STUDY OF HYDROXYAPATITE COATINGS ON TITANIUM FOAMS USING PULSED LASER DEPOSITION (PLD)

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CERTIFICATE

This is to certify that work in this thesis has been completed by **Ms. Eeman-e-Maryam**, **Ms. Iqra Abid** and **Mr. Fahad Bin Aziz** under the supervision of Dr. Usman Liaqat and Dr. Khurram Yaqoob at the School of Chemical and Materials Engineering (SCME), National University of Sciences and Technology, H-12, Islamabad, Pakistan.

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DEDICATION

With profound reverence,

We dedicate this project to our **beloved parents** and **respected teachers** whose guidance and unflinching support helped us throughout the process.

ACKNOWLEDGEMENTS

Praise be to Almighty Allah who made us capable to do our best and get through this project.

For the successful completion and execution of our final year project, we sincerely acknowledge all the advice and support we have received from our Supervisor Dr. Usman Liaqat. Without his diligence, we would not have been able to project our true capabilities for this strenuous task.

Furthermore, all the guidance our Co-Supervisor Dr. Khurram Yaqoob provided us with, helped us achieve a better understanding and problem-solving approach. We thank him for the provision of all the required material samples.

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We pay our sincerest regard to all the staff and faculty members of the Materials Engineering department for guiding and helping us at various stages throughout the process.

ABSTRACT

This study primarily focuses on the synthesis of Hydroxyapatite powder, the development of its coating on titanium foams using the pulsed laser deposition coating technique and the testing of coating parameters i.e. surface roughness, hydrophilicity, topography etc. followed by thorough evaluation for their use as biomedical implants.

In the biomedical field, metallic prosthesis and implants are extensively used. Among these, titanium and its alloys are most widely used. But there are some of its short-comings such as stress-shielding induced aseptic loosening which can be overcome by coating it. It is often used for implantation purposes in the form of foams which reduces its density and Young's Modulus. In this research, titanium foams have been coated with hydroxyapatite which is originally a ceramic material having excellent biocompatibility and bioactivity as it resembles the bone apatite in terms of its composition. Hydroxyapatite has been synthesized by the wet/homogeneous precipitation method with Calcium hydroxide, phosphoric acid, and deionized water as the primary precursors. HA has been characterized for purity and particle size determination and morphology using Scanning Electron Microscopy (SEM), Fourier Transform Infrared Spectroscopy (FTIR), Energy Dispersive X-Ray Spectroscopy (EDS), and Raman Analysis.

The coating has been done using Pulsed Laser Deposition (PLD) as the process is economically feasible and has optimum coating thickness control. Additionally, the coating has been tested for hydrophilicity (contact angle), surface roughness, and the surface morphology using Drop Shape Analyzer, Optical Profilometry, and Scanning Electron Microscopy (SEM) respectively. The results prove the effectiveness of Hydroxyapatite coating as it imparts enhanced biocompatibility to titanium foams and helps prevent stress-shielding induced aseptic loosening.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

1.1 Biomedical Implants and Devices

The first chapter discusses the history of biomaterials and how far they have come in terms of advancement and technology today. The major classes of materials that can be used in the biomedical field will be reviewed next, with the main focus being metals, polymers, ceramics, and composites. Metallic implant types will be elaborated on in detail, followed by the problem statement and the major issues faced in bioimplants. Various generalized terms and their long-term effects related to biomedical implants will be explained, alongside the need to prolong opting for revision surgery as much as possible.

1.1.1 Historic Advancement of Biomaterials

By using biomaterials, the medical field has made quantum leaps in helping people receive temporary as well as permanent solutions to numerous bodily injuries that result in a loss of function of a particular organ or body part.

With the rate at which advancement in technology is increasing at an exponential rate in the world, it is no surprise that there have been historic breakthroughs in the field of medicine and biomedical devices. However, the use of biomaterials to completely replace or provide temporary support in case of bodily injury is not a recent discovery.

1.1.2 Types of Implant Materials

A wide variety of natural and synthetic materials can be used to serve the purpose of biomedical implants and devices. With each material comes an assortment of benefits and shortcomings, which is what we will discuss in the following subsections.

1.1.2.1 Metals

One of the oldest materials known to man, metals have been around for thousands of years. Metals were first introduced as biomaterials in the late 19th century when they were used as fixtures to mend bone fractures. They have some generally well-known properties like high malleability and ductility, good electrical conductivity due to the presence of delocalized electrons, good conductors of heat, hard and opaque with a shiny exterior, and relatively higher densities. They have a 'metallic bond', which basically means that they are positive centers surrounded by a sea of delocalized electrons. Metals as standalone materials do not have very superior properties, which is why they are alloyed with other metallic or non-metallic elements.

Another property that makes metals attractive choices for biomedical implants is their load-bearing ability. This comes in handy especially when replacing body parts like femurs and hip-joints. Because metals have a high Young's Modulus and yield strength, they can be used to replace body parts like these as they will not deform and break easily. Even though there are multitudes of metals present in the periodic table, only a handful of them are suitable enough to be used as biomedical devices and implants. The most popular ones are stainless steel, titanium alloys, and cobalt alloys.

1.1.2.2 Ceramics

Ceramics are inorganic compounds that may have ionic or covalent bonding and are made up of combinations of metals and non-metals. They have a few characteristic properties that make them stand out from other materials. Ceramics are extremely hard with a brittle nature. They have high melting points and are very stable in extreme temperature conditions. Since they have no delocalized electrons within their structures, ceramics are generally poor conductors of electricity and heat. They are also extremely stable and unreactive in chemical environments. These reasons are why they are popularly used as refractory materials. They have high compressive strengths but poor tensile properties.

Ceramics are one of the most biocompatible materials known to man. Due to their relative inertness, they can easily be implanted in the body without the fear of rejection. There are three major classes of bio ceramics. The first ones are bioactive ceramics, which includes bio glasses. These form strong chemical bonds with the host tissue and are considered the efficient materials for tissue regeneration. They also form an interface between the implant and the bone tissue very quickly, hence reducing overall healing time.

The second class is composed of bioresorbable ceramics which are Calcium phosphate based. Common examples are hydroxyapatite and tricalcium phosphate. Hydroxyapatite is similar in composition to the inorganic phase of the bone. It is popularly used in bioimplants due to its biomimetic nature, as well as high wear resistance, and its ability to promote cell adhesion by creating a strong anchorage of the implant with the host tissue. Since all of these are bioresorbable, they degrade as the calcium to phosphorus ratio decreases over time. Hydroxyapatite is known to degrade at a slower rate than tricalcium phosphate, therefore leaving adequate time for healing.

The third and last class is composed of bioinert ceramics, which include zirconia, alumina etc. These ceramics are highly inert and very stable to corrosive environments, which make them ideal implant choices. However, they also have a high Young's modulus, which may lead to stress-shielding problems and consequently bone atrophy.

1.1.2.3 Polymers

Polymers are long-chain molecules that are made up of smaller blocks called monomers. They are formed via two modes of polymerization, namely addition polymerization and condensation polymerization. Polymers can be divided into two types i.e., natural and synthetic. Natural polymers include polysaccharides (chitin, cellulose, starch, agar etc.), proteins (collagen, fibrin, silk etc.), and natural rubber (amongst many others). Synthetic polymers are manufactured and commonly derived from oils (petroleum). Common examples of these include teflon, nylon, polyethylene, polyvinyl chloride (PVC) etc.

Polymers as biomaterials have both their pros and cons. On one hand, they are easy to fabricate and resilient. They have apt flexibility, reasonable biocompatibility, are lightweight and many are biodegradable as well. The issues with polymers are their general lack of mechanical strength, which makes them unsuitable for load-bearing applications. They are also relatively difficult to sterilize. This is because when sterilization of polymers is attempted via UV rays, they photo-oxidize and degrade. This leads to a change in their inherent properties. Polymers also have poor thermal properties.

Biodegradable polymers play a huge role in the field of medicine. From biodegradable sutures to tissue scaffolds, they have a wide range of uses. The basic mechanism of biodegradation is that the backbone of the polymers are targeted (bulk degradation). This way, the degradation rate is faster than if the branches of the polymer were targeted first (surface degradation).

Applications of polymers in biomaterials consist of temporary scaffolds, sutures, as temporary barriers to prevent surgical adhesion of tissues during healing, and in recent times, as drug delivery agents and drug eluting stents.

1.1.2.4 Composites

Composites are heterogeneous materials. They are a physical mixture of two or more constituents that have been bonded together such that they retain their individual superior properties. They consist of a continuous phase called the matrix and a discontinuous phase called the reinforcement. The matrix binds the constituents together, protects the reinforcements from environmental damage, and transfers load to the reinforcement. The reinforcement, on the other hand, provides strength and stiffness to the composite due to their load-bearing abilities. Both the matrix and the reinforcement can be present in large quantities, and composites in general have a high strength-to-weight ratio.

Generally, composites are well-known because of their excellent mechanical properties. However, in the field of biomaterials, they are used mostly because of their degradation and regeneration properties.

Composites are divided into three subcategories depending on the type of matrix used. These subcategories are Metal Matrix Composites (MMC's), Polymer Matrix Composites (PMC's), and Ceramic Matrix Composites (CMC's). All of these materials as standalone choices for biomedical applications are faced with problems. For example, polymers have lower mechanical strengths, Young's modulus, and a reduced thermal and chemical resistance. To cater to this issue, they are formed into polymer-based composites which can give them the load-bearing qualities they need to be used in for medical purposes like implants.

Similarly, metals are prone to corrosion, whereas ceramics are difficult to fabricate on an industrial scale. The answer to all these issues is the use of biocomposites, which gives a plethora of impressive properties and increases the efficiency of materials as well.

The biggest example of a composite in the human body is the bone. Made up of an organic phase called collagen which represents the soft and spongy cancelous bone, whereas the hydroxyapatite (calcium phosphate) inorganic phase makes up the cortical bone, which is hard, strong, and compact in nature.

Some examples of the applications of biocomposites are dental crowns used after root canals, which are made up of carbon fiber reinforced epoxies. Chitosan-based biocomposites are used for scaffolds in bone tissue engineering. The purpose of these scaffolds is to provide temporary support to the bones as they are healing after some trauma or injury. They can also be used as prosthetics and as orthopedic implants.

1.2 Categories of Metallic Implants

Metals are usually alloyed with other elements to achieve superior properties. However, due to a number of factors, only a few alloys are deemed fit to be used as biomaterials. These factors include unpredictability in biodegradation (for example a Magnesium-based alloys, infection, inflammation, leaching of toxic ions into the bloodstream, stress-shielding and aseptic loosening, and corrosion amongst many others. [1]

Popular metal alloys used in biomedical applications (specifically for orthopedic and dental implants) include stainless steels, titanium and its alloys, and cobalt-based alloys. More recent advancements in this field consist of biodegradable magnesium alloys, nitinol (shape-memory alloys), and tantalum alloys.

1.2.1 Stainless Steels

Stainless steels are iron-based alloys. The main constituents present in this (other than iron) are chromium, nickel, cobalt, molybdenum, and carbon. The amount of chromium present is 10.5% w/w, and above, because stainless steel has self-healing chromium oxide layers above this percentage that protect it from corrosion. The amount of carbon is less than 1.2% w/w, and it is kept low so as to avoid the precipitation of chromium carbide in the regions of grain boundaries. Carbide precipitation makes a material more susceptible to corrosion and lowered overall fracture strength. The presence of nickel is crucial for adequate toughness, tensile strength, and ductility.

The most common types of stainless steels used are 316 and 316L, which contain 0.08% and 0.03% carbon respectively. They serve as great choices for internal fixation components (bone plates, screws, pins, nails, etc.) and can even be used as total hip implants.

1.2.2 Titanium Alloys

Titanium and its alloys are the most widely used biomaterials for a number of reasons. They have excellent biocompatibility and a low density of 4.5 g/cm^3. Furthermore, it has superior osseointegration properties. This means that there are more chances of a formation of a strong interface between the implant and the bone tissue, and there is adequate anchoring achieved as well. They have a relatively lower elastic modulus as compared to other metal alloys (with a value of approximately 110 - 120 GPa). Due to this reason, they act more like natural joints as opposed to artificial ones. They also have superior corrosion resistance, which paves way for long-term usage inside the body.

Commercially available pure Titanium (cp-Ti) is used in applications that do not require very high mechanical strengths and load-bearing abilities. Other than

that, the most popular Titanium alloy is Ti-6Al-4V, which consists of 6% Aluminium and 4% Vanadium. It has a higher strength than pure titanium. They are commonly used as orthopedic implants, as dental wires for braces, pacemakers as mesh implants for skull defects, amongst many other applications.

1.2.3 Cobalt Alloys

The most common alloys belonging to this subcategory are the Cobalt-Chromium alloys. They have upto 65 w/w % Cobalt and about 35 w/w % Chromium. Molybdenum is often added to provide higher strength by the introduction of fine, small grains in the alloy mixture.

Cobalt-chromium alloys have a high strength, good stability at elevated temperatures, and a relatively higher wear and abrasion resistance. For these reasons, they are used as implants for joint replacements. They can be used for extended periods of times, especially in cases of fracture. Moreover, they can be used as dental and orthopedic implants. The one problem faced when it comes to cobalt-chromium alloys is their manufacturability. They are very rigid alloys; therefore, it is difficult to make them into rod-like shapes because of their low ductility.

1.2.4 Pros and Cons Associated with Metallic Implants

Every material has its pros and cons, and this brief paragraph will discuss both with regards to the metallic biomaterials discussed above.

Stainless steels have a moderate to high strength, have good formability and weldability, and are readily available. They are also on the cheaper side due to ease of fabrication and wider usage. However, they cannot be used in the body for longer periods of time due to fear of leaching of toxic ions like nickel, which can cause allergies, cancer, and genotoxic or mutagenic activities. They usually fail because of pitting corrosion, which greatly reduces their efficiency as well.

Titanium and its alloys have lots of desirable qualities. From excellent biocompatibility, bio-inertness, osseointegration, to its lower density and relatively low Young's modulus, every aspect makes it the best option for

biomedical applications. However, they are difficult to fabricate due to their high chemical reactivities at elevated temperatures. This also makes them extremely expensive. Pure titanium also has a low wear resistance, which is unacceptable if the material is being used inside the body. They are also not considered viable for cemented hip implants due to their relatively lower elastic modulus (as compared to stainless steels and cobalt alloys).

Cobalt-based alloys have lower ductility and are very rigid, which can cause them to go towards sudden fracture if exposed to very high loads. Despite this, they have excellent strength, wear resistance, and high temperature stability.

Most of these metallic implant materials are said to have good corrosion resistance, low wear and tear, they can still face issues like degradation, and the release of toxic ions into the bloodstream that may lead to osteolysis and aseptic loosening. They may also stimulate metal sensitivity, which causes pain, swelling, inflammation, and tissue necrosis.

1.3 Problem Statement

The problem identified at the beginning of our project was that Titanium, when used as a biomedical implant, tends to undergo Stress-shielding Induced Aseptic Loosening. This phenomenon will be discussed in detail in the next section.

1.3.1 Stress-Shielding Induced Aseptic Loosening

Stress-shielding is the improper transfer of mechanical loads between the bone and the implant. It occurs when there is a significant difference in the stiffness (Young's modulus) of the bone and the implant. Generally, metallic implants have a very high elastic modulus as compared to that of the bone. For example, let us consider the elastic modulus values of Titanium and the bone. Titanium has an elastic modulus of around 120 GPa, whereas the bone has an elastic modulus of around 10 - 30 GPa. When used as bioimplants, the metals tend to bear all of the load, leaving very little to no load transfer for the bone. The bone needs apt load transfer so that it can heal by tissue regeneration. However, when stress-shielding occurs, the bone does not heal properly. It grows to become highly porous and has very low density, which paves way for bone atrophy. [2]

There are several problems that arise due to stress-shielding. The bone may lack proper alignment and density due to uneven stress distribution. Chances of refracture occurring become higher, and one would have to opt for a revision surgery.

The stress-shielding can also lead to aseptic loosening of the implant inside the body. [3] This is the failure of a proper bond formation between the implant and the bone tissue. It occurs due to several reasons like improper mechanical fixation or wear and tear, resorption of the bone, and inflammation due to osteolysis.

1.3.2 Inflammatory Response

The inflammatory response is basically a defense mechanism that flares up when the body is exposed to a foreign species or a biomedical device such as implants and pacemakers. [4]

1.3.3 Biocompatibility

Biocompatibility is one of the most significant properties attributed to biomaterials and one that plays a huge role in the success rate of biomedical implants. In simple words, biocompatibility can be described as the ability of a material to perform with an appropriate host response from the body in a specific application. This is not to be confused with bio-inertness. The term 'biocompatibility' has come a long way in terms of research. Instead of wanting to introduce a material into the body that is completely passive, it is better to introduce one that initiates a positive response from the body. When addressing bioimplants in particular, if the body generates a suitable response, there will be an increase in protein attachment to the implant, therefore leading to cell attachment, and in turn, tissue regeneration. This will facilitate the bone healing process and speed it up as well.

1.3.4 Bioactivity

Bioactivity is the property of a material that elicits a relevant response when the material is exposed to a biological stimulus. Unlike inert materials, they can interact with their surroundings and act accordingly. A popular class of bioactive

materials are bioceramics, specifically calcium phosphate ceramics. This will be elaborated upon in the upcoming sections.

1.3.5 Osseointegration

Osseointegration is the structural and functional linkage formed between the bone and the implant surface such that the bone tissue connects and integrates with the implant. When the bone heals completely, the osseointegrated implant is anchored to the bone, so there is no movement which can potentially cause harm to the fixation of the implant. [5]

1.4 Objectives

The main objective of this project is to coat the Titanium foam samples with hydroxyapatite to impart higher biocompatibility and make them more biomimetic, hence leading to better structural anchoring between the implants and the bone which will reduce the need for revision surgeries in the future.

1.4.1 Hydroxyapatite Coating on Titanium Foams

Hydroxyapatite is the inorganic phase of the bone and is a highly biocompatible ceramic material that promotes osseointegration between the bone tissue and the implant. In biological media, hydroxyapatite reacts with the ions present in the body fluid and forms a surface apatite coat which induces protein adsorption and cell attachment and leads to bone formation and resorption of the biomaterial, hence promoting osseointegration.

According to literature study, hydroxyapatite-coated implants show faster healing and bone attachment as compared to non-coated implants by altering the surface properties. This is because it is the most stable form of calcium phosphide found in the human body, hence it makes implants more biomimetic and reduces the need for revision surgery. Similar is the case with titanium implants when they are coated with hydroxyapatite.

1.4.2 Biomimetic Nature of Hydroxyapatite

Hydroxyapatite has the empirical formula $Ca_5(PO_4)_3(OH)$, which means its calcium to phosphorus ratio is 1.67, which is comparable to that of the bone

apatite. It gives bones rigidity and has load-bearing properties. Therefore, it is biomimetic, and this property has a very positive effect on the osseointegration properties of the implant. It has the ability to integrate in bone structures and assists in the growth of the bone without dissolving or breaking down. When implants are coated with hydroxyapatite, there are lesser chances of rejection and this accelerates the healing process.

1.4.3 Evaluation of Coating Parameters for Implant Efficiency

There are numerous coating parameters that can be studied to determine the efficiency of the implant. In our case, we took into consideration the surface roughness of the coating, the hydrophilicity of the coated implants, and the surface topography.

1.4.3.1 Surface Roughness

Surface roughness is defined as the amount of texture present on a surface. We can quantify surface roughness by calculating how much deviations a surface has from that of an ideal one. Large amounts of deviations indicate a rougher surface, and vice versa. Usually, the surface roughness value of a sample is the arithmetic mean of all the deviations of the surface from the mean line. It is typically measured in microns (μm) or micro-inches (μ-in).

1.4.3.2 Hydrophilicity

In Layman terms, hydrophilicity can be defined as the ability of a surface to attract water. Hydrophilic materials have a high surface energy; therefore they attract water and increase the overall wettability. Hydrophilicity is not limited to water only. Any liquid that wets a surface adequately is called hydrophilic. The contact angle formed in hydrophilic materials is always less than 90°. The smaller the angle, the more hydrophilic the surface of the material and vice versa.

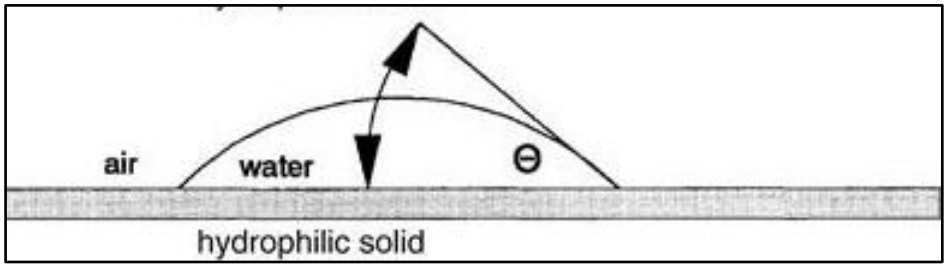

Figure 1: Schematic diagram of a Hydrophilic Substance

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

The major emphasis of this chapter is the elaboration on mechanical and biological properties of Titanium metal, the types and synthesis methods of metallic foams specifically titanium foams. The chemical composition, properties and synthesis methods of Hydroxyapatite have also been discussed in detail. A brief description of the coating deposition methods along with their advantages and disadvantages is provided. Furthermore, the reasons for employing the Pulsed Laser Deposition (PLD) technique for Hydroxyapatite coating on Titanium foams have been enlisted and described.

2.2 Titanium Metal

Titanium is a transition metal having a shiny silver appearance. It is lightweight, strong and possesses significant corrosion resistance properties. It occurs naturally in the form of oxides in mineral ores including rutile, ilmenite, and leucoxene. The major constituents of these mineral ores are listed in the table below:

Table 1 - Titanium Ores

Mineral Ore	**Constituents**
Rutile	TiO_2, Cr_2O_3, Fe_2O_3, SiO_2, ZrO_2, V_2O_5
Ilmenite	$FeTiO_3$
Leucoxene	Ilmenite, rutile, and titanite

These ores are present in rocks, soil, and water bodies. After mining and comminution (crushing, grinding etc.) of the ore, it is concentrated and separated into the useful mineral ore (concentrate) and waste/residue called tailing. Then, titanium is commercially extracted from the concentrate via the Kroll process. Hunter process is also used. [6]

The extraction process consists of heating the rutile ore with chlorine and coke at 900^0C to yield titanium tetrachloride ($TiCl_4$) followed by its reduction with liquid magnesium which results in the production of pure spongy titanium metal. The reduction reaction is carried out in a stainless-steel crucible at 800-900^0C. Titanium foam/sponge is then further processed in vacuum to form solid and homogenous titanium ingots.

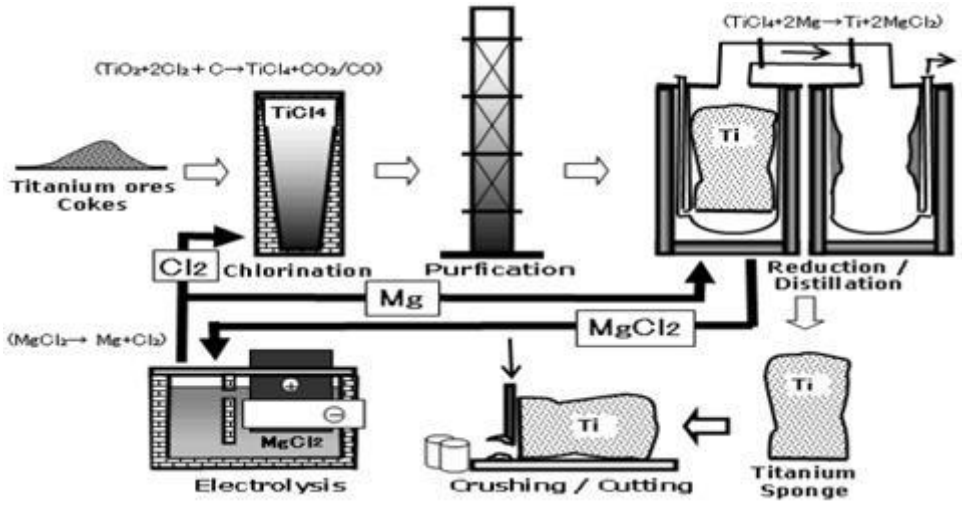

Figure 2 - Kroll Process for Extraction of Titanium

Some important physical, chemical, and atomic properties of titanium metal are listed below:

Table 2 - Physical and Chemical Properties of Titanium

Properties	**Value**
Atomic Mass	47.867 Daltons
Atomic Number	22
Density	4.5 g/cm^3
Color	Silvery white or greyish
Melting Point	1668^0C
Boiling Point	3287^0C
Crystal Structure	hcp (hexagonal close packed)

2.2.1 Mechanical Properties of Titanium

Mechanical properties refer to the behavioral response of materials under the application of load. These properties mainly include strength, elasticity, hardness, fatigue, fracture toughness, creep etc. [7] Titanium has versatile mechanical properties which are favorable for its use in the biomedical industry. Some of the important mechanical properties of titanium are mentioned in the following table.

Table 3 - Mechanical Properties of Titanium

Mechanical Properties	**Value**
Young's Modulus (E)	116 GPa
Vickers Hardness	830-3420 MPa
Brinell Hardness	716-2770 MPa
Tensile Strength	240-450 MPa
Tensile Strength/Density	$107x10^{6}$ $Nmkg^{-1}$
Fracture Toughness	28-108 MPa
Poisson Ratio	0.32

2.2.2 Biological Properties of Titanium

Titanium and its alloys fall in one of the categories of metallic materials which have been approved by U.S. Food and Drug Administration (FDA) to be used safely as part of bioimplants. Besides having excellent mechanical properties, titanium and its alloys also possess superior properties when employed as implants in physiological media. Some of these important properties are elaborated below:

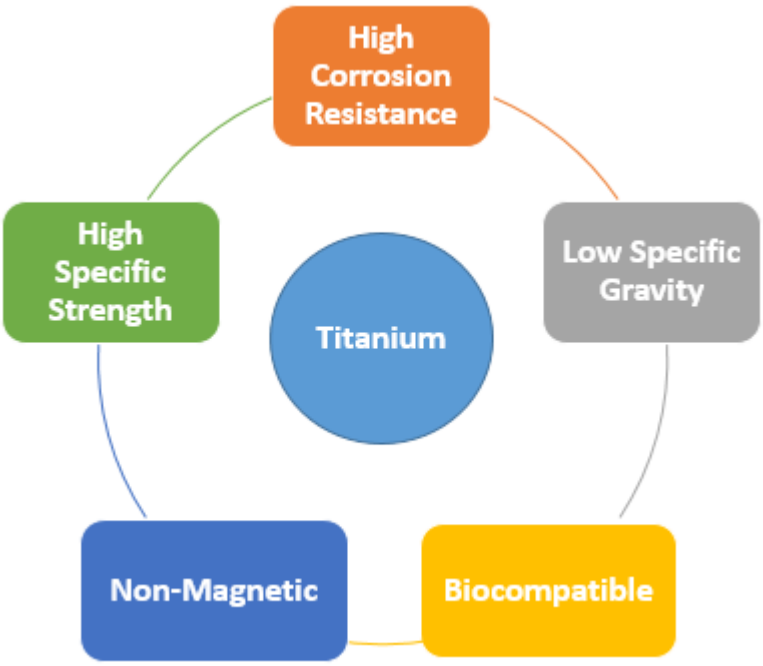

Figure 3 - Advantages of Titanium Implants

2.2.2.1 Biocompatibility

The term biocompatibility refers to the ability of a material which enables it to bind and coordinate naturally with the surrounding living tissues and cells without causing any toxicity, inflammatory or immunological response in the tissues. Titanium is regarded as a highly biocompatible material as it does not produce any negative responses when it comes in contact with the bodily fluids. Studies have shown that titanium, when placed in simulated body fluids, does not support the formation of free radicals which are detrimental to tissue cells. [8]

2.2.2.2 Bio-inertness and Corrosion Resistance

Biological fluids are considered to be aggressive fluids because they contain various enzymes, ions, proteins, and blood cells etc. Many materials cannot tolerate such aggressive contact. But there are certain materials which are inert towards these fluids.

Bioinert materials are those materials which do not react or combine with biological tissues to generate any response, either positive or negative. Titanium is bioinert to a large extent as it does not participate in any adverse reactions

within the body. [9] Moreover, titanium is said to have excellent corrosion resistance properties due to the formation of titanium oxide layer on the surface when exposed to atmospheric conditions. This oxide layer provides protection to the metal beneath it and protects it from further corrosion. Titanium and its alloys when immersed in simulated body fluids, show a very low rate of corrosion as opposed to other metallic materials. [10]

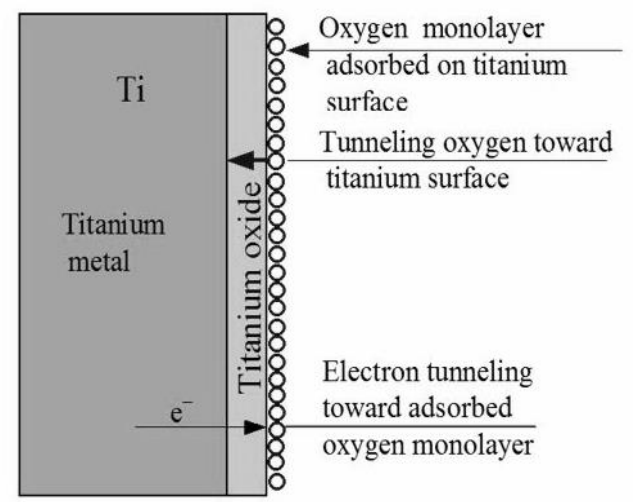

Figure 4 - Oxide Film Formation on Titanium

2.2.2.3 Low Density

Titanium and its alloys have a low specific density as compared to other metals and alloys like stainless steels and cobalt based alloys. This low-density feature enables it to be used as orthopedic implants because its density closely resembles that of the bone as compared to other metallic implants. [11] Titanium has a density of 4.5 grams per cubic centimeter. Whereas the density of cortical bone lies between 1.6 to 2 grams per cubic centimeter. This indicates that there is no significantly drastic difference between the densities of titanium and the bone tissue. This makes titanium a biomimetic material in terms of physical properties i.e. it imitates some properties of natural bone.

2.2.2.4 Low Young's Modulus

Another important property which has a significant influence on the functionality of implants is Young's Modulus or elastic modulus which is correlated with the stiffness of the material. The density of titanium is

comparable to that of the bone modulus. Although bone's modulus is much lower, titanium's modulus is in close proximity to it when compared with moduli of other materials. [12]

A comparison between moduli of cortical bone and some important classes of metallic biomaterials is given in the following table.

Table 4 - Young's Moduli of Biomaterials and Human bone

Materials	**Young's Modulus (GPa)**
Cobalt based Alloys	240
Stainless Steel (316L)	200
Ti Alloys	105-125
Mg Alloys	40-45
Cortical Bone	10-30

The closeness in the moduli of cortical bone and titanium eliminates the possibility of stress-shielding effect. This effect occurs if the stiffness of the implant is way larger than that of the bone. So, the implant tends to bear all the load and shields the bone from all the stress, causing a halt to proper bone growth (which essentially grows in response to the stress applied to it), resulting in loss of implant functionality. Titanium, however, does not withstand all load by itself because of its low density and elastic modulus and continually transmits the load to the bone preventing bone atrophy i.e. rate of bone resorption becomes greater than rate of bone formation. [13]

2.2.2.5 Osseointegration and Osteoconduction

Due to superb biocompatibility and surface properties of titanium, it shows two extremely important phenomena for successful and functional implantation. These phenomena are osseointegration and osteoconduction. The term 'Osseo' refers to bone. In orthopedics, osseointegration means the anchoring of the implant with the host tissue by the formation of a fibrous interface between the implant surface and the tissue. This anchoring promotes an efficient structural, functional, and biological attachment between the implant and the living tissue and allows for unhindered relative movement. Titanium exhibits this phenomenon. [14]

Furthermore, it also possesses a property called osteoconduction, which means that the bone starts growing at the implant surface due to its high biocompatibility and bioactivity.

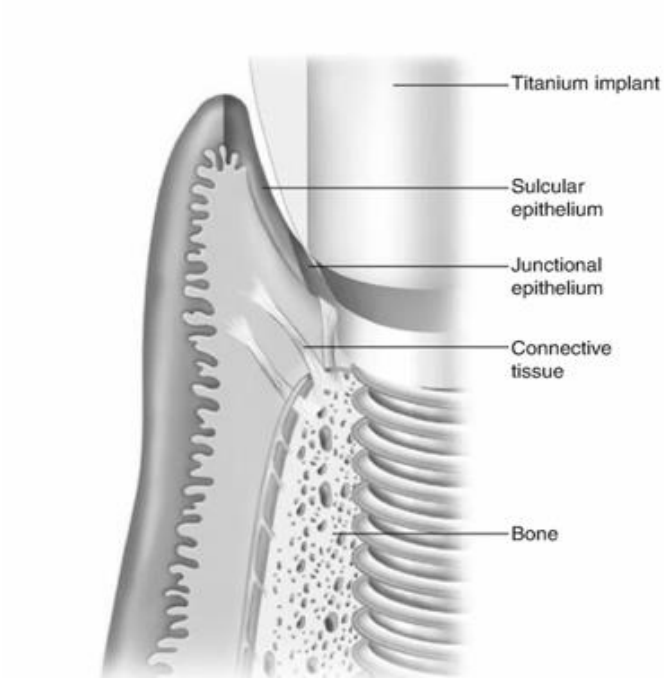

Figure 5 - Osseointegration Phenomenon

2.3 Metallic Foams

Metallic foams are defined as spongy porous metals having a large proportion of air spaces or voids which are evenly distributed throughout the material. The key feature of these materials is the presence of a large number of pores typically

75-95% of the overall volume of the material while the remaining 5-25% is the metal. Some of the basic physical and chemical properties of the base metal are retained by the foams. The presence of porosity imparts a blend of useful properties to the material for different applications.

However, foams show immensely different mechanical behavior compared to the base metal. The deformation and fracture mechanisms, tensile strength, elastic modulus, fracture toughness etc. differ greatly from that of the base metal. [15]

2.3.1 Types of Metallic Foams

Based on the nature of the pores/voids and the manner of the distribution of the porosity, there are four basic types of metallic foams. Those types are briefly described below:

1. **Open-Cell Foams:**

   These foams contain open pores which are connected to each other and have a maze-like connection between them. The major fabrication methods used to produce these foams include powder metallurgy and polyurethane casting.

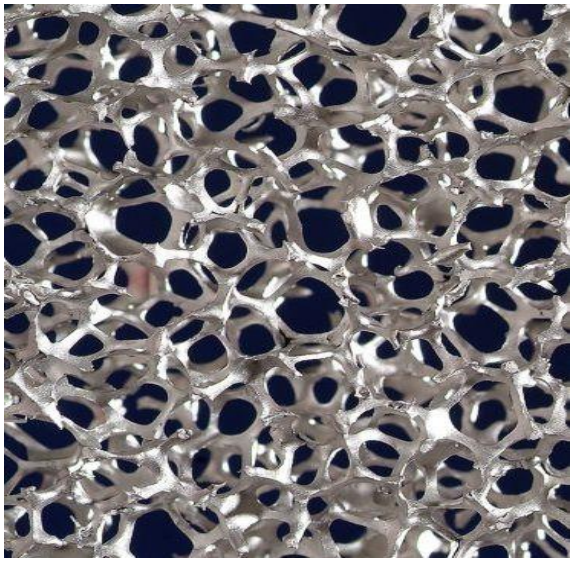

Figure 6 - Open-Cell Foam

2. **Closed-Cell Foams:**

In these foams, the porosity is not interconnected, and each pore is closed and encapsulated with a wall of the base metal. These foams can be fabricated by introducing gas bubbles within the molten metal, forming gas within the melt by adding gas-releasing agents or by the evolution of gas dissolved in the melt. [16]

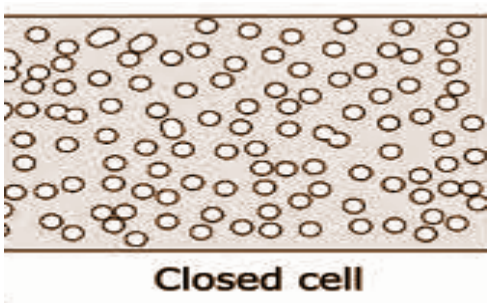

Figure 7 - Closed-Cell Foam

3. **Stochastic Foams:**

The foams containing unevenly dispersed porosity are called stochastic foams. The distribution and order of pores depends on the fabrication technique used. These foams are manufactured by Chemical Vapour Deposition, solid or liquid foaming or casting.

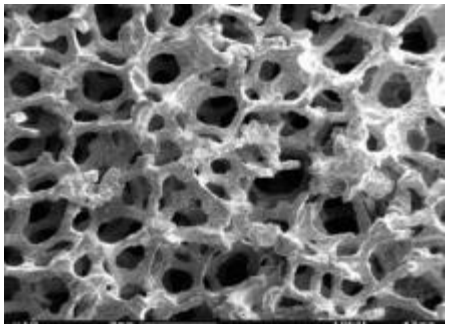

Figure 8 - Stochastic Foam

4. **Regular Foams:**

Regular foams, as the name suggests, contain evenly dispersed and ordered pores. Fabrication methods generally include additive manufacturing, casting, direct molding etc.

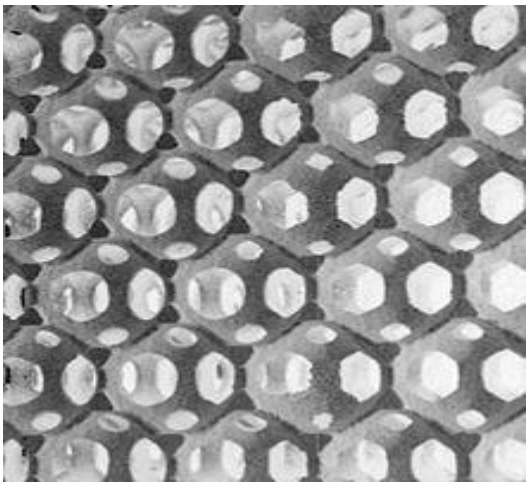

Figure 9 - Regular Foam

2.3.2 Mechanical Properties

The mechanical properties of metallic foams are significantly different from those of the metal from which the foams are fabricated. The major mechanical properties which change include Young's modulus, stiffness, toughness, creep, and fatigue mechanisms. The elastic modulus, density, toughness, and fracture strength decrease largely upon foaming of metals. These alterations in properties are tremendously important in certain applications where reduction in specific density and modulus is required, such as orthopedic implants.

2.3.3 Applications of Metallic Foams

The important applications of metallic foams are listed below:

- Metallic foams are used in structural applications where relatively high strength is required at a lower density.
- They are used in shock/impact absorbing devices as well as acoustic dampers.
- Catalysts are generally metallic foams to provide for large surface area with minimal usage of material.

- Filtration devices also employ metallic foams.
- They are widely used in implants and prosthetic devices.
- They are also used in heat exchangers and heat sinks etc. [17]

Figure 10 - Metallic Foam Hip Implant

2.3.4 Titanium Foams for Bioimplants

Titanium foams are regarded as one of the most biocompatible and effective materials available. These foams contain open interconnected pores which makes them highly beneficial for tissue regeneration as the interconnectivity of pores allows for better vascularization i.e. growth of veins within the tissue growing on the implant for provision of nutrients to the newly forming tissue.

Additionally, the primary reason why titanium foams are utilized for implantation purposes is that the presence of open-cell porosity leads to the reduction of the specific density of the implant as well as a decrease in its stiffness, which is highly favorable to get its mechanical properties closer to that of the bone. [18]

2.3.5 Synthesis Methods of Ti Foams

Titanium foams are synthesized by a number of different methods, but these methods are primarily categorized broadly as the powder metallurgy synthesis route and the chemical or physical synthesis method. [19]

2.3.5.1 Powder Metallurgy Route:

In this fabrication technique, titanium or titanium alloy powder is milled to attain uniformly sized particles. Some space holder materials such as sugar, tapioca starch, salt or acrawax etc. are used to create voids within the matrix. The space holding materials are used in the form of beads or agglomerates and are mixed in a required proportion into the metallic powder. Then, the powder is compacted and sintered. The thermal degradation or burning of the organic space holding material leads to the creation of the desired pores in the metal matrix. [20]

Other powder metallurgy processes involve the expansion of pressurized gas bubbles within the metal powder mixture during the sintering process.

2.3.5.2 Other Fabrication Methods

Titanium metal, when in molten state, is highly reactive and tends to readily react with the oxygen or other gases present in the atmosphere, thus its processing at high temperatures is generally avoided or is done in high quality vacuum conditions. Titanium foams are thus mostly fabricated using solid-state processes. However, some superplastic expansion methods involving sudden and drastic temperature changes are also employed to obtain high porosity titanium foams.

2.3.6 Scale and Percentage of Porosity

The scale and percentage of porosity in titanium foams varies with the processing methods employed, types of pore-creating materials used, their relative amounts and the process conditions.

Some studies carried out to determine the effect of the space holder concentration on the total volume of porosity produced in the metal show that

the space holder concentration or volume % is directly proportional to the amount of porosity formed. [21]

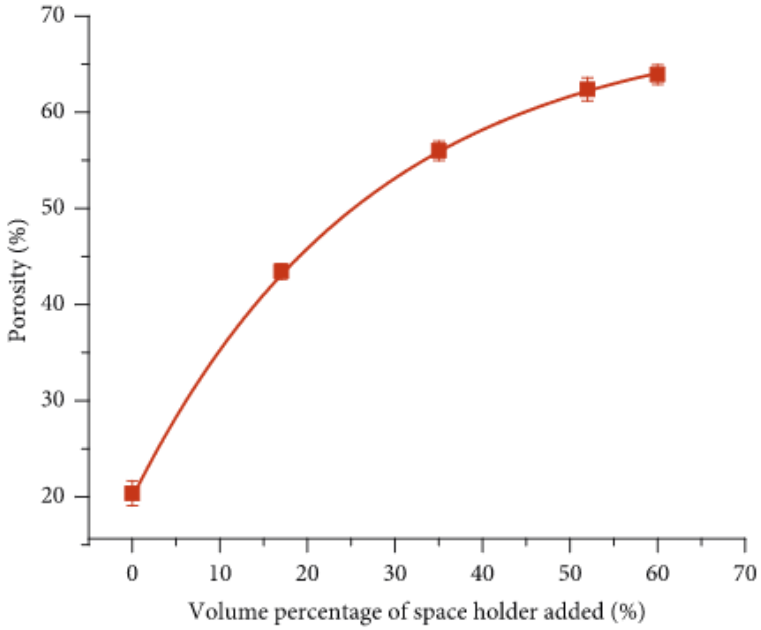

Figure 11 - Effect of Space holder volume on % Porosity

2.4 Hydroxyapatite

Hydroxyapatite is an inorganic ceramic material, chemically composed of calcium and phosphate, that has properties and composition resembling that of the bone apatite. It occurs naturally in human bone and tooth tissues in a modified form.

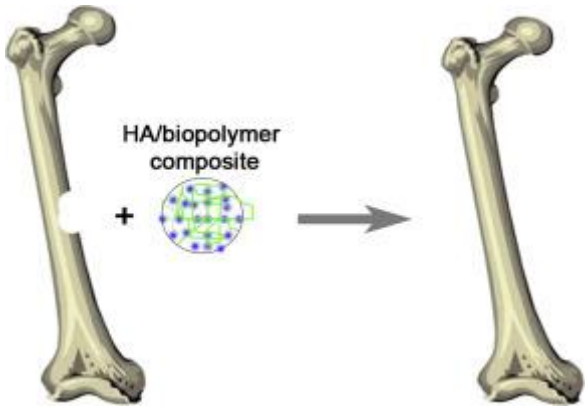

Figure 12 - Hydroxyapatite-Bone Mineral

2.4.1 Chemical Composition of HA

Hydroxyapatite is mainly composed of calcium, phosphorus, oxygen, and hydrogen. It constitutes a major part of the human bones as part of the cortical bone tissue. It is also found in dental enamel and dentin. Hydroxyapatite is basically a derivative of apatite which are phosphate-based minerals, with hydroxyl group attached to it. It has a chemical formula $Ca_{10}(PO_4)_6(OH)_2$.

2.4.2 Properties of HA

Hydroxyapatite has superior biological properties like high biocompatibility, excellent bioactivity, osseointegration and osteoconductive properties. It can be used as bone replacement and repair material. Some of the important properties of hydroxyapatite are given below:

Table 5 - Properties of Hydroxyapatite

Properties	**Value**
Empirical Formula	$Ca_5(PO_4)_3(OH)$
Formula Mass	502.31 g/mol
Color	White
Crystal Structure	Hexagonal
Moh's Hardness	5
Density	3.14-3.16 g/cm^3
Tensile Strength	18 MPa
Compressive Strength	174 MPa

Young's Modulus	6 GPa

Hydroxyapatite is a well-suited material for biomedical implantation purposes, but it has some limitations to it. One of these limitations is a low strength. As it is originally a ceramic material, it performs poorly under tensile loads and is highly brittle. Moreover, it has poor toughness. It also has difficult formability.

However, it is one of the best candidates for implant coatings as it imparts higher biocompatibility, bioactivity and makes the implant more biomimetic in terms of composition and properties like strength, stiffness, and Young's Modulus.

2.5 Synthesis of Hydroxyapatite

Although hydroxyapatite is a naturally occurring bone mineral, it can also be synthesized artificially by a number of different methods which are mostly chemical methods. Some of these methods are explained in the following sections.

2.5.1 Methods

There are three basic methods of hydroxyapatite synthesis, including hydrothermal method, solvothermal method, homogeneous/wet precipitation method. These methods are devised to obtain uniformly shaped HA particles having homogeneous composition, narrow particle size distribution, no agglomeration and higher surface areas. [22] These methods are briefly described below:

2.5.1.2 Hydrothermal Method

This method involves the use of an airtight autoclave maintained at a high temperature and pressure with the use of different reactants to produce fine grained hydroxyapatite. Different precursors or reactants are used depending on the material availability, type of hydroxyapatite they produce and the ease of handling of the materials. In this process, the reactant solutions are mixed together in the required proportions and are then fed into the autoclave at

specified temperature for a predetermined time (different for different reactants). After the formation of required HA particles, the solution is filtered, and HA particles are washed and dried.

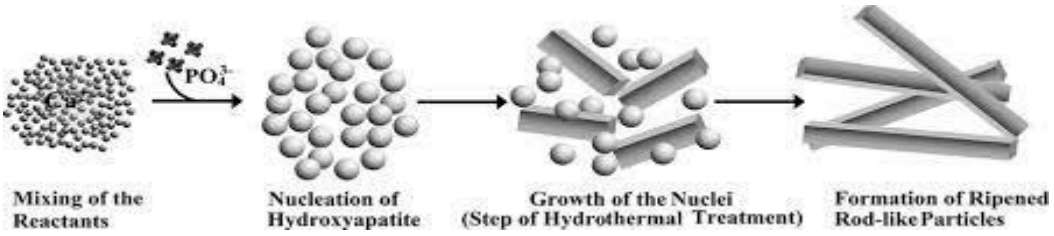

Figure 13 - Hydrothermal Method

The reactants may be $Ca(EDTA)^{2-}$ and $NH_4(2HPO_4)$, phosphogypsum waste (PG) and potassium dihydrogen phosphate (KH_2PO_4) or $Ca(NO_3)_2.4H_2O$ and $(NH_4)_2HPO_4$ etc. The process does not require high temperature post treatments.

2.5.1.2 Solvothermal Method

In this method, an autoclave is used at a certain temperature and reaction conditions to form hydroxyapatite using Calcium citrate and phosphoric acid solution. The aging of the solution gives a gel which in turn is dried and calcined to give nanosized crystalline hydroxyapatite particles.

2.5.1.3 Wet/Homogeneous Precipitation Method

This method is carried out at a specific temperature and ph. The Calcium-based salt and phosphorus-based acid is formed into a solution and mixed to attain a certain basic pH after which the mixture is continuously stirred. Precipitation occurs and after a few hours the solution is filtered, and Hydroxyapatite particles are dried and calcined at specified temperatures.

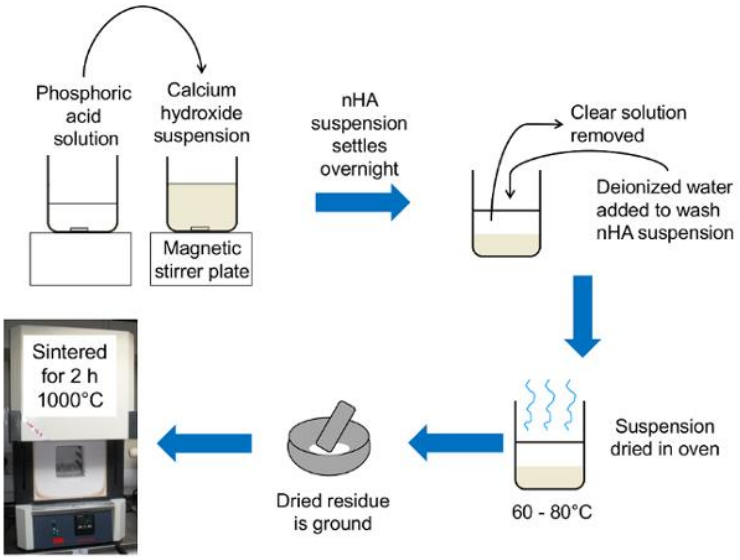

Figure 14 - Schematic Diagram of Wet Precipitation Method

CHAPTER 3

METHODOLOGY

This chapter is solely focused on the methodology that has been designed and implemented to carry out the process of Hydroxyapatite synthesis, its characterization, and the preparation of titanium foam specimens for coating with Hydroxyapatite via Pulsed Laser Deposition.

3.1 Lab Scale Synthesis of HA

Hydroxyapatite has been synthesized using the wet/homogeneous precipitation method. The materials used, the equipment, apparatus and the process is explained in this section.

3.1.1 Materials Utilized

- Calcium Hydroxide $Ca(OH)_2$
- Phosphoric Acid (H_3PO_4)
- Deionized Water
- Aluminium foil

3.1.2 Apparatus and Equipment

- Round bottom Flask
- Thermometer
- Hot plate
- Clamping stand
- Condenser tube
- Beaker
- Magnetic stirrer
- Centrifuge machine

- Test tubes
- Petri dish
- Drying oven
- Ceramic crucible
- Spatula
- Mortar and pestle
- Muffle furnace

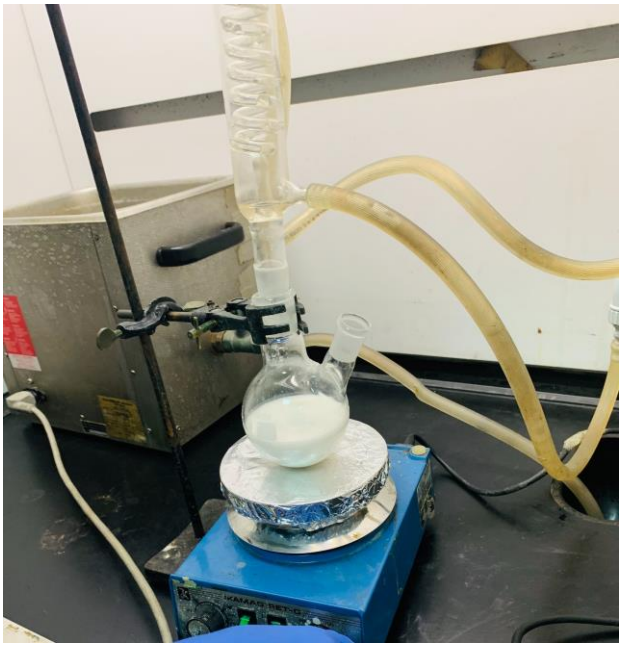

Figure 15 - Apparatus for HA Synthesis

3.1.3 Procedure:

The procedure followed for the wet precipitation method for HA synthesis is explained as follows:

- The precursors used were calcium hydroxide, phosphoric acid, and deionized water. The process began with the preparation of a solution of 8.17 grams calcium hydroxide in 30ml deionized water. The solution was added in a round bottom flask, which was then fixed to sit on a hot plate maintained at 100^0C.

- To keep the water from evaporating, a condenser tube was fixed at the opening of the flask. A thermometer was inserted to monitor the temperature simultaneously. The solution was continuously stirred using a magnetic stirrer for an hour. The whole apparatus was placed in a fume hood to ensure safety.
- In a measuring tube, 9.1ml of phosphoric acid was added in 90.9ml of deionized water to form a 100 ml solution. Then, 5ml of the acid solution was added to the round bottom flask and the pH was determined using pH strips.
- The initial pH was 13. The process continued with the addition of 5ml of acid solution into the flask at 5 minutes' intervals followed by pH determination at each step until the pH reached a value of 10. At this pH, HA formed has a Ca/P ratio of 1.67 which is comparable to that of the bone apatite. The change in the pH at each interval is shown in the following table.

Table 6 - pH for Amount of Acid Added in each Interval

Amount of Acid Added	pH
5	13
10	13
15	13
20	13
25	12
30	12
35	12
40	11
45	11
50	10

- The figure shows the pH strips used.

Figure 16 - pH Strips for each acid-addition interval

- Then, sufficient time was allowed for precipitation to occur. i.e. nearly 3-4 hours. Hydroxyapatite precipitates as a result of the following chemical reaction.

$$10\ Ca(OH)_2 + 6\ H_3PO_4 \rightarrow Ca_{10}(PO_4)_6(OH)_2 + 18\ H_2O$$

- The post-treatment consists of separating the HA particles from the solution followed by drying, grinding and calcination.
- Centrifuge machine was used for separation of HA particles. The solution was centrifuged 5-7 times at 40,000 rpm for 10 minutes in each cycle. The product was then put in a Petri dish and dried overnight in an oven at 60^0C.
- After completely drying, it was calcined at 950^0C in a muffle furnace for 4-5 hours. Pure and ultrafine hydroxyapatite powder was obtained by this process.
- Then for the hydroxyapatite coating by Pulsed Laser Deposition, the HA powder was formed into pellets using hydraulic press. The press used a load of 7 tons for 3 minutes to form each pellet. The pellets were around 1cm in diameter and 3-4mm thickness.

3.2 Why Wet Precipitation Method?

The reasons for which wet/homogeneous precipitation method was chosen for HA synthesis are as follows:

- This method is highly recommended for obtaining the required Calcium to Phosphorus ratio (Ca/P) e.g. 1.67 which is comparable to the Ca/P ratio of human bone because there can be a better stoichiometric and pH control in the process.
- The method is essentially simple and does not require numerous precursors and specialized apparatuses like autoclave which is required for the other two synthesis methods i.e. the hydrothermal and solvothermal method.
- There are less process control parameters involved in the process i.e. only temperature and pH which are relatively easier to control and monitor. Also, the process does not require very high temperatures for synthesis.
- The apparatus and reagents for the process are readily available and are low cost. So overall, the process is economical.
- The crystallinity and fineness of the HA particles produced by this method are also comparatively better. [23]

3.3 Characterization of HA

The characterization of Hydroxyapatite powder was done for its identification, determination of the presence of phosphate functional groups and observation of the morphology of the particles and presence of agglomeration in HA powder. The characterization techniques and their modes utilized are elaborated in the following subsections.

3.3.1 Fourier Transform Infrared Spectroscopy (FTIR)

Fourier Transform Infrared Spectroscopy (FTIR) is a technique which is essentially used for the identification of functional groups present in a molecule depending on the vibrations induced, energy absorbed or emitted in a certain functional group when it is exposed to infrared radiations. It is generally

determined in a wavenumber range 4000-400 cm^{-1} of the infrared light. It is recorded in terms of percentage transmittance i.e. the amount of infrared radiation absorbed by the material with respect to a reference, and the wavenumber (cm^{-1}).

The main elements of an FTIR spectrometer include a source, interferometer, detector, amplifier, and a software to generate results. The source generates infrared radiations and the interferometer directs them and measures the amount and wavelengths of the incoming radiations falling on the sample. The light either is absorbed or transmitted through the sample and is detected by the detector which then amplifies the signals. The amplified signals are in turn converted into the required format and a software generates an IR spectrum for that material.

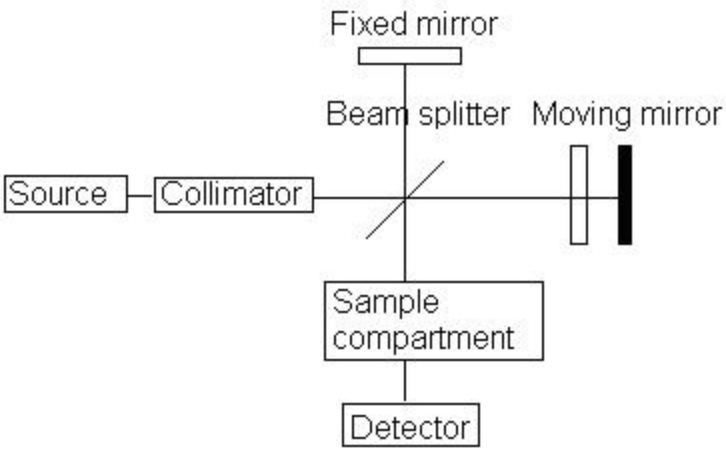

Figure 17 - Schematic Diagram of FTIR Spectrometer

The generated FTIR spectrum is then compared with the database for different materials as a particular peak at a certain wavenumber corresponds to a specific functional group. [24]

FTIR spectroscopy has been employed in this process for the detection of the functional groups that are characteristic of hydroxyapatite. The major functional group which is crucial to its detection is the phosphate group. Other functional groups like hydroxyl groups are also detected.

3.3.2 Scanning Electron Microscopy

Scanning Electron Microscope is an instrument which uses an electron beam to scan and image a conductive material for visualizing the topography and determination of the sample composition. This identification is based on the principle of secondary electron emission as a concentrated electron beam hits a specimen. [25] The number of secondary electrons produced is measured in terms of intensity which is then converted into a 2D image. The information regarding the secondary electrons is also used for the determination of composition of a material as the number of secondary electrons produced is different for different materials.

A typical arrangement of a scanning electron microscope consists of an electron source which ejects high energy electron beam, condenser/focusing lenses to focus the beam at a spot, the sample compartment and a detector which detects and amplifies the signals. The whole chamber is evacuated with vacuum pumps to attain high vacuum. Vacuum is necessary for this microscopy as the presence of air molecules may deflect the incoming electron beam and electrons may ionize the air molecules and which may diminish the propagation of the electron beam.

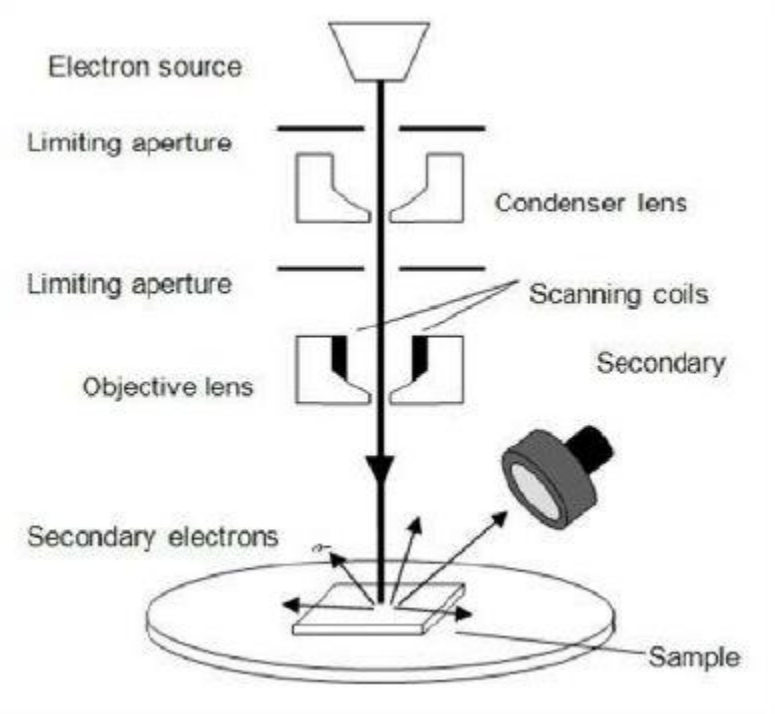

Figure 18 - Schematic of Scanning Electron Microscope

In this study, SEM has been employed to examine the topography of the Hydroxyapatite powder synthesized, to determine the morphology of the ultrafine particles and checking for signs of any agglomeration present.

The sample preparation for SEM is of paramount importance. The samples must be small and conductive to allow the flow of electrons through its surface. [26] In this case, the hydroxyapatite powder was sprinkled on a carbon conductive tape which was attached to a conductive stub, the stub was then grounded when placed in the sample holder to avoid charge build up.

3.3.3 Energy Dispersive Spectroscopy/ Energy Dispersive X-Ray Analysis

This technique is used in parallel with the Scanning electron microscope. Its principle is based on the generation of x-rays when an electron is knocked out of the energy levels of an atom. These x-rays emitted are characteristic of a specific material.

The electron beam, when bombarded on the specimens, generates secondary electrons, backscattered, or reflected electrons and x-rays. X-rays are produced

when secondary electrons are ejected as a result of bombardment by high energy electrons. [27] These x-rays are detected and quantified by a detector. These signals are used for chemical analysis and determination of the amounts of certain chemical elements present in the material. [28]

The apparatus is quite similar to the scanning electron microscope with additional x-ray detectors and amplifiers attached to it. These detectors are mostly made of silicon wafers.

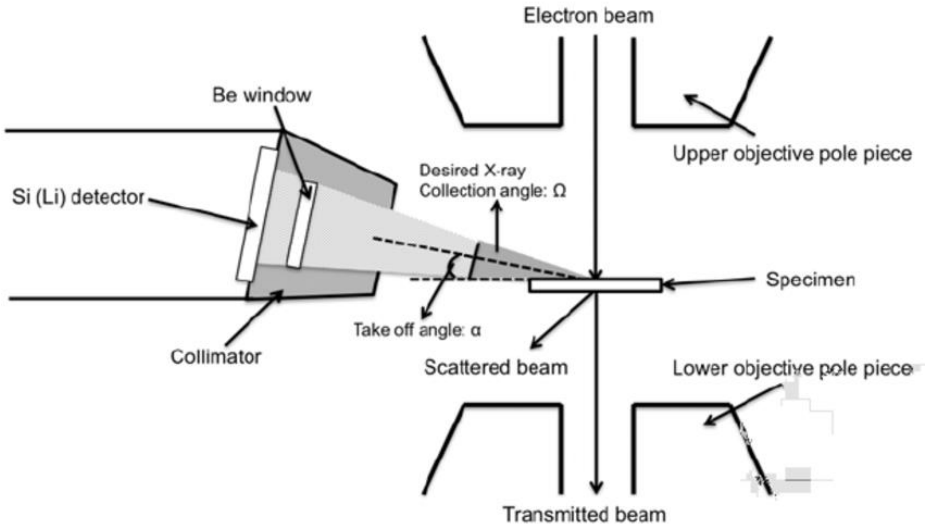

Figure 19 - Energy Dispersive Spectroscopy

In this project, EDS has been used for the detection of major elements present in hydroxyapatite samples like calcium, oxygen, phosphorus etc. The EDS spectrum is plotted as x-ray count on the x-axis and kilo-electron volt energy on the y-axis. And each peak at a certain value of energy signifies a particular element.

3.3.4 Raman Spectroscopy

Raman Spectroscopy is a characterization technique that is particularly utilized for the determination of chemical structure of molecules, phases, and crystallinity of materials. The basic principle of this technique is the vibrational movements of molecules when they absorb energy of incident photons.

Basically, the process involves the scattering of light as it falls on molecules and the electromagnetic fields of photons induce vibrational motion within the bonds of the molecule. Mostly, the energy of incident and scattered photons remains

the same. However, in some interactions, the photons impart some of their energy to the molecular bonds and they tend to vibrate. The vibrational modes depend on the structure and geometry of the molecule. This characteristic of molecules is used for the formation of the Raman spectrum. [29]

The spectrum is plotted with Raman shift on the x-axis and Raman intensity on the y-axis. Raman shift is the change in the wavelength of the incident and scattered electron in a Raman scattering.

In this study, Raman spectroscopy is used for the structural and chemical analysis of hydroxyapatite powder.

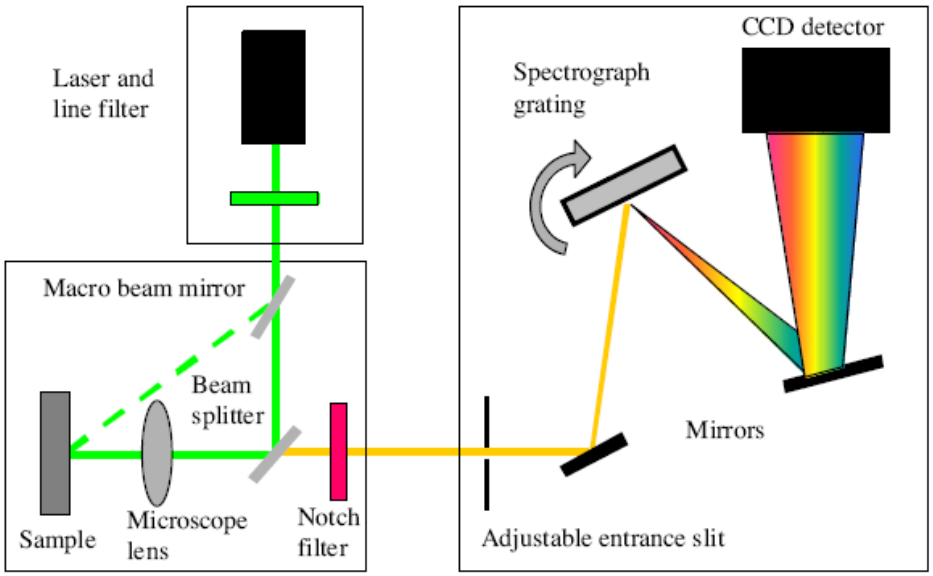

Figure 20 - Schematic Representation of a Raman Spectrophotometer

3.4 Sample Preparation

The titanium foam specimens have been sectioned and prepared for coating with hydroxyapatite via Pulsed laser deposition. The methods adopted for this purpose have been described in the following sections.

3.4.1 Sectioning

Initially, the titanium foams were in the form of elliptical discs which needed to be cut into smaller fragments so that they can be used correctly in the fixture of

the PLD machine. For this purpose, the samples were cut into sections of dimensions:

- Length= 1 cm
- Breadth= 1 cm
- Thickness= 1.99 mm

The Wire Electrical Discharge Machining process was employed for sectioning of titanium foams as it is a hard material which can be difficult to cut using other cutting techniques and tools.

Figure 21 - Titanium Foam Substrates

3.4.2 Wire Electrical Discharge Machining

The Wire Electrical Discharge Machining process consists of a thin conducting wire to which a voltage is applied so that it acts as an electrode, while the workpiece is attached to the opposite potential. The cutting force is provided by the potential difference between the two electrodes which causes an electrical discharge. The wire used is harder than the material which needs to be cut. The process is done in the fluid medium, often deionized water. The cutting mechanism involves erosion of the workpiece material by the sparking due to electrical discharge. The wire is clamped between two wire supply rolls which are fed continuously, and their movement is guided by computer numeric control.

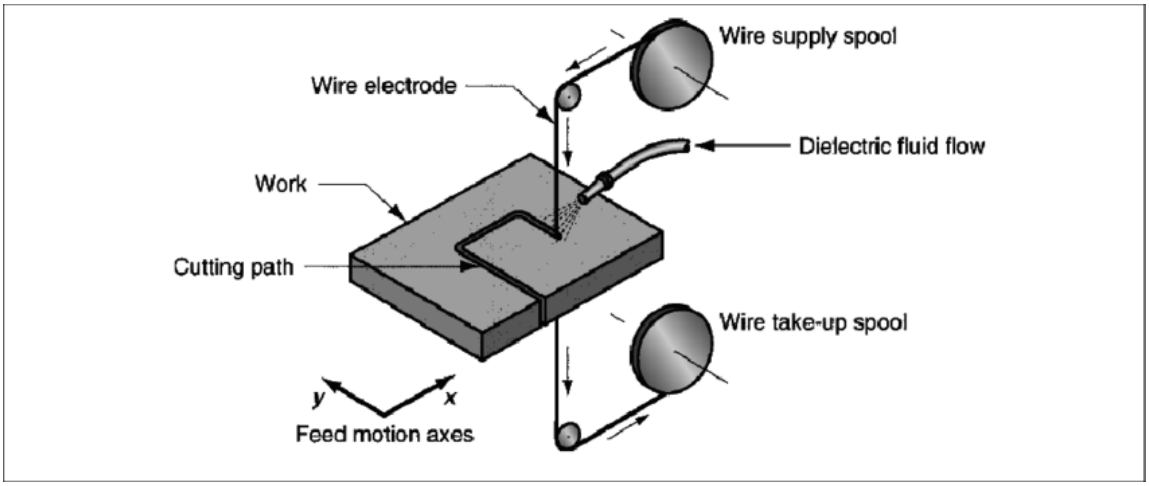

Figure 22 - Illustrative Representation of Wire EDM Process

The reason for using deionized water in the process is that it acts as a lubricant and absorbs the excessive heat and sparks generated by the electrical discharge process which may soften or melt the wire or the specimen. It also flushes away any eroded material particles from the workpiece.[30] Moreover it does not conduct electrical currents due to the mobility of ions in it, thus eliminating any chances of corrosion.

Wire EDM process is used for the cutting of Titanium forms as they are hard enough to be cut by any other cutting technique. The wire used for cutting of Ti foams was a Molybdenum alloy wire which is significantly harder than titanium.

3.4.3 Metallographic Surface Preparation

After cutting of Titanium foam samples into the desired dimensions, the metallographic surface preparation was applied to them. The process consists of grinding the surface with emery papers of different grit sizes, followed by polishing of the samples on a polishing wheel in alumina slurry. The steps followed and important terminology is explained as follows:

- First of all, the samples were ground using 240 grit Silicon Carbide emery paper. The grit size refers to the number of SiC particles per inch of the grinding paper. These papers are available in a range of 60 to 1200 grit sizes. [31]

- Then the samples were ground further on the grinding wheel using 320, 400, 600, 800, 1200 grit size emery papers respectively for 3-5 minutes.
- After grinding, the samples were polished on a polishing wheel for 4-5 minutes. Ultrafine alumina powder slurry was used as a polishing medium.

Figure 23 - Polishing Wheel

- The samples were then washed and dried using an air dryer.

3.5 Coating Techniques

There are many aspects that pave the way for the success of bioimplants and of those aspects is the coating, which in our case is hydroxyapatite. There are numerous methods to coat implants. The following subsections will elaborate on Plasma Spray Coating, Sputter Deposition, and finally, Pulsed Laser Deposition (PLD). [32] The reasons to opt for Pulsed Laser Deposition as our coating method will also be discussed in detail.

3.5.1 Plasma Spray Coating

Plasma Spray Coating is a process in which a plasma jet is generated by passing a gas mixture of argon/hydrogen through a strong electrical discharge that is created between the gap of an anode and a cathode. The release of robust energy heats up the gas mixture swiftly. The fine powder of the coating material is injected into the plasma jet and this leads to the creation of molten drops, which

are then accelerated towards the substrate surface. Once these drops hit the surface, they solidify and form the coating.

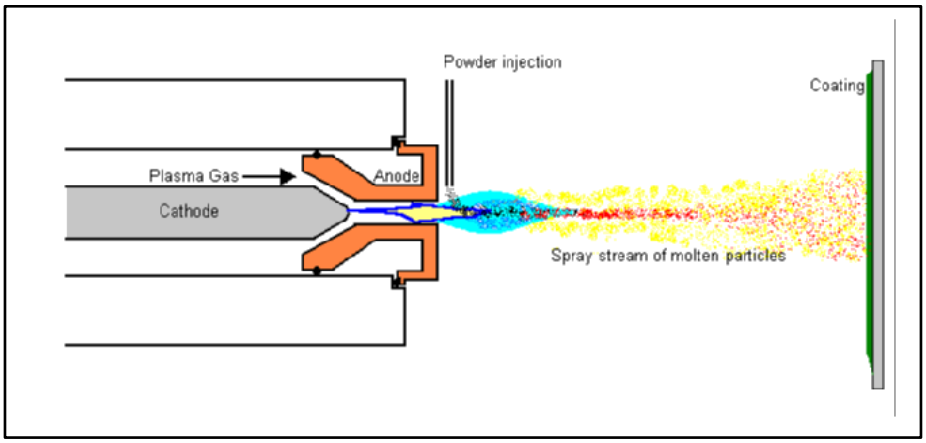

Figure 24 - Plasma Spray Coating Schematic Diagram

The Plasma Spray Coating process is the most widely used technique for hydroxyapatite coatings.

One can achieve high quality coatings that have very little porosity and satisfactory adhesion strength by this process. However, it is an expensive process because the surface needs a lot of preparation before coating (shot peening, grit blasting etc.). Moreover, there are chances of the substrate getting overheated during the process which can alter its mechanical properties.

3.5.2 Sputter Deposition

Sputtering is a thin-film deposition technique. A gas (most commonly argon) is purged into the chamber which is placed under vacuum. A potential is applied such that the target (coating material) has a negative potential and the substrate has a positive potential. This results in the ionization of argon and it gets attracted towards the target. They strike the target so that atoms of the coating material get ejected out due to high number of collisions. These atoms form a vapor stream or a plasma which moves towards the substrate and sticks to it as a coating.

Good control can be exercised in terms of deposition rate as the deposition occurs atom by atom. However, the deposition rate is quite slow, hence it takes a

lot of time to coat the material. The surface also requires pre-cleaning for better adherence of the coating with the substrate.

3.5.3 Pulsed Laser Deposition (PLD)

Pulsed Laser Deposition is a technique that holds a lot of promise for the coating of bioimplants in the future. This process uses a high-intensity pulsed laser to melt, ionize, and evaporate material from the target coating material. This is called ablation. Eventually, a luminous plasma plume is formed that expands towards the substrate where it condenses to form a high-quality thin film.

With Pulsed Laser Deposition, film-thickness can be controlled very accurately, while simultaneously maintaining a high level of purity. The coating formed is very smooth, with little to no porosity, and it adheres to the substrate strongly. Because it uses a laser power source, there are lesser chances of environmental and safety hazards. It is a relatively economical process as it requires no extra surface preparation other than metallography. It is also a clean process because no by-products form during the coating. [33]

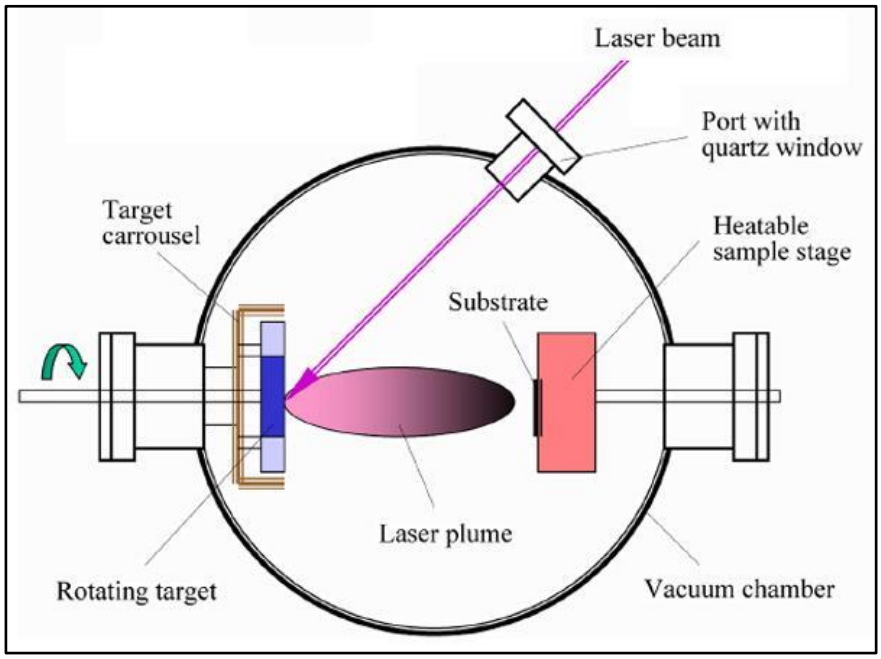

Figure 25 - Pulsed Laser Deposition Schematic Diagram

CHAPTER 4

RESULTS AND DISCUSSION

This section deals with the results obtained from all the characterizations performed on Hydroxyapatite and the coating of HA over Titanium foam substrates.

4.1 Development of HA Coating via PLD

Our prepared Titanium foam samples and hydroxyapatite pellets were sent to the National Center of Physics, Islamabad. Pulsed laser deposition was carried out, and the Titanium foam samples were coated with the Hydroxyapatite.

The target to substrate distance was fixed at 4cm. This is because larger distances generally contribute to more uniform coatings which have a higher quality. The temperature of the substrates (Titanium foams) was kept constant at 400℃. A high temperature was chosen keeping in mind the fact that the films deposited at higher temperatures are more crystalline than the ones deposited at lower temperatures. We desired a more crystalline coating for our substrates. The deposition time was 40 minutes. The reason for choosing this particular deposition time was that slow deposition rates lead to better control of the coating parameters like the coating thickness. The laser energy was 14 mJ, and the whole process took place in a high vacuum of 10^{-7} mbar.

4.2 Testing and Study of Coating Parameters

After the coated samples were collected, its properties were studied carefully to judge how well it would perform as a bioimplant.

4.2.1 Surface Roughness and Coating Thickness

The surface roughness and the thickness of the hydroxyapatite coating were measured using an optical profilometer. It is a type of microscope that uses light instead of a physical probe to measure the deviations from the mean line of a sample which are then converted to the arithmetic mean value of the roughness

(Ra). The light from the lamp source is split into two paths by a beam splitter such that one beam covers the surface of the sample being tested while the other directs the light to a reference mirror.

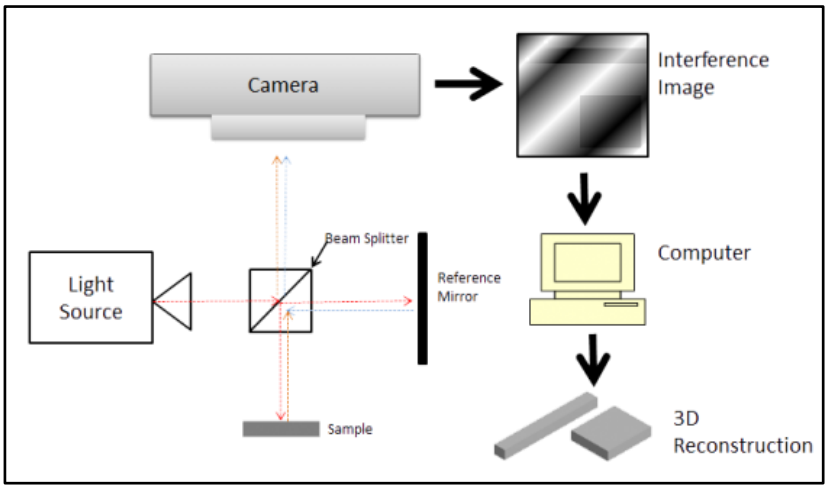

Figure 26 - Schematic Diagram of Optical Profilometer

To measure the surface thickness, we removed half of the HA coating from the titanium sample using tape and ethanol. The sample was then assessed via an optical profilometer. Here the difference in step height was recorded as the coating thickness.

4.2.2 Hydrophilicity

The hydrophilicity of the coating was determined by contact angle measurement, which was done with the help of the Drop Shape Analyzer. The hydroxyapatite-coated Titanium foam samples were placed on the stage and the position of the stage was adjusted so that it was level with the camera and the light source. A sessile drop of water was gently placed on the substrate surface by the tip of a needle that was attached to a syringe. The amount of water and the placement was controlled by the Kruss software. The Infrared light source allowed the camera to record the contours of the drop and give us a grey-scale image that clearly showcased the contact angle. The sample surface is referred to as the baseline and the contact angle is given by the angle between the baseline and the drop function.

4.2.3 Surface Topography

The surface topography of the hydroxyapatite-coated Titanium foams was analyzed via Scanning Electron Microscopy.

4.3 Results of Coating Parameters

The results of the Coating Parameters are discussed below.

The surface roughness of the hydroxyapatite-coated Titanium foams was measured via an optical profilometer and the Ra value obtained was 0.374 microns (µm).

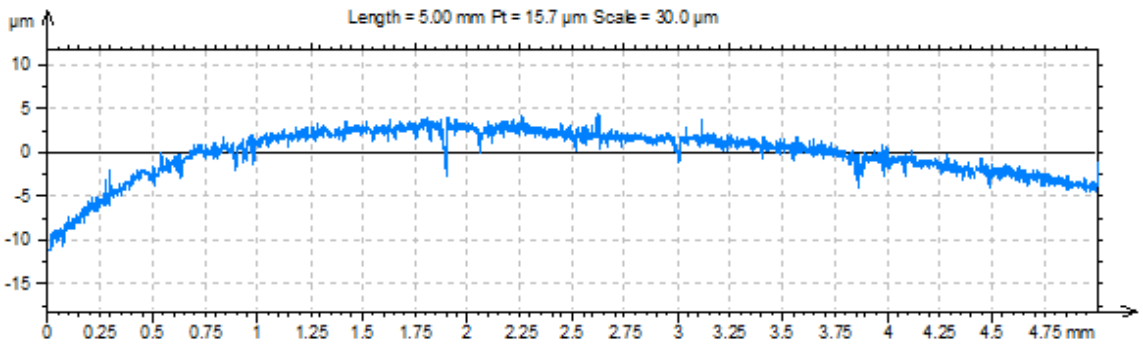

Figure 27 - Step Profile of HA-coated Ti foam

ISO 4287			
Amplitude parameters - Roughness profile			
Rp	2.05	µm	*Gaussian filter, 0.8 mm*
Rv	3.35	µm	*Gaussian filter, 0.8 mm*
Rz	5.40	µm	*Gaussian filter, 0.8 mm*
Rc	1.23	µm	*Gaussian filter, 0.8 mm*
Rt	7.95	µm	*Gaussian filter, 0.8 mm*
Ra	0.374	µm	*Gaussian filter, 0.8 mm*
Rq	0.558	µm	*Gaussian filter, 0.8 mm*
Rsk	-1.38		*Gaussian filter, 0.8 mm*
Rku	10.5		*Gaussian filter, 0.8 mm*
Material Ratio parameters - Roughness profile			
Rmr	0.402	%	*c = 1 µm under the highest peak, Gaussian filter, 0.8 mm*
Rdc	0.655	µm	*p = 20%, q = 80%, Gaussian filter, 0.8 mm*

Figure 28 - Amplitude parameters - Roughness Profile

The thickness of the HA-coated Ti foams was also measured via the optical profilometer. Half of the coating was removed with the help of tape and ethanol.

The mean difference in step height was found to be 6.59 microns (µm), as shown below.

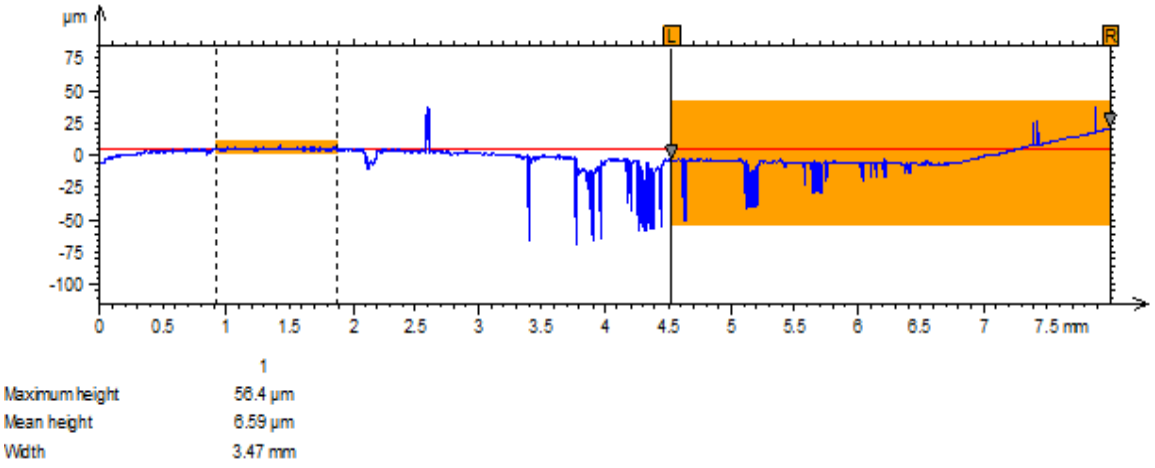

Figure 29 - Mean Step Height of the HA-coated Ti foam

Coming towards the contact angle measurement to determine the hydrophilicity of the HA-coated Titanium foams. This was done using the drop shape analyzer and the contact angle was found to be 50.7°.

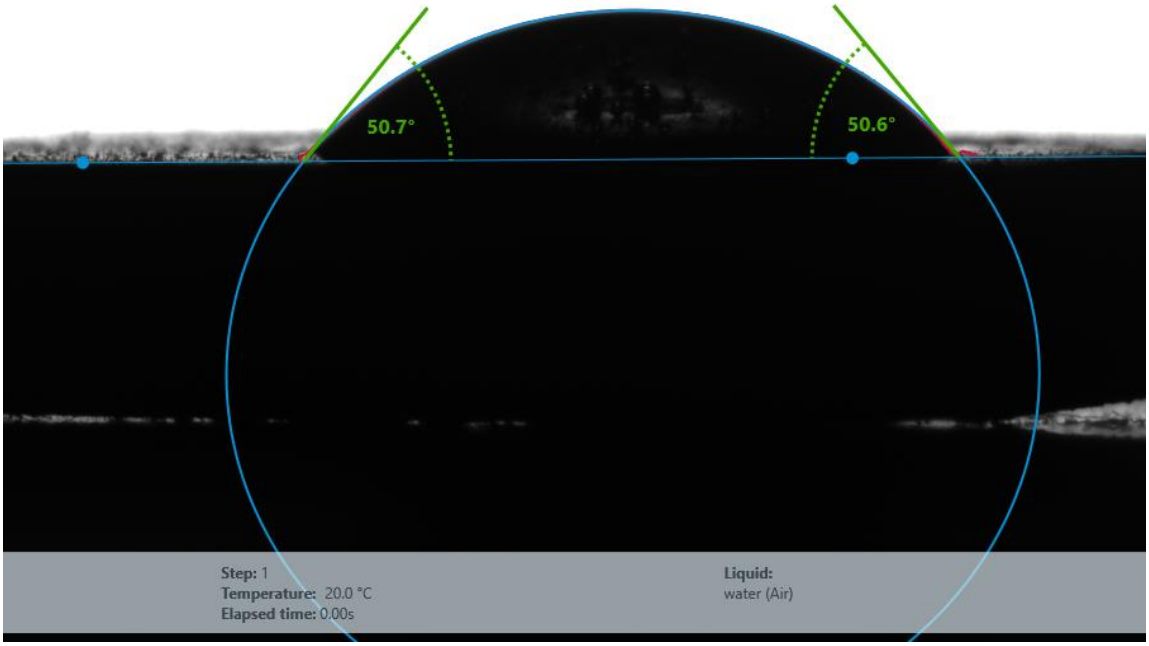

Figure 30 - Contact Angle of the HA-coated Ti foam

Lastly, the surface topography of the HA-coated Ti foams was observed via Scanning Electron Microscopy. The results of the SEM images are shown below.

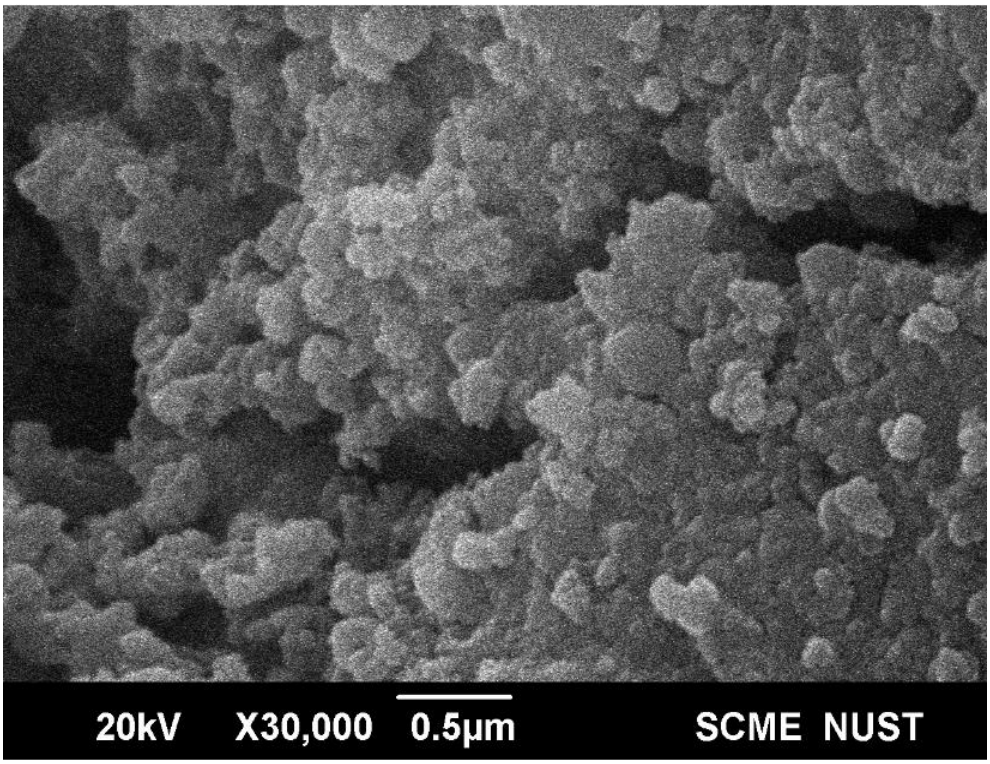

Figure 31 - SEM image of HA-Coating at 30,000x magnification

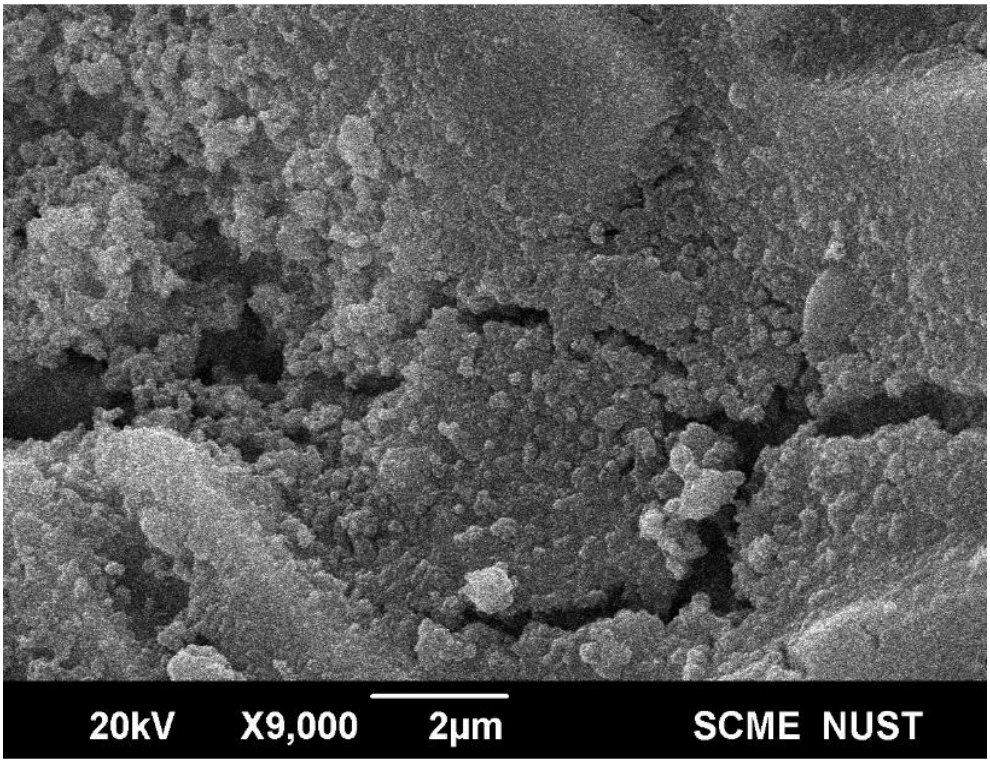

Figure 32 - SEM image of HA-Coating at 9000x magnification

4.4 Conclusions of Coating Parameters

Now that the results of the tests carried out to observe the coating parameters have been covered, we will move forward to their conclusions and what they signify.

The rougher surface of the Hydroxyapatite coating provides a larger surface area which leads to a greater number of cells anchoring the implant to the corresponding host tissue. Thus, the fixation between the implant and bone tissue is optimal and revision surgery can be avoided. The roughness value we obtained via the optical profilometer (0.374 microns) was quite low due to the fact that the coating was performed via Pulsed Laser Deposition, which generally forms smoother coatings on substrates.

The thickness of the Hydroxyapatite coating was found to be 6.59 microns, which falls into the general category of thickness values obtained after Pulsed Laser Deposition.

The contact angle was found to be 50.7°, therefore, hydrophilic in nature. This is characteristic of hydroxyapatite coatings because of the presence of phosphate and hydroxyl groups. The hydrophilic nature of the coating promotes good cell adhesion through the support of surrounding body fluid if any. This is because a hydrophilic surface tends to attach more proteins to itself, which paves way for cell attachment, and the process of tissue regeneration is escalated.

Lastly, the surface topography was observed via Scanning Electron Microscopy and the images showed a great deal of roughness with respect to the Hydroxyapatite coating, with many dips and grooves along the surface. These irregularities become sites for protein attachment, which further promotes tissue regeneration and bone healing by providing a strong interface between the implant and tissue.

4.5 Results of HA Characterization

The Hydroxyapatite powder we synthesized in the initial stages of our project was characterized via a number of techniques including Raman, Fourier

Transform Infrared Spectroscopy (FTIR), and Scanning Electron Microscopy (SEM).

Raman spectroscopy was used for the structural and chemical analysis of hydroxyapatite powder on the basis of their chemical structure. The results for Raman Analysis are shown below:

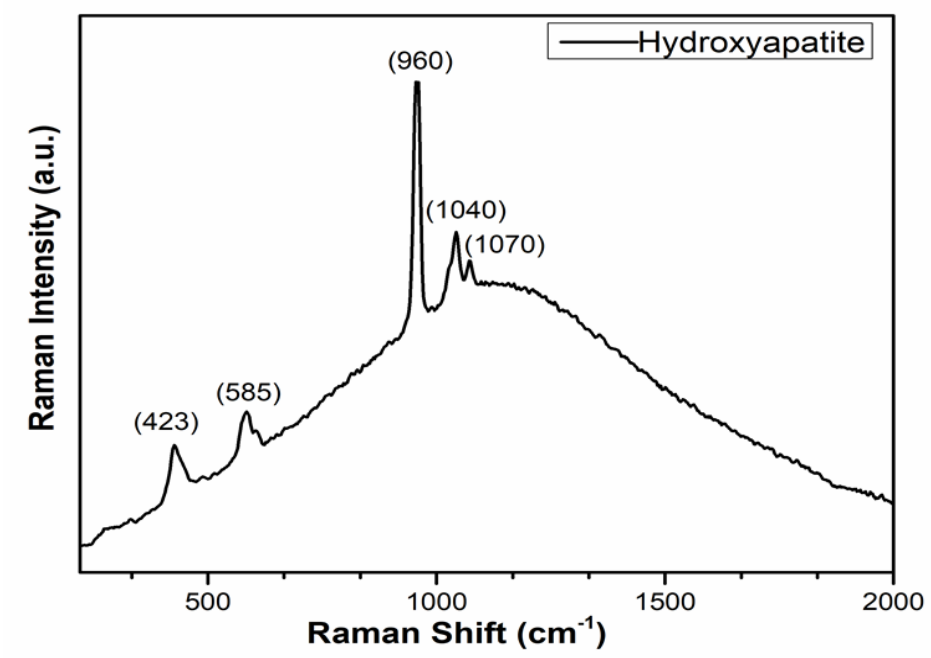

Figure 33 - Raman Spectrum of Hydroxyapatite

Fourier Transform Infrared Spectroscopy (FTIR) was performed for the determination of the major functional groups present in the synthesized material.

The results are given below:

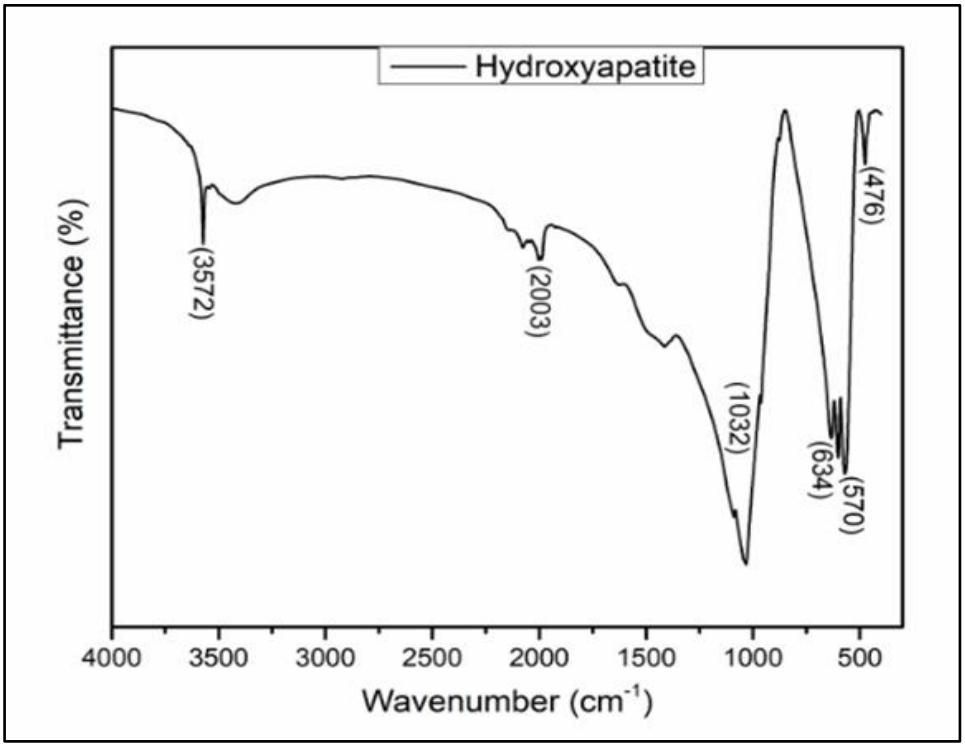

Figure 34 - FTIR analysis for HA Powder

Lastly, Scanning Electron Microscopy (SEM) was performed on the hydroxyapatite powder to analyze the morphology of the HA particles, and Energy Dispersive Spectroscopy (EDS) was done in parallel with the Scanning Electron Microscopy (SEM). It was done for the confirmation of the elemental constituents of Hydroxyapatite.

The SEM results are as follows:

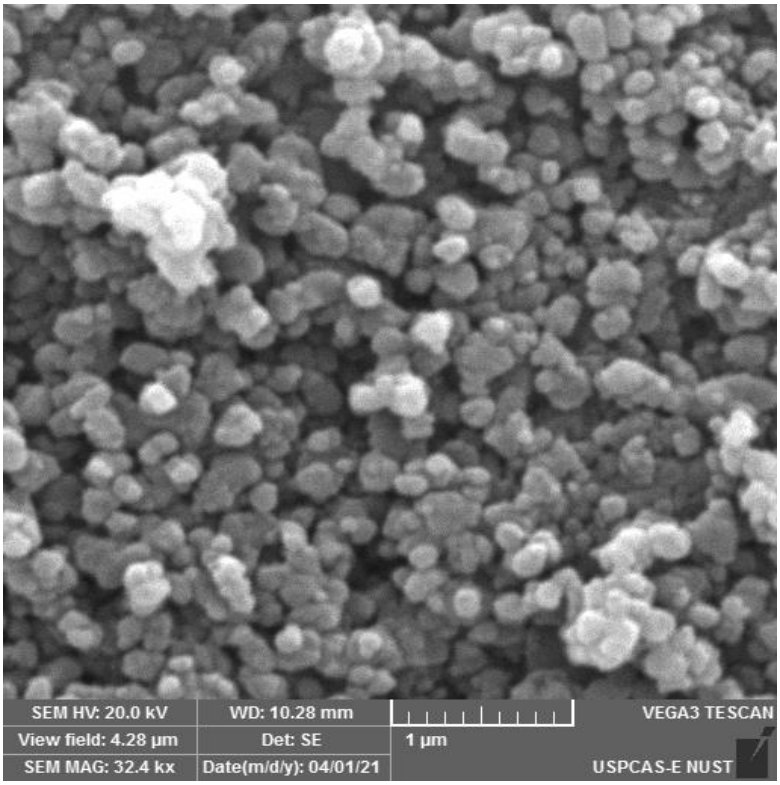

Figure 35 - SEM Analysis of HA powder at 32,400x magnification

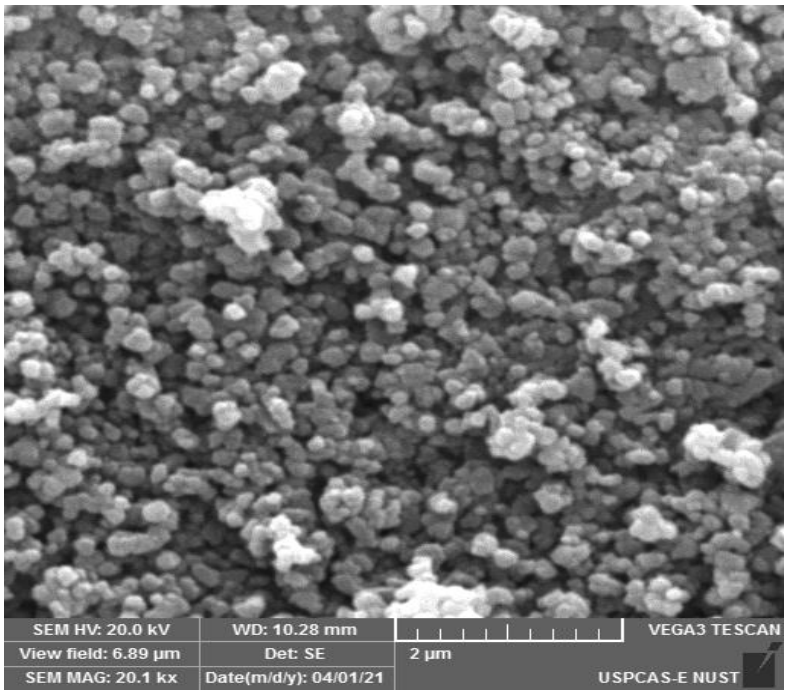

Figure 36 - SEM analysis of HA powder at 20,100x magnification

The EDS Spectrum obtained via Electron Dispersive Spectroscopy is as follows:

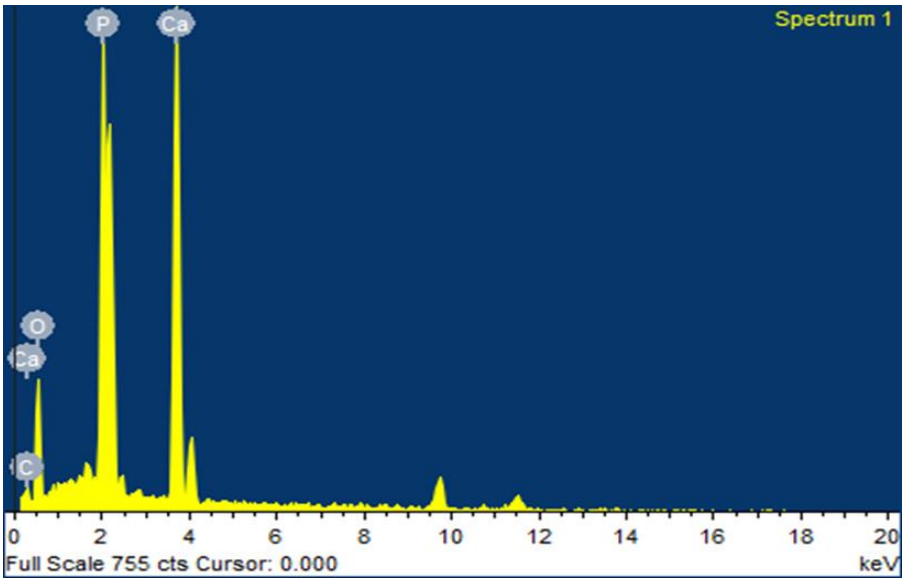

Figure 37 - Energy Dispersive X-ray Analysis

4.5.1 Conclusions of Raman Analysis for Hydroxyapatite

After a comparison of the graph obtained with the existing Raman spectrum of hydroxyapatite, we observed that the first peak with a wavenumber of 423 cm^{-1} is for v2 phosphate. The second peak at 585 cm^{-1} indicates v4 phosphate, and the highest peak at 90 cm^{-1} shows v1 phosphate. For hydroxyapatite, this value is always between 956 to 966 cm^{-1}.

Peaks 4 and 5 indicate the presence of carbonate or phosphate.

V stands for vibrational modes, and these differ according to the shift in the laser during the process. V1 stands for symmetric stretch, while v2 and v4 stand for symmetric bend mode and antisymmetric bend mode respectively. We confirmed the presence of phosphate groups in our HA sample using this technique.

4.5.2 Conclusions of FTIR

By comparing our FTIR results with the existing HA FTIR spectrum, we found out that the first two peaks at the wavenumbers 3572 cm^{-1} and 2003 cm^{-1} indicate the presence of hydroxyl group. The third major peak at 1032 cm^{-1} and the peak at 570 cm^{-1} indicate the presence of a phosphate group while the peak at 634 cm^{-1} corresponds to the hydrogen-phosphate group. The presence of the phosphate functional group is essentially the confirmation that the synthesized material is hydroxyapatite.

4.5.3 Conclusions of SEM

Both the SEM images of the Hydroxyapatite powder show overall spherical particles of HA with some angular irregularities. Moreover, a lot of agglomeration can be observed, which occurs due to the hygroscopic nature of Hydroxyapatite. As the samples were sent to USPCAS-E, NUST for the Scanning Electron Microscopy and were tested late, agglomeration became more pronounced. Furthermore, there were no surfactants used during the synthesis of hydroxyapatite due to their toxic nature, and the samples were not sonicated prior to SEM, which are also other possible reasons for the agglomeration.

The results of Electron Dispersive Spectroscopy indicate the presence of Calcium, phosphorus and oxygen at the corresponding peaks, which ultimately verify the elemental constituents of HA. The smaller initial peak for carbon is a residual peak as carbon dioxide gets adsorbed into HA during post-treatment.

4.6 Further Testing

Discussed in this section are a few tests that can further aid in the study of Hydroxyapatite-coated Titanium foams and analyze them for their potential use in the biomedical field as implants.

4.6.1 Compression Test

The compression test can be performed via the Universal Testing Machine (UTM). The samples are subjected to compressive loads and a stress-strain curve is obtained until the fracture point.

This is a suitable test to be performed because bioimplants when placed in the body have to bear extensive compressive loads. Thus, it is useful to determine how much load an implant can withstand before it is placed in the body.

4.6.2 Bioactivity Test

The bioactivity test can be performed by soaking the samples in a SBF (Simulated Body Fluids) solution. This is done for a number of days and after adequate soaking, the samples are dried in an oven. Then, the weight loss of the samples is calculated.

This test is crucial to judge how bioactive a material is and how long it will last as an implant in the body.

4.6.3 Fatigue Study

A fatigue test is performed to determine the fatigue strength of a sample or its ability to withstand cyclic loading conditions. This test is performed with the help of a fatigue tester into which the sample is loaded and subjected to cyclic loading. They have various standards and the one we felt will be suitable for this study is ASTM F1800.

Fatigue study is important because the implant also undergoes major cyclic stresses when placed in the human body.

REFERENCES

[1] Prasad, K., Bazaka, O., Chua, M., Rochford, M., Fedrick, L., Spoor, J., Symes, R., Tieppo, M., Collins, C., Cao, A., Markwell, D., Ostrikov, K., & Bazaka, K. (2017). Metallic biomaterials: Current challenges and opportunities. Materials, 10(8). (Page 3)

[2] Huiskes, R., Weinans, H., & Van Rietbergen, B. (1992). The relationship between stress shielding and bone resorption around total hip stems and the effects of flexible materials. Clinical Orthopaedics and Related Research, 274, 124–134.

[3] Abu-Amer, Y., Darwech, I., & Clohisy, J. C. (2007). Aseptic loosening of total joint replacements: Mechanisms underlying osteolysis and potential therapies. Arthritis Research and Therapy, 9(SUPPL.1), 1–7.

[4] Tang, L., & Eaton, J. W. (1995). Inflammatory responses to biomaterials. American Journal of Clinical Pathology, 103(4), 466–471.

[5] Science, O., Ito, K., Nanba, K., & Mura, S. (1998). Comparison coated and into of osseointegration uncoated threaded between titanium implants tibia dental placed defect. 40(1), 37–41.

[6] Lide, D. R., ed. (2005). CRC Handbook of Chemistry and Physics (86th ed.). Boca Raton (FL): CRC Press.

[7] Kashef, S., Asgari, A., Hilditch, T. B., Yan, W., Goel, V. K., & Hodgson, P. D. (2010). Fracture toughness of titanium foams for medical applications. Materials Science and Engineering A, 527(29–30), 7689–7693.

[8] Tengvall, P., Elwing, H., Sjöqvist, L., Lundström, I., & Bjursten, L. M. (1989). Interaction between hydrogen peroxide and titanium: a possible role in the biocompatibility of titanium. Biomaterials, 10(2), 118–120.

[9] Goharian, Amirhossein & Abdullah, Mohamed. (2017). Bioinert Metals (Stainless Steel, Titanium, Cobalt Chromium). 10.1016/B978-0-12-804634-0.00007-0.

[10] C. Vasilescu, S.I. Drob, E.I. Neacsu, J.C. Mirza Rosca. Surface analysis and corrosion resistance of a new titanium base alloy in simulated body fluids, Corrosion Science, Volume 65 (2012), Pages 431-440.

[11] Oldani, Carlos & Dominguez, Alejandro. (2012). Titanium as a Biomaterial for Implants. 10.5772/27413.

[12] Mohammed, Mohsin & Khan, Zahid & Siddiquee, Arshad. (2014). Beta Titanium Alloys: The Lowest Elastic Modulus for Biomedical Applications: A Review. International Journal of Chemical, Nuclear, Metallurgical and Materials Engineering. 8. 726-731.

[13] Niinomi, M., & Nakai, M. (2011). Titanium-based biomaterials for preventing stress shielding between implant devices and bone. International Journal of Biomaterials, 2011.

[14] Carlsson, L., Röstlund, T., Albrektsson, B., Albrektsson, T., & Brånemark, P. I. (1986). Osseointegration of titanium implants. Acta Orthopaedica, 57(4), 285–289.

[15] Gibson, L. J. (2000). Mechanical Behavior of Metallic Foams. Annual Review of Materials Science, 30(1), 191–227.

[16] Alexandra Byakova, Yuri Bezim'yanny, Svyatoslav Gnyloskurenko, Takashi Nakamura, Fabrication Method for Closed-cell Aluminium Foam with Improved Sound Absorption Ability, Procedia Materials Science, Volume 4, 2014, Pages 9-14, ISSN 2211-8128.

[17] Rehman, T. ur, & Ali, H. M. (2020). Thermal performance analysis of metallic foam-based heat sinks embedded with RT-54HC paraffin: an experimental investigation for electronic cooling. Journal of Thermal Analysis and Calorimetry, 140(3), 979–990.

[18] Singh, R., Lee, P. D., Dashwood, R. J., & Lindley, T. C. (2010). Titanium foams for biomedical applications: a review. Materials Technology, 25(3–4), 127–136.

[19] Xiao, J. & Qiu, G.. (2017). Research Progress in Preparation Methods of Titanium Foams or Porous Titanium. Xiyou Jinshu Cailiao Yu Gongcheng/Rare Metal Materials and Engineering. 46. 1734-1748.

[20] Sharma, M., Gupta, G. K., Dasgupta, R., Kumar, M., & Kumar, P. (2018). Titanium Foams Processed Through Powder Metallurgy Route Using Lubricant Acrawax as Space Holder Material. Transactions of the Indian Institute of Metals, 71(8), 1933–1940.

[21] Shbeh, M., Oner, E., Al-Rubaye, A., & Goodall, R. (2019). Production and Digital Image Correlation Analysis of Titanium Foams with Different Pore Morphologies as a Bone-Substitute Material. Advances in Materials Science and Engineering, 2019, 1670837.

[22] Ma, G. (2019). Three common preparation methods of hydroxyapatite. IOP Conference Series: Materials Science and Engineering, 688(3).

[23] Rodríguez-Lugo, V., Karthik, T. V. K., Mendoza-Anaya, D., Rubio-Rosas, E., Villaseñor Cerón, L. S., Reyes-Valderrama, M. I., & Salinas-Rodríguez, E. (2018). Wet chemical synthesis of nanocrystalline hydroxyapatite flakes: Effect of pH and sintering temperature on structural and morphological properties. Royal Society Open Science, 5(8).

[24] Schmitt, J., & Flemming, H. (1996). FTlR-spectroscopy in microbial and material analysis, Microbially Influenced Corrosion of Materials, January.

[25] Akhtar, K., Khan, S. A., Khan, S. B., & Asiri, A. M. (2018). Scanning electron microscopy: Principle and applications in nanomaterials characterization. In Handbook of Materials Characterization.

[26] Fischer, E. R., Hansen, B. T., Nair, V., Hoyt, F. H., & Dorward, D. W. (2012). Scanning electron microscopy. Current protocols in microbiology, Chapter 2, Unit2B.2–2B.2.

[27] Ngo, P. D. (1999). Energy Dispersive Spectroscopy. Failure Analysis of Integrated Circuits, 205–215.

[28] Seifollah Nasrazadani, Shokrollah Hassani, in Handbook of Materials Failure Analysis with Case Studies from the Oil and Gas Industry, 2016.

[29] C.V. Raman & K. S. Krishnan, A New Type of Secondary Radiation, Nature 121, 501- 502 (1928)

[30] Spedding, T. A., & Wang, Z. Q. (1997). Study on modeling of wire EDM process. Journal of Materials Processing Technology, 69(1–3), 18–28.

[31] Di-Ameter, M. (n.d.). Standard ANSI grit European (P-Grade).

[32] Physics, R. (2003). I :: 5227, 312–317.

[33] Anil, S., Venkatesan, J., Shim, M. S., Chalisserry, E. P., & Kim, S. K. (2017). Bone response to calcium phosphate coatings for dental implants. In Bone Response to Dental Implant Materials. Elsevier Ltd., 64-67.