP and MEP have similar linear relationship with cells as in the case of CPTi. Hydroxyapatite shows adverse reaction in the case of the Ti6Al4V.

Similarly the ionized media of Ti6AL4V-ELI results are shown in the figure 34. The graph shows that the Hydroxyapatite has almost the same behavior as in Ti6Al4V. The EP and MEP shows better viability while titanium coated ionized media shows the variations in the cell viability.

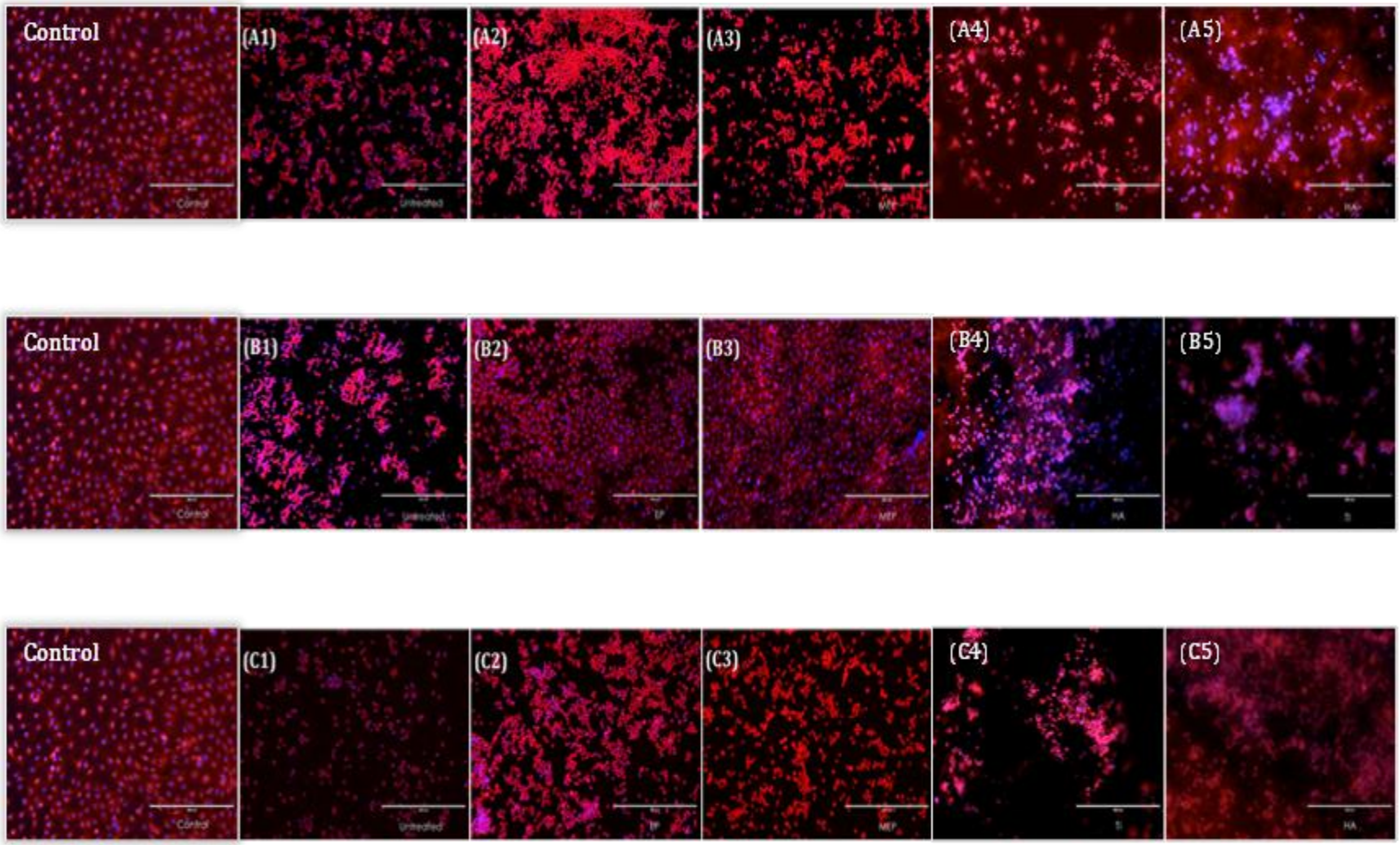


Figure 34: MC3T3 Pre Osteoblast cell Proliferation on the surface; (A1) CPTi Untreated; (A2) CPTi EP; (A3) CPTi MEP; (A4) Ti; (A5) HA; (B1) Ti6Al4V Untreated; (B2) Ti6Al4V EP; (B3) Ti6Al4V MEP; (B4)Ti6Al4V Ti; (B5) Ti6Al4V HA; (C1) Ti6Al4V-ELI Untreated; (C2) Ti6Al4V-ELI EP; (C3) Ti6Al4V-ELI MEP; (C4) Ti6Al4V-ELI Ti; (C5) Ti6Al4V-ELI Ti

CHAPTER VII

CONCLUSIONS

This works presents a novel approach to enhancing the corrosion resistance and biocompatibility of Titanium alloys for biomedical applications. Enhancing the biocompatibility of medical devices implies seemless integration into the body for increase health and longevity of the patient. In this investigation the titanium alloys CPTi, Ti6Al4V and Ti6Al4V-ELI has been subjected to different surface treatments i.e. Electropolishing, Magnetoelectroplishing, Titanium coating and Hydroxyapatite coatings. All coatings provide stable oxide passive film to the alloys.

Surface morphology, chemistry, roughness and wettability were analyzed. The ions released by the surface treated alloys of titanium and their effects of MC3T3 cells were investigated in this study.

All surface treated titanium alloys were more resistant to pitting corrosion as compared to the bare surface. This study clearly indicates that EP and MEP are more effective technologies than Ti and HA coatings. EP changes the surface chemistry and surface morphology by providing a porous morphological structure to the surface, while MEP provides a micro-granular structure. HA provides more porous structure but it is not resistant to pitting and crevice corrosion. EP and MEP morphologies provide new podium for osteoblast proliferation. From roughness and wettability studies it can be concluded that the surface chemistry is playing a

more dominate role in wettability of surface in contrast of roughness. These surface modifications make the alloys more corrosion resistant in biological environment hence indicates more life span for implants. From the cell proliferation experiments it was concluded that MC3T3 cells were proliferating better on EP and MEP surfaces when compared to untreated surfaces.

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APPENDIX A

APPENDIX A

KITAZAKI HATA AVERAGE VALUES SURFACE FREE ENERGIES, CONTACT ANGLES AND WORK OF ADHESION OF SURFACE MODIFIED TITANIUM ALLOYS

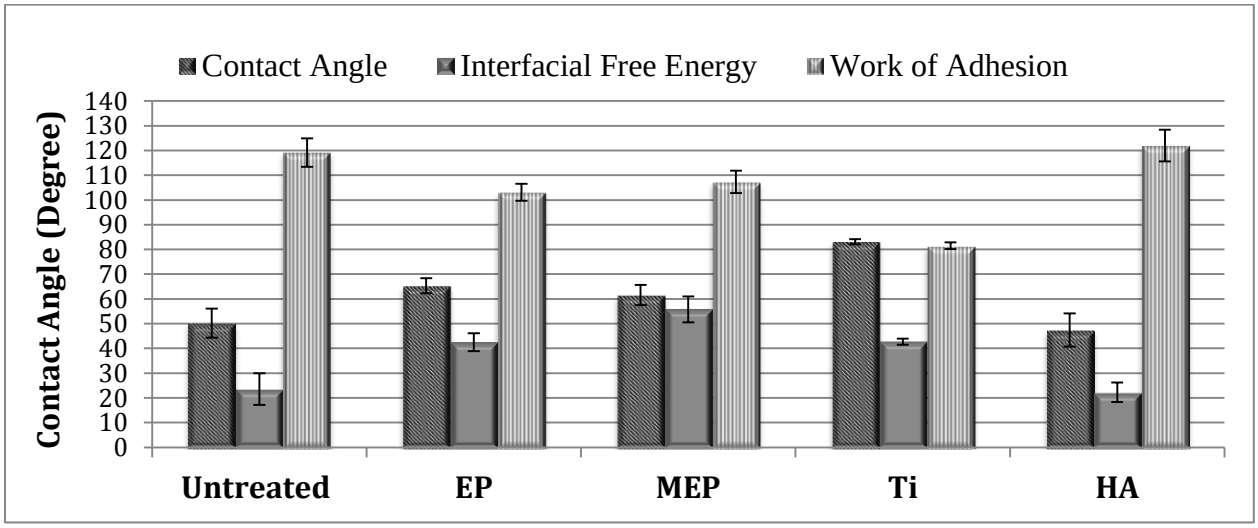

Figure 35: Kitazaki Hata average values of CPTI

Table 12: Kitazaki Hata average value of CPTI

Sample	Contact Angles (Deg)			Interfacial Free Energy (mJ/m^2)			Work of Adhesion (mJ/m^2)		
	Water	Diiodo-methane	Ethylene Glycol	Water	Diiodo-methane	Ethylene Glycol	Water	Diiodo-methane	Ethylene Glycol
Untreated	50.22	36.3	25.7	23.56	29.06	26.98	119.16	91.66	90.64
EP	65.34	34.98	52.48	42.52	31.26	43.8	103.12	92.38	76.74
MEP	61.6	30.66	51.38	55.72	46.58	60.64	107.34	94.48	77.32
Ti	83.16	21.54	45.2	42.72	4.42	17.82	81.48	97.96	81.28
HA	47.4	29.1	15.05	22.225	26.98	25.3	121.98	95.13	93.7

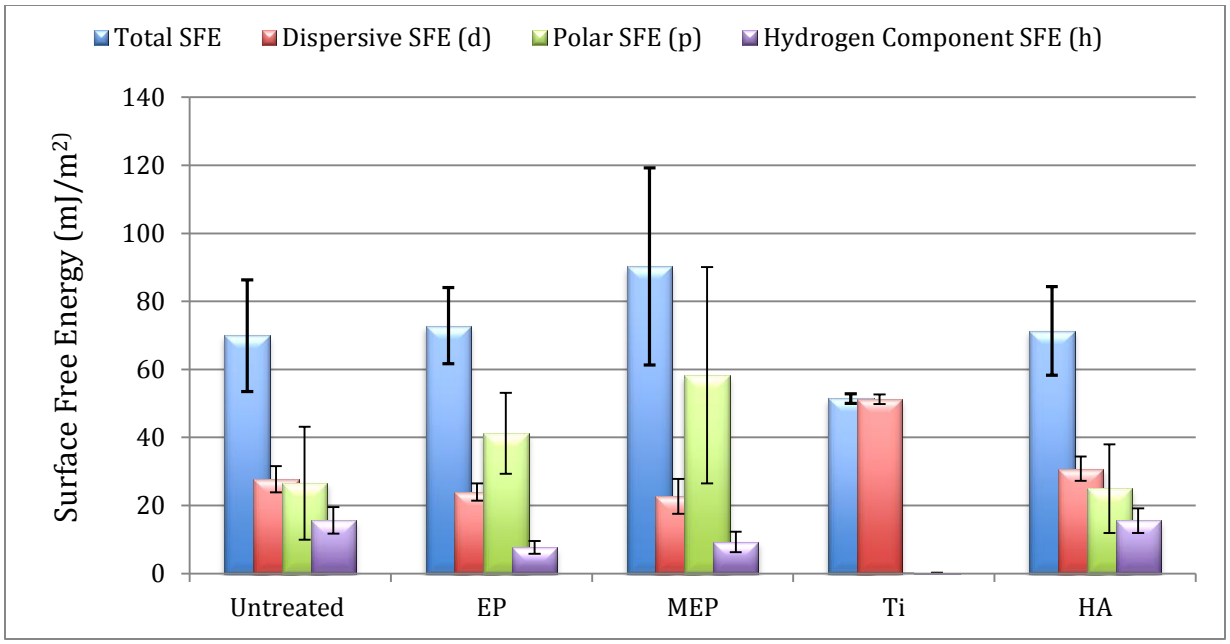

Figure 36: Kitazaki Hata average values of surface free energy components of CPTi

Table 13: Kitazaki Hata average values of surface free energy components of CPTi

Surface Free Energy Components (mJ/m^2)				
Sample	Dispersion (d)	Polar (p)	Hydrogen Component (h)	Total
Untreated	27.72	26.56	15.64	69.92
EP	23.96	41.22	7.66	72.84
MEP	22.68	58.28	9.3	90.26
Ti	51.26	0	0.14	51.4
HA	30.8	24.95	15.55	71.3

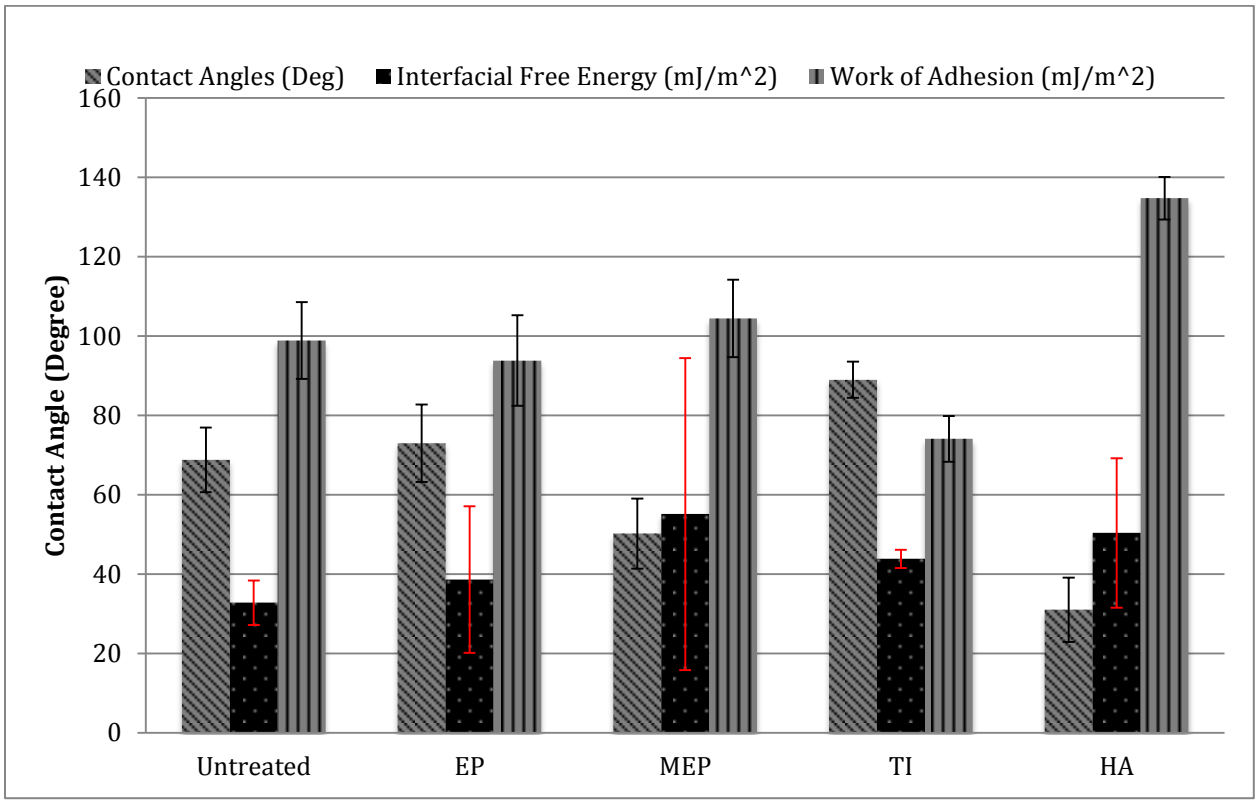

Figure 37: Kitazaki Hata average values of Ti6Al4V

Table 14: Kitazaki Hata average values of Ti6Al4V

Sample	Contact Angles (Deg)			Interfacial Free Energy (mJ/m^2)			Work of Adhesion (mJ/m^2)		
	Water	Diiodo-methane	Ethylene Glycol	Water	Diiodo-methane	Ethylene Glycol	Water	Diiodo-methane	Ethylene Glycol
Untreated	68.82	37.4	50.35	32.8	18.52	28.41	98.9	91.17	78.12
EP	72.98	33.2	52.44	38.6	17.16	30.58	93.82	93.26	78.2
MEP	50.2	35.2	58.4	55.12	46.7	58.1	104.42	90.84	76.34
Ti	88.98	34.56	62.2	43.84	3.54	18.46	74.08	92.38	74.36
HA	31	26.1	12.26	50.38	66.74	65.78	134.74	96.38	94.24

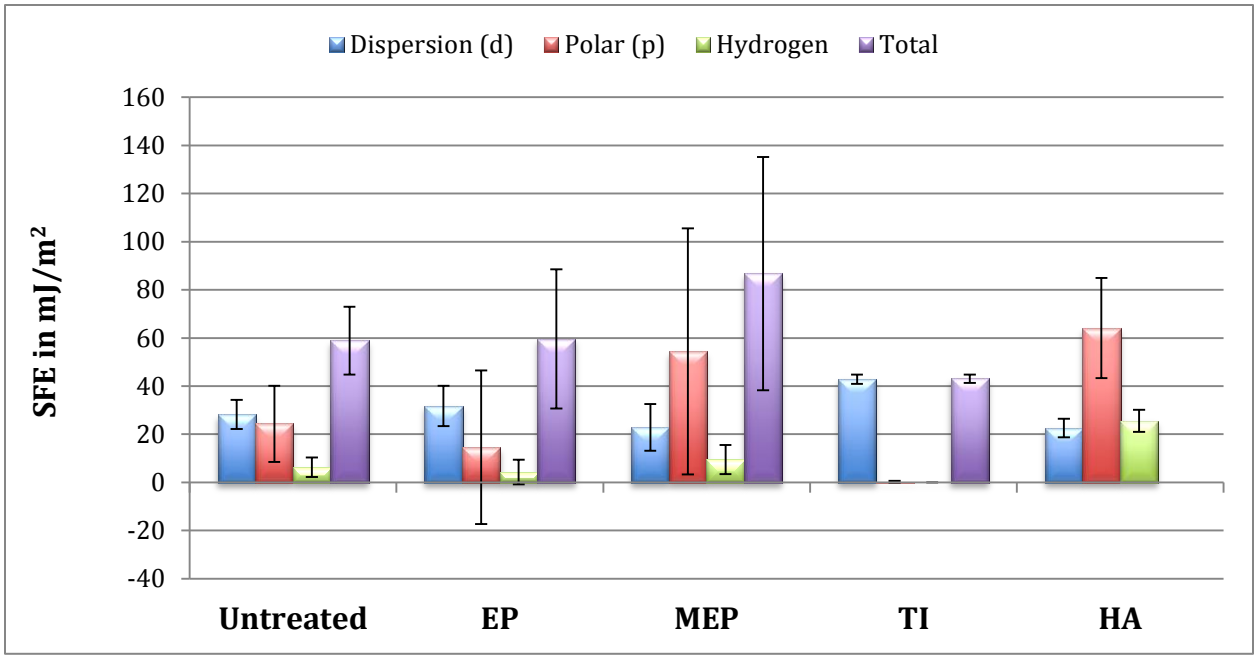

Figure 38: Figure 37: Kitazaki Hata SFE components of Ti6Al4V

Table 15: Kitazaki Hata SFE components of Ti6Al4V

Sample	Dispersion (d)	Polar (p)	Hydrogen Component (h)	Total
Untreated	28.25	24.35	6.3	58.9
EP	31.74	14.56	4.32	59.62
MEP	22.86	54.38	9.5	86.74
Ti	42.88	0.2	0	43.08
HA	22.58	64.12	25.62	112.32

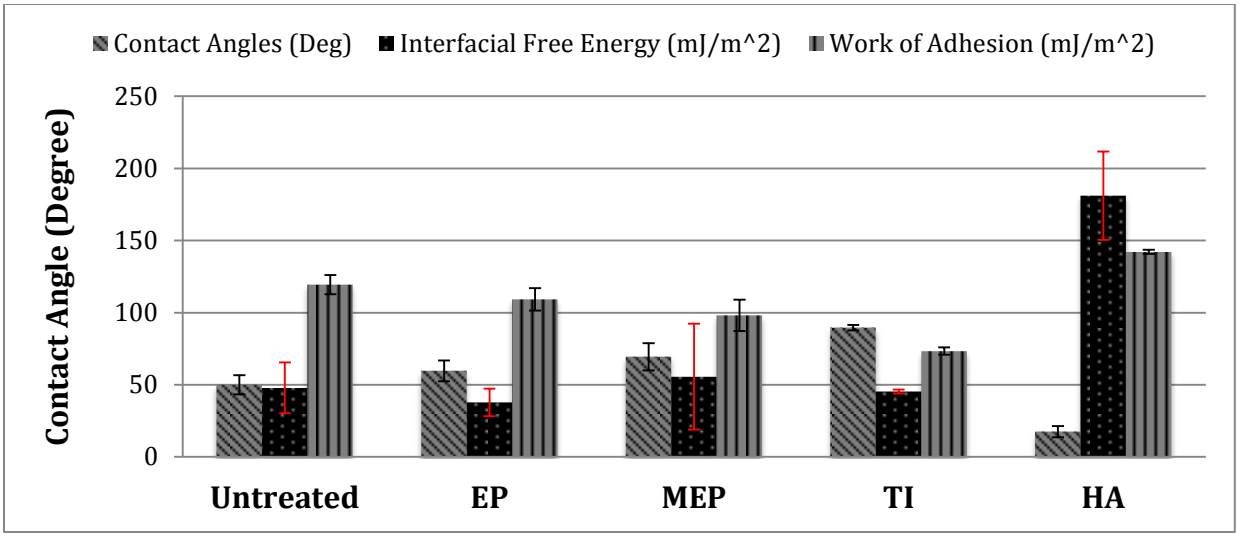

Figure 39: Kitazaki Hata average values of Ti6Al4V-ELI

Table: Figure 16: Kitazaki Hata average values of Ti6Al4V-ELI

Sample	Contact Angles (Deg)			Interfacial Free Energy (mJ/m^2)			Work of Adhesion (mJ/m^2)		
	Water	Diiodo-methane	Ethylene Glycol	Water	Diiodo-methane	Ethylene Glycol	Water	Diiodo-methane	Ethylene Glycol
Untreated	50	39.96	35.6	47.85	54.7	58.82	119.36	91.72	86.26
EP	59.72	39.52	46.62	37.78	35.12	41.52	109.26	89.92	80.42
MEP	69.44	35.02	56.44	55.62	39.32	54.58	98.02	92.4	73.96
Ti	89.58	32.9	56.44	45.23	3.3	19.48	73.36	93.26	73.98
HA	17.52	39.62	46.72	181.06	211.26	217.7	142.1	89.9	80.36

BIOGRAPHICAL SKETCH

Zia ur Rahman was born in January 24 1985 in a valley of Swat at Khyber Pukhtoonkhwa province Pakistan. He received his early education from his valley and after high school or Fsc, he got admission at Balochistan University of Information Technology, Engineering and Management Sciences (BUITEMS) Quetta Baluchistan Pakistan in 2005. From this institute he got his Bachelor of Science degree in Petroleum and gas engineering in 2009. In 2010, he got a chance to work with Oil and Gas Development Company limited (OGDCL) Pakistan. This company is playing a vital role in the annual budget of Pakistan. Mr. Rahman was attached to the department of petroleum production and performed his duties at Chanda, Mela, Sheikhan and Sahibgul wells and its processing facilities. Moreover, he learned about the different operations of drilling engineering, production operations, flow and production management. He also supervised the servicing companies like Halliburton, Sprint, and Schlumberger during different operations at different well sites and fields. After finishing one year of contract with company, he got admission at University of Texas Pan American (UTPA) Edinburg Texas USA. He came to United States in August 2012 and continued his studies for masters in Mechanical Engineering. During his Masters he presented at various conferences and won a prize at the TMS Conference in San Antonio 2013. He also presented his research data at NACE conference at San Antonio. In May 2014 he accomplished his masters successfully.

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