THE EFFECTS OF SURFACE ROUGHNESS ON THE FUNCTIONALITY OF TITANIUM BASED ALLOY Ti13Nb13Zr ORTHOPEDIC IMPLANTS

A Dissertation
Submitted to the Graduate Faculty
of the
North Dakota State University
of Agriculture and Applied Science

By

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In Partial Fulfillment of the Requirements
for the Degree of
DOCTOR OF PHILOSOPHY

Major Department:
Mechanical Engineering

June 2021

Fargo, North Dakota

North Dakota State University

Graduate School

Title

The Effects of Surface Roughness on The Functionality of Titanium Based Alloy Ti13Zb13Zr Orthopedic Implants

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ABSTRACT

In this study, the effects of surface roughness on the wettability, cell attachment, and mechanical properties of titanium-based Ti13Nb13Zr orthopedic implants have been investigated. The aim of this multidisciplinary research was to find an optimum range of surface roughness for Ti13Nb13Zr orthopedic implants that could maximize the attachment and the proliferation of cells and improve the wettability of the surface, without adversely affecting the mechanical strength of the implants. There have been some published research works that support the existence of relations between roughness and the functionality of implants, but still, an optimum roughness that can satisfy all of the orthopedic requirements, either is not fully studied or not published.

It was seen that the performance of orthopedic implants depends on multiple paradoxical parameters. The results of this study on Ti13Nb13Zr show, even though increasing the surface roughness can increase the initial phase of cell attachment onto the surface of implants, other functions such as wettability and mechanical properties can be influenced adversely. Through an experimental methodology, this study proposes an optimum range of roughness, which meets all three major functions of cell attachment, mechanical properties, and wettability. In respect to the recent serious health concerns reported over the implants made of Ti6Al4V which is a common material in the implant industry, researchers are currently working to introduce a better biomaterial. In this study, Ti13Nb13Zr which is a new and advanced biomaterial with improved biocompatibility and more desired mechanical properties was studied. The reason for this selection backs to the fact that Ti13Nb13Zr does not release toxic ions (such as Al and V ions) and its mechanical properties are closer to the bone in comparison to many titanium alloys such as Ti6Al4V.

Different ranges of surface roughness were created on the samples by surface treatment methods. To investigate the behavior of Ti13Nb13Zr with different roughness values, three categories of tests were performed including contact angle measurement, three-point bending, and cell culture tests. It was noted that surface roughness plays a complicated behavior. It was observed that Ti13Nb13Zr samples with roughness range between 20μm to 25μm show better performance.

ACKNOWLEDGMENTS

My profound thanks go to my advisor, **Dr. Xinnan Wang**, for guiding and supporting me to be a better version of myself in my professional and personal life. I would like to express my special thanks to **Dr. Ying Huang** and **Dr. Long Jiang** to be in my Ph.D. committee and, supporting me with their valuable comments.

I would like to extend my sincere gratitude to **Dr. Karami** and **Dr. Kallmeyer** for all their great mentorship and sincere support since 2014 to complete my Master's and Ph.D. degrees. I owe them a lot, as they helped me to experience the most fruitful chapter of my life at NDSU.

I also deeply thank the scientists and experts who supported me to successfully run and complete my multidisciplinary Ph.D. research.

Dr. Amanda Brooks, Associate Professor of Molecular Biology at RVU, Ivins, UT.

Dr. Jared Aelony, Orthopedic Surgeon, Essentia Health, Detroit Lakes, MN.

Sahar Tabatabaie, QC leader, Pharmascience Co., Montreal, Quebec, Canada.

Dr. Raquib Hasan, Research Scientist, Vertex Pharmaceuticals Co, Boston, MA.

Dr. Wally Puckett, Director of Orthopedics research, Smith & Nephew Co, Memphis, TN.

DEDICATION

Learning is a long and adventurous journey, but rewarding!

This journey would not have been possible without the dedicated support of my loved ones, who I shall now thank as best as I am able:

My father, my mother, my wife, my brothers and my son!

Baba, Zahra, Behnaz, Amin, Javid, Saeed, Reza and Bryan!

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CHAPTER 1. INTRODUCTION

1.1. Motivation

1.1.1. Bone Health Crisis in the United States and North Dakota

In 2034, for the first time in US history as shown in Figure 1, the number of older Americans over 65 years old will exceed the number of children under 18 years old [1]. According to the American Academy of Orthopedic Surgeons (AAOS), by 2030, total hip and knee replacements are expected to grow up to 171% and 189%, respectively [2]. The true picture of bone and joint health in the United States is even worse if the number of civic, sport, and military incidents added to the current statistics. Bone is the second most transplanted tissue after the blood. Annually more than 1.6 million bone graft operations are performed in the USA, which is the highest in the world [3, 4]. Bone health is a significant key to the quality of life and overall health because bones keep the body in the appropriate frame for static and dynamic conditions. Moreover, bones serve as a depot for minerals that are necessary for the proper functions of many vital organisms inside the body. The aging US population, a rise in obesity, and an inactive lifestyle complicate the treatment of bone diseases and creates a national healthcare crisis. Current methods to address bone-related issues, appear inefficient or in some cases are incompatible with the prolonged life expectancy in modem societies. Numerous studies have shown the serious limitations and complications of conventional treatments for bone diseases [6-11]. Unfortunately, as shown in Figure 2, the state of North Dakota, along with Iowa and Wisconsin has the highest number of total hip and total knee replacements in the nation per capita [12].

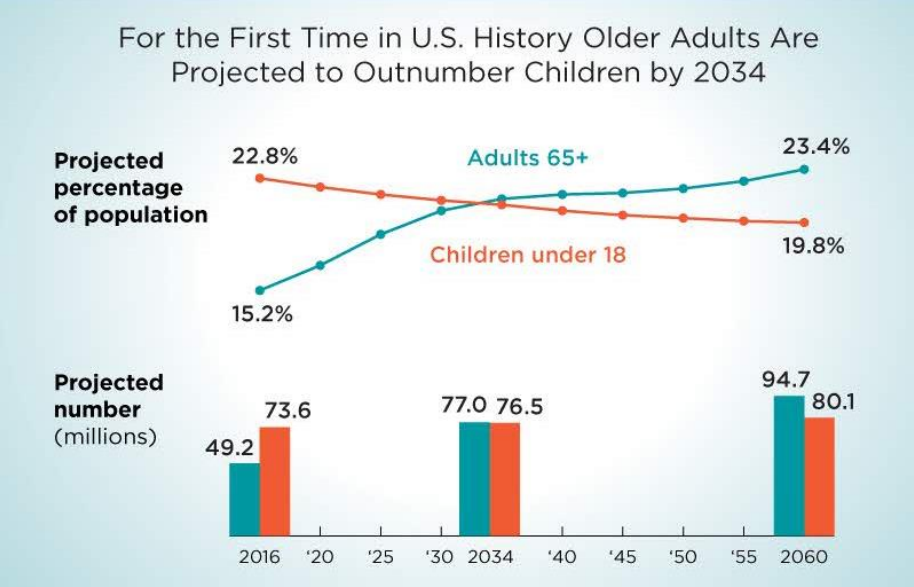

Figure 1: According to the US Census Bureau, the United States will be considered an old nation by 2034 [1].

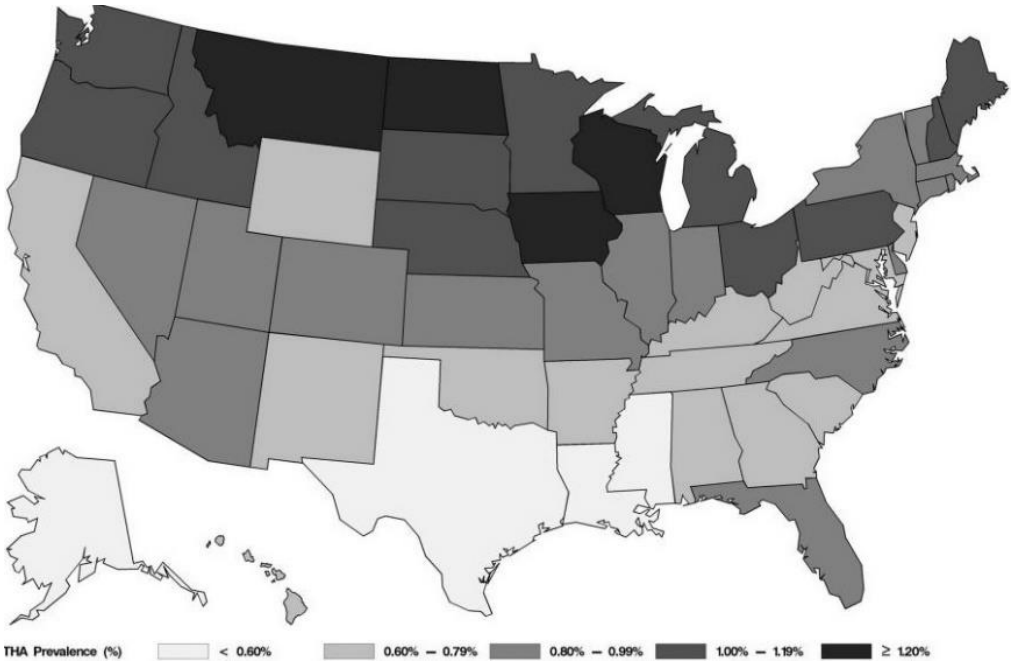

Figure 2: North Dakota, Iowa, and Wisconsin have the highest numbers of total hip and total knee replacements in the nation per capita [12].

1.1.2. The Issue of Bone and Implant Separation

Based on a comprehensive clinical data collection on the failed hip and knee surgeries within the first year of the operation in several countries (Great Britain, Sweden, Australia, New

Zealand, and the US), several causes of failure were observed which three major ones are loosening and separation of bone and implants (41.4%), excruciating pain (23%) and infection (18.4%) [13]. On the other hand, it was observed that the quality of the bone-implant interface is directly influenced by the implant surface roughness [14-18], which, since the early 80s, has always been recognized as one of the main features that are particularly essential for integrating the implant into the bone. Morphology and surface roughness not only affect the cell attachment, proliferation but also can affect the synthesis of extracellular matrix and even cell shape [19]. In addition, roughness not only enables the retaining of bone cells but also allows them to migrate over the implant surface. The first interaction of bone and implant is controlled by physical and chemical properties such as roughness, wetting, structure, defects, and oxide thickness and is critical for long-term implant success [20-22].

1.2. Research Objectives and Scope

With a view to overcoming persisting clinical problems, this study was designed to improve the functionality of orthopedic implants with a particular focus on the surface of implants. Within the scope of this multidisciplinary research, the work was concentrated on Ti-13Nb-13Zr which is the next generation of Titanium alloys with beta microstructure. This advanced Titanium alloy was specifically designed to substitute the old generation of Ti-based implants such as Ti-6Al-4V which has raised many concerns due to releasing toxic Aluminum and Vanadium ions. With suitable surface modifications, it's possible to promote desirable physiochemical properties of the implants. In this research, the main goal is to determine the effects of surface roughness on the functionality of implants. In this regard, we focused on the study of wettability, cell attachment, and mechanical properties (fracture toughness) as three functions that can be affected by roughness. By results from experiments, we introduced a range of surface roughness that meets

all implant requirements in terms of good wettability, high cell attachment rate, and better mechanical properties.

CHAPTER 2. LITERATURE SURVEY

2.1. Systemic Review on Similar Works

Topography is considered a major factor in the performance and life span of orthopedic and dental implants, and this fact has been supported by several studies. For instance, Lange et al. [23], Linez-Batailon et al. [24], Kirbs et al [25], and many others [26-30], believe that there is a direct relation between surface roughness and cell attachment and eventually bone and implant integration. In this regard, the science and engineering of orthopedic implants have recently received a considerable amount of attention. The phenomenon of surface roughness has received particular attention due to emerging new technologies such as additive manufacturing (AM) methods, which are capable to fabricate individual implants with desired roughness values based on each patient. Generally, surface roughness can alter some physical properties of metals, such as adhesion, friction, and contact conductance (thermal and electrical), but the surface roughness of metallic implants plays a more important role.

Hoffman and her colleagues [31], created roughness ranging from Ra=0.07 μm to 6 μm on commercially pure titanium (CpTi) and Ti6Al4V samples. They showed that increasing the surface roughness is in favor of better integration and can increase the chance of mechanical interlocking at the interface of bone and implant. On the other hand, Schuh et al. [32], believe that increasing the surface roughness, is not necessarily in favor of better integration and might increase the possibility of abrasion, which eventually ends in poor bone-metal bonding. Many researchers such as Albrektsson [33], Szmukler-Moncler [34], Kieswetter [35], and Rupp [36] have suggested that the surface roughness of orthopedic implants is not a singular feature, but it is an interrelated feature that should be studied from different angles through a multidisciplinary approach. Lampin [37] and Deligianni [38] highlighted the effect of surface roughness on the first critical steps of

implantation in which the blood touches the implant and, protein adsorption occurs. Rupp et al. [39] showed that surface roughness enhances osseointegration of titanium implants and, can affect the wettability behavior. Despite the existence of some research data supporting the positive influence of surface roughness on bone and implant integration, the problem is, there is no suggested optimal range of roughness values that can provide maximum protein adsorption and cell attachment. To the best knowledge of the author, a wide range of surface roughness from Ra=0.07 μm to 100 μm [31, 40] were studied by different researchers, but still, the lack of research data on the optimum range of roughness is sensible.

Over five hundreds peer-reviewed research papers and several books have been carefully reviewed, which brought to the author's attention that there is a common and repetitive discrepancy in the currently available literature. This discrepancy arises from the fact that surface roughness can play a dual role in the performance of orthopedic implants. For instance, Cooper et al. [41] by *in vitro* and *in vivo* studies concluded that, an increase in the surface roughness of commercially pure titanium (CpTi) implants improved bone integration at the interface. Similarly, Buser et al. [42] indicated that there is a tendency for an increase of bone-implant contact due to increased surface roughness. On the other hand, some researchers, such as London [43], Novaes [44], Carlsson [45], Gotfredsen [46], and Vercaigne [47], could not confirm any meaningful relation between surface roughness and bone and metal integration. Some researchers such as, Elias [48] and Wennerberg et al. [49-51] believe increasing the surface roughness may cause an intolerant bone response which leads to mechanical failure.

Depending on the scale of irregularities of the material surface, surface roughness can be categorized into three main groups; macro roughness (>100 μm), micro roughness (100 nm-100 μm), and nano roughness (<100 nm), any range of roughness can influence the cell response to the

implant [52]. It has shown that the micro-topography can maximize the interlocking between bone and the surface of the implant [53]. Wen et al. [54] have shown that some surface properties, such as surface roughness affect the mechanical stability of the implant-tissue interface. Basically, the mechanical properties of implants should be suited and compatible with receiving tissue surfaces. Oshida [55, 56] proposed that if the roughness could be manipulated to be in the range of 1 to 20 μm, the implant's survival rate would be acceptable, he attributed this finding to morphological compatibility of the surface. Ratner et al [57] and Baro et al [58] showed that in micron-level (roughness > 10 μm) roughness can influence the mechanical properties of the titanium-bone interface, the mechanical interlocking of the interface, and the biocompatibility of the implant. Kasemo et al. [59] showed that surface roughness in the range of from 10 nm to 10 um may also influence the interfacial biology since it is the same order as the size of the cells and large biomolecules. Micro-roughness at this level includes materials defects, such as grain boundaries, steps and kinks, and vacancies that are active sites for adsorption, therefore influence the bonding of biomolecules to the surface of the implant [60]. Keller et al [61] believe micro rough surfaces promote significantly better apposition than smooth surfaces, resulting in a higher percentage of bone in contact with the implant. Micro rough surfaces may influence the mechanical properties of the interface, stress distribution, and bone remodeling. Increasing the contact area and eventually increasing the mechanical interlocking of bone to the implant, can decrease stress concentrations resulting in decreased bone resorption.

Rich and Harris [62] reported that some cells exhibit *rugophilia*, or an affinity for rough surfaces, whereas some cells failed to readily adhere to these same surfaces. Studies by Brunette [63] and Chehrouhdi et al. [64,65] have demonstrated that cells tend to attach and orient themselves in the grooves with specific dimensions on the roughened surfaces. Michaels et al. [66] and Keller

et al. [67] determined that cells are more likely to attach to rough CpTi surfaces produced by sand-blasting than smoother surfaces polished with 1μm diamond paste. During the surgical procedure of implantation, the implant most likely will encounter blood. Almost instantly following contact with blood, the implant surface will be covered with plasma proteins that become adsorbed onto the surface [68, 69]. It is reported that as Ti surface exposes to the blood, it takes about 10 minutes for protein-adhesion (polymorphonuclear granulocytes) to happen on the surface [70]. During the first week after implantation of Ti, a fluid space, which contains proteins [71-73] and other inflammatory cells [74], separates the implant surface from the tissue. The majority of cells in the fluid space are not in direct contact with the surface during the first week of the healing [75]. However, during later time intervals, macrophages are adherent to the surface of Ti [74, 76]. Macrophages (type of cells) are active around the implant and release mediators which act as messenger cells, transmitting information about the implant surface structure and composition to the surrounding tissue [77- 79].

Ramazanoglu et al. [80] have stated that: although various studies have shown that surface alterations, such as roughness, have improved the outcome of osseointegration, it is still poorly understood that either this enhancement was caused by topographical reasons or fabrication-related changes in surface composition and wettability characteristics. Furthermore, the majority of published papers lack an adequate surface characterization, as stated in the literature that makes the evaluation of the effect of unique surface properties on osseointegration. However, general observations using different in vitro and in vivo studies can be still made to evaluate the effect of surface properties (topography, composition, crystal structure, and wettability) on osseointegration.

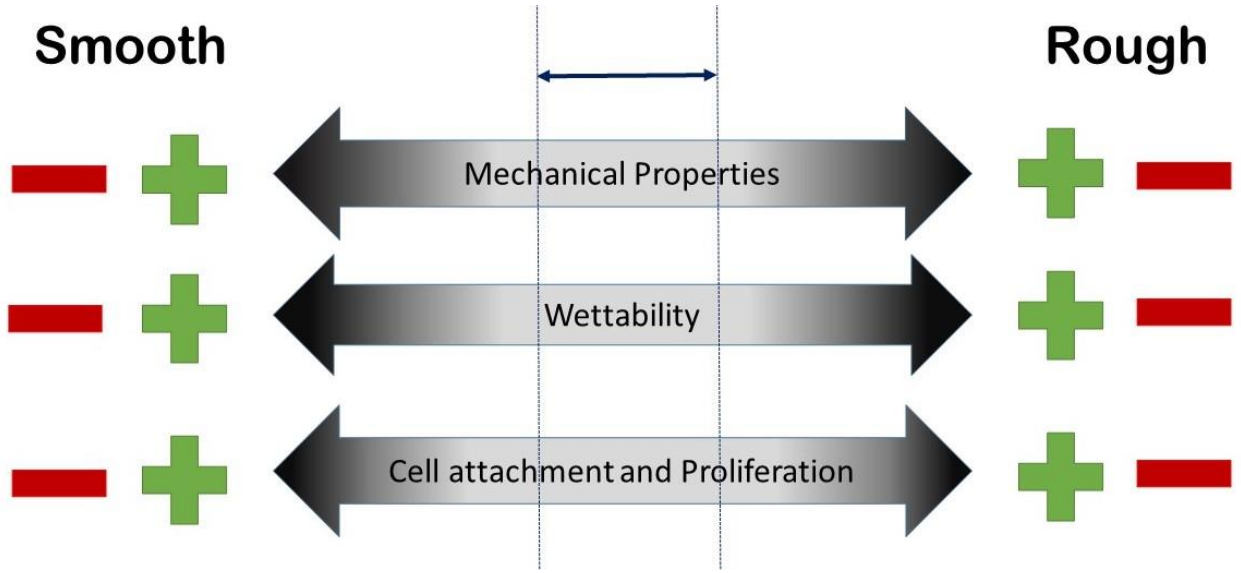

Figure 3: This study aims to find positive or negative effects of surface roughness on mechanical properties, wettability, and cell behavior on the Ti13Nb13Zr implant.

There is little precise knowledge on the implant-bone interface and on the factors which influence host response and the long-term integrity. More fundamental research is needed on both materials and design [81]. There is little information on the effects of the surface topography particularly roughness on implant-tissue interaction [82]. Increasing surface roughness will create more surface area which eventually increases the susceptible corrosion cites and eventually adverse effect on bone-implant integration [83-86]. Increasing surface roughness is also associated with surface breakdown, wear, and debris: [87-90]. Murray et al. concluded that increasing surface roughness can increase surface energy which *in vitro* can release mediators that stimulate cell attachment [91]. Kasemo and Lausmaa [92] believe that a lack of understanding of the interactions at the bone-implant interface is the major barrier to fabricate more effective orthopedic and dental implants. Various explanations have been given to rationalize the above-mentioned discrepancies in the effects of substrate surface roughness on the reaction of the human body to the implants, but still more studies seem essential to cover the existing discrepancies. Separation of implants from surrounding bone has always been recognized as one of the leading causes of failure of orthopedic

and dental implants. A better understanding of surface roughness and its links to properties such as hydrophilic characteristics, cell attachment, and fracture of implant, can help us to improve the functionality of implants [93-96]. Recently due to emerging of advanced 3D bio-printers for the fabrication of implants, lots of studies have been initiated to improve the functionality of implants. One of the main drawbacks of studies done so far is, they don't consider the roughness as a multi-aspect feature. Variation of surface roughness can be in favor of some properties and at the same time adversely affect some other properties of implants. It is known that the surface roughness of orthopedic implants can affect cell adhesion, proliferation, and eventually bone-implants bonding [97-102]. Figure 1 is a simplified illustration of the effects of roughness on the functionality of orthopedic implants. We believe even though surface roughness can change the cell attachment rate but there are other important properties that can be affected by surface roughness and eventually influence the performance of the implant. In this regard, we study the effects of the roughness of implants through three key criteria. To aim this goal, in the experiment phase of this study, three sets of experiments will be performed:

- Contact angle measurement (wettability test) at different roughness values.
- Cell attachment studies at different roughness values.
- Mechanical properties of surface at different roughness values.

The results of the above experiments will help us to determine the variation of wettability, cell attachment, and mechanical properties as the functions of roughness and then introduce a surface roughness value, which at the same time provides the best wettability, maximum cell attachment as well as longer durability.

2.2. Bone Healing

2.2.1. The Structure and Treatment

The human skeletal system contains over 200 unique bones. From an engineering standpoint, bone is a composite material, with a remarkable hierarchical structure, and unique mechanical properties enabling individuals to be statically stable and dynamically in motion. From the biological standpoint, Bone is a complex tissue, which is highly responsive to its environment. The main components of bone are classified in Figure 2. Bone is made up of a calcium phosphate ceramic matrix, (hydroxyapatite), making up 65%–70% of the bone mass and 25%–30% protein, (mainly collagen) [103]. There are four types of cells in osseous tissues: osteogenic cells, osteoblasts, osteocytes, and osteoclasts. Osteogenic cells undergo cell division and develop into osteoblasts. Osteoblasts play a role in bone formation and collagen secretion. As osteoblasts secrete extracellular matrix, then osteoblasts evolve into osteocytes. Osteocytes, also known as mature bone cells, are responsible for nutrients and waste exchange with the blood. Osteoclasts are bone- destroying cells and responsible for bone resorption. Bone also consists of bone lining cells, fibroblasts, and fibrocytes. Bone lining cells control the movement of ions between bone and the surrounding tissue [104]. Two distinct types of bone exist within the body: cortical and trabecular bone. Cortical bone is a dense form of bone with Young's modulus of 4 up to 30 GPa, which appears at the outer edges of long bones. Trabecular bone, on the other hand, is a very porous structure, made up of small compartments, known as trabeculae with Young's modulus of 0.2 up to 0.5 GPa. In addition to structure, bone serves as a reservoir for many important metabolic ions, such as calcium and phosphates. These ions play important roles in signaling and in molecular energy storage. To regulate the level of ions systemically, bone must interact with many other organs within the body, such as muscle, kidneys, and liver [105].

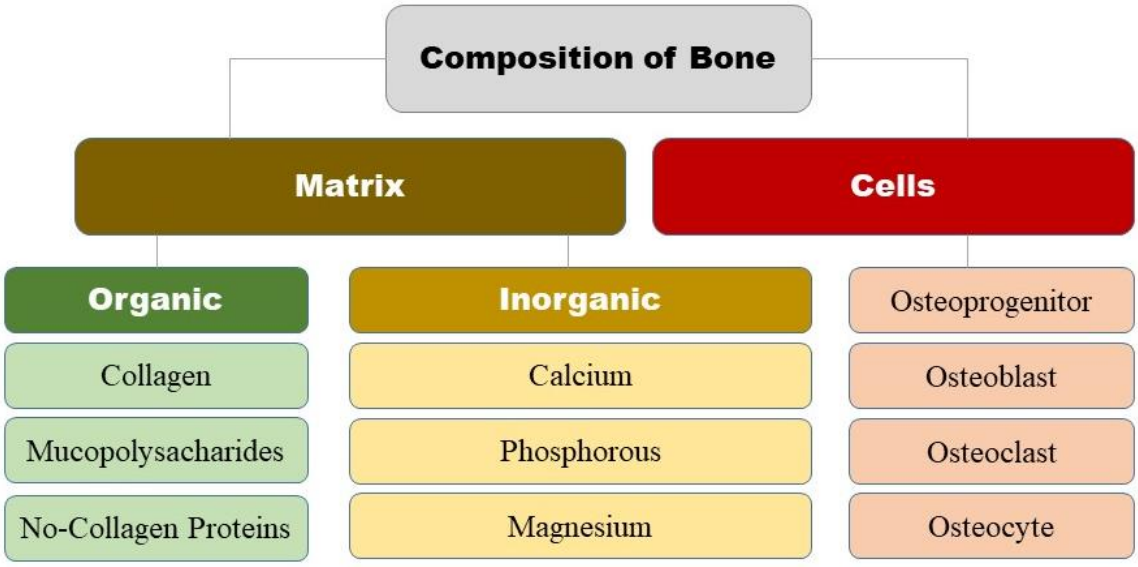

Figure 4: Components of the bone as a composite.

From a structural point, Bone consists of macro, micro, and nanoscale porosities that have different functions and characteristics. Macro-scale porosity gives rise to mechanical anisotropy. Micro-scale porosity provides sufficient vascularization and cell migration, while nanoscale features act as a framework for cell and mineral binding. According to Branemark et al [106], osseointegration could be defined as the "continuing structural and functional co-existence, possibly in a symbiotic manner, between differentiated, adequately remodeled, biologic tissues, and strictly defined and controlled synthetic components, providing lasting, specific clinical functions without initiating rejection mechanisms". Bone has a major advantage compared to engineering structural materials which is self-repairing and can alter its properties and geometry in response to changes in mechanical and metabolic demand. Bone properties also change from species to species. Bone physical properties differ from one person to another but also within one individual from one location to another. Owing to its different apparent porosity, the mechanical properties of trabecular bone and cortical bone are clearly different. The mechanical properties are fundamentally anisotropic and can be described as a two-phase material in which the interstitial

fluid can play a determinant role in the mechanical response depending on the type of loading applied. Many factors can play a role in the mechanical properties of bone; strain rate and age effects are very relevant for bone repair and its study with biomaterials [107-109]. As it can be seen in Figure 3, several types of damage are found in bone: traverse cracks (1), linear (2), oblique non-displaced (3), oblique displaced (4), spiral (5), greenstick (6), and communicated cracks (7) [110].

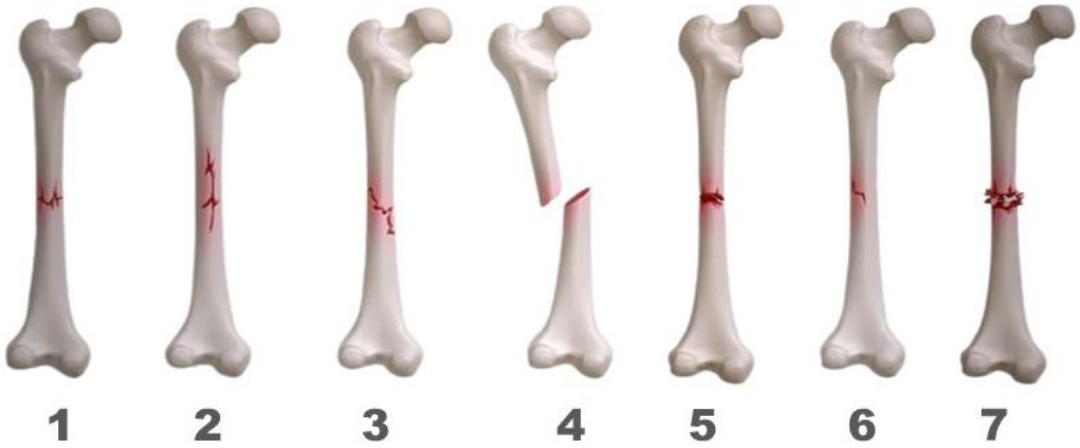

Figure 5: Bone as composite is subjected to different mechanical failures [111].

Bone-related conditions can become a huge burden to society, due to the prevalence of the diseases. This burden includes the cost of healthcare and lost productivity, as well as the intangible cost of pain and reduced mobility for an individual. With an aging population, there is increasing pressure to alleviate some of the burdens by reducing costs and increasing the quality of life after treatment. Some of the main consequences of the bone problems are summarized in Figure 6. One of the key challenges for driving bone repair forward is the translation of new technologies to the clinic. The needs identified by clinical doctors and by researchers are not the same. Clinicians often raise issues with infection around the implant, the ability to provide adequate fixation, and the overall cost of the device. On the other hand, researchers are more concerned with the interaction of the implant with native bone and its integration at the site [112].

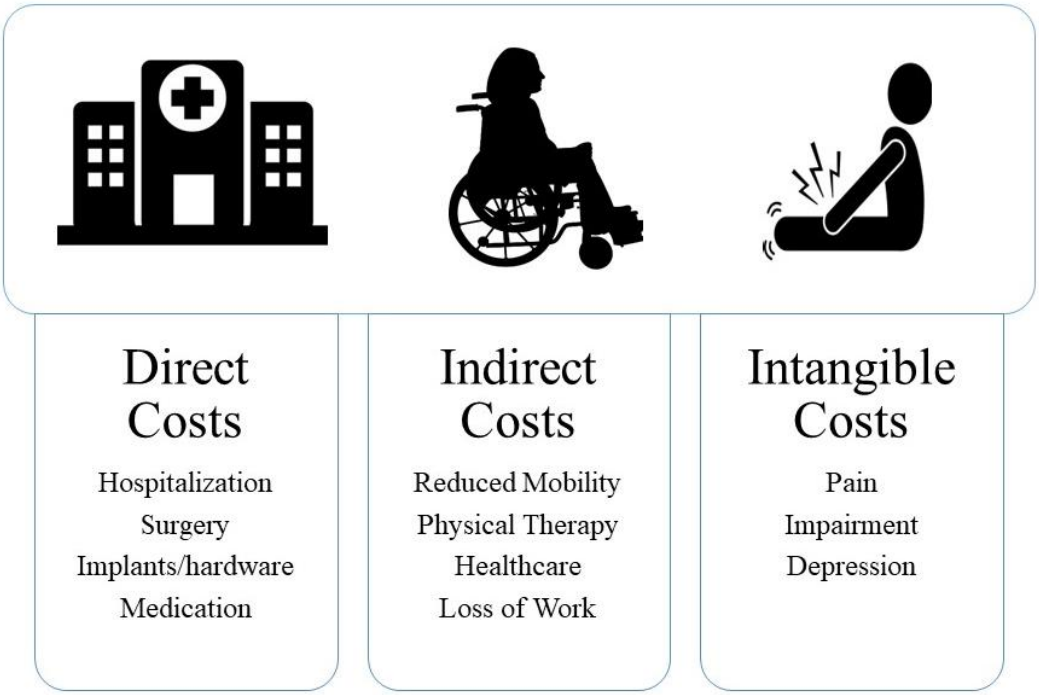

Figure 6: Some of the main consequences of the bone problems.

2.2.2. Orthopedic and Dental Implantation

Within the last decades, biomaterials have emerged to direct the repair of tissue after disease or trauma by directing the regeneration of healthy native tissue at the site. Materials that function in this way are not merely fulfilling a mechanical or engineering requirement but are interacting with various cell types in the bone space. The cellular response can be directed by many different cues inherent in the material and its structure. Thus, it is important to consider all aspects of implant and material design when considering bone repair [113]. Cells are naturally responsive to their surroundings. They respond to environmental features at a range of scales from macro down to molecular. Cell-surface interactions are mediated by the cell membrane. They interact with the surrounding surface primarily through proteins by the formation of focal points that join the cells to binding sites [114].

With regard to bone healing, immediately after placing an imlant in the body, there three distinct and crucial steps will occur: (1): the first and the most important healing phase, *Osteoconduction*, relies on the recruitment and migration of *Osteogenic* cells to the implant surface, through the blood clot on the surface of implant which facilitates cell migration. (2): The second healing phase is, *de novo* bone formation, which results in the formation of a mineralized interfacial matrix. These two healing phases, Osteoconduction, and *de novo* bone formation result in contact *Osteogenesis* and, given an appropriate implant surface, bone bonding, and (3): the third healing phase, bone remodeling, which is a slower process called *Osseointegration*. This process can be defined as a direct structural and functional connection between bone and the surface of the implant and is a time-depending process [115,116]. Osseointegration is critical for implant stability, and is considered a prerequisite for implant loading and long-term clinical success of implants. The process of osseointegration involves an initial interlocking between bone and the implant body, and later, biological fixation through continuous bone apposition and remodeling toward the implant. The process itself is very complex and there are many factors that influence the formation and integration of bone onto the implant surface [117, 118].

2.2.3. Cell Adhesion and Differentiation

Adhesion plays an integral role in cell communication and regulation and is of fundamental importance in the development and maintenance of tissues. *In vitro*, most mammalian cells are anchorage-dependent and attach firmly to the substrate [119]. According to the “cell adhesion model”, the more a cell sticks the more it shows the greater number of chemical bonds it has on its surface [120,121]. Cell adhesion is involved in stimulating signals that regulate cell differentiation, cell cycle, cell migration, and cell survival [122]. The affinity of cells to substrate

is a crucial consideration in biomaterial design and development. Figure 5 shows the schematic illustration of cells and surface interaction and adhesion.

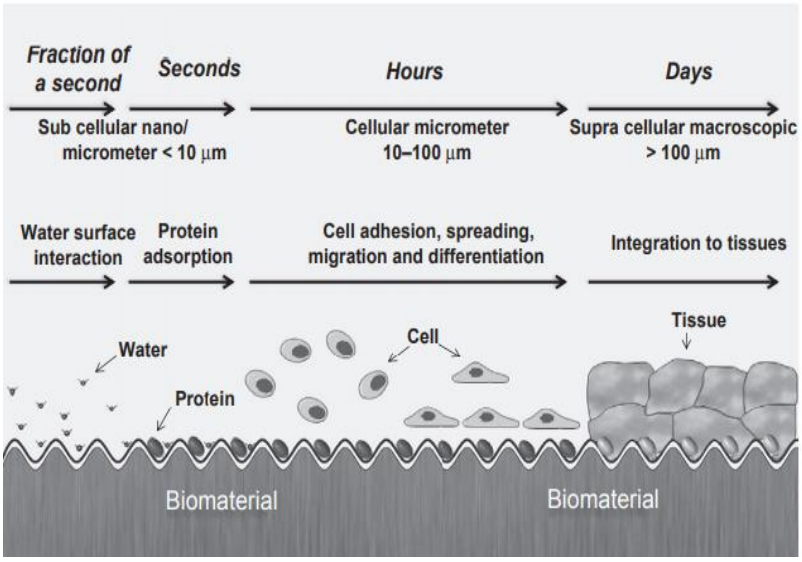

Figure 7: Tissue biomaterial interactions: illustration of events occurring at the interface between biological systems and biomaterial surface at varying time and length scales [123].

When a biomaterial is implanted into the body, the first molecules to reach its surface are water molecules. The water interaction and binding on surfaces are influenced by the surface characteristics, subsequently influencing the proteins and other molecules that arrive later [124]. Depending on the location of the implanted biomaterial in the body, the implants are surrounded by different types of tissues. In the case of artificial joints, bone fixation devices, and dental implants, the surface of the material attaches to both hard and soft tissues [125].

In the aqueous medium such as inside the body which containing both proteins and cells, the smaller protein molecules cover the implant surface before the cells arrive. Hence, as a matter of fact, the cells adhere onto an adsorbed protein layer [126,127]. These proteins first come from blood and tissue fluids at the wound site and later from cellular activity in the region [128,129]. With sufficient passing of time, molecules with greater affinity for the surface arrive, but with a slower rate (because of lower concentration and/or larger size) of approach. Finally, cellular

contact and interaction with the adsorbed proteins on the surface of the implant is the third stage, which usually takes from minutes up to days. The adsorbed proteins act as a translator between the surface properties of the material and the cell receptors, determining the fate of the implant in the biological environment [130]. This third stage is highly influenced by complex interactions among the different types of proteins, surface chemistry and topography (porosity, pore size and geometry, interconnectivity) [131]. The behavior of proteins at surfaces plays a vital role in determining the nature of the tissue implant interface. Adsorbed proteins affect blood coagulation, complement activation and bacterial, and cell adhesion [132-134]. Apart from contact, cell adhesion also involves spreading of cells over the surface, followed by the subsequent differentiation and growth of cells. Once the proteins are adsorbed onto the implant surface, they act as the interface between the surface and the cells. As a result, cells interact indirectly with the biomaterial surface through the adsorbed layer of proteins (not to the native surface) [135-137].

2.3. Implantable Materials

As the first response, an implanted object inside the body will be perceived as a threat by the host tissue and the immune system will attempt to eliminate it. This will not happen if the biomaterials are inert and cannot be degraded. Finally, inert biomaterial will be integrated into the tissue. The choice of materials used for designing a medical implant is governed by biocompatibility, bioadhesion, biofunctionality, corrosion resistance, etc. Understanding liquid-solid interactions through the behavior of the liquid-solid interface are very important in biomedical implants. Liquid-solid interaction may or may not include chemical reactions, and the degree of liquid spreading over the solid surface (wetting) may vary based on the chemical properties of the materials involved (surface free energy) and the topography of the solid surface (roughness). The wetting of solid surfaces by biological fluids is often necessary for a chain of

biological events to unfurl (expand) so that a foreign material may be accepted in vivo and thus become bioactive [138].

Ideally, the best materials for the fabrication of bone implants are materials that can mimic the natural environment and function of human bone. To achieve this goal, the ideal biomaterials for bone implant applications should have the following functions.

- Promote cell adhesion and migration
- Enhance vascularization
- Facilitate diffusion of vital cell nutrients and secreted products
- Support mechanical and biological functions

In this regard, different categories of materials such as metals, ceramics, polymers, and their composites have been investigated [139]. According to Food and Drug Administration (FDA), in the section of US medical device amendment, all the materials are reviewed for safety and effectiveness and classified as Class I, II and III. As it can be seen in Figure 6. Class I materials are those considered to be of low risk in causing adverse reactions. Materials in Class II must satisfy more restrictive regulations than Class I. The Class III must pass full safety tests and efficacy assessments before going to the market. Many factors contribute to the choice and suitability of implant materials such biocompatibility, corrosion and wear resistance, longevity, strength and toxicity, etc. We will discuss implantable materials in metallic, polymeric, ceramic, and composite forms.

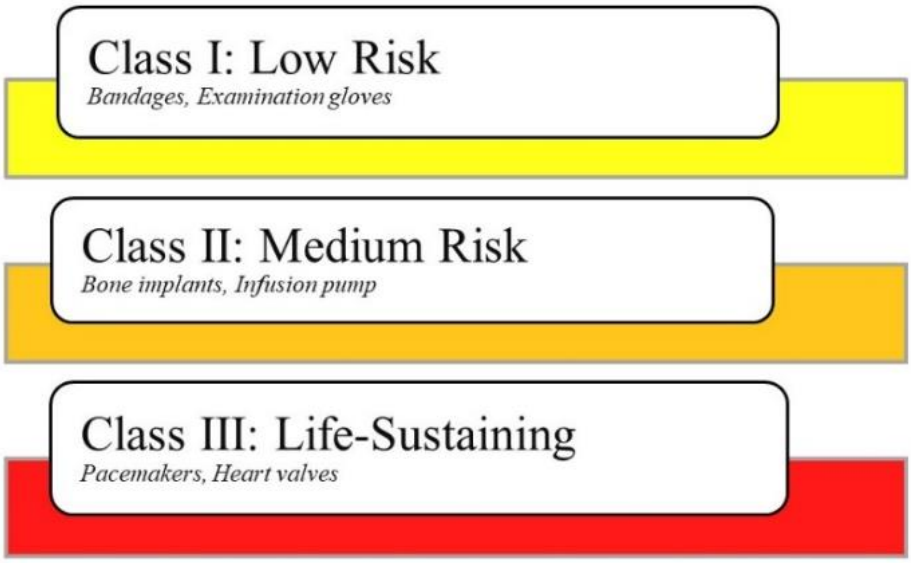

Figure 8: FDA classifications of biomaterials.

2.3.1. Ceramics

Ceramics have been used in orthopedic applications as either implants or coatings for implants. Ceramics such as hydroxyapatite and tricalcium phosphate (TCP) are among the most widely used materials for bone substitution due to their compositional similarity to bone mineral and excellent chemical biocompatibility [140,141]. Hydroxyapatite supports the growth of bone cells (osteoconduction) along its surface or within the pores by providing a porous structure similar to that of natural bone [142]. Moreover, as a hydroxyapatite scaffold degrades in the body over the time, it releases calcium and phosphate ions, which stimulates new bone formation (osteoinduction) [143]. One of the great advantages of TCP and hydroxyapatite for bone implant applications is that they have shown minimal immunogenicity or toxic consequences [144]. Ceramics present high compressive strength, close to that of trabecular bone [145] and can be manufactured into highly interconnected macroporous structures [146,147] thereby increasing vascularization, nutrient delivery and bone ingrowth [148-150]. Calcium phosphate ceramics such as hydroxyapatite can present more adaptable biodegradability compared to other ceramics [151].

One of the biocompatible ceramics, which has recently been developed for bone tissue engineering and drug delivery system, is Laponite® (LAP). LAP is a kind of silicate based nanoparticles with chemical formula $(Mg, Li)_3Si_4O_{10}(OH)_2Na_3$ and disc-shaped morphology in nanoscale. LAP consists of negative face charge and weakly positive edge charge leading to strong interaction with other materials to develop nanocomposites with excellent physical and mechanical properties. Previous studies revealed that clay based nanocomposites could simultaneously improve durability, mechanical strength, surface characteristics and biocompatibility making them promising for bone tissue engineering applications. For instance, researches demonstrated that LAP nanoplates could promote cell proliferation and encourage the osteogenic differentiation in bone repair applications. Along with the mentioned advantages, ceramics have disadvantages too, for example poor performance in load-bearing conditions and brittleness have limited the use of ceramics in loaded bone applications [152]. One solution is, to use composites of ceramics instead of ceramic alone. For instance, a composite of ceramic and polymer can exhibit the excellent biocompatibility of ceramics as well as the durability of polymers.

2.3.2. Polymers

Polymers are considered very good choices for bone tissue engineering due to their excellent tunable and adaptable characteristics. In particular, biodegradable polymers, can be designed to support tissue growth until needed and then be resorbed by the body [153].

2.3.2.1. Synthetic Polymers

Common synthetic polymers for bone tissue applications include poly (glycolic acid), poly (lactic acid), copolymers of poly (DL-lactic-glycolic acid) (PLGA), polycaprolactone (PCL) and many others [154]. Fabrication of implants using synthetic polymers has many advantages such as the possibility of tuning the mechanical properties and fabrication of complex structures as well as

controlling the degradation rate [104]. Despite the wide range of properties provided by organic polymers, the lack of bioactivity is a major drawback of these types of materials [155]. To solve this issue, synthetic polymers have been used as a composite with other materials. For example, cellulose nanocrystals (CNCs) were added to polyacrylamide (PAAm) polymers matrix to improve bioactivity by increasing the biomineralization rate [156]. Another problem associated with synthetic materials for scaffold applications is their acidic degradation or toxic degradation byproducts, which can adversely affect the tissue growth. [151, 154]. Physical characteristics of synthetic polymers can also limit their application as bone implants. For example, poly (L-lactic acid) or PLLA is hydrophobic which consequently causes the lack of homogeneous integration of proteins and poor cell attachment to the surface [157].

2.3.2.2. Natural Polymers

Natural polymers such as collagen, silk fibroin, chitosan, alginate and hyaluronic acid have recently received attention as bone scaffolds and implants due to their superior chemical biocompatibility, low immunogenicity, and proven ability to facilitate cell growth [154]. One additional important advantage of natural polymers is the possibility of tuning and optimization of process factors such as concentration, charge, chemical addition in order to get desired properties [158]. Also, the presence of ligands such as bone morphogenetic protein 2 (BMP-2) in natural polymers has been shown to facilitate bone cell adhesion [141]. Natural polymer hydrogels can create 3D structures with the ability to absorb large amounts of water [159,160]. With their soft and flexible structure, hydrogels can be used for cell encapsulation, minimizing the amount of damage to the host tissue [161,162]. Gelatin methacryloyl (GelMA) hydrogels have been widely used for various biomedical applications due to their suitable biological properties and tunable physical characteristics. Three dimensional (3D) GelMA hydrogels closely resemble some

essential properties of native extracellular matrix (ECM) due to the presence of cell-attaching and matrix metalloproteinase responsive peptide motifs, which allow cells to proliferate and spread. GelMA is also versatile from a processing perspective. It crosslinks when exposed to light irradiation to form hydrogels with tunable mechanical properties which mimic the native ECM. It can also be microfabricated using different methodologies including micromolding, photomasking, bioprinting, self-assembly, and microfluidic techniques to generate constructs with controlled architectures. Hybrid hydrogel systems can also be formed by mixing GelMA with nanoparticles such as carbon nanotubes and graphene oxide, and other polymers to form networks with desired combined properties and characteristics for specific biological applications. Recent research has demonstrated the proficiency of GelMA-based hydrogels in a wide range of applications including engineering of bone, cartilage, cardiac, and vascular tissues, among others. Other applications of GelMA hydrogels, besides tissue engineering, include fundamental single-single cell research, cell signaling, drug and gene delivery, and bio-sensing. Some limitations of natural polymers are the difficulty in controlling their degradation rate as well as low mechanical stability [163]. Since collagen is abundant in bone tissue [141], collagen hydrogels are inherently biocompatible and biodegradable, highly porous, minimally antigenic and can easily be combined with other materials [164]. A limitation of collagen-based materials is, the relatively poor mechanical properties. Silk fibroins offer excellent mechanical properties, biocompatibility and versatility in processing [148]. Silk fibroin is environmentally stable, flexible and degradable by proteolytic enzymes [165]. Chitosan can be extracted from skeletal materials of crustaceans, mushroom envelopes, green algae cell walls and yeast [162]. It has been shown that chitosan has a superior ability to promote cell adhesion and growth [158,166]. Moreover, it can show

antibacterial properties [167,168]. Like other natural polymers, the limitation of chitosan for use in bone applications is its poor mechanical strength.

2.3.3. Composites

There are several types of hybrid or composites implantable materials. A fiber-reinforced composite was prepared from PMMA and E-glass to enhance the bone bonding proper ties of the materials [169]. Chitosan-clay-based bio-nanocomposites were developed as advanced functional materials [170]. The adhesion of proteins involved in osteoblast cells was examined on composites polyurethane/$CaCO_3$ surface with promising results [171]. Bond coats based on bioinert ceramics materials such as titania and ZrO_2 were developed to increase the adhesion strength of the coating system HA-bond coat to Ti-6Al-4V alloy surfaces used for hip endoprostheses and dental root implants. It was reported that the bond coats improved the adhesion strength, as measured by modified ASTM D 3167-76 peel test, by up to 100%. Further, the resorption resistance, as determined by in vitro leaching in simulated protein-free body fluid, endured for up to 28 days [172]. A glass in the SiO_2-Al_2O_3-CaO-CaF_2-K_2O-B_2O_3-La_2O_3 system was prepared. The composite possesses a modulus of elasticity of 96±3 GPa and a fracture toughness (K_{1C}) OF 4.77±0.27 $MPam^{0.5}$. The attachment and proliferation test osteoblastic cells were evaluated, and it was found that cells exhibited acceptable attachment, spreading, and proliferation, and thus exhibited excellent biocompatibility [173]. The carbon fiber-reinforced polyetheretherketone (PEEK) was evaluated for dental implants. It was reported that although PEEK could represent a viable alternative material for dental implants, further experimental studies on the chemical modulation of PEEK seem to be necessary, mainly to increase the bone-implant contact ratio and to minimize the stress distribution to the peri-implant bone [174].

2.3.4. Metals

Due to formability and mechanical strength specifically in load-bearing applications, metals have always been attractive candidates for biomaterial applications. Metals such as titanium, magnesium or stainless steel have been used for joint prostheses, plates and screws [145]. Even though metals have big advantages for bone implant applications, it should be noted that since most metals have elastic moduli much higher than those of human bones, stress shielding and resorption of the surrounding bone tissue can be a deleterious consequence [175]. This drawback has been solved to some extent by increasing the porosity in the microstructure of implants, which also can have positive effect on vascularization and cell adhesion on the implants. For example, He et al. decreased the elastic modulus of a titanium from 1.22 to 0.18 Gpa, which is close to elastic modulus of cancellous bone, by increasing the porosity from 44.2% to 65.1% [176]. Unfortunately, the long-term presence of some metals such as Al, within the body creates health issues such as alzheimer, infertility, neurological and cardiological symptoms [177]. In case of temporary implants, one solution includes the fabrication of magnesium alloy-based highly porous metals, which can degrade *in vivo* by corrosion. However, even though magnesium degradation has been shown to stimulate bone-healing, concerns remain regarding the inflammatory response to the degradation of metals *in vivo*, as well as, the body's ability to clear the corrosion products [178]. Fatigue, ion-releasing, corrosion, lack of integration with host tissue, which can lead to the formation of fibrous tissue, and risk of infection are other issues of metallic implants [140].These different materials and their key advantages and limitations are summarized in Table 1.

Table 1: Different types of materials for orthopedic implant applications

Material	Advantage	Disadvantage
Metals		
Titanium alloys	Biocompatible	High elastic modulus
Tantalum	Ductile	Low corrosion resistance (Mg)
Magnesium	Cytocompatibility	No direct bonding to tissue
Stainless steel	High corrosion resistance (Ti & Ta)	
Cobalt chromium	Biodegradable (Mg)	
	Excellent mechanical strength	
Ceramics		
Bioglass	Biocompatible	Brittle
Hydroxyapatite	Bioactive	Weak in tension
Aluminium Oxide	Strong in compression	Low impact resistance
Polymers		
Polyester	Biodegradable	Low mechanical strength
PUL	Ductile	Bioinert
PMMA	Easy to fabricate	
PLLA	Light weight	
PEG		
Composites		
	Light weight	Separation of components
	Easy to fabricate	Low Osteointegration
	Good mechanical strength	Not many clinical data available

2.3.4.1. Titanium Alloys

Titanium alloys are now the most attractive metallic materials for biomedical applications. In medicine, they are used for implant devices replacing failed hard tissue. Examples include artificial hip joints, artificial knee joints, bone plates, screws for fracture fixation, cardiac valve prostheses, pacemakers, and artificial hearts. Basically, titanium and titanium-based alloys can be classified into alloy groups:

- α type (HCP: hexagonal-closed packed crystalline structure)
- (α + β) type

- β type (BCC: body-centered cubic crystalline structure)

Alloying elements added to titanium are divided into two groups: alpha (α) stabilizers and beta (β) stabilizers. Elements, such as Al, Sn, Ga, Zr, and interstitial elements, dissolve into the titanium matrix and are strong solid solution strengtheners. Hence, they are known as α-stabilizers and exhibit good high-temperature performance. Alloying elements such as transition metals V, Mo, Nb, Ta, and Cr, providing much β phase, are referred to as β-stabilizer [179]. Figure 7 shows different types of titanium alloys.

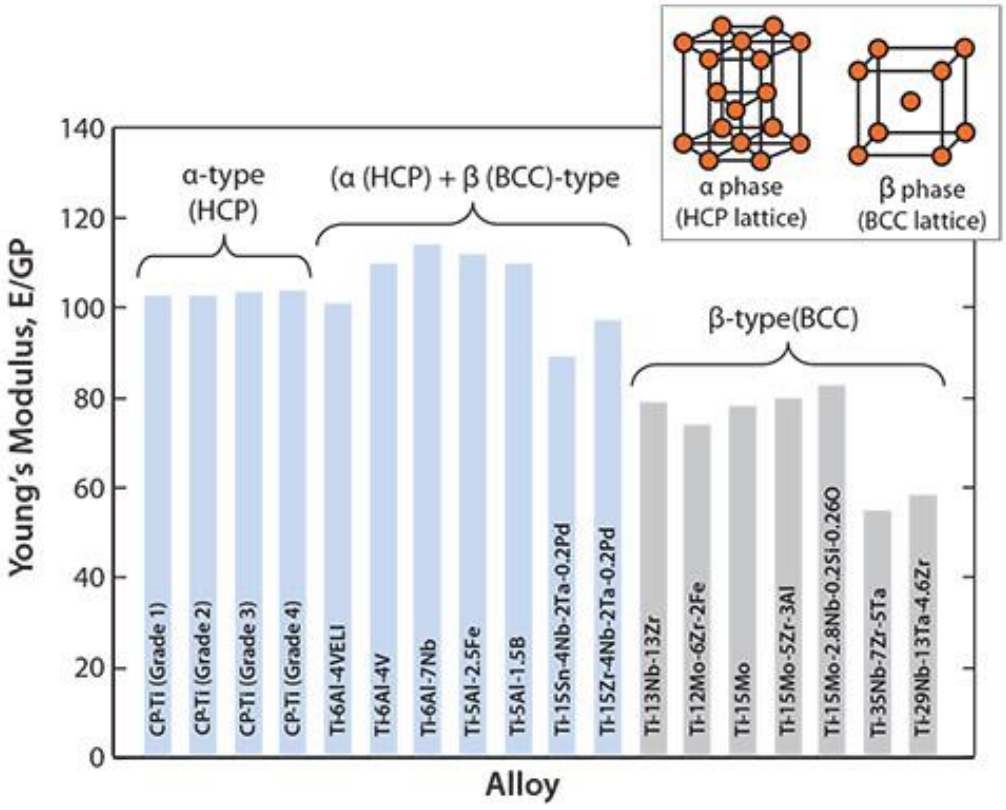

Figure 9: Classification of titanium alloys based on constituting phases [180].

The basic expectation of implants, is to create osseointegration or biointegration between bone and implant. Although a wide variety of potential materials have been used for this purpose, only a few have achieved the expected requirements which most important ones are commercial

pure (Cp) Ti and Ti-based alloys. One of the main reason for the popularity of Titanium alloys is, Ti alloys create an oxide film on the surface. This oxide layer limits the dissolution of elements and facilitates the deposition of biological molecules. Although Ti and its alloys are currently considered the most attractive metals for orthopedic and dental applications, the tensile strength of CpTi is not enough for hip replacement applications, pin or screws [181,182].Moreover, wear resistance is also inferior to that of stainless steels and cobalt alloys [182]. The frequent occurrence of wear debris from Ti has been associated with inflammation, bone resorption and pain [183-187]. To improve mechanical strength and wear resistance, various elements have been added to create new Ti alloys. As a comparison Ti-6Al-4V is a high-strength Ti alloys, but its biocompatibility is considered lower than CpTi. The wear and corrosion resistance of Ti-6Al-4V are lower than Ti. The fact is Ti-6Al-4V releases compounds and wear debris containing Al or V ions, both of which are toxic [183]. In this regard, new V-free titanium alloys were developed with strength similar to Ti-6Al-4V. Accordingly, Ti-6Al-7Nb and Ti-5Al-2.5Fe [188] have been developed and used in service. Recently Ti-13Nb-13Zr [189] and Ti-29Nb-13Ta-4.5Zr [190] have been developed for orthopedic application in order to avoid “stress shielding” effect caused by modulus mismatch between the implant and the bone. The Ti-Zr binary alloy has better corrosion resistance and mechanical properties than CpTi. Ikarashiet al. [191] implanted Ti-Zr samples in rats for eight months. It was reported that all Ti-Zr samples showed no systemic toxicity and even better biocompatibility than Ti for use as an orthopedic implants [192-194]. Table 2 summarizes all Ti alloys with possible applications in orthopedic applications.

Table 2: Titanium alloys with potential application in orthopedic implants

Ti Alloys		Young's Modulus (GPa)	Reference
Ti, Commercially pure (Cp)	Grade 1	103	[195-199]
	Grade 2	103	[195-199]
	Grade 3	103	[195-199]
	Grade 4	104	[195-199]
	Grade 7	105	[195-199]
	Grade 11	110	[195-199]
Ti-6Al-4V Grade 5		114	[200]
Ti-6Al-4V ELI		114	[201]
Ti-15Zr-4Nb-0.2Pd-0.2O-0.05N		100	[202]
Ti-15Zr-4Nb-4Ta-4Mo		112	[202]
Ti-16Nb-13Ta-4Mo		91	[203]
Ti-15Sn-4Nb-2Ta-0.2Pd		89	[202]
Ti-15Sn-4Nb-0.2Pd-0.2O		86	[202]
Ti-15Zr-10Cr		80	[204]
Ti-13Nb-13Zr		75	[205]
Ti6Al-7Nb		74	[206]
Ti-12Mo6Zr		74	[207]
Ti-29Nb-13Ta-4Mo		74	[208]
Ti-29Nb-13Ta-6Sn		74	[208]
Ti-29Nb-13Ta-2Sn		62	[208]
Ti-19Zr-10Nb-1Fe		59	[209]
Ti-29Nb-13Ta		80	[210]
Ti-29Nb-13Ta-7Zr		53	[205]
Ti-10Zr-5Nb-5Ta		43	[210]

To overcome to the problem of toxicity of titanium-based implants, scientists have developed several options. One of the most successful and the newest accomplishments is Ti-13Nb-13Zr, that was developed in late 90's. Ti-13Nb-13Zr (ASTM F 1713) is a beta titanium alloy

developed for use in biomedical implants that combines a low elastic modulus, high strength, excellent hot and cold workability, and superior corrosion resistance [211].

Generally, besides the cytotoxicity, implant materials must have two requirements in terms of mechanical properties: high strength and low Young's modulus. The latter is the main interest, because it is much more difficult to control in comparison to the former. Young's modulus of human bone tissue is reported to be 10–30 GPa. [212]. Although Ti and its alloys have been used for biomedical applications, their elastic moduli are still higher than that of bone tissue [213]. Excessive Young's moduli of traditional biomedical metals (e.g., Co-Cr-Mo, Grade 4 Ti, Ti-6Al-4V, and stainless steels) lead to a stress-shielding effect; they decrease an amount of stress applied to bone tissue near an implant, resulting in the weakening of human bone [214]. Compared with those materials, Ti-13Nb-13Zr is also beneficial to biomedical applications due to its moderately low Young's modulus [215-217]. Compared to Ti alloys for orthopedic implants, Ti-13Nb-13Zr has very recently received attention to be used as the best candidate for orthopedic implants and dominate the market in the future [218-222]. The Ti-13Nb-13Zr alloy used in this study possessed a chemical composition of 13.8 mass% Nb, 14.0 mass% Zr, 0.06 mass% Fe, 0.07 mass% O, 0.01 mass% C, 0.007 mass% N, and balance Ti.

2.4. Orthopedic Implants

2.4.1. History

Historically, the first attempts of tissue transplantation into humans, backs to the time of Hippocrates (approximately 400 BC), while they are some other evidence that show Egyptians and ancient Hindus have also involved in these types of procedures. As described in Sanskrit texts of India, the transplantation therapies were described around 3000 years ago when skin grafting was used to reconstruct noses that were amputated as a means of judicial punishment. However, it was

the Dutch surgeon Job Van Meekren in the 1600s who has used part of a dog's skull to fill a defect in a soldier's cranium and therefore has been documented as the first transplantation operation [223-227].

2.4.2. Overview of the Implant Technology

Human-kind of 21st century uses a tremendous number of artificial organs or medical devices that help to main patients' quality of life. They include hydrocephalus shunts, ocular and contact lenses, orbital floors, artificial ears, cochlear implants, nasal implants artificial chins, mandibular mesh , artificial skin, blood substitutes, heart valves, pacemakers, breast prostheses, pectus implants, glucose biosensors, dialysis shunts and catheters, absorbable pins, temporary tendons, birth control implants vascular grafts, spinal fixations, finger joints cartilage, joint replacements (hip, knee, ankle), artificial legs, bone plate and bars, Harington spinal bars, and etc. Development of new implants or modification of current ones, is one of the great examples of multidisciplinary scientific research, because it needs a collective knowledge from a broad range of different disciplines. In order to place an implantable material inside the body, the host's hard tissue has to be traumatized. In other words, Injured or diseased tissues will be removed to some extent in the process of implantation. The success of the entire operation depends on the type and degree of tissue response to the implant during the healing process. Following the implantation, bone adaption or integration of an implant which is a series of biological reactions starts with bone turnover at the interface [228-230]. The long-term success of implant therapy is dependent on many interrelated factors which the most important ones include Biological (or in short, biocompatibility), Morphological and Biomechanical characteristics. In other words, these factors must be successfully satisfied to create and maintain a sufficient volume of connective tissue with minimal inflammatory consequences [231].

In order to increase the functionality of implants, significant efforts have gone into the development of implant biomaterials that hold the promise of improving clinical success. The technologies have evolved from simple modification of the oxide surface to precise nanoscale modification technologies that involve the formation of a uniform and consistent surface that leads to altered cellular response. Further, there are developing technologies that utilize changes in surface chemistry. Also, biologists, are also being added to the oxide surface in order to assist in stability of implant in the live environment of human body [232-235].

The successful outcome of any implant procedure is mainly depend on the interrelationship of the various factors, including biocompatibility of the implant material, macroscopic and microscopic nature of the implant surface and designs, the status of the implant in both a health and a morphology (bone quality) context, the surgical technique, the health of patient, the undisturbed healing phase, and loading conditions [236, 237].

2.4.3. Orthopedic Implants

Orthopedic implants can be used in several parts of human skeleton and can be categorized into two groups including permanent joint replacements and temporary fracture fixation devices. Permanent orthopedic implants include the hip, knee, ankle, shoulder, elbow, wrist, and finger joints, which are expected to serve in the human body throughout the life span of the patients. On the other hand, temporary orthopedic implants including plates, screws, pins, wires, and intramedullary nails are needed to fix broken or fractured bones and are supposed to serve for a relatively short time just long enough to let bones heal [238].

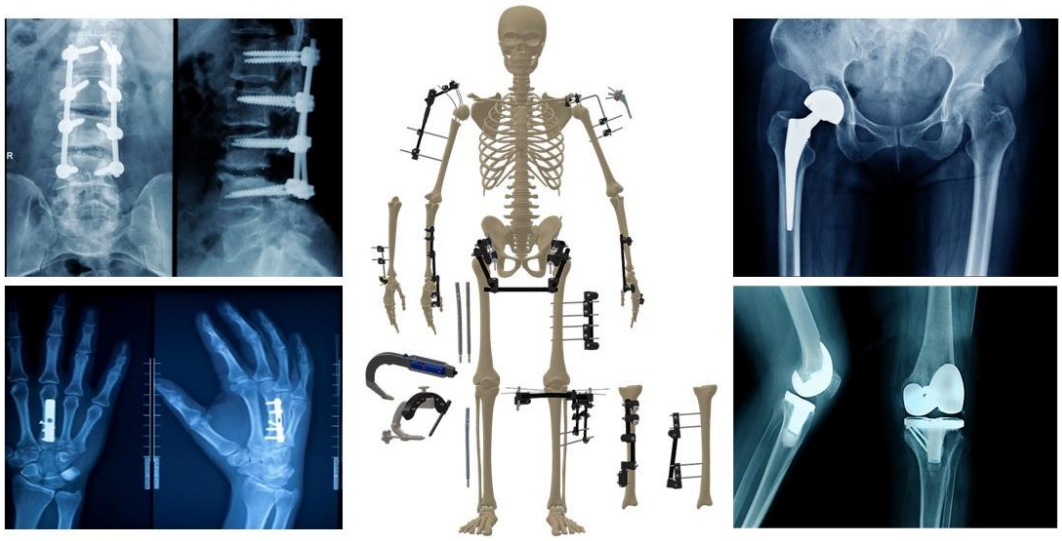

Figure 10: The variety of Orthopedic Implants in human body [239,240].

The global orthopedic implants market size was $46.5 Billion and is forecasted to reach to $64 Billion in 2026. The United States had almost half of the market share ($22.6 Billion) in 2018 and is expected to still dominate the global market share in this industry, due to the increasing incidence of osteoarthritis in the country. Recent exponential increase in technological innovations and extra government investments in the medical industry are anticipated to spur the orthopedic implants worldwide [241].

An ideal bone implant material should have osteoconductive, osteoinductive and osseointegration ability. Furthermore, other key criteria for implant performance include biocompatibility and mechanical compatibility. In addition, the implant waste after degradation should not cause harmful effects to the body. Recent trends in bone tissue engineering studies have revealed that bone implants may also serve as a drug delivery system if they are appropriately designed [242]. The expectation on future implants is long-term clinical performance with zero revision surgery. Revision surgery is undesirable due to health, social and economic implications, and is sometimes more complex than the primary surgery. The main reasons behind revision

surgery are poor osseointegration at the implant-bone interface, aseptic loosening and infections [243].

2.4.4. Orthopedic Implants Failure

Orthopedic implants are subjected to several interaction factors whenever they come in contact with physiologic systems such as blood, immune, musculosketal and nerves. The boy physiologic fluids contain several ions and bio-molecules, proteins and cells [244].

Implants can fail due to couple of reasons, including failure to integrate, implant fracture, implant malposition causing damage to vital structures (such as the inferior alveolar nerve, sinus membrane, a natural tooth, or an adjacent implant), and advanced loss of bone around an integrated, loaded implant, resulting in implant mobility and/or removal. Most clinicians simply classify implant failure into two categories: early failure (occurring before implant osseointegration) and late failure (occurring after implant integration and loading).Loosening and bacterial infection (or both) are the most common risks of implantation [245-248], moreover implants can also fail due to wear, fatigue, chemical degradation, corrosion [249].

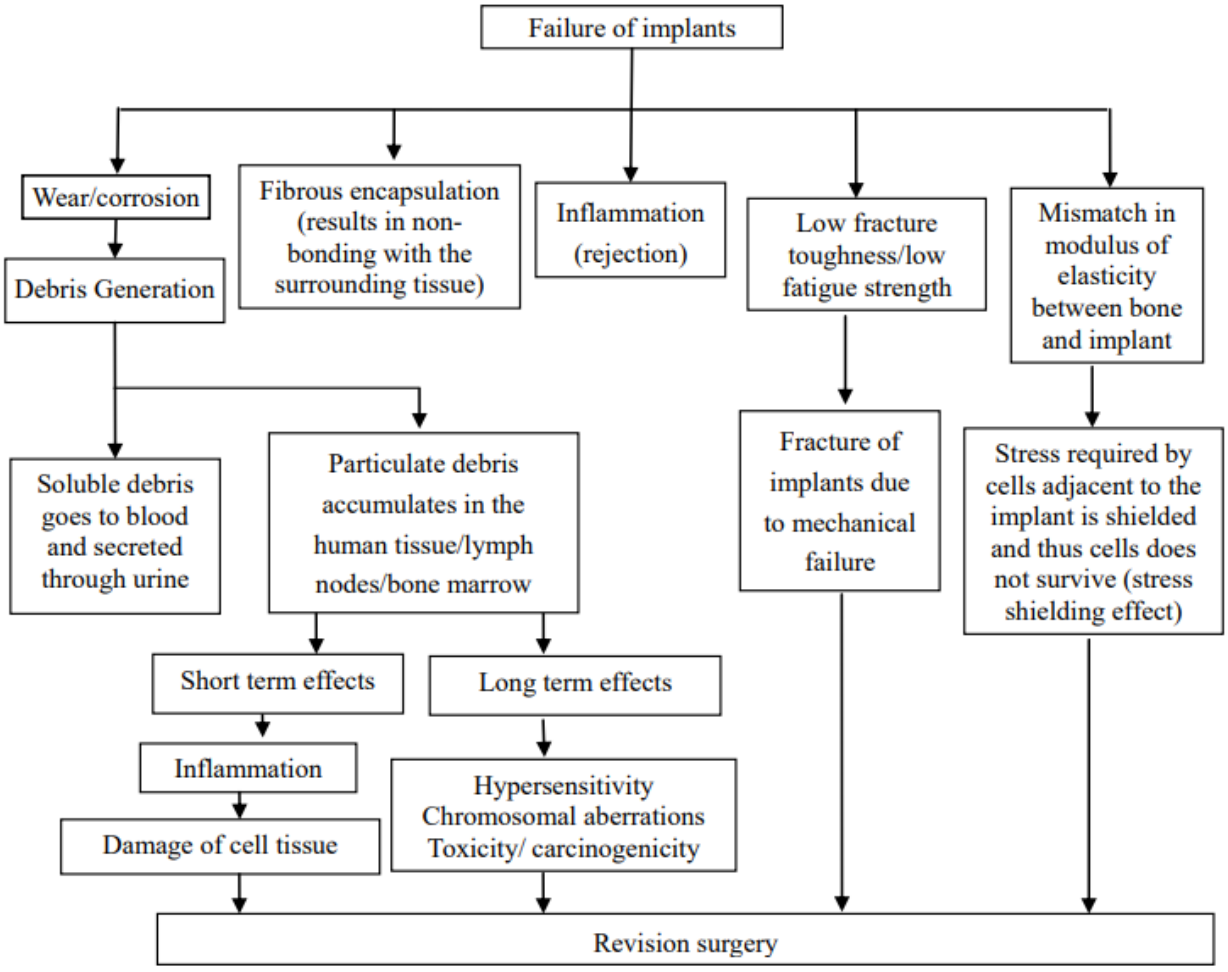

Figure 11: Different modes of failure in orthopedic implants [250].

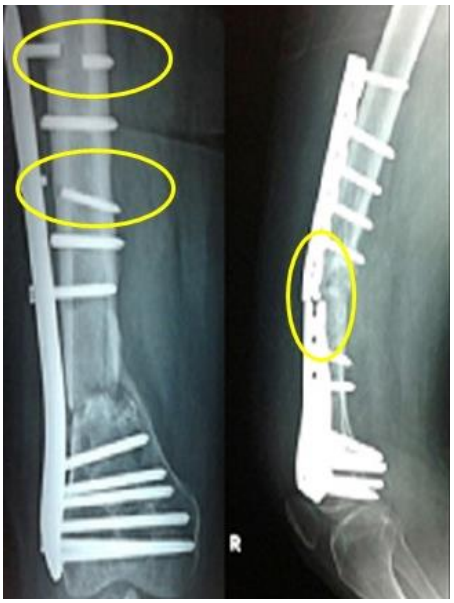

Figure 12: X-ray graph of Mechanical failure in orthopedic implant [251].

Overall, a leading cause of titanium-based implant failure is their loosening following implantation. Loosening may happen for several reasons, such as frequent displacements and movements, overload, contamination of the implant surface, or systematic problems interfering with osseointegration. Finally, lack of initial stability is also a source of early failure. This can be caused by placement of the implant in poor-quality bone or in insufficient natural bone. An implant must possess both structural and surface compatibility with the host tissue. With particular reference to bone implants, mechanical and physico-chemical compatibility is required [252, 253].

2.4.5. Biological Considerations

There are three levels which should be considered as dealing with the design considerations from biological approaches.

a. The quality of not having toxicity or injuries effects on biological systems.
b. The level of acceptance of the implant by the host tissue.
c. The ability to perform a specific function that stimulates an appropriate host response.

Beside these major considerations, there are other important factors in orthopedic implants which are beyond the scope of this work such as: age, sex, race, obesity, smoking and alcohol consumption, surgical indications, diabetes and metabolic syndrome [254].

The biological behavior of metallic implant surfaces is governed mainly by the interfacial kinetics associated with metal ion release and protein binding. The kinetics of metal release, and protein biding to metals (particularly Co-Cr-Mo and Ti implants) in human serum is well studied [63]. The nature of the interfacial kinetics, is a function of contact load and surface residual stress.

There are several methods have been proposed to control biological performance of orthopedic implants and increase biocompatibility. Li et al. [255] coated a thin Hydoxyapetite (HA) layer on a micro-arc oxidized titanium substrate by means of the sol-gel method. The HA sol was aged fully to obtain a stable and pure phase of HA, and the sol concentration was varied to alter the coating thickness. It was reported that (a) the micro arc oxidation increased the biocompatibility of the titanium, and the bioactivity was improved further by the sol-gel HA coating on the anodized Ti, and (b) the proliferation and alkaline phosphatase activity of the osteoblast-like cells on the microarc oxidized Ti/HA sol-gel treated Ti were greatly higher than those on the microarc oxidized Ti without the HA sol-gel treatment [255]. Schwarz et al. [256] evaluated the influence of plaque biofilm removal on the mitochondrial activity of human osteoblasts grown on titanium surfaces. Surface preparation techniques have been shown to have significant effect on the biocompatibility of the alloy [257-259]. The cytotoxicity and cytocompatibility of all treated surfaces were favorable compared to the untreated controls. Filip et al. [260] characterized the surface and the bulk structure of TiNi implants and concluded the surface passivation has a great effect on the degree of Ni loosening.

2.4.6. Mechanical Considerations

Aseptic loosening that may occur after long term implantation is mainly due to (i) the biomechanical mismatch of the implant and surrounding tissues, and (ii) fibrous tissue formation that leads to implant mobility. One way to overcome the biomechanical mismatch is to lower the elastic modulus by introducing porous structures into the biomaterials or by designing novel materials.

Orthopedic implants must function under biomechanical environments. If the implants and the receiving host bone are incompatible, the intended biofunctionality will not be achieved. In design of orthopedic implants, the engineers should consider physiological loads such as axial rotation, flexion extension, and lateral bending. In other words the implant should have sufficient structural integrity to endure the mentioned forces [261]. Figure 11 shows a linear relationship between strength (in terms of yield strength) and rigidity (in terms of Young modulus) among the various types of biomaterials , where **P**: polymeric materials, **B**: bone, **D**: dentin, **HSP**: high strength polymers (e.g., Kevlar), **E**: enamel, **TCP**: tricalcium phosphate, **HAP**: hydroxyapatite, **TI**: commercially pure titanium, **TA**: titanium alloys (e.g., Ti-6Al-4V), **S**: 304-series stainless steel, **PSZ**: partially stabilized zirconia, **A**: alumina, **CF**: carbon fiber.

Right after the implant installation into the body, the surface of implant exhibits solid osseointegration with surrounding tissue over the time. As the first stages of treatment passed, both implant and the bone are ready to be subjected to forces. When the combination of bone and implant as a one system, carry the applied load, even though the system deform elastically (strain continuity condition) each part will experience different stress levels, because each one possess a different Young's modulus (E). According to Figure 11, the Young modulus of E is approximately

15 GPa, while the Young's modulus of Titanium is around 150 GPa, the difference between on titanium (T) and Bone (B) will be expressed as

$$\Delta\sigma = \sigma_T - \sigma_B$$

This mechanical situation is explained as "discrete stress". When the stress difference happens ($\Delta\sigma$), happens to be bigger than interfacial bonding (or fused), the interfacial will be debonded and failure happens.

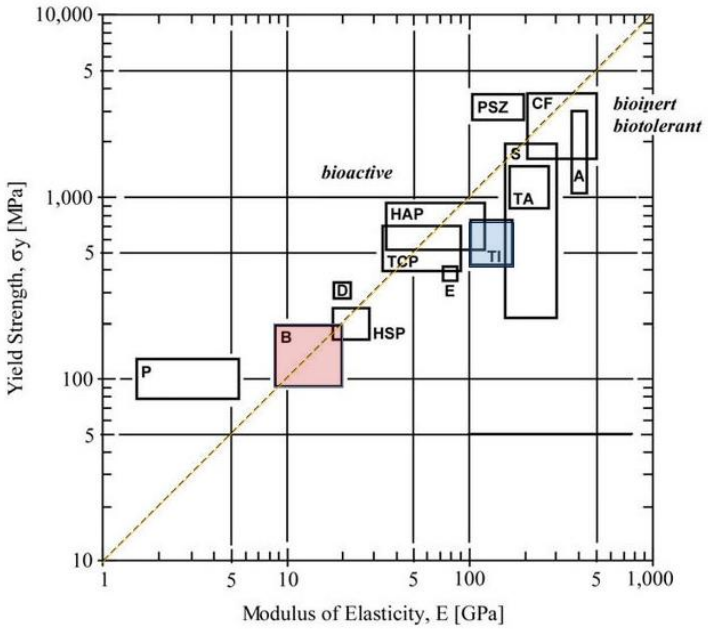

Figure 13: Relationship between yield strength and modulus of elasticity of various biomaterials [262].

To solve this mechanical issue, several research have been done and some solutions have been reported. For instance, Hydroxyapetite (HA) coating on orthopedic implants substrates will help and promote the primary stages of osseointegration, due to the similar chemistry to natural bone. Back to Figure 11, we will see that HA is located on the graph somewhere between Titanium and Bone materials. This indicates that HA can play a role of mechanical buffering function. Referring to Figure 11, it can be seen that ceramics (such as PSZ and Alumina) are located further

away Bone than Titanium. This means that potential interfacial interface stress ($\Delta\sigma$), could be much higher than $\Delta\sigma$ created between bone and Titanium implants. This is a crucial design consideration in case of working with ceramic implants.

As discussed one of the main factors that decreases the lifespan of load-bearing implants are the mismatch of Young's modulus and the weak interfacial bond between bone and metallic implants. Besides that, another restrictive factors on the longevity of metallic implants is wear-induced Ostelysis and aseptic loosening [263].

An innovative engineering solution to reduce the effect of Young's modulus mismatch and strengthen the interfacial bond, is using of porous metals or applying modified roughness on the surface of implants. These mentioned techniques, can effectively decrease the effect of Young's modulus mismatch and make pathways for bone in-growth into the pores for stable anchorage or biological fixation of the implant [263]. From mechanical engineering point of view, metals with high yield strength, low elastic modulus and less cytotoxic alloying elements, would be preferred materials for orthopedic implants. In this regard, one of the best candidates which has been developed so far, is Ti-13Nb-13Zr Due to its excellent mechanical properties and biological compatibilities [264]. One important parameter in the role of bone remodeling is the difference in Young's modulus between bone and its replacement [265]. Basically the larger the difference, the more rapidly bone resorption takes place. Ti is rapidly good in this regard because of its lower Young's modulus. Sutherland et al. [266], however, pointed out that this not true when the above mechanical environment is concerned. Medical grade titanium samples were examined using XPS before and after immersion in different types of proteins.

The surface layer of implants should be biomechanically compatible with the mechanical properties of vital soft/hard tissues. As it was discussed, coating of orthopedic and dental implants

with materials such as HAP is a very effective technique to satisfy biomechanical requirements. Asaoka and et al. [267] suggested another method. They prepared porous titanium samples made from titanium granular powder with diameter 420-500 microns, their results show that even though the mechanical properties decreased to some extent but a thick layer of ingrowth of tissues is formed.

There are different methods of initially fixation of implants to the bone, such as:

- Modifying the surface: Creating a texture to promote the fixation by mechanical interlocking on the macro or micro scale. Plasma-sprayed coating of metals or ceramics which increase the surface irregularity (roughness) is one example for this method.
- Using some type of bioactive biomaterials as the coating layer or bulk, to create strong adhesion or attachment between the bone and implant via chemical or physical bonding.
- Osseointegration concept, which includes both macro and micro interlocking [268-273].

2.4.7. Morphological Considerations

Surface has a crucial function in biological interactions for four reasons:

- Surface is the only part of an implant in direct touch with bio-environment.
- The surface of an implant is almost different with the rest of the bulk materials due to molecular rearrangement, surface reactions and contaminations.
- The surface characteristics of the implant govern the biological response.
- The surface topography, affect the stability of the implant/tissue interface [274-276]. It has been studied the rough-surface implants have significantly better functionality compared with smooth surfaces [277-280].

Bone implants are in close contact with cells for long periods of time, so they should possess biological characteristics in favorable of surrounding tissues. For example, they must

stimulate and facilitate the cell attachment because both connective and epithelial cells (with which implants are in touch) are anchorage-dependent, and therefore need a supportive surface which could promote attachment, division and differentiation of cells. It has been observed that surface preparation can significantly improve the biological behaviors of implants [281-283].

The results of several studies show that the success or failure of implants not only depend the chemical structure of the surface but also depend to the morphology such as surface roughness [284-287]. The analysis of clinical research on the performance of installed orthopedic implants show that regardless of material's type, if the surface roughness could be maintained in the range of 1 to 50 microns and average pore size of 10-500 microns, the success rate of implant in terms of bond strength and longevity is higher [288]. These results highlight the importance of morphological compatibilities [289]. In other worlds, the overall interactions between implant and cells, are governed by the surface morphology of implant, which can later guarantee the longevity and the rigidity of bone/implant bonding [290-293]. Xue et al. [294], by using Laser Engineered Net Shaping (LENS), successfully fabricated Ti samples with 17% up to 58% porosity, pore size up to 800 microns. Comparison between porous and polished titanium samples showed that cell spread more evenly, proliferated more and eventually created stronger bond with implant on the porous surface. Xue and his team concluded that minimum pore size of 200 microns, is critical to have enough ingrowth cells bonds. Other studies also have also shown that surface roughness can influence the mechanical properties of interlocking bonding and consequently the performance of the implant [295, 296]. Some researchers indicated that roughness in the range of 10 nm to 10 μm might influence the biological behavior of the implant due to the size similarity of roughness and cells [297]. Microroughness at this level includes materials defects (grain boundaries, steps, kinks and vacancies) that preferred sites for attracting biomolecules to the implant surface [298].

Roughened surfaces promote significantly better cell adhesion than smooth surfaces, which result in a higher percentage of bone in contact with the implant. The point is, even though roughness can positively influence the cell adhesion, mechanical properties might be negatively affected due to stress distribution [299-302].

2.5. The Concept of Interface

Nowadays, the concept of interface is mostly used for the components of a computing system, where the exchange of data happens. In chemistry, interface mostly refers to discontinuity between two systems such as the surface between different phases (or different matters). In chemistry, unlike the computing sciences, the concept of interface does not mean a place for exchanging the information, but it's a boundary. In biomedical engineering, the concept of interface falls somewhere in between two conditions explained above. The interface is the surface where the material comes into contact with the cells. Here, cells and the materials are two separate entities, however, unlike in the case of the chemical interface, there is a definitive exchange of information. The fact is, at the interface, the cell processes the signals from the material and decides how to interact with it according to its own physical, chemical, and biological properties [303]. The fundamental requirement of a biomaterial is that the material and the surrounding physiological environment should coexist without having any undesirable effect on one another. Because the surface is the interface where the biomaterials meet and interact with the biological environment (i.e., bone, soft tissue, blood), the surface properties are the major factors that ultimately determine the rejection or acceptance of a biomaterial in the body. The biological events that regulate host responses to materials such as protein adsorption and cell adhesion occur at the biomaterial-tissue interface and are modulated by the physicochemical properties of the materials [304].

Because the top layer of surface atoms is those atoms that are in immediate contact with the other phases (i.e., gas, liquid, or solid), this top layer of surface atoms could be regarded as the surface. On the other hand, the structure and chemistry of that top layer of atoms or molecules are significantly determined by the atoms or molecules immediately below. Atoms at the surface of metallic materials are considered relatively reactive to the environment because atomic configuration terminates at the surface. For example, in the presence of oxygen atoms, oxygen and metal atoms chemically bond together to form an oxide layer [305]. From biological standpoint, Cell/material interface is a self-explanatory which indicates how cells, predominantly human cells, interact with a surface designed for biomedical applications [306]. In research projects like this, which engineering concepts touch biological science, it is necessary to clarify what scale size we are talking about. As seen in Figure 12, at different scales, different interactions are happening. At each level, it requires an in depth understanding of the physical and chemical underlying principles of the cell/material interactions and the main governing parameters that decide the outcome [307-309].

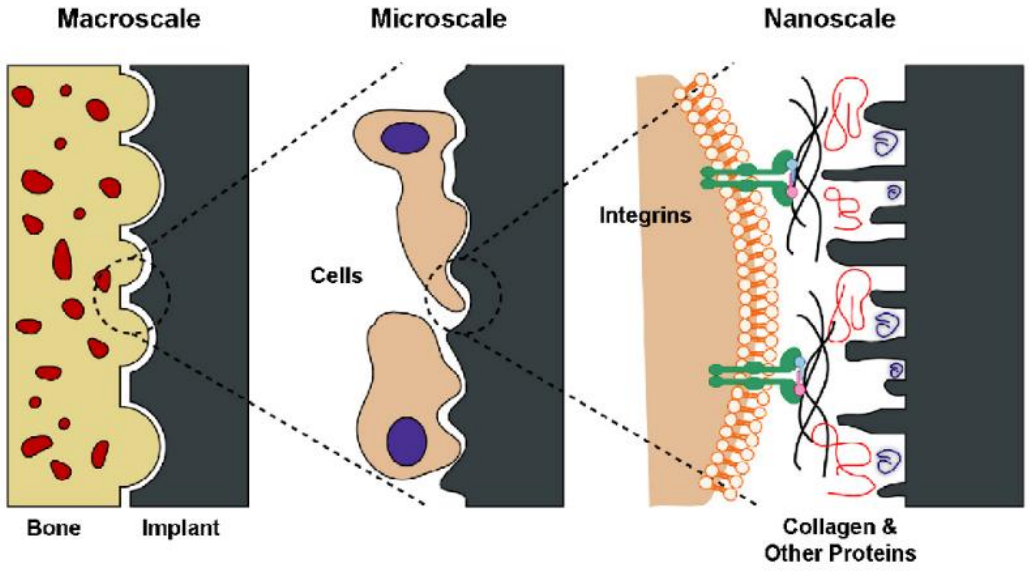

Figure 14: Illustration of bone-implant interface at macro, micro and nano scales [310].

2.5.1. Surface Characteristics of Metallic Biomaterials

Surface characteristics determines not only aesthetic appearance but also interaction with the environment, including mechanical interactions such as friction and wear and chemical interactions such as adherence and biocorrosion. The implant surfaces must have some specifications to be able to perform their required task in the body. Figure 13 shows some of basic topographical requirements of orthopedic implants. Each of these specifications will be discussed in the following.

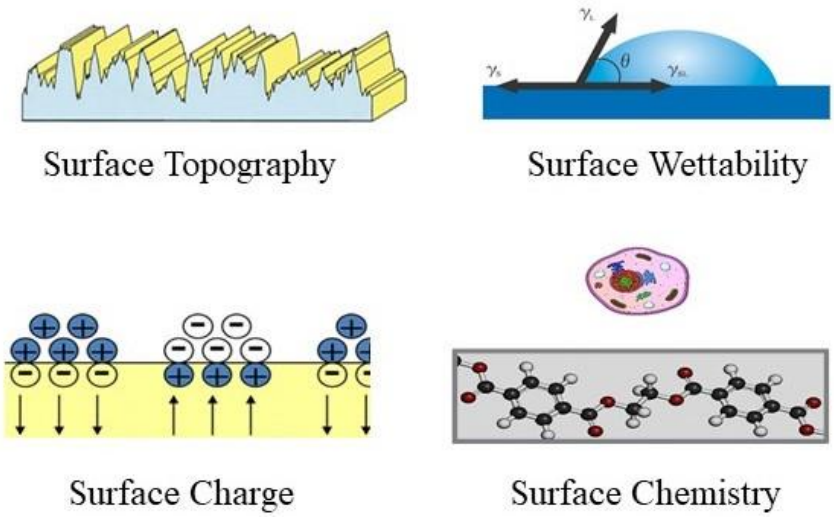

Figure 15: The variety of surface characteristics that influence the biological response to an implanted material.

2.5.1.1. Surface Chemistry

Surface chemical composition is an important surface characteristic in the design of biomaterials because it determines which functional groups are available for interaction with the biomolecules. Depending on the type of species available and its exposure, the biomolecules may have different affinities for various surfaces [311]. The most straightforward approach to modify the surface chemistry of an implant is the application of coatings. The surface chemistry can also be regulated by the deposition of another material with a suitable adhesion onto the implant. Such

coatings can be realized with organic or mineral layers. In titanium surfaces, the biological effects of surface chemistry are related mainly to the architecture of the titanium oxide (titania, TiO_2) layer [312]. The addition of ions such as calcium [313], sulfur [314], phosphorus [314], or magnesium [315] to the native titanium oxide layer through electrochemical oxidation methods has been used extensively to modify the surface chemistry of the metallic implants [312-315].

2.5.1.2. Surface Charge

Considering the role of electrostatic interactions in many biological events, the presence of surface charge at the solid solution interface is one of the major metallic properties that modulate both protein adsorption and cell adhesion [316,317]. Positively charged surfaces promote cell adhesion, while negatively charged surfaces reduce it [318]. It is notable that the cell adhesion on negatively charged surfaces becomes low or negligible at low ionic strength, when the range of electrostatic force is long. On the other hand, cell adhesion is strong at high ionic strength when electrostatic repulsion becomes negligible and van der Waals attraction dominates [319]. In another study investigating the adsorption of albumin onto metal oxides, it was reported that on positively charged metal oxides, i.e., TiO_2, ZrO_2, and Al_2O_3, the adsorption was dependent on the surface charge density [320]. The adsorption of albumin onto metal oxides showed an increasing trend from TiO_2 to ZrO_2 and finally to Al_2O_3. In general, proteins adsorb to positively charged surfaces and stimulate osteoblast attachment and spreading [321].

2.5.1.3. Surface Wettability

The surface of a material is a termination of an extended, three-dimensional structure and thus generally represents an increase in energy, i.e., the surface energy. On the atomic scale, this energy is present as unterminated chemical bonds. Because the bonding energy of the surface molecule is less than the bonding energy associated with a bulk phase molecule, the chemical

bonds will “dangle” into the space outside the solid material and will result in the surface atoms having higher energy than the atoms in the bulk. As a result, surface atoms will attempt to reduce free energy by rearranging and/or bonding to any available reactive molecules, such as air or water at a metal surface, to reach a more favorable energy state [322-324]. Therefore, the surface of a material usually has a different chemical composition than its bulk. Well-known examples are the presence of oxide layers on almost all metals and hydroxylated ceramic surfaces. If a surface is exposed to a new environment such as the tissue, it is likely to react again to further lower the energy of the system [325]. The term surface energy is also closely linked with wettability. Whereas surface energy describes interactions with a range of materials, surface wettability (i.e., hydrophilicity and hydrophobicity) describes these interactions with liquid only. The wettability is the extent to which a solid surface is wetted by a liquid, usually expressed as wetting angle [326]. Since liquids (e.g., water) have a huge capacity for bonding, a material of high surface energy can enter into more interactions with liquids and consequently will be more hydrophilic. Hydrophilic surfaces such as glass, therefore, have high surface energies, whereas hydrophobic surfaces such as polytetrafluoroethylene or polystyrene have low surface energies. Indeed, the hydrophilicity or hydrophobicity of a material surface is a result of its surface energy, which in turn is associated with the distribution of electrical charges. Concurrently, the distribution of electrical charges is a consequence of the surface chemistry and crystallinity, and all these properties are affected by the surface topography [327]. Contact angle measurements are probably the most frequently used technique to measure the average wettability of a surface. A zero degree (0°) wetting angle corresponds to complete wetting, while angles higher than 70° or 80° correspond to poor wetting [328]. For titanium implant surfaces, contact angle measurements give values ranging from 0° (hydrophilic) to 140° (hydrophobic) [329]. The wettability of a material’s

surface is vitally important for the adsorption of proteins and the cell adhesion. When an implant material is placed inside a human body, the wetting of the implant material is among the first and foremost plethora of events that take place by the physiological fluids. This further controls the adsorption of proteins, followed by attachment of cells to the implant surface. Hence, surface wettability is considered an important criterion that can dictate the biocompatibility of the implant material [330]. The three most important factors that affect the wettability of a surface are its chemical composition, microstructural topography, and surface charge [331]. Many studies mentioned that different cell behaviors, related to different surface wettability, may be mediated by protein absorption, because surface wettability modified the type and the quantity of adsorbed cell adhesion molecules. Low energy (hydrophobic) surfaces tend to adsorb more proteins, while high energy (hydrophilic) surfaces tend to resist protein adsorption [332-335]. Apart from various hydrophilic biomaterials that have been shown to reduce platelet adhesion and thrombus formation [336], as compared to more hydrophobic biomaterials, it has also been reported that very highly hydrophobic surfaces suppress protein and platelet adsorption and thus ensure good blood compatibility [337,338]. The reason for high affinity of protein adsorption on hydrophobic surfaces is not well investigated, but is believed to be related to lower water heat of adsorption on hydrophobic surfaces and increased unfolding of adsorbed proteins, due to interactions between internal hydrophobic protein domains and the hydrophobic surface [339]. These interactions lead to greater denaturation and increased internal protein entropy. In general, protein molecules change their conformations to a larger extent on hydrophobic surfaces than on hydrophilic surfaces. Proteins are generally structured with their hydrophobic residues buried within the core of the protein and their hydrophilic residues (charged and polar) lining the protein's solvent accessible surface. If the surface is hydrophobic, the protein will tend to adsorb by the various hydrophobic

patches of residues present on the protein's amphiphilic surface, with the protein then tending to unfold and spread its hydrophobic core over the surface. This is due to the thermodynamic driving force acting to reduce the net hydrophobic surface area of the system exposed to the solvent. On the other hand, hydrophilic surfaces tend to interact with the charged and polar functional groups of the protein's surface, thus influencing adsorbed protein orientation, but with a lower tendency to cause the protein to unfold and spread over the surface [340,341]. On the contrary, numerous studies have shown that a hydrophilic surface experiences a significant increase in cell attachment, spreading, proliferation, and differentiation [342-346]. For example, in an investigation on the relationship between surface characteristics of titanium and initial interactions of titanium-osteoblasts [347], reported greater numbers of adhered osteoblasts and higher cell activity on the surfaces with higher surface energy (hydrophilic) and surfaces with more hydroxyl groups. It has also been confirmed in another study that the differentiation of osteoblasts is higher on hydrophilic substrates (OH^- and NH_2- terminated self-assembled monolayers (SAMs)) than on hydrophobic substrates ($COOH^-$ and CH_3^- terminated SAMs) [348]. By using SAMs of alkanethiols on gold with different terminal functional groups (COOH, OH, and CH_3) [349], it was observed thicker fibrous capsules formed around the implanted surfaces coated with $CH3^-$ terminated SAMs compared to $COOH^-$ and OH^- terminated SAMs. Some in vitro studies have compared osteoblastic cell adhesion and proliferation on different biomaterials with almost the same finish treatment. These studies revealed better results on the more hydrophilic materials with a higher protein adsorption [350]. Taking these studies into account, it can be concluded that osteoblast cells generally adhere to and proliferate better on moderate to highly hydrophilic substrates, and this probably explains the higher adsorption of proteins observed, such as fibronectin [351]. Osteoblast adhesion was reported to decrease when the contact angle of surface increased from 0° to 106°.

Fibroblasts were found to have maximum adhesion when contact angles were between 60° and 80° [352,353]. Nebe et al. [354] compared the influence of roughness and surface energy of polymeric and metal surfaces on fibroblast adhesion and concluded that surface energy was more important than surface roughness in determining cell adhesion and proliferation. This finding suggests that the influence of roughness on cell adhesion strength may be secondary to the surface energy for high surface energy materials (i.e., metals). At the nanoscale, the surface energy can be increased by a more textured surface topography. One of the methods to obtain a high surface energy in metallic biomaterials is the refinement of coarse grains into the nanometer scale. The resulting nanograined materials are materials in which the atoms are clustered to such a degree that each grain consists of only few atoms with the grain size in the nanometer scale when compared to the conventional materials that have grain sizes in the micron scale. It is worth noting that despite the fact that nanograined materials have lesser number of atoms in each grain, the number of atoms on the surface is very high, and hence it possesses a large surface energy [355].

2.5.1.4. Surface Roughness and Topography

The topography of the implant surface is a key factor affecting cellular morphology, self-orientation, proliferation, and differentiation and has a significant influence on cell-cell signaling of implant-adherent cell [356]. Topographical modification of biomaterial surfaces is aimed at creating three-dimensional features in the form of micropores and nanopores, gratings, columns, microgroove, dots, pits, and random surface roughness. The surface topography is typically characterized by a succession of peaks and valleys, which can be quantified using either two dimensional profiles or three-dimensional parameters. All implant materials intended for bioactive applications must contain complex topographical features. This aspect is inspired by biological processes that occur naturally in bone remodeling. The resorption surface of old bone by

osteoclasts provides a highly topographically complex surface into which the new bone matrix will be deposited and subsequently becomes interdigitated and interlocked [357]. Similarly, rough, textured, and porous surfaces could stimulate cell attachment and formation of ECM [358].

Substrates with more topographical features will expose more surface area for possible interaction with proteins. For example, surfaces with grooves or pores have greater surface area compared with smooth surfaces. Other surface features, such as machine marks introduced during processing, provide additional sites for protein interaction [359]. Presence of surface roughness provides initial stabilization until bone can grow and attach to the implant surface to provide further improvement in implant bonding [360]. On the contrary, very smooth materials (i.e., endothelium-mimicking surfaces) are typically used in blood-contact applications [361]. Surface topography is such an important characteristic that one cannot expect bone bonding to occur to artificial biomaterials (even in the case of calcium phosphate biomaterials) without any surface topographical features [362]. Nevertheless, nonbonding materials can be rendered bone bonding by modifying their surface topography [363]. The role of a specific surface characteristic has to be investigated in the context of the other characteristics. For instance [364], it was observed lower cell viability on a smooth titanium substrate than on plastics, although both substrates have similar hydrophilicity and roughness. This lower cell viability is ascribed to a different surface composition. The role of surface topography versus surface chemistry in controlling biological responses is still under debate. While chemical hypotheses have generally been adopted in the open literature to explain bone bonding, there are experimental evidences demonstrating that bonding is achieved by micro-mechanical interdigitating of the highly conserved extracellular interfacial matrix, known as cement line, with the highly topographically complex surface at the submicron level [365-367]. Depending on the scale of irregularities of the material surface, surface

roughness can be categorized into three main groups—macroroughness (100 mmemilimeters), microroughness (100 nme100 mm), and nanoroughness (<100 nm)—each with its specific influence on cell response [368]. The macroroughness scale is directly related to implant geometry, with threaded screw and macroporous surface treatments giving surface roughness of more than 10 mm. The microtopographic maximizes the interlocking between mineralized bone and the surface of the implant [369]. Surface profiles in the nanometer range play an important role in the adsorption of proteins, adhesion of osteoblastic cells, and thus the rate of osseointegration [370]. To meet the requirements for an enhanced bone implant contact formation, various methods have been developed to create micro- and nano-featured surfaces. Some of these methods include blasting, acid-etching, anodization, and plasma-spraying [371,372]. The response of cells to microtopographic or nanotopographic features depends mainly on the type and size of the cell. For larger cells, such as osteoblasts and neurons, macroscopic descriptions of the surface roughness could be reasonable [373]. It has also been demonstrated that metal surfaces with low microscale to nanophase topography enhance adhesion of osteoblasts [374,375] investigated the enhanced functions of osteoblasts on nanophase metals and indicated that more calcium and phosphorus deposition is observed on the surface of nanophase metals. Because more particle boundaries are present on the surface of nanophase compared to conventional metals, this may be an explanation for the measured increase in osteoblast adhesion. For smaller cells, such as human vein endothelial cells, surface roughness at nanometer scale (100 nm) could enhance cell adhesion and growth [376]. However, because proteins are entities smaller than cells, microscale topographic structures are too large and cannot be sensed by proteins. Thus, proteins can be stimulated by using smaller features, in this case, nanotopographic features are closer to the dimension scale of proteins and adjust better to their size [377]. Cells commonly show different shapes when cultured on substrates

with different roughness and topographic features [378]. When cells are cultured on an anisotropic topography such as a groove, cells orientate themselves along the axis of the groove to find a more suitable place for their further development. This phenomenon is called “contact guidance” and has been only observed in vitro [379]. When the surface is polished in only one direction, the edges can give a clear orientation to the cells like moving guides [380]. When the surface consists of an organized array of pillars, the cells are able to identify this organization and orient themselves in the orthogonal direction [381]. It was shown that osteoblast cells also react by changing their morphology to suit the environment that they grow in. Osteoblasts maintain a rounded shape without extensions when they are on a flat titanium surface, whereas the titanium nanotube array causes the cells to become elongated, showing an increased number of extensions and filopodia [382]. However, despite the effect of surface anisotropic or isotropic features on cell shape and orientation, there is no considerable difference in bone bonding and osseointegration between these two features [383]. It was considered isotropic and anisotropic implant titanium surfaces with similar roughness levels, reported that titanium implants prepared with isotropic and anisotropic surfaces of similar roughness integrate similarly to bone during the three months after implantation. Nevertheless, it is difficult to define the topographic features for implant surfaces prepared by classical processes, such as plasma spraying or sandblasting, that display rather isotropic surfaces [384]. Human mesenchymal stem cells (hMSCs) were shown to differentiate better on surfaces presenting slight disorder of 120-nm-diameter nanopits (displaced square placements) compared to a hexagonal, square, or even random placement [385].

Last but not least, cells may also sense the viscoelastic properties of substrata. Soft, flexible substrata may inhibit attachment and spreading, while stiff, solid surfaces may promote it [386]. Indeed, altering the surface topography will concomitantly lead to the modification of the surface

chemistry [387]. One possibility to overcome this difficulty is to process model materials, i.e., materials with homogenous composition (not alloyed), and use a process that does not apply too strong an amount of energy. The approaches based, for example, on nano- and micro-patterning are effectively very useful for understanding the mechanisms of cell response to topography, but they produce surface morphology and surface chemistry very different from what is observed on a real implant surface [388]. Another possibility to discriminate the relative influence of topography and chemistry is to cover the surface with a thin layer of another material to control the surface chemistry, but without changing the surface topography (at least at the micrometer scale) [389].The surface topography can be altered by creating porous structures throughout the bulk or only on the surface (e.g., porous coatings) of the metallic biomaterials. As a result, the porous implant material can be strongly bonded to bone by infiltrating the bone into the interconnected pores. The bone ingrowth into porous surface can cause strong interlocking between the porous implant and the surrounding bone tissue, providing greater mechanical stability at this critical interface. The porous biomaterials also possess a low modulus of elasticity, which minimize or eliminate the stress shielding effect, preventing loosening of the implants [390]. The detailed studies made by several authors lead us to conclude that the size of the pores should be in the range of 200-500 mm for better osseointegration [391,392].

2.5.2. Biomedical Surface Modification

Depending on a specific application, a suitable surface modification method may result in an increase or reduction of protein adsorption, control of cell adhesion, growth and differentiation, modulation of fibrous encapsulation, etc. Surface treatment of biomaterials offers the ability to improve material and biological responses through changes in a material's surface chemistry,

topography, energy, and charge, while still maintaining the bulk properties of the implant. Surface modifications can broadly be classified into three categories [393]:

a. Addition of materials of desirable functions to the surface;
b. Conversion of the existing surface into more desirable compositions and/or topographies.
c. Removal of material from the existing surface to create specific topographies

There are several parameters that can dictate how cells behave against the inserted biomaterial into the body. These parameters include: physical and chemical properties of the material, including wettability, surface topography, functional groups, pH and electrical charge, among other factors. One of the biggest challenges in the determination of the effects of interfacial properties is the high level of interdependence of several properties with each other. Chemical composition of the surface directly affects the hydrophilicity and protein absorption properties. Similarly surface roughness has a direct effect on the protein absorption and hydrophilicity [394-397]. Various modifications of the implant surface can alter the percentage of Osseointegration. New types of reinforcements for implants and the use of growth factors to augment bone regeneration have been developed [398]. As mentioned before, osseointegrations a major factor influencing the success of implants. As it can be seen in Figure 14, to achieve strong and durable bonding between implant and bone, we need to focus on parameters that can stimulate osseointegration, such as topographical parameters.

Figure 16: Long-term and reliable functionality of orthopedic implants on proper bone-implant integration [399].

Current implants research has studied the interaction between bone and implant surfaces in order to understand and improve the Osseointegration process. The implant surface treatment, chemical or topographic modifications, and cellular interactions can affect bone healing, promote accelerated osteogenesis, and increase bone-implant contact and bone strength. As it can be seen in the following table, there several methods of surface modification:

Table 3: Biomedical surface modification

Mechanical	Chemical	Physical
Grinding	Cid/Alkali Treatment	Thermal Spraying
Polishing	Sol-Gel Coatings	Physical Vapor Deposition
Blasting	Anodic Oxidation	Plasma Treatment
Machining	Chemical Vapor Deposition	Ion Implantation
	Biochemical Surface Modification	

Coatings, claddings, thin films, and other surface modifications may be categorized in many ways. One of these is principally by the form of the material deposited at any given instant on a small area of the surface. Such forms include atoms or molecules, particles, bulk, and others. Atomic deposition include electroplating, CVD (chemical vapor deposition), PVD (physical vapor deposition), ion implantation, vacuum evaporation, molecular beam epitaxy, plasma and spray pyrolysis. Particulate deposition, includes thermal spray, flame spray, high velocity oxyfuel spray, impact packing, fusion coating, enameling, and electrophoresis. Bulk coating/cladding should include wetting processes such as painting and dip coating, electrostatic spaying such as spin coating, laser cladding, and high temperature synthesis. In addition to these, shot peening and laser peening can included as mechanical modification [400,401]. Surface transformations (such as ceramization, nitrization, etc.) can be performed on metals in order to combine their load-bearing properties to the inertness and wear resistance of ceramics. In a joint prosthesis, metals are useful

for their high fatigue strength and ductility, but they are more sensitive to superficial corrosion and wear than ceramics. Coating a ceramic on metal surface will improve the qualities of the metallic component [402].

With surface modification, chemical and mechanical durability and tissue compatibility of a surface layer would be improved. From an economical point of view, surface modification is considered an inexpensive process because only the surface layer needs to be modified. In other words, with surface modification, the key physical properties of a biomaterial can be retained while only the outermost surface is modified to tailor to the biointeractions. The chief purpose of surface modification is to improve corrosion resistance, wear resistance, antibacterial property, bioadhesion (bone ingrowth), and biocompatibility, while other important requirements such as adequate mechanical strength and processability are governed by the bulk material properties. Surface modifications should provide distinct properties of interaction with biomolecules or cells of the biological environment. These would promote, for example, the adaptation or in growth of cells onto the surface of fixation elements of artificial joints, or prevention of cellular interaction with the surface to inhibit endothelial cell proliferation to provide cardiovascular devices with a suitable blood compatible surface [403]. There is no universal technique for the surface modification that can be applied to all biomaterials, and variations exist depending on the application and the type of materials. For instance, in bone implant materials, rapid bone conductivity is required; materials of cardiac stents have to be structured to avoid cell proliferation provoking restenosis; in cardiovascular devices, blood compatibility or anti-thrombogenecity is required; in dental implants, soft-tissue compatibility is required to prevent bacteria invasion from the crevice, which is between the dental implant and gingival epithelium. The choice of a suitable method is dependent on many factors, including the substrate material, component design and

geometry, cost, and the end applications in which two aspects of the surface engineering process, coating thickness and process temperature, are often highlighted [404].

CHAPTER 3. EXPERIMENTAL PROCEDURES

3.1. Material

One novelty of this research is the material, Ti13Nb13Zr as the newest member of the titanium-alloy family was studied in this research. The reason for selecting this material backs to the fact that the American Food and Drug Administration (FDA) raised concerns about the toxic effects of the dominant material in the market, Ti6Al4V. Recently it has been found Ti6Al4V releases aluminum and vanadium ions in the body, causing long-term health problems such as peripheral neuropathy, Osteomalacia and, Alzheimer diseases [405,406]. Currently, orthopedic implant manufactures are searching for materials to substitute Ti6Al4V. In this regard, Ti13Nb13Zr is considered one of the main candidates to be used for future orthopedic and dental applications. Besides better compatibility, Ti13Nb13Zr has a lower Young's modulus (E=75 GPa) than Ti6Al4V (E=114 GPa) which is a big advantage for orthopedic applications. The Ti13Zr13Nb alloy (Xi'an SAITE Metal Materials Development Co., Ltd., Xi'an, China) with the chemical composition of Ti13Nb13Zr is13.0 wt.%Nb, 13.0 wt.%Zr, 0.086 wt.%O, 0.009 wt.%N, 0.0012 wt.%H, and balance Ti.

3.2. Sample Preparation

In order to create desired surface roughness on Ti13Nb13Zr samples, they were subjected to different surface finishing techniques as shown in Table 4. Once the desired surface roughness was achieved, the samples were cleaned with ethanol and distilled water to remove any particulates. One of the common problems in performing experiments associated with surface roughness is creating relatively accurate range of roughness. In fact, researchers such as Dr. Seewig at Technical University of Kaiserslautern [407], believe that some extent of discrepancies in roughness values are inevitable. This problem was observed in this study too. By reviewing

some similar works and related technical reports, it was found that in order to create a particular roughness, the surface finishing parameters should be found experimentally.

Table 4: Different techniques of surface modification

Surface Finishing	Technique	Principle
Mechanical	Machining	Mechanical friction
	Grinding & polishing	
	Sandblasting	Projection micro-particles onto substrate
Chemical	Acid etching	Chemical corrosion.

3.2.1. Machining

Machining is one of the common techniques to create roughness on the surface of metals. The main trend in many industrial applications is reaching smooth surfaces. In this regard, the machining parameters will be set to reach the smooth surfaces, however it is possible to intentionally set the parameters to reach desired roughness. In machining process, the resultant surface roughness strongly depends on several parameters, but the major parameters are the feed rate of the specimen and the speed of the machine's spindle as shown in Figure 17. It is observed that the surface roughness increases with increased feed rate and lower speed. Table 5 shows the machining parameters and the resultant surface roughness in this study.

Figure 17: ACER milling machine is grinding the samples.

Table 5: Machining parameters and the resultant surface roughness.

Resultant Roughness (µm)		Spindle Speed (rpm)			
		150	200	250	350
Feed Rate (mm/rev)	0.45	75±3.6	63±2.5	60±2.5	47±2.2
	0.3	71±3.2	60±2.5	54±2.2	40±2.2
	0.25	65±2.5	58±2.5	52±2.0	36±2.0
	0.2	61±2.2	58±2.5	49±2.0	35±2.0

3.2.2. Sandblasting

Sandblasting or grit blasting is one of the typical physical surface modification techniques that force abrasive particles (i.e., sand, alumina, hydroxyapatite, TiO_2) against the surface through a pressurized projection by means of compressed air in a closed chamber as seen in Figure 18. Due to simplicity and cost efficiency, this method is a very common and popular method in orthopedic industry. Factors that need to be taken into consideration for sandblasting include particle size, exposure time, nozzle size, working distance, blasting pressure and blasting angle. Several

combinations of parameters as seen in Table 6, were performed on samples and a wide range of roughness between 10 to 50 microns were created.

Figure 18: TRINCO sandblasting machine.

Table 6: The parameters of sandblasting were used in this research.

Particle size (grits)	Exposure time (min)	Blasting pressure (psi)	Blasting angle (°)	Working distance (cm)
280	1.5	40	60	10
100	2	60	90	20
60	3	100		
54	5			
30	8			

3.2.3. Grinding and Polishing

Samples polished with silicon carbide abrasive papers with grit numbers 24, 50, 150, 180, 400, 600, 800, and 1200. The speed of the machine was 100 to 200 rpm. The samples were then cleaned with ethanol and distilled water for approximately 2 minutes. A range of surface roughness numbers between 5 to 30 microns was created by this technique.

Figure 19: ALLIED METPREP polishing machine and sand papers with different grit numbers.

3.2.4. Acid-Etching

Acid-etching is a procedure of roughening titanium implant surfaces with strong acid solutions including hydrochloric acid (HCl), nitric acid (HNO_3), sulfuric acid (H_2SO_4), hydrofluoric acid (HF), and other combined acid solutions. As it can be seen in Figure 18, this method can be done by immersion at the acid solution in different exposure times. Acid-etching usually fabricates the structure of the micro-pits with pit sizes in the range of 0.5–2 μm [408,409]. In the experiment, a solution of HCl/H_2SO_4 was prepared. The etching solution was used at room temperature under the hood for different exposure times from 30 seconds up to 5 minutes and surface roughness between 3 to 8 micron created. Roughness values increased with the increase of the etching time.

Figure 20: Acid etching

3.2.5. Quality Control

In order to make sure that after each surface processing, the desired roughness is achieved, a sequential quality control (QC) was performed as shown in Figure 21. In fact, the samples were examined with Hirox® profilometer microscope (Figure 22). For each sample, 10 line profile scans were taken (each) in the longitudinal and transverse directions to produce an average Ra (mean surface profile roughness). The line profile scans were 4 mm in length and complied with ISO 4288-1996. Overall, fifty acceptable samples in ten different surface roughness values fabricated for the study as shown in Table 7.

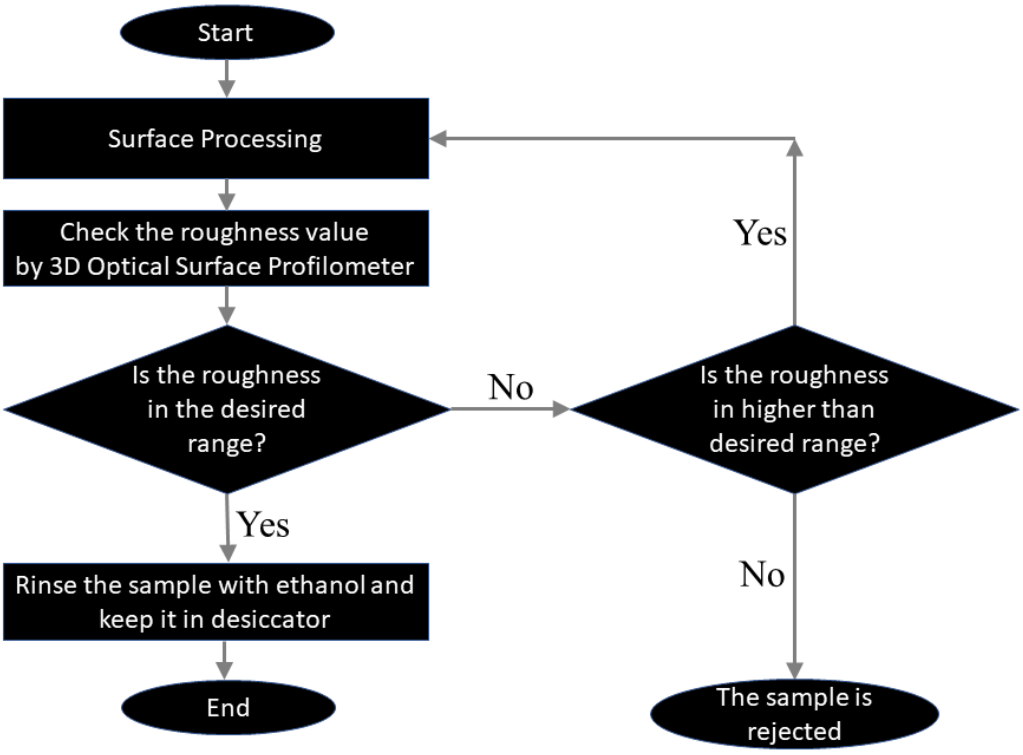

Figure 21: The procedure of controlling the roughness of the samples.

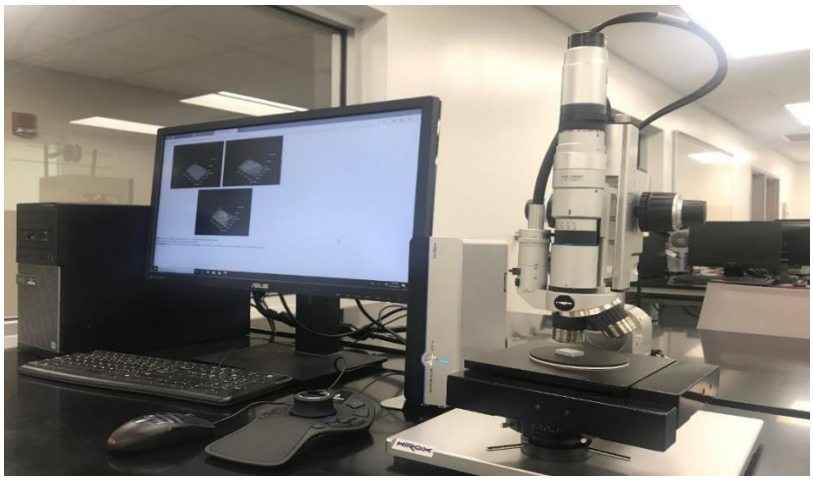

Figure 22: Hirox® profilometer microscope

At least fifty samples with ten different surface roughness values (five samples for each group) were fabricated as shown in Table 7.

Table 7: Samples with different surface roughness.

Group No.	Surface Roughness (μm)	Surface Roughening Technique(s)
1	<5	Acid etching, Smooth polishing
2	7-10±2.5	Grinding and polishing
3	13-18±3	Grinding and polishing
4	22-27±3	Grinding and Sandblasting
5	31-36±3	Grinding and Sandblasting
6	42-47±5.5	Grinding and Sandblasting
7	51-57±5.2	Coarse Grinding
8	63-66±7.1	Coarse Grinding and Machining
9	>70	Machining

CHAPTER 4. EXPERIMENTAL PROCEDURE AND RESULTS

4.1. Bending Test

Orthopedic implants as foreign materials within the human body which are supposed to endure the different types of mechanical conditions in the corrosive environment of the body in a long term. Currently, over 80% of all orthopedic prosthesis are made from metals [410]. Similar to all metallic parts, the metallic orthopedic implants are susceptible to different types of mechanical failures such as initiation and propagation of cracks, fatigue, deformation, wear, and fracture. Since orthopedic implants in the body will be subjected to different static and dynamic load conditions, their ductility and resistance to deformation and crack initiation, is the most crucial mechanical consideration in design. Bending is the most important mechanical test of metallic implants. Bending characteristics of the implant play a key role in evaluation of implants in both the short and long terms.

- Since implants are responsible to provide support and fixation to the damaged bones or joints, any poor behavior in terms of bending strength, can cause catastrophic consequences for the patient.
- Bending is a very sensitive test to the surface topography of the sample such as roughness [411]. Since all orthopedic implants have roughness to some extent, this test is very common in this field.
- During the bending test, multiple force conditions including compression, tension and shear are active. For this reason, the bending test is considered one of the important mechanical testing methods, which can evaluate the mechanical response of a specimen to the overall combination of forces. This is very similar to the force conditions of the implants in real-world scenario.

4.1.1. Theory of Bending Test

Bending tests are conducted by placing a length of material across a span and pushing down along the span to bend the material until failure. Bending tests reveal the elastic modulus of bending, flexural stress, and flexural strain of a material. 3-Point bending involves placing the material across a span supported on either end of the material and bringing down a point source to the center of the span and bending the material until failure while recording applied force and crosshead displacement (Figure 23). Hard materials like bone and implants can be tested using bending tests as a measure of the tissue or material in both tension (the bottom of the sample as it is tested) and compression (the top of the material as it is tested).

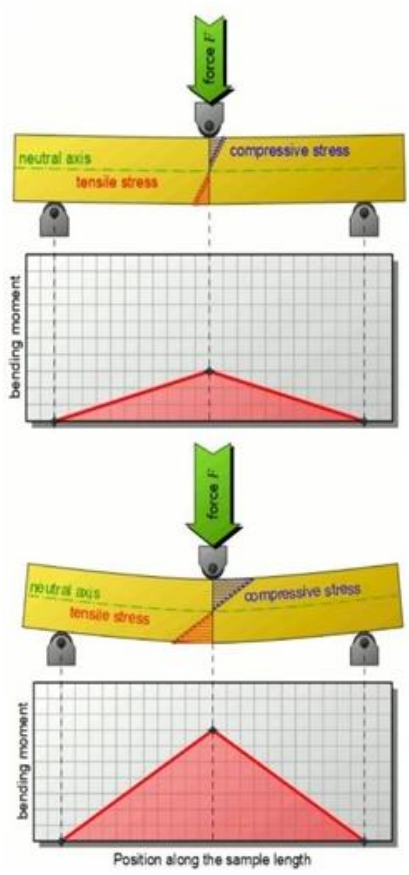

Figure 23: Schematic illustration on 3-point bending test (flexural test) [412].

As seen in Figure 24 The stress values are highest at the surface layer of the material due to the maximum compression or strain.

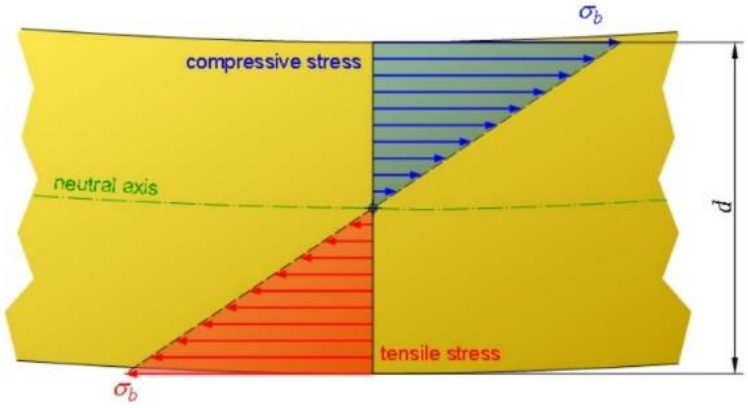

Figure 24: Maximum tension and compression stress are active on upper and lower surfaces respectively [412].

Typically, universal testing machines are used to perform the bending tests and the test variables such as specimen dimensions (mostly rectangular and cylindrical shapes) and test speed are determined by ASTM or ISO standards. Different types of rigid materials such metals, ceramics, plastics and wood can be tested. This can be achieved by managing the span to depth ratio (S/d). For most materials, the acceptable ratio is 16 and for some materials this ratio is 32 to 64 in order to minimize the shear stress. Maximum stress and maximum strain are determined for load increments and the results will be plotted on a stress-strain diagram.

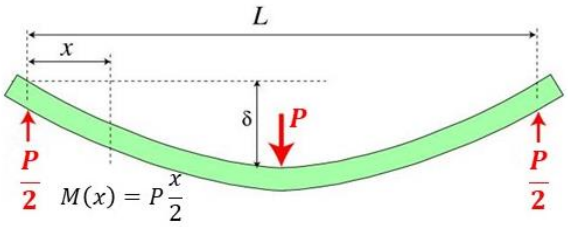

Figure 25: The maximum deflection occurs in middle of beam.

By using differential equation, deflection of the sample under three point bending test can be calculated by two time integration of the moment $M(x)$

$$y(x) = \frac{1}{EI} \iint M(x)\, dx \qquad \text{(Eq.1)}$$

By applying boundary conditions and differentiation, the deflection of the sample would be:

$$y(x)\,.\; EI = \frac{P\,x^3}{12} - \frac{P\,L^2}{16}x \qquad \text{(Eq.2)}$$

And maximum deflection in the middle of the span:

$$y_{max} = -\frac{1}{48EI}\,\frac{P}{L^3} \qquad \text{(Eq.3)}$$

4.1.2. Bending Experiment

The test was performed based on ASTM E290 [413], using INSTRON 5567 Universal testing machine as seen Figure 26. The results for all nine groups of samples are presented in Figures 27 to 35.

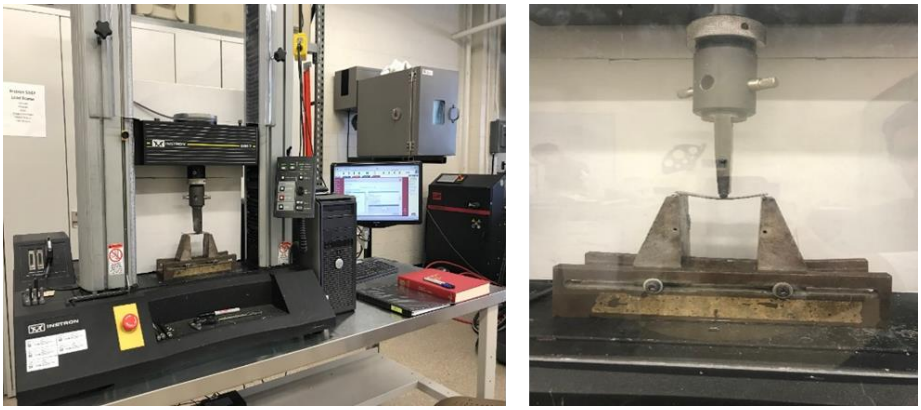

Figure 26: The instrument and set up of bending test.

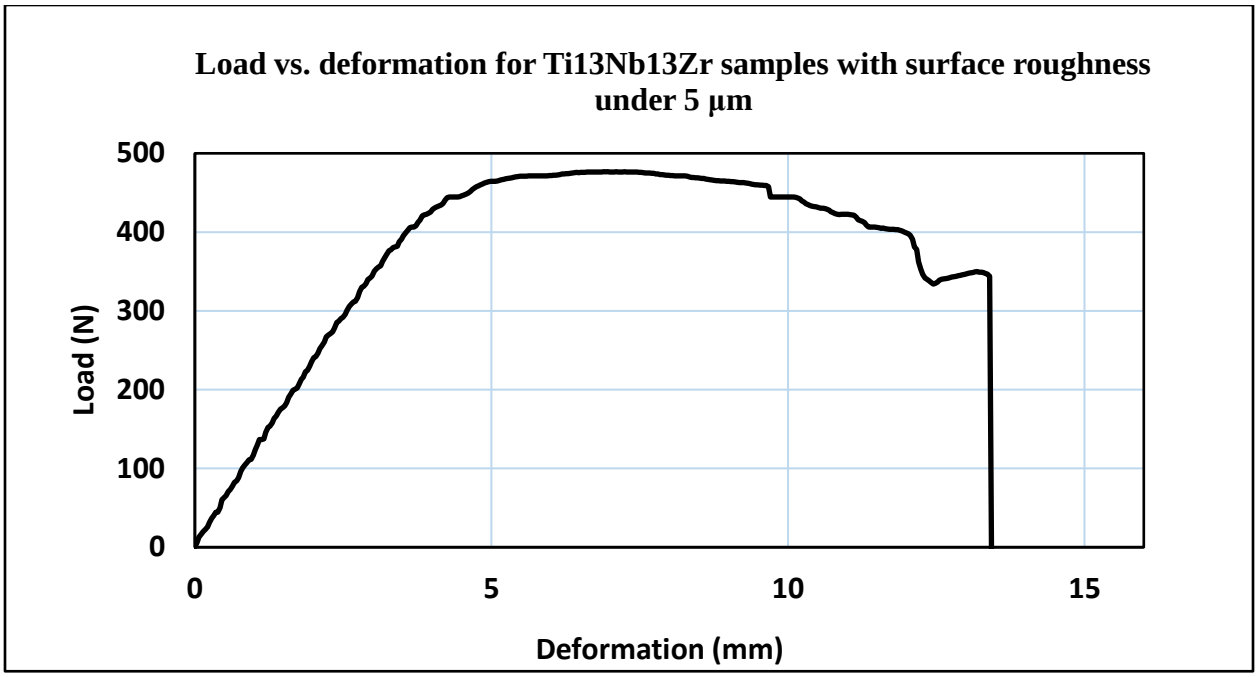

Figure 27: Bending test results for samples with surface roughness under 5µm (Group No.1).

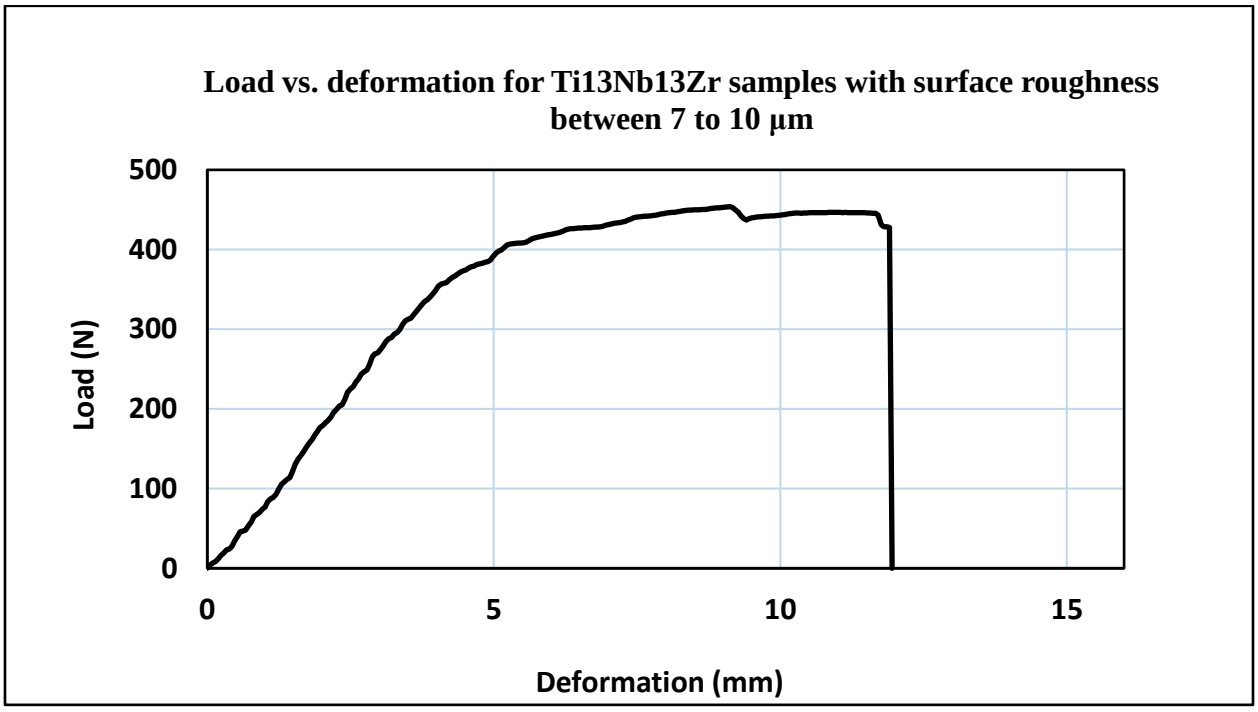

Figure 28: Bending test results for samples with surface roughness between 7 to 10µm (Group No.2).

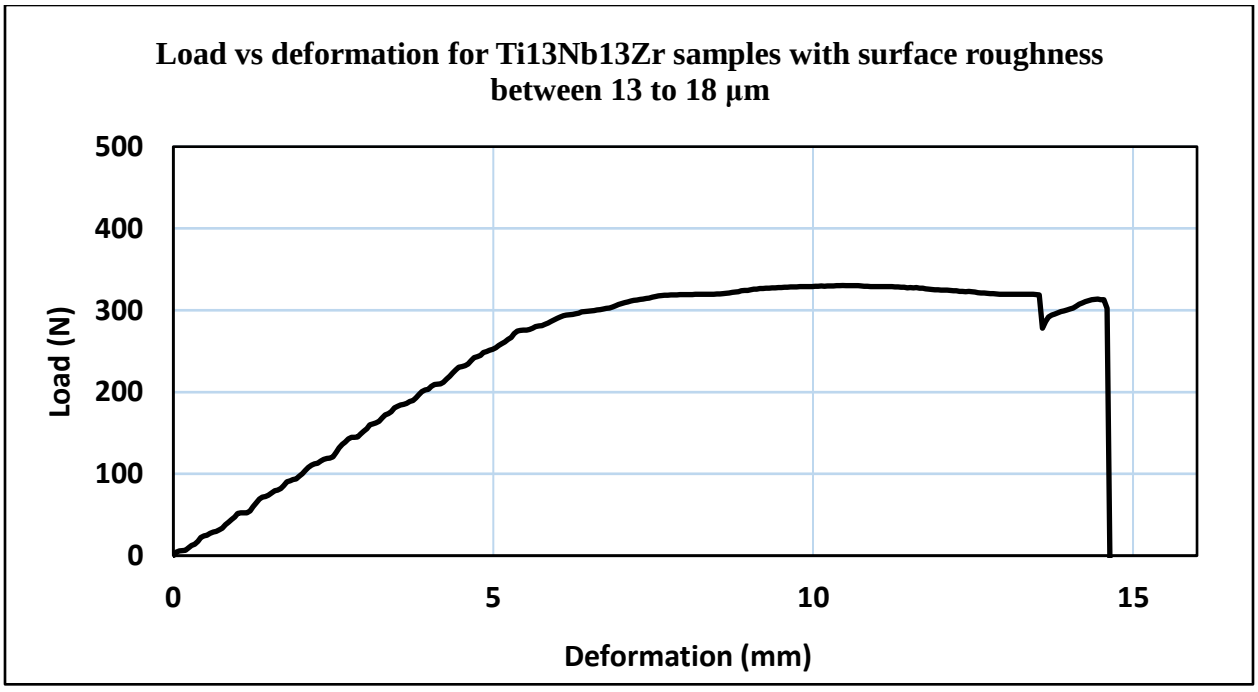

Figure 29: Bending test results for samples with surface roughness between 13 to 18µm (Group No.3).

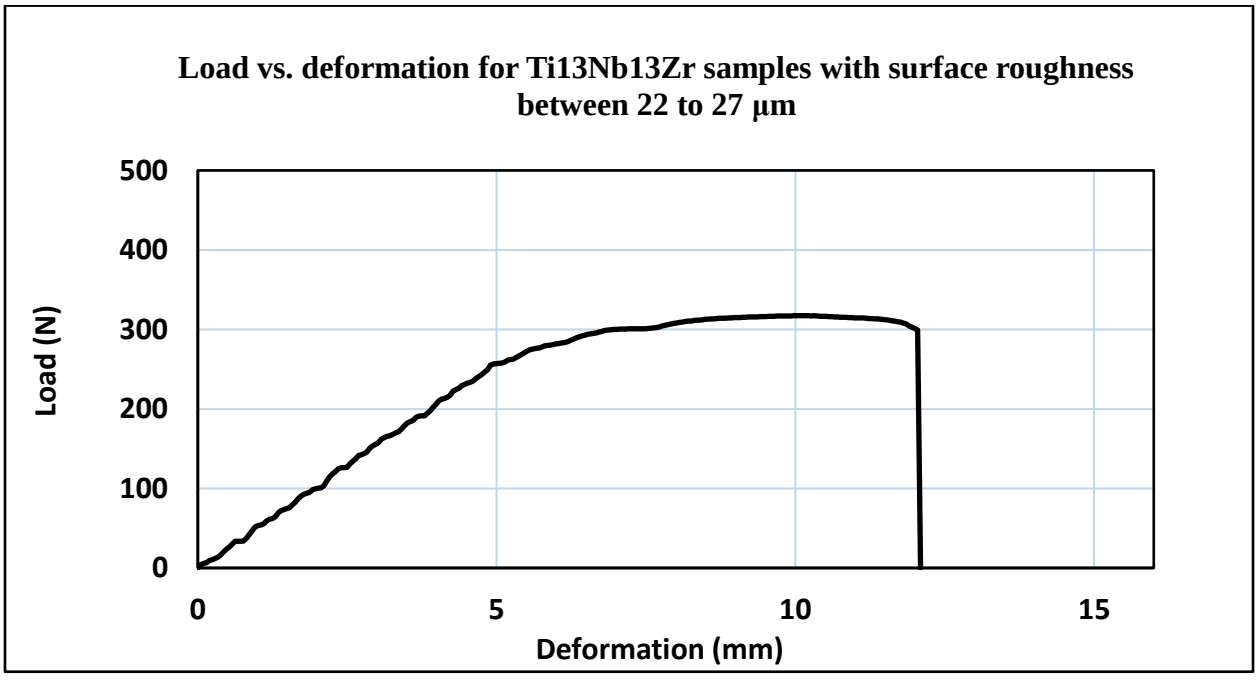

Figure 30: Bending test results for samples with surface roughness between 22 to 27µm (Group No.4).

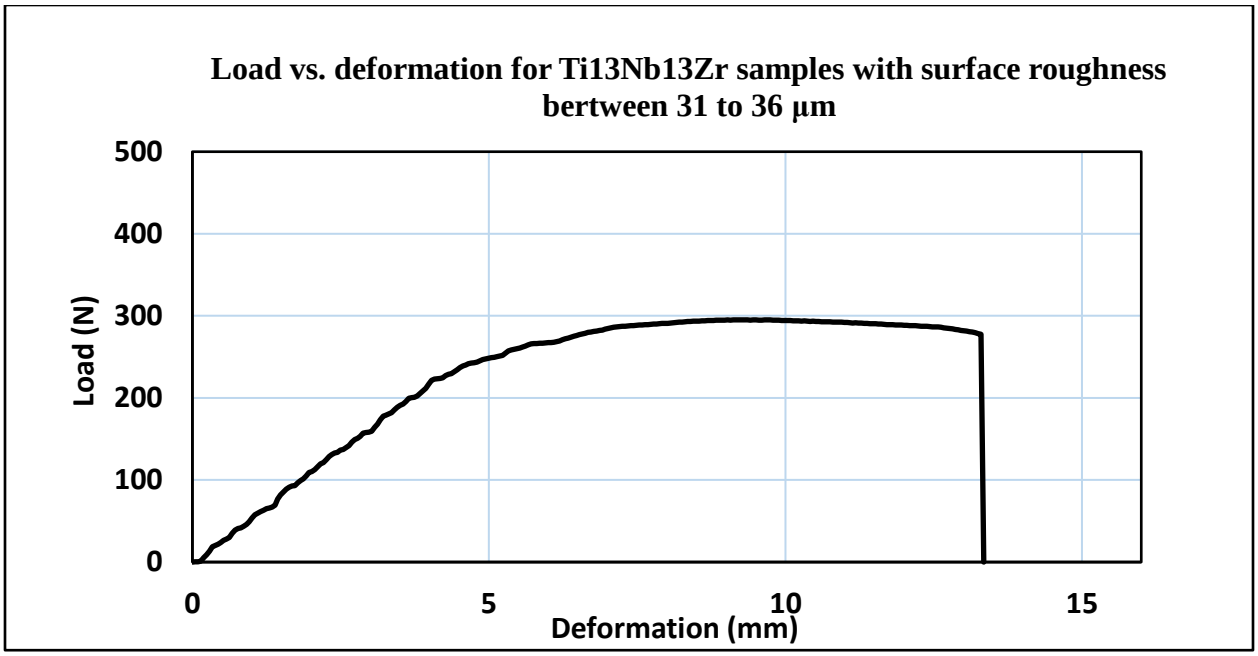

Figure 31: Bending test results for samples with surface roughness between 31 to 36µm (Group No.5).

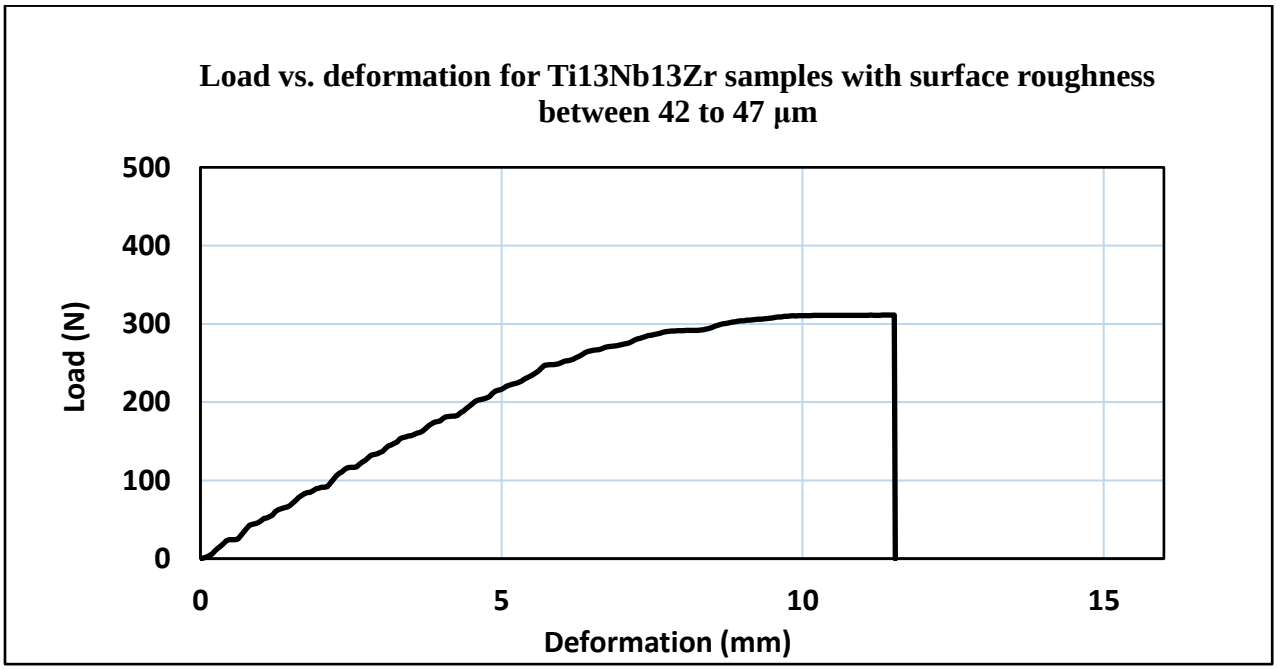

Figure 32: Bending test results for samples with surface roughness between 42 to 47µm (Group No.6).

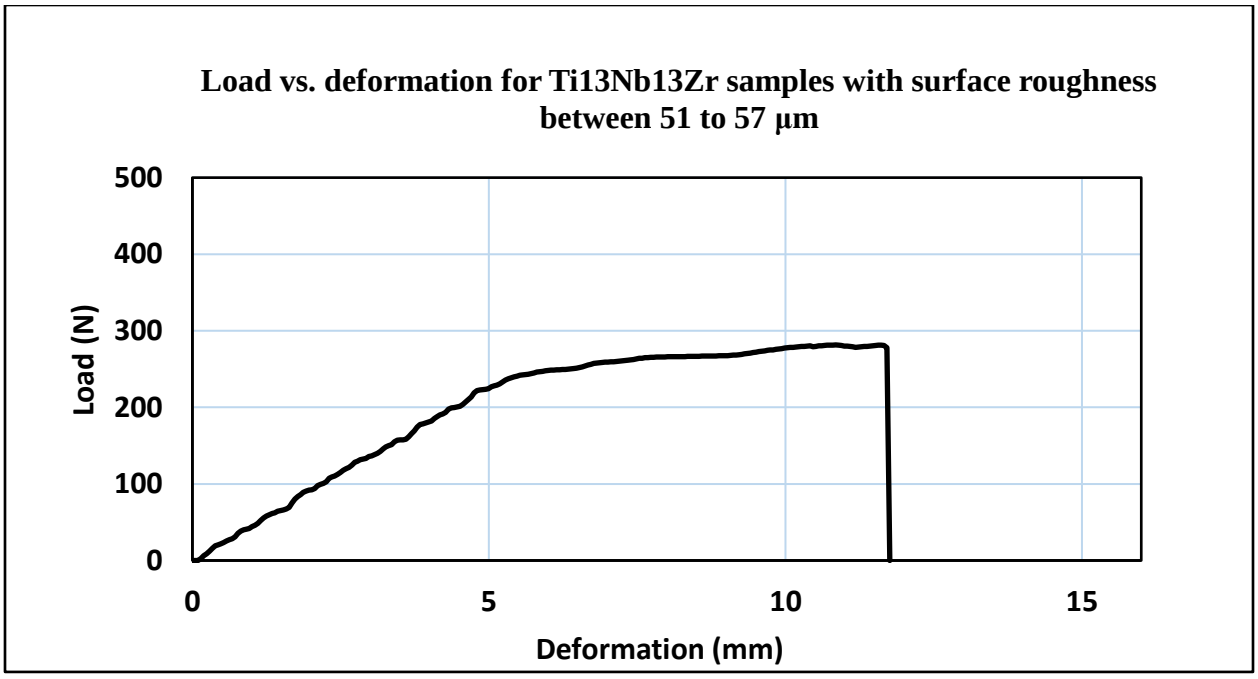

Figure 33: Bending test results for samples with surface roughness between 51 to 57µm (Group No.7).

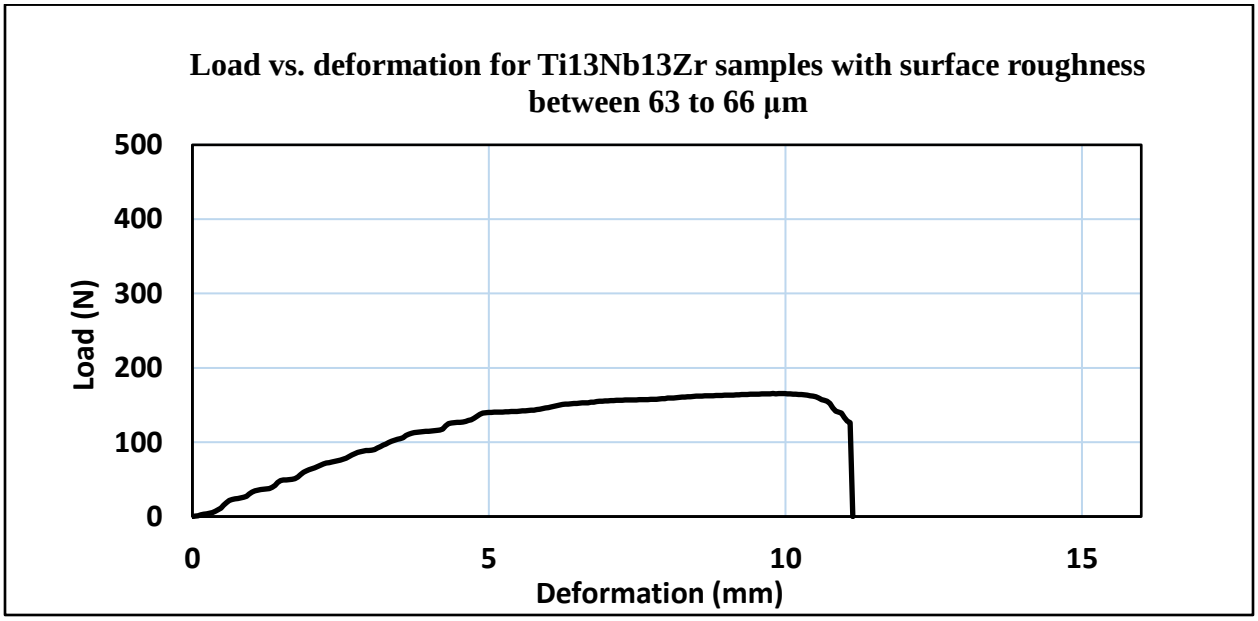

Figure 34: Bending test results for samples with surface roughness between 63 to 66µm (Group No.8).

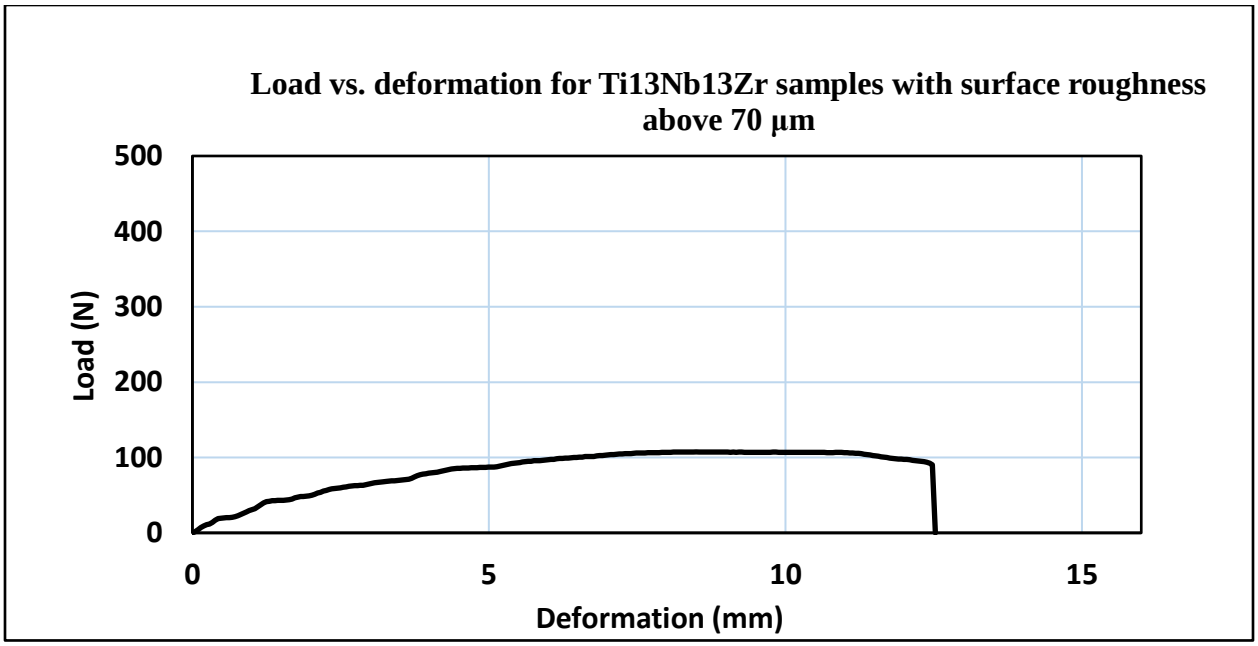

Figure 35: Bending test results for samples with surface roughness higher than 70µm (Group No.9).

The overall and comparative results of all samples are presented in Figure 36.

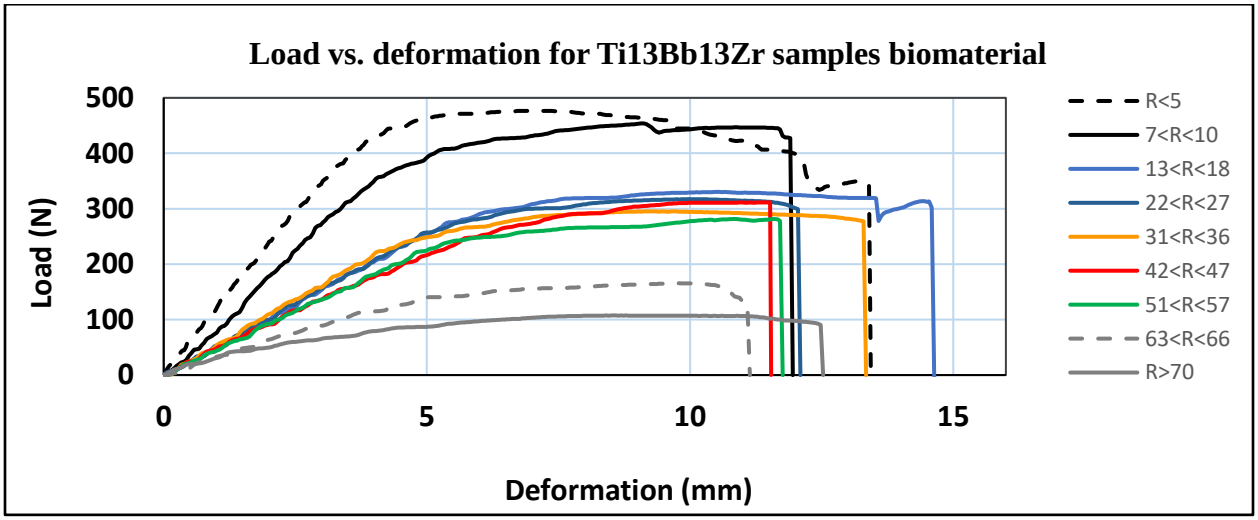

Figure 36: Comparison of the mechanical behavior of all Ti13Nb13Zr samples in 3-point bending test.

The results showed that the strength of samples decreased obviously in two segments. The first drop-off takes place when the roughness increases more than 10 µm and the second drop-off takes place when the surface roughness goes higher than 57 µm. In this regard, due to three reasons,

the Ti13Nb13Zr implants with surface roughness above 60 μm were not recommended for load-bearing orthopedic applications.

First, according to FDA requirements, the metallic materials must be able to carry the bending and compression stresses at least over 130 MPa before yield point [FDA] and this approximate number is subjected the exact location and function of the implant. Considering the Safety factor of 4.6 for most metallic load carrying implants [415], they must be able to stand flexural and compression load at least 498 MPa. Referring to Figure 36, samples with roughness above 60 μm exhibit flexural strength less than 498 MPa and therefore are not acceptable. The second reason is that, the amount of errors induced to roughness measurement is higher at the bigger values of roughness, this should alarm us – as designers or engineers- that the data at higher values of surface roughness are subjected to more errors in measurements. It should be noticed that, in orthopedic industry, as surface roughness increases, more conservative approaches are taken due to deteriorating effects of higher roughness on adjacent tissues and nerves. For example, the destructive effects of micro-motion of implant increase as roughness increases.

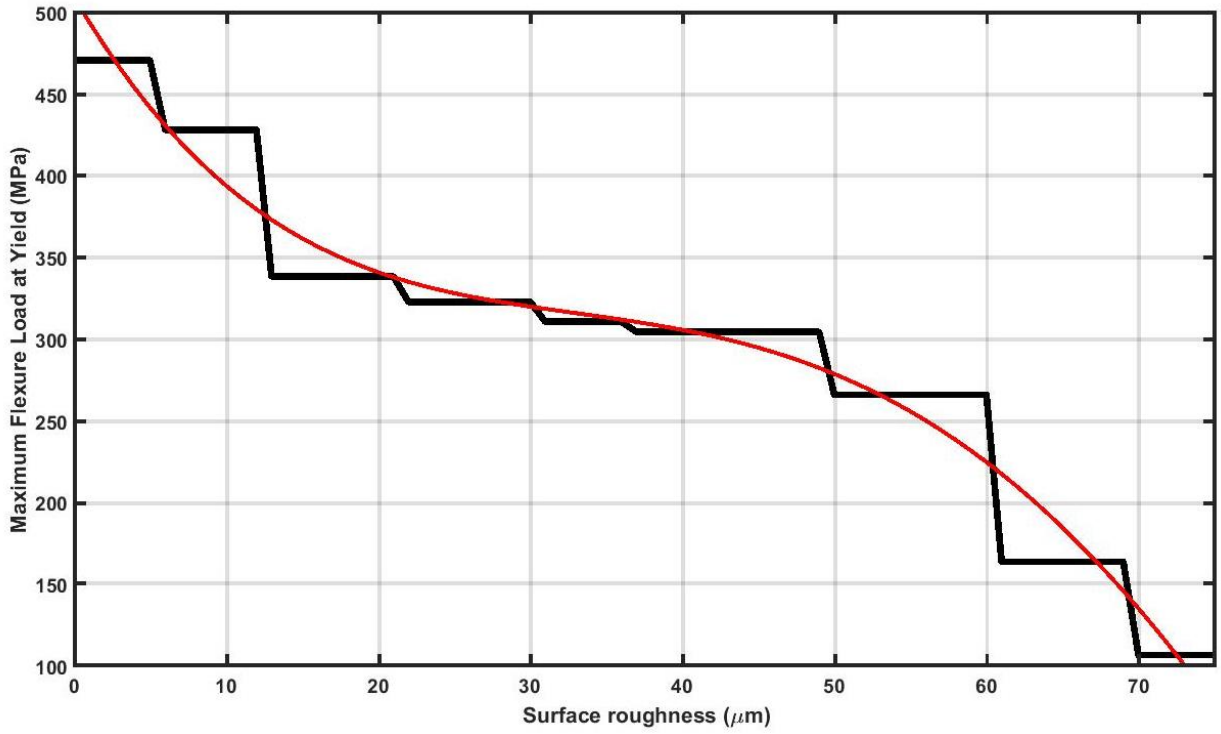

Figure 37: Reduction in maximum flexural load at yielding for Ti13Nb13Zr samples with increasing the surface roughness

It was observed that bending test results and the failure of Ti13Nb13Zr are highly sensitive to the magnitude of roughness. This observation can be attributed to the fact that surface roughness can act as stress concertation cites.

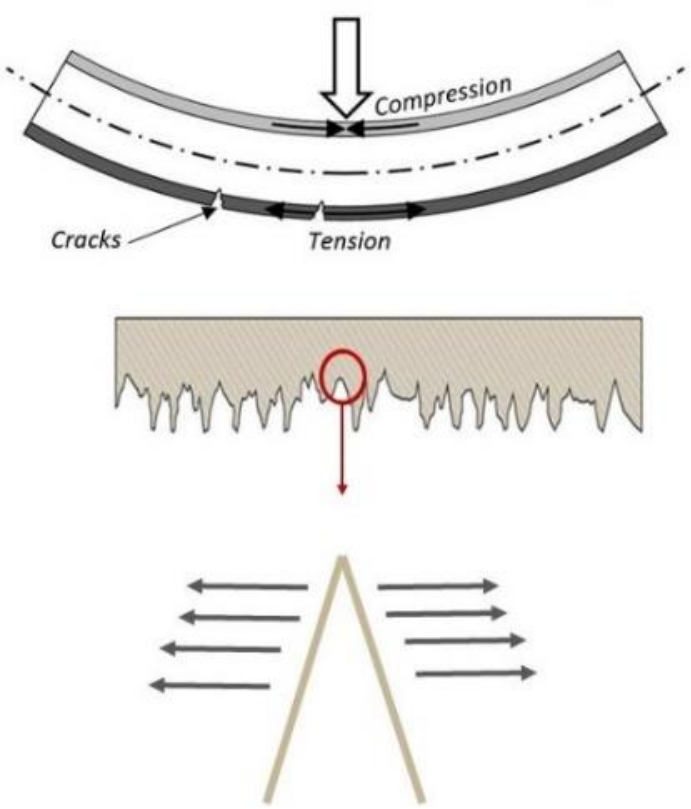

Figure 38: The tension forces on a roughened surface significantly expedite the crack propagation.

Due to increasing roughness, the magnitude of asperities will increase on the surface which will induce higher stress intensity on the rougher surfaces. This phenomena would cause early crack nucleation and initiation of crack propagation. In applications such as orthopedic implants which surface roughness is needed, a very important mechanical design consideration is surface integrity. As it was seen in the experiments that surface integrity in rougher samples is very vulnerable and causes premature failure in orthopedic implants. The conclusion from this section is that failure strain (and hence energy absorption) for Ti13Nb13Zr is highly sensitive to surface roughness. In fact, the failure strain significantly drops off with increasing of average surface roughness.

4.2. Wettability Measurement

Wettability describes the attempt of a solid to form a common interface with a liquid that comes into contact with it. One way of measurement of the wettability is the contact angle test which was described earlier. A low contact angle (less than 90°) indicates a hydrophilic surface in which the liquid will subsequently spread over the surface. A large contact angle (more than 90°) indicates the surface is hydrophobic leading to formation of a droplet on the interface (Figure 39). However, this reaction is controlled by other features such as the molecular interaction between the different phases [37, 38].

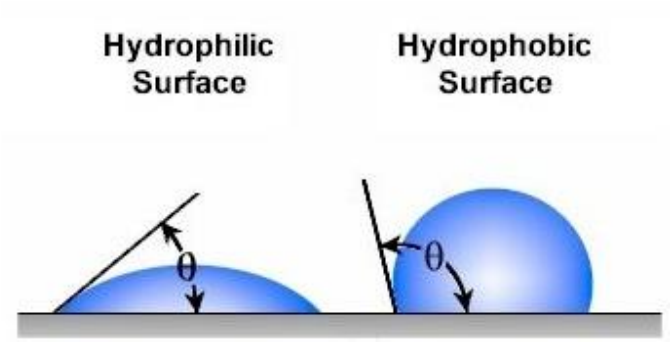

Figure 39: Hydrophilic and hydrophobic surfaces

4.2.1. The Importance of Wetting Test in Orthopedic Applications

Wettability of the implants has a vital role in bone-implant attachment and integration. This importance backs to the fact that liquid forms of proteins are the first materials that touch implants as they are inserted into the body. As seen in Figure 40, in successful implantation, as soon as an implant as a foreign object goes into the body, the first reaction of the body is to evaluate if the foreign body is a suitable substrate to sit or not through the wettability characteristic of the implant (part a). In the second phase, the immune system of the body evaluates the biocompatibility of the implant in terms of safety and toxicity (part b). As the implant was accepted by immune system releases biochemical signals to the appropriate cell tissues to come and attach to the implant (part

c). Bone cells are coming and attach to the implant to build integration (part d). In 3 up to 4 weeks, the first layer of new bone has bonded to the implant.

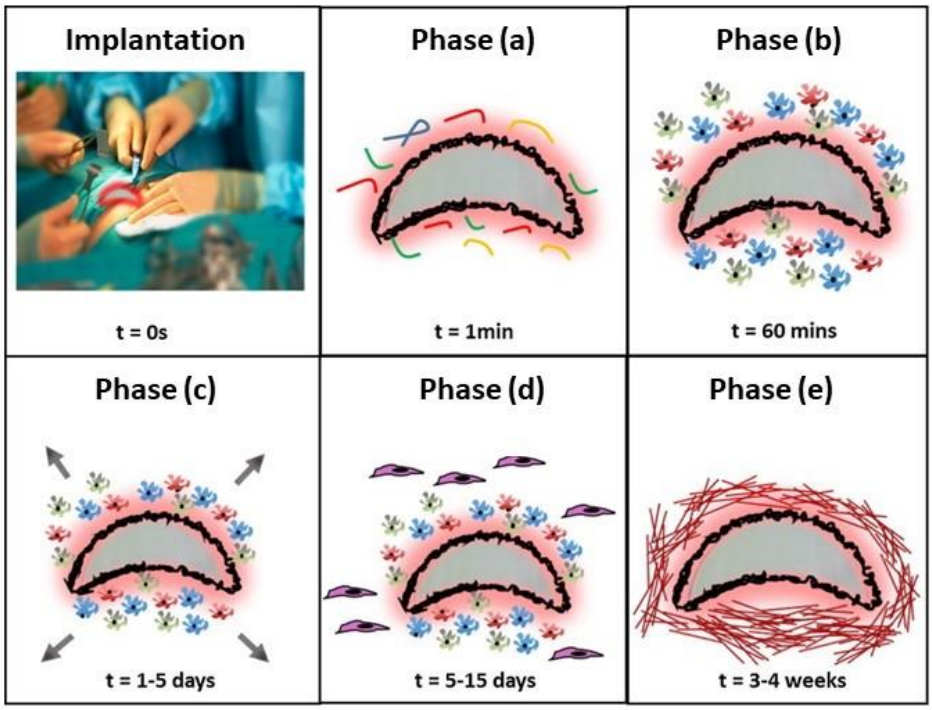

Figure 40: Natural innate response to implantation. Following the implantation procedure, a layer of proteins from the surrounding vasculature adsorbs onto the biomaterial surface. This leads to infiltration and adherence of cells such as platelets, monocytes and macrophages. These cells in turn release cytokines and chemokines that recruit tissue repair cells [416].

As explained above, the implantation process inside the body starts with reaction of proteins on the surface of biomaterials in liquid atmosphere. If the process of adhesion goes well, then the initial cells will be attached to the proteins. In case the situation is in favor of initial cells, they release chemical signals to attract the main repairing cells to come and attach to the implant which can be considered the beginning of integration of surrounding tissue and the implant. This fact shows the significant role of wettability to dominate the process of implantation. Any change in surface wettability will affect protein adsorption that consequently changes cell adhesion through receptors [417]. Increasing the surface wettability may enhance the adhesion and provide contact guidance for osteoblast migration along the surface [418].

4.2.2. Simplified Model of Wetting

As it was already mentioned, surface plays a crucial role on a successful implantation, but the fact is surface is not a singular phenomenon and involves different parameters. For example, hydrophobicity and hydrophilicity are crucial factors in facilitating or hindering the protein attachment leading to success or failure in cell adhesion. One of the main concepts that help determine the behavior of the surface toward the liquids is surface energy. For two macroscopic flat surfaces, free energy (energy per unit area) ΔW changes by the interfacial energy of solid A and liquid B and can be expressed as [419]:

$$\Delta W = -2\,\gamma_{AB} \qquad \text{(Eq.4)}$$

Interfacial energy per unit area (γ_{AB}) can be expressed in the form:

$$\gamma_{AB} = \gamma_A + \gamma_B - W_{AB} \qquad \text{(Eq.5)}$$

Where W_{AB} is the energy change of bringing unit area A into contact with unit area B in liquid form. Also, γ_A and γ_B are surface tensions of solid A and liquid B, respectively. They are very useful for deriving surface energy terms in a complex system and are often used for obtaining approximate values which cannot be easily measured. Equation 5 can be rewritten as by [419]:

$$\gamma_{AB} = \gamma_A + \gamma_B - 2\sqrt{\gamma_A \gamma_B} \qquad \text{(Eq.6)}$$

Equation 3 shows that interfacial energy (γ_{AB}) can be described entirely in terms of the intrinsic properties of material A and material B. All biological interactions require water on solid surfaces. If one considers a specific system where material A is solid, B is vapor and C is a liquid (Figure 3), then from Young's equation [420], the interfacial tension relation of solid A, medium B, liquid C can be represented by:

$$\gamma_{AC} + \gamma_{BC}\cos\theta = \gamma_{AB} \qquad \text{(Eq.7)}$$

Where γ_{AC} is the surface tension between solid A and liquid C, γ_{BC} is the surface tension between medium B and liquid C, and γ_{AB} is the surface tension between solid A and medium B.

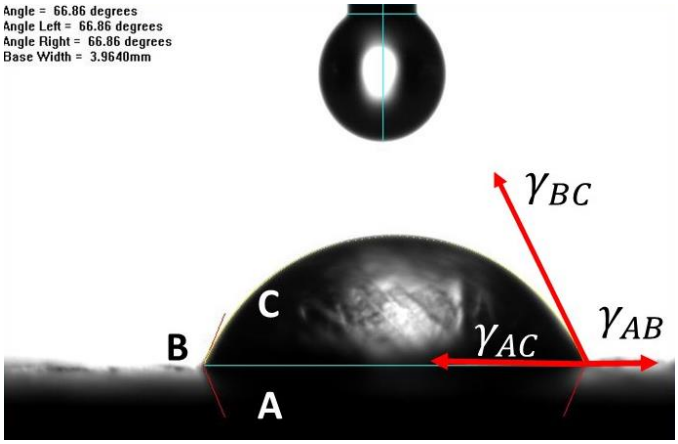

Figure 41: Intermediate partial wetting: Surface tension between solid A and liquid C determines the contact angle.

The Young-Dupre equation can be written in the form as [420]:

$$\gamma_{BC}(1 + cos\,\theta) = \Delta W_{ABC} \quad \text{(Eq.8)}$$

Where ΔW_{ABC} is the adhesion energy per unit area of surfaces A and C, adhering in medium B. Interfacial and surface energies determine how macroscopic liquid droplets deform when they are adherent to a surface. Usually, the solid-medium (or vapor) and liquid-medium energy has negligible effects [421, 422]. This implies that $\gamma_{AB} = \gamma_A$ and $\gamma_{BC} = \gamma_C$. Denoting γ_A as γ_S and γ_C as γ_L, and also ΔW_{ABC} as ΔW_{SL}, it gives:

$$\gamma_{SL} = \gamma_S + \gamma_L - W_{SL} \quad \text{(Eq.9)}$$

If the van der Waals force (dispersion force) is dominant for the interaction between media 1 (solid) and 2 (liquid), a good approximation is [419]:

$$W_{12} \approx \sqrt{W_{11}^d W_{22}^d} \approx 2\sqrt{\gamma_1^d \gamma_2^d} \quad \text{(Eq.10)}$$

where W^d and γ^d are dispersion parts of total surface tension of W and γ. So, the equation has the same form as equation 3.

$$\gamma_{12} \approx \gamma_1 + \gamma_2 - 2\sqrt{\gamma_1^d \gamma_2^d} \quad \text{(Eq.11)}$$

While dispersion forces exist in all atoms and any surfaces, polar forces (Coulombic or H-bonding) can be another source for total interfacial tension. According to this model, the polar fraction of surface tension is considered by:

$$\gamma = \gamma^d + \gamma^p \quad \text{(Eq.12)}$$

where $\gamma^d$the dispersion is term and γ^p is the polar term. Then, each phase can be spilt up into dispersion (d) and polar (p) parts:

$$\gamma_l = \gamma_l^d + \gamma_l^p \quad \text{(Eq. 13)}$$

$$\gamma_s = \gamma_s^d + \gamma_s^p \quad \text{(Eq.14)}$$

Interfacial tension (Eq.12) between two solid-liquid interfaces can be expressed [423] by:

$$\gamma_{sl} = \gamma_s + \gamma_l - 2(\sqrt{\gamma_s^d \gamma_l^d} + \sqrt{\gamma_s^p \gamma_l^p}) \quad \text{(Eq.15)}$$

Young's equation 7 can be written as

$$\gamma_s = \gamma_{sl} + \gamma_l \cos\theta \quad \text{(Eq.16)}$$

After removing γ_{sl} equations 15 and 16 can be reduced as:

$$1 + \gamma_l \cos\theta = 2(\sqrt{\gamma_s^d \gamma_l^d} + \sqrt{\gamma_s^p \gamma_l^p}) \quad \text{(Eq.17)}$$

This formula is useful when determining unknown total surface tension $\gamma_s = \gamma_s^d + \gamma_s^p$ with the aid of knowledge of known dispersion and polar tensions of liquids. The total surface tension of a solid can be obtained after calculating at least two contact angles from different solvents in ideal experimental conditions.

Equation 14 can also be expressed by

$$\frac{(1+\gamma_l\, cos\theta)}{2\sqrt{\gamma_l^d}}.\gamma_l = \sqrt{\gamma_s^p}.\sqrt{\frac{\gamma_l^p}{\gamma_l^d}} + \sqrt{\gamma_s^d} \qquad \text{(Eq.18)}$$

This formula is in an identical form with $y = ax + b$; here y equals the left section of equation 18 and

$$a = \sqrt{\frac{\gamma_l^p}{\gamma_l^d}}\ ,\ x = \sqrt{\gamma_s^p}\ , b = \sqrt{\gamma_s^d} \qquad \text{(Eq.18a)}$$

The linear plotting of the Eq.18 provides direct information on polar and non-polar surface tension for unknown solid surfaces. In Figure 42, Dots represent experimental values by contact angle measurement and the slope represents the square root of polar tension of the surfaces.

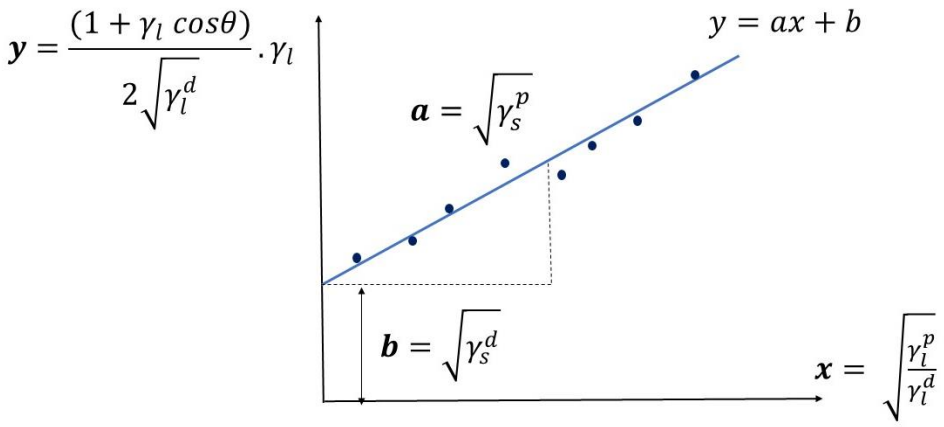

Figure 42: Measurement of contact angle according to surface tension between solid and liquid.

To obtain surface tension γ_s from contact angles with known γ_l , the surface tension terms can be described as:

$$\gamma_s = f(\gamma_{sl}, \gamma_l) \qquad \text{(Eq.19)}$$

Equation 19 is referred to as an equation of state (EOS). By using large amounts of contact angle data, the required equation of state was recently formulated [424,425] as:

$$\gamma_{sl} = \gamma_s + \gamma_l - 2\sqrt{\gamma_s^d \gamma_l^d}\ .e^{-\beta(\gamma_l-\gamma_s)^2} \quad \text{(Eq.20)}$$

where, $\beta = 0.0001246\ (m^2/mJ)^2$

From Young's equation (Eq.13), equation 20 can be described as

$$cos\theta = -1 + \sqrt{\frac{\gamma_s}{\gamma_l}}\ .\ e^{-\beta(\gamma_l-\gamma_s)^2} \quad \text{(Eq.21)}$$

4.2.3. The Procedure of Wetting Experiment

In practice, a droplet is placed on the solid surface and the image of the drop is recorded. The static contact angle is then defined by fitting Young-Laplace equation around the sitting droplet. Schematic and actual set up of wettability test are shown in Figures 43 and 44 respectively.

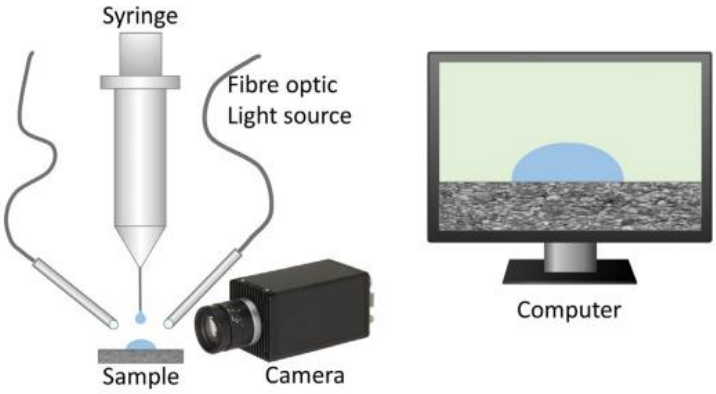

Figure 43: Schematic Goniometer experimental set-up [426].

Figure 44: Actual Goniometer experimental set-up

Figures 45 to 53 show the actual wetting behavior of all groups of samples, and the results are summarized in Table 8.

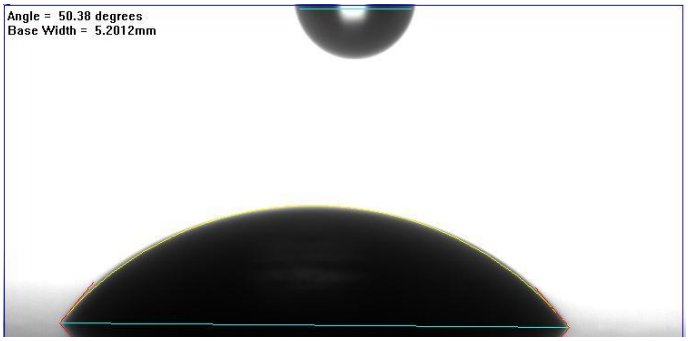

Figure 45: The image of contact angle measurement. A drop has a volume of 10µl and lays on the Ti13Nb13Zr surface with surface roughness less than 5 µm.

Figure 46: The image of contact angle measurement. A drop has a volume of 10µl and lays on the Ti13Nb13Zr surface with surface roughness between 7 to 10 µm.

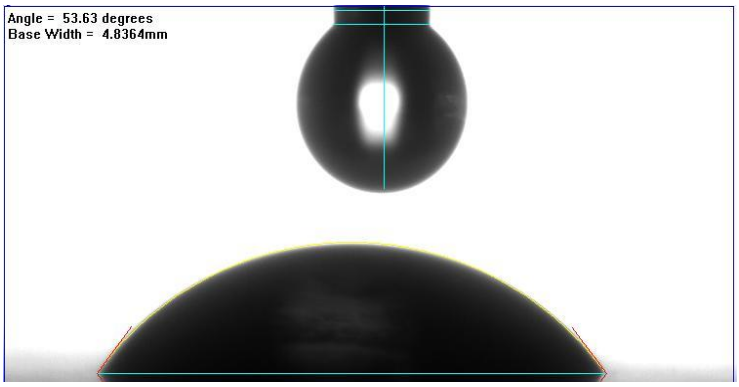

Figure 47: The image of contact angle measurement. A drop has a volume of 10µl and lays on the Ti13Nb13Zr surface with surface roughness between 13 to 18 µm.

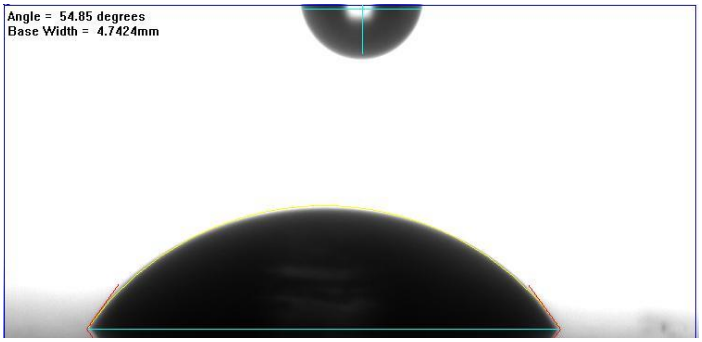

Figure 48: The image of contact angle measurement. A drop has a volume of 10µl and lays on the Ti13Nb13Zr surface with surface roughness between 22 to 27 µm.

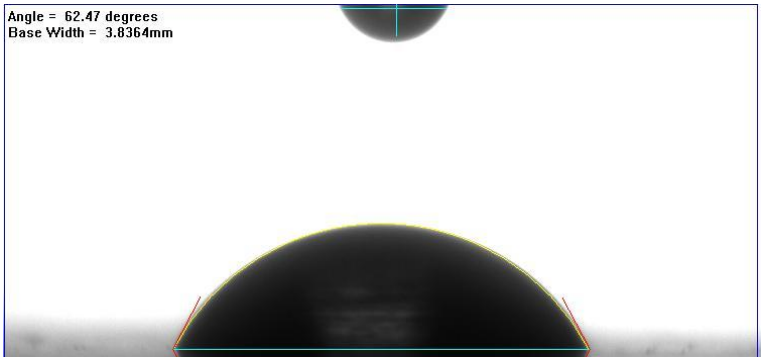

Figure 49: The image of contact angle measurement. A drop has a volume of 10µl and lays on the Ti13Nb13Zr surface with surface roughness between 31 to 36 µm.

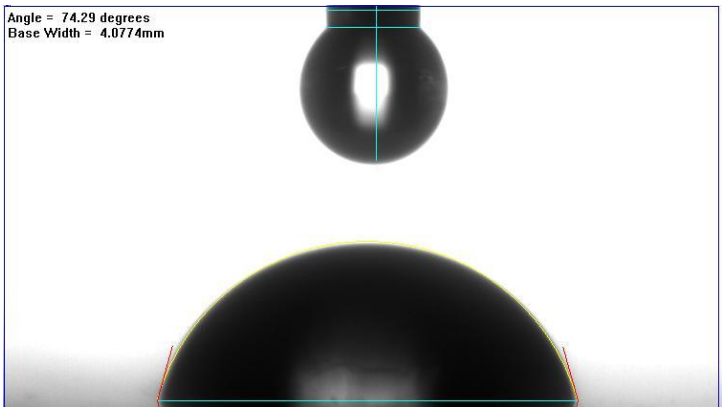

Figure 50: The image of contact angle measurement. A drop has a volume of 10µl and lays on the Ti13Nb13Zr surface with surface roughness between 42 to 47 µm.

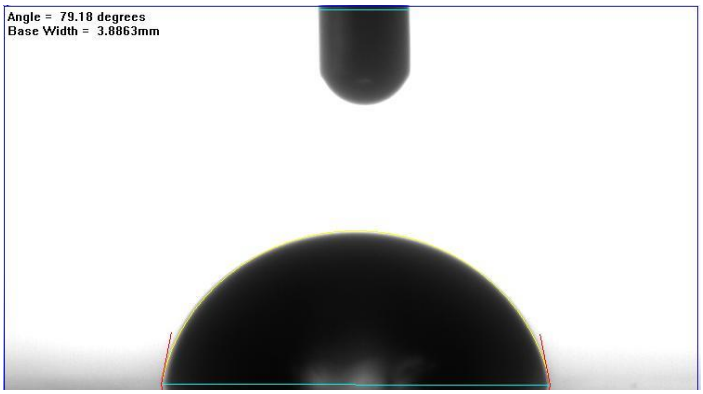

Figure 51: The image of contact angle measurement. A drop has a volume of 10µl and lays on the Ti13Nb13Zr surface with surface roughness between 51 to 57 µm.

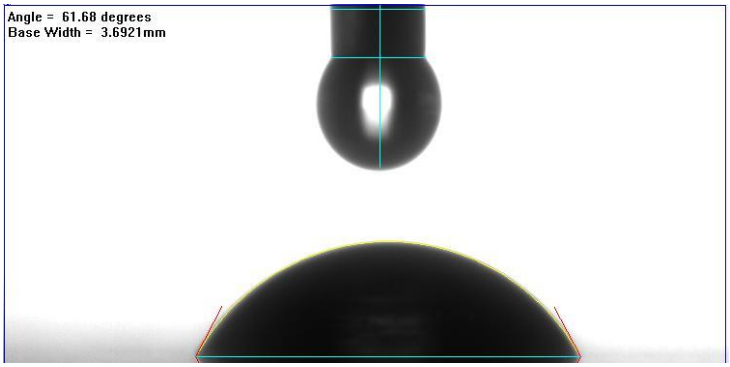

Figure 52: The image of contact angle measurement. A drop has a volume of 10µl and lays on the Ti13Nb13Zr surface with surface roughness between 63 to 66 µm.

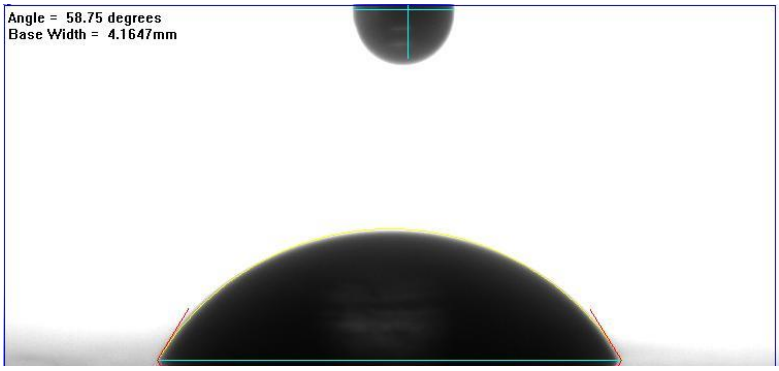

Figure 53: The image of contact angle measurement. A drop has a volume of 10µl and lays on the Ti13Nb13Zr surface with surface roughness higher than 70 µm.

Table 8: Contact angle of Ti13Nb13Zr samples with different surface roughness.

Group No.	Surface Roughness (µm)	Contact angle (°C)	Actual Illustration
1	<5	50.38	
2	(7-10)±2.5	53.23	
3	(13-18)±3	53.63	
4	(22-27)±3	54.85	
5	(31-36)±3	62.47	
6	(42-47)±5.5	74.29	
7	(51-57)±5.2	79.18	
8	(63-66)±7.1	61.68	
9	>70	58.75	

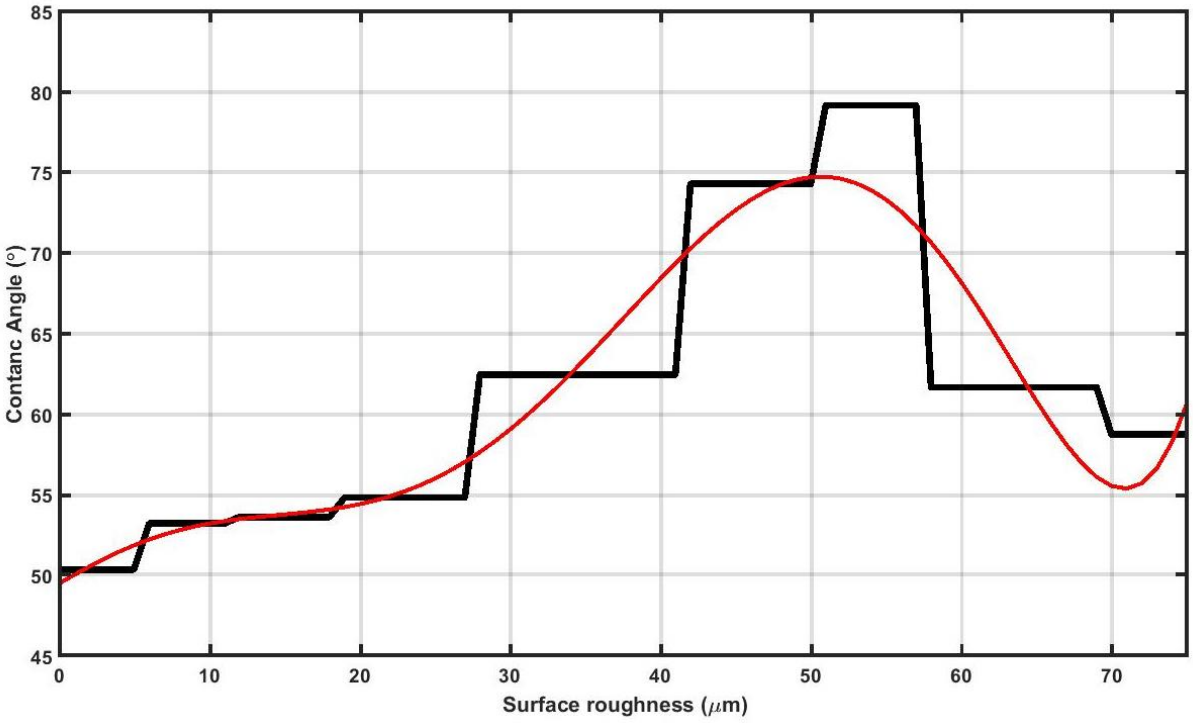

Figure 54: The wetting characteristics of Ti13Nb13Zr samples for different roughness values.

4.3. Cell Adhesion and Proliferation on Implants

The major factor of bone and implant separation is referred to several reasons. The most important one is limited initial cell attachment. Poor initial cell attachment will cause poor bone ingrowth and inadequate integration and eventual separation of bone and implant [427]. As adhesion phase completes, cell division takes place which is a basic biologic process that results in increasing of cell numbers over time. The term “proliferation” applies to the increasing volume of a cellular population and is measured in units of volume (eg, cubic centimeters) or weight (eg, milligrams). Figure 55 shows schematic illustration of cell adhesion and proliferation. There are different experimental setups to study the cell adhesion and proliferation on a surface. One of the fundamental method is the “wash off” technique. Basically not-attached cells are washed off and are counted either manually or by instruments. Cell proliferation and the number of cells can be determined directly by counting manually (e.g., hemocytometer) or automatically (e.g., Coulter

counter or flow cytometer). Hemocytometer is counting chamber (pattern) which consists of a thick glass microscope slide with a grid of perpendicular lines etched in the middle. The grid has specified dimensions so that the area covered by the lines is known, which makes it possible to count the number of cells in a specific volume of solution. The automated cell counting can be done by Coulter counter or flow cytometer which work based on the detection and measurement of changes in electrical resistance produced by cells as they traverse a small aperture. Cells suspended in an electrically conductive diluent such as saline are pulled through an aperture (orifice) in a glass tube.

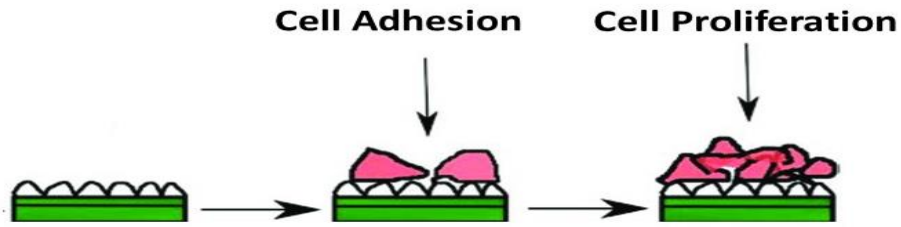

Figure 55: Schematic illustration of cell adhesion and cell proliferation

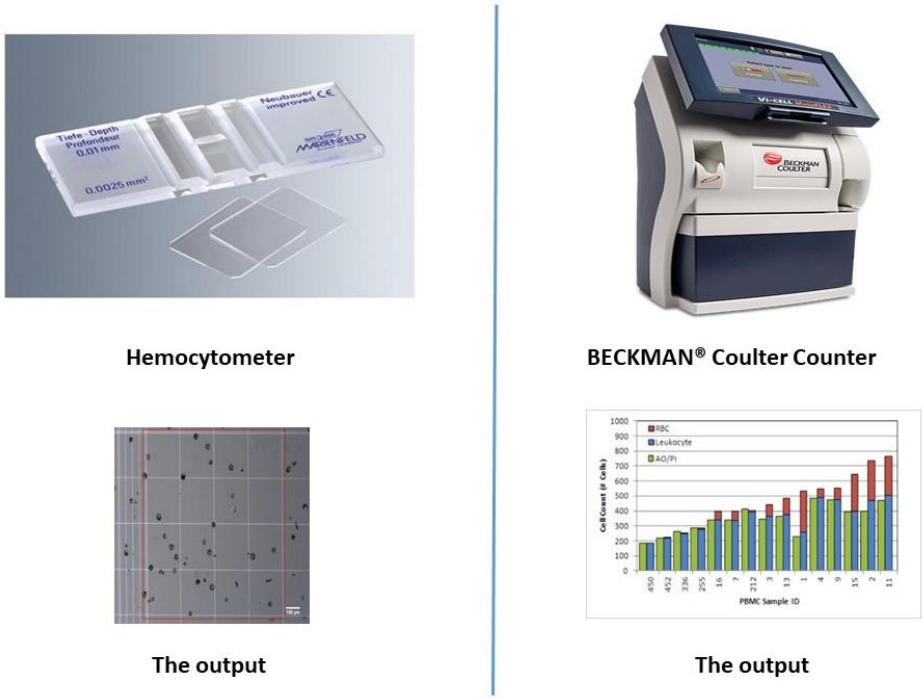

Figure 56: The common methods to count the cells in vitro: (a) Hemocytometer and (b) Coulter Counter

4.3.1. Experimental Procedure of Cell Counting

Cell culture Human MG-63 osteoblastic cells (Sigma-Aldrich, USA) were cultured in Dulbecco's Modified Eagle's Medium (DMEM); ThermoFisher Scietific, USA) with 10 % fetal bovine serum (FBS) (Sigma-Aldrich, USA), at 37 °C, in a 5 % CO_2 humid atmosphere. DMDEM contains four fold concentration of amino acids and vitamins, and as an quick explanation, it should be said that FBS it is used extensively as a supplement to basal growth medium in cell culture applications. For cell adhesion study,Ti13Nb13Zr samples were placed individually into the sterile 24-well plates and the cell suspension at a density of 5×104 cells/ml (100 µl) was pipetted onto the surface of each sample. After 4 h, 500 µl complete medium was added to each well and cells cultured for 48 hours. The samples were rinsed three times with phosphate buffered saline (PBS) following the removal of the medium. For cell proliferation, suspended MG-63 cells in DMEM with 10 % FCS (5×10^4 cells/0.3 ml) were seeded onto Ti13Nb13Zr samples. In order to

prevent touching the lateral parts of the samples, Ti13Nb13Zr samples were covered by tape. After 10 min to allow cell sedimentation and adhesion to the non-adherent cells (i.e. cells that did not adhere to the surface within the given period of time) was then drawn up with a pipette, transferred into 12 × 75 mm test tubes and analyzed by flow cytometry for quantitative assessment. The results are shown in Figures 57 and 58.

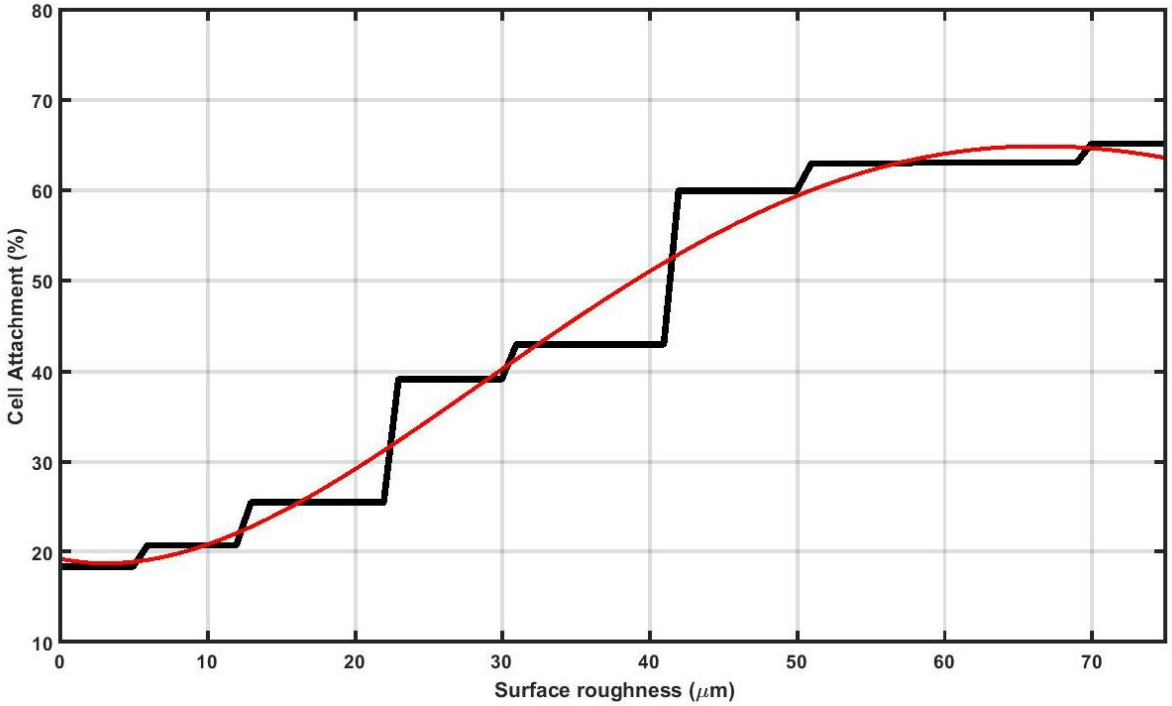

Figure 57: Increasing the surface roughness of Ti13Nb13Zr sample provides a more suitable site for cell attachment.

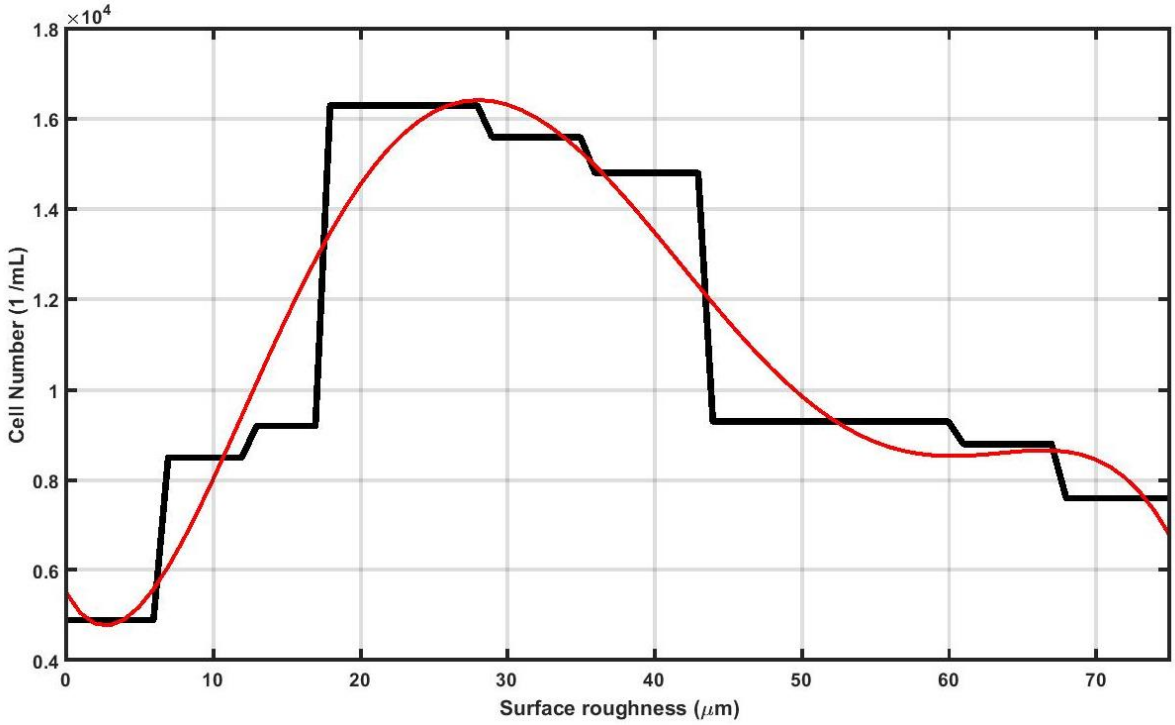

Figure 58: Increasing the surface roughness of Ti13Nb13Zr samples supports the cell proliferation to a particular level and then adversely affects it.

The results show that there is a noteworthy correlation between the surface roughness of Ti1313Nb13Zr and the cell attachment. As it can be seen in Figure 57, surface roughness can obviously endorse the initial cell adhesion. It is observed that as the roughness increases, the number of attached cells to Ti13Nb13Zr increases. This phenomenon can be attributed to the fact that a rougher surface has a greater surface area which can provide more binding sites. The integration process of bone cells and implants consists of two major phases: adhesion and proliferation. It was observed in Figure 58 that cell proliferation on Ti13Nb13Zr samples increases with increasing of surface roughness to a certain range of roughness and then it decreases. It is concluded that there is an optimum range of surface roughness that provides better proliferation.

CHAPTER 5. RESULTS AND DISCUSSION

As discussed in the literature review, there is an existing scientific gap on the role(s) of surface roughness on the functionality of orthopedic and dental implants. One of the reasons which might justify this poor understanding can be attributed to the multi-aspect and paradoxical effects of surface roughness on the functionality of orthopedic implants. One of the main drawbacks in many published research works is the single approach to the surface roughness. The majority of current literature has been done by biologists and medical experts, and is very much focused on understanding the correlation between surface roughness and cell adhesion, proliferation, and morphology. Even though it's well accepted that surface roughness can influence the number of adhered cells, still it is not well documented what value of surface roughness can maximize the cell adhesion. In the real scenario, the implant is not responsible only for cell adhesion, but first, it should provide a hydrophilic surface to be accepted by the immune system and also tolerate biomechanical conditions for a long period of time.

This study shows that wettability, cell adhesion, and proliferation and mechanical properties should be considered as a set of connection parameters, the optimum range of surface roughness, should satisfy these connected factors. All of these three major criteria are summarized in Figure 59.

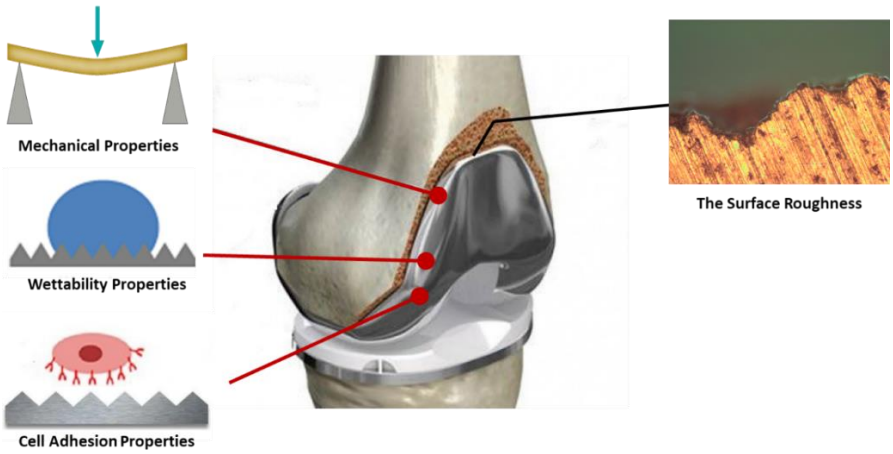

Figure 59: This study was designed to answer this question that what is the optimum surface roughness which can satisfy major criteria of an orthopedic implant: high mechanical strength, high wettability, and maximum cell attachment.

Besides the multidisciplinary nature of this research, another novelty of this research is the material used in this study. Ti13Nb13Zr is the newest member in the family of Ti-based orthopedic alloys. The problem is Ti6Al4V releases aluminum and vanadium ions which are found toxic in the body for the long term. Ti13Nb13Zr is not only superior to Ti64 in terms of biocompatibility, but also has a significant mechanical advantage which is its lower Young's modulus than Ti64. There is a design consideration for metallic orthopedic implants, which is called mechanical biocompatibility. To satisfy the mechanical biocompatibility, metals used for implants must be mechanically harmonized with hard tissues as much as possible. Due to the difference in Young's modulus, the load transferred between metallic implants and bone is not equal, which results in reducing stress stimulation of the bone and eventually loses the density of bone and fracture, this effect is known as the stress shielding effect.

The methodology of this research is based on the overlapping of three groups of results to find the optimum range of surface roughness. The investigation of mechanical properties, first of all, was noticed that Ti13Nb13Zr is highly sensitive to the roughness of samples. The results of three-point bending tests show flexural strength of Ti13Nb13Zr samples decreases as the value of

surface roughness increases. To explain this phenomenon, it should be said that surface as the outermost layer of an implant is the place that cracks can form and propagate throughout the entire object, and increasing the surface roughness can significantly increase the stress concentration sites.

This sensitivity might need some extra conservative considerations when this material is supposed to be used in orthopedic load-carrying joints and implants. Our results on deteriorative effects of surface roughness on the flexural strength is in good agreement with similar works [428,429], but this research showed that Ti13Nb13Zr, is even more sensitive and susceptible to the negative effects of cracks due to the surface roughness. This correlation is attributed to the fact that surface roughness in a way that was fabricated in this study, is considered a subtractive and mechanically aggressive method. The nature of the subtractive method is to remove some parts of the surface by mechanical force, which creates numerous micron level defects as well as high amount of residual stress on the surface. These imposed irregularities on the surface, threaten the surface integrity and eventually the stability of the sample. To fabricate rougher surfaces, more forces are applied on the samples, therefore more defects are imposed on the surface which makes them more susceptible for crack initiation. Even though the corrosion test was not in the scope of this test, it is worth mentioning that more inconsistency and irregularities on the surface, increase the possibility of corrosion due to inducing more residual stress generated on the surface. Bending test data are particularly useful for implants because it is supposed to be used as a supportive structure where the product is supposed to carry the applied loads and return to the original shape in case of any bending. In this regard, desired roughness on an orthopedic implant must be flexible enough to be bent to some extent without permanent deformation, and simultaneously it must be rigid enough to perform its function properly to hold the applied load.

In terms of wettability measurement, it is found that roughness has a complicated relationship with wettability. It was observed that upon increasing the roughness, the contact angle increases which means lower hydrophilicity and eventually lower wettability which is not favorable for orthopedic implants. This trend was observed for the Ti13Nb13Zr samples with surface roughness less than 5μm up to 57 μm. It was observed that upon increasing the surface roughness by more than 57 μm, the wetting characteristic of the Ti13Nb13Zr samples are changed and lower contact angles were recorded. In fact, samples with roughness higher than 57 μm showing more hydrophilicity. To explain why contact angle increases with increasing the surface roughness of Ti13Nb13Zr, it should be noted that as surface roughness increases, the liquid loses enough contact of base material and liquid does not enter inside the roughness and as matter of fact, a water drop sits on a patchwork of Ti13Nb13Zr and air which leads to contact angles increase. But when roughness is increased higher than 57 μm, the factor of surface energy dominates the interaction and higher roughness means higher surface energy which is in favor of wettability.

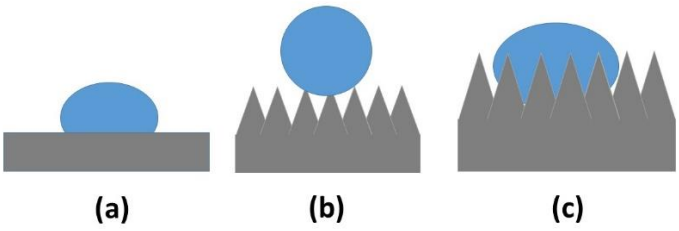

Figure 60: Wettability of Ti13Nb13Zr declines with increasing surface roughness to a certain level, and after that, increasing the roughness, improves the wettability.

The cell study was performed in two separate sections cell adhesion and cell proliferation. It was observed the percentage of attached cells on the surface of Ti13Nb13Zr increases as the roughness increases. This correlation between cell adhesion and roughness can be attributed to the fact that increasing surface roughness of Ti13Nb13Zr means more sites for the samples to attach.

In other words, rougher surface has more surface area, also rougher surface has more potential holes and grooves which facilitates both attachment and then stabilization of the cells. The fact is in the real scenario, cell adhesion is only one phase of cell growth. Upon the cell adhesion completed, the cell proliferation should start. In this regard, the surface of an implant not should support cell attachment, but it also should facilitate the cell proliferation. The cell proliferation results show that surface roughness showed more complicated behavior. Cell proliferation of Ti13Nb13Zr increases with increasing the surface roughness up to 20 μm and then it decreases as surface roughness increases. This variation can attribute to the fact that roughness up to 20 μm, acts as supportive chambers that hold enough cells to get connected and grow. As the surface roughness increases more than 20, the surface acts like walls that isolate the cells and, the rate of cell connection declines. In order to introduce the optimum roughness for better performance, the experimental results of this study are considered as related parameters. In other words, we assumed that the performance will be improved if all criteria will be satisfied in terms of surface roughness. In this regard, all results as seen in Figure 61 are summarized and it was observed that a surface roughness between 20 to 25 μm is the surface roughness range that satisfies all the criteria.

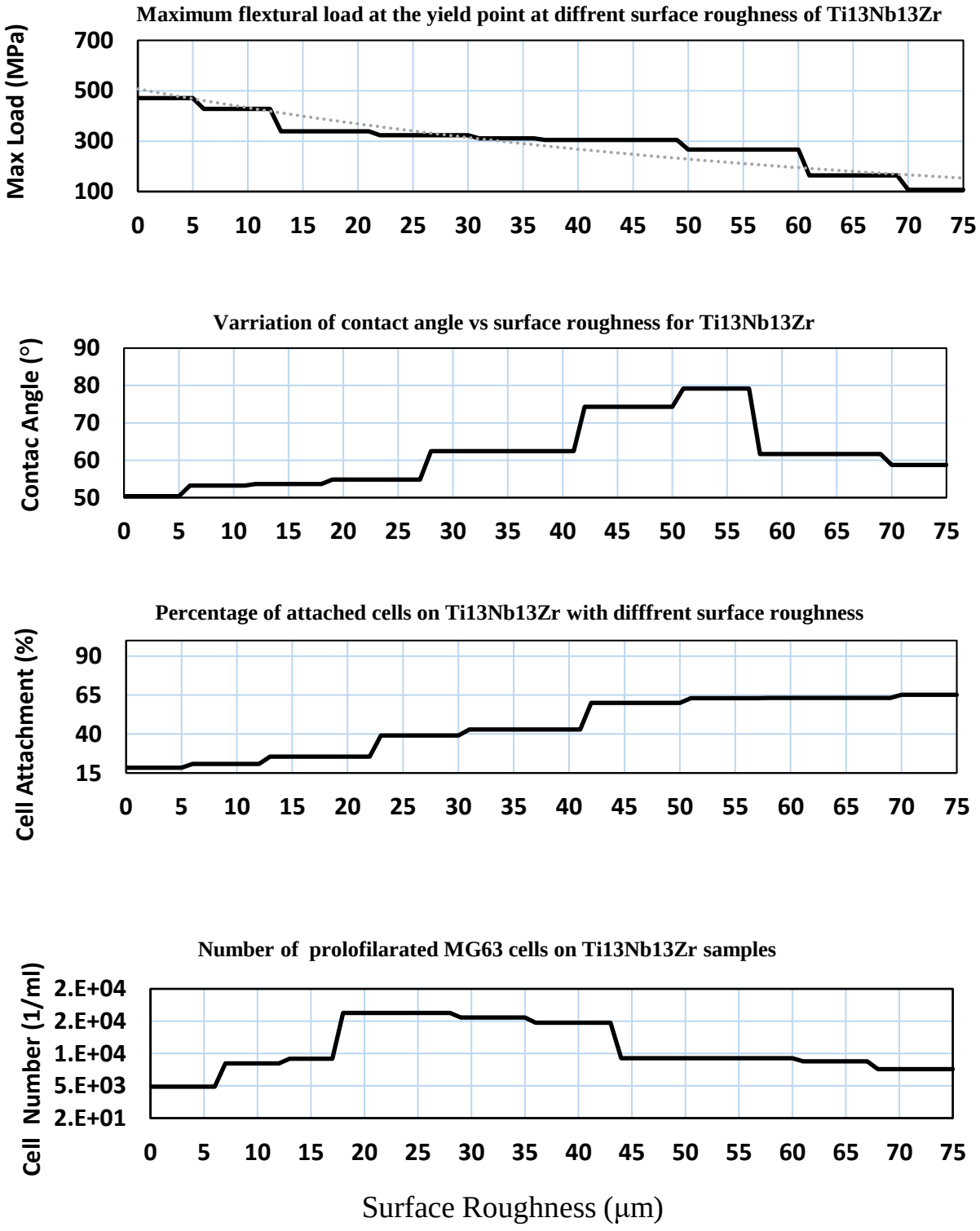

Figure 61: The effects of surface roughness on mechanical, wetting, cell adhesion, and cell proliferation of Ti13Nb13Zr samples with different surface roughness values.

5.1. Conclusion

In orthopedic implants, an ideal surface, not only should play a substantial role to absorb nutrients and cells on itself, but it should actively resist any crack formation as well. The situation even becomes more complicated if it is considered that increasing the surface roughness in favor of more cell and nutrients attraction, will increase the stress concentration sites and eventually crack formation as well as increasing the possibility of corrosion on the surface. In fact, by creating roughness on the surface of a sample, the ductility and bending strength of the specimen will be influenced. The results of this study on Ti13Nb13Zr with different surface roughness values show that surface roughness between 20 μm to 25 μm can satisfy the major requirements of a metallic orthopedic implant including cell adhesion and proliferation, bending strength, and wettability. Fulfillment of these requirements will guarantee the better functionality of implants inside the body.

5.2. Further Studies

This research and its results can pave the way for further research in this field. The issue of bone and implant separation is a subject that demands much more studies because it is an old problem with new solutions and motivations. New technologies such as advanced 3D printers which can print implants with desired surface roughness, as well as emerging new biomaterials such as Ti13Nb13zr, are pushing this industry forward. On the other hand, the high demand of aging Americans for more effective bone treatments highlights the need of more research. In this regard, the following areas are some of the potential areas that need more investigation.

1. Modification of mechanical properties of Ti13Nb13Zr to have properties more similar to the bone, such as creating porous samples.

2. Functionalization of surfaces such as coating Hydroxyapatite on the surface of Ti13Nb13Zr to increase cell attachment and proliferation.
3. Performing *in vivo* studies on animals.

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