Fabrication and Characterization of Carbon Coating on Biomedical Implant

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September, 2021

Fabrication and Characterization of Carbon Coating on Biomedical Implant

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A thesis submitted in partial fulfillment of the requirements for the degree of

MS Biomedical Sciences

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September 2021

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Acknowledgment

I am grateful to Allah Subhana-Watala, my Creator, for guiding me through this endeavor at every step and for every new concept He implanted in my head to help me improve my work. Without Your invaluable assistance and direction, I would have been unable to do anything. It was Your will that whoever supported me over the duration of my thesis, whether it was my parents or anybody else, therefore no one else is worthy of praise but You.

I would also like to thank my supervisor, Dr. Murtaza Najabat Ali, for his unwavering support and advice during my thesis, as well as for providing me with resources for my research. I would like to express my gratitude to him for assisting me in learning and for giving me his precious time and attention.

I would also like to express my gratitude to Sadia Hassan for her tremendous support and cooperation. She was my guide and inspiration during my research work. I sought her guidance whenever I got stuck in something. I would not have been able to complete my thesis without her assistance. I appreciate her compassion and assistance throughout my research work.

I would also like to pay special thanks to S.U Khan for his unwavering support and encouragement. I always looked up to him for inspiration and advice. He has always rendered valuable assistance to my study.

I would also like to thank my siblings, particularly Erum Younis, for their unwavering faith in me. She has always been a tremendous source of inspiration and provided me the financial assistance as and when required.

I am grateful to my friends Afeera Zara Naz, Wafa Arif, Khola Ikram, kainate kamal khan, Iqra Shafiq, Nadia Sadiq, and Misbah Rasool for being with me through every thick and thin during my master's degree.

Dedication

Dedicated to my lovely parents, Muhammad Younis Nadeem and Kishwar Sultana, whose unwavering support, selfless love, cooperation, and a lot of prayers helped me to achieve this wonderful accomplishment.

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Abstract

Biomedical implants are devices that are implanted into the human body to maintain, reinforce, or even replace a portion or an entire biological structure to help in the healing process, supply medication, and monitor the body's function. An implant's performance decreasing below a particular tolerable level after implantation is a major problem that can compromise the implant's targeted therapeutic duration and lead to surgical adjustments. The biocompatibility, functionality, and endurance of the implant material should be encouraged, with a focus on the material's interaction with the target host. Different problems, such as poor mechanical qualities, metal ion leaching, hemolysis, and so on, can all contribute to implant failure by producing toxicity, allergic response, unfavorable local tissue reaction, and restenosis. All these difficulties were resolved by utilizing the physical vapor deposition technique to deposit a pure carbon coating layer on a graphite substrate on a stainless-steel implant. The thickness of the coating layer was measured using optical profilometry. Scanning electron microscopy was used to examine the microstructure. AFM was used to examine the topography and roughness of the coated layer. SEM EDX was used to determine the elemental composition, and micro indentation testing was used to determine the hardness of the coated layer. Leach and hemolytic tests were used in the biological examination of the coated sample. The sample with a 1.5 μm thick coating was found to be mechanically strengthened, non-hemolytic, and biocompatible.

CHAPTER NO 1: INTRODUCTION

The term "implant" encompasses a broad range of biomedical implants, ranging from bioimplants made up of metals like orthopedic plates and screws to bioelectronics like stents and neurostimulators. Several implants that can be removed or replaced when they have served their purpose are called primary implants, whereas remaining are intended to be permanent (i.e., they remain in the body for a lifetime). The definition of biomedical devices are, the medical devices that are inserted in the cavity of the human body that can either be formed surgically or naturally and are meant to reside there for a long span following the treatment. The focus of this analysis is on implants that include metal parts or components that come into interaction with human tissue. (Uhthoff, Poitras, & Backman, 2006).

1.1: Functions of biomedical implants:

For the following purposes, they support, strengthen, or even replace a part or entire biological structure:

They provide the body with strength and support.

They aid in the healing process.

Medication is delivered through them.

They are also utilizable in keeping track of the body's various functions.

1.2: Brief history of biomedical implants :

Humans were formerly treated with conventional medication and operations, but owing to their limitations and inefficiency, biomedical implants are now utilized to treat a variety of disorders, including cardiovascular diseases, bones, joints, and dental issues. The area of biomaterials is not

new; as early as 4000 years ago, the Egyptians and Romans used material such as linen, wood, gold, and iron for different purposes i.e.as sutures, toe repair and dental applications. Nylon, Teflon, silicone, stainless steel, and titanium were among the other materials employed after World War ii. Bioimplants have taken the place of traditional drug delivery techniques in the fields of dentistry, orthopedics, cardiology, immunology, histopathology, neurology, plastic and reconstructive surgery, and ophthalmology owing to the availability of improved diagnostic instruments and breakthroughs and improvements in the field of materials as well as surgical techniques. Stainless steel, titanium, cobalt-chromium molybdenum alloys, metals, alloys, polymers, ceramics, and composites are among the materials utilized in the formulation and fabrication of implants. Because implants function in the human body, they are exposed to a variety of biological environments of varying physicochemical nature, and their contact with tissues, bones, and hostile bodily fluids frequently result in corrosion, fracture, implant failure, cytotoxicity, inflammation, poor mechanical strength, and thrombogenesis.

The biomaterial's acceptability by the human body is a most important consideration. The material implanted in the body of a living organism should not cause any harmful impact such as allergy, inflammation, or toxicity, either prior to surgery or during the recovery period. Besides fatigue strength and fracture toughness, biomaterials must be comprised of the following characteristics such as adequate mechanical strength to retain the structure when force is applied to it, high corrosion and wear resistance in extremely corrosive environmental conditions and varying loading conditions and it should last for a prolonged period and should not break down till the person dies.

For all these issues the best possible solution is to coat biomedical implants with a material that improves biocompatibility, hemocompatibility, corrosion resistance, mechanical strength, and reduces cytotoxicity. so, we can conclude that an ideal coating should have the following characteristics

1.3: Characteristics of an ideal implant :

- excellent mechanical and physical properties over wide temperature ranges
- Good compact coating layer
- Hardness and wear resistance
- Chemically inert and so that it could not react with oxides, and it could not cause corrosion of the implant.
- resistant to erosion
- reduced cytotoxicity
- biocompatibility
- hemocompatibility

different coating techniques have been used to deposit a deposition layer on bioimplants to increase the mechanical properties , hemocompatibility and biocompatibility of medical implants .

CHAPTER NO 2 : LITERATURE REVIEW

2.1: Commonly used biomaterials in the fabrication of biomedical implants :

Metals and metal alloys (a mixture of metal elements) have been widely utilizable for a variety of biomedical applications in most medical disciplines for more than a century. Iron-based alloys such as stainless steel, cobalt-based alloys, pure titanium, and its alloys (Ti-6Al-4V) have been utilizable in implants in the past. Various metals that don't easily corrode or fuse are called refractory metals. These metals have also been utilizable in the fabrication of implants as an element of alloys. Examples of refractory metals are as following tantalum (Ta), tungsten (W), and molybdenum (Mo). Biomedical implants containing electronic components commonly use noble metals. Examples of noble metals are gold (Au), silver (Ag), platinum (Pt), and iridium (Ir). (Khan, Muntimadugu, Jaffe, & Domb, 2014).

2.1.1: Stainless Steel :

The application of implant determines which metal or metallic alloy need to be employed in the fabrication of biomedical implant. For example, stainless steel(surgical grade) has high mechanical strength and is ductile, but the integration of stainless steel to soft tissues of the body or bone might not be favorable .(Khan et al., 2014)

Depending upon the application of implant and properties of the material it was concluded that stainless steel is frequently utilized in devices used in the fixation of fractures and/or temporary implants that will be removed in the future.

2.1.2: Co-Based Alloys:

Co-based alloys such as Co-Cr-Mo, Co-Cr-W-Ni, on the other hand, have better mechanical properties i.e., strength and hardness, they also show extreme corrosion resistance

properties, but due to low ductility, these types of implants cannot be processed and worked by machine.(Matusiewicz, 2014)

So, it is concluded that areas where implants with high wear resistance are required such as hip prostheses, artificial joints, and permanently implanted devices are commonly made up of co-based alloys because of the characteristic's properties of co-based alloys.(Khan et al., 2014)

2.1.3: Nitinol:

Another Nickle titanium alloy called nitinol (Ni-Ti) is also more often used in implant fabrication because of its properties such as thermal stability (i.e., the characteristic feature of a material that changes back to its actual form as the temperature changes(Elahinia, Hashemi, Tabesh, & Bhaduri, 2012), it's ultra-elastic properties (i.e., property of a material to regain its actual form when the applied stress is removed) and its biocompatible nature. Due to useful characteristics of Ni-Ti, it is currently utilized in many applications such as vascular stent applications and orthopedic fixing devices.

Table 2.1 lists the properties, benefits, and drawbacks of frequently used biomaterials for implants. (Pandey, Awasthi, & Saxena, 2020; Prasad et al., 2017)

Metals	Composition	Advantages	Disadvantages	Applications
Stainless steel	Fe; 16-18.5% Cr; 10-14% Ni; 2-3% Mo; <2% Mn; <1% Si; <0.003%	Highly resistant to wear and corrosion	High modulus of elasticity delays the process of bone regeneration	• Stents • Artificial valves • Others
Titanium and its alloys	Ti; 0.05% N; 0.1% C; 0.5% Fe; 0.015% H; 0.4%	Biocompatible High tensile strength Fracture toughness high osteointegration rate	Cytotoxic Low corrosion resistance Low wear resistance High cost	• Orthodontic wires • Fillings • Others

Co-Cr and its alloys	Co; 19-21% Cr; 14-16% W; 9-11%	Highest mechanical strength	Cytotoxic due to Co, Cr, and Ni Low corrosion resistance Low wear resistance Low friction resistance	• Artificial plates • Bones fixations (screws, pins , plates) • Others

Table 2. 1: Properties , Advantages , Disadvantages, and Applications of commonly used Biomaterials

2.2: Challenges and issues arise due to the biomaterial used in fabrication of biomedical implant:

After implantation, an implant's performance dropping below a certain reasonable threshold is a serious concern that can impair the implant's desired therapeutic period and eventually result in surgical modifications. The implant material's biocompatibility, functioning, and durability should be promoted, with an emphasis on the material's interface with the target host (Nasab, Hassan, & Sahari, 2010).

Different issues, such as corrosion and wear caused by thrombus formation, particulate matter, insufficient bone integration, infection, low fracture and fatigue strength, low mechanical properties, variation in the elastic modulus of the material of implant and surrounding bones (stress protection), and inflammation can significantly contribute to the implant failure.

2.3: Few significant problems leading to implant failure include:

Corrosion of metal implant

Leaching of metal ions from implant

Hemolysis due to metal implant

2.3.1: Corrosion of Metal Implant:

Due to corrosive implantation medium and cyclic loading, metal implants are susceptible to corrosion during their working. (Geringer, Forest, & Combrade, 2005)

Corrosion is a process in which metal degrades due to an electrochemical reaction between the metal implant and its working medium. These oxidation-reduction reactions can cause the leaching of metal ions from the surface of an implant, leading to the failure of the implant. Corrosion repercussions depend on various aspects such as level, intensity, and kinds of corrosion, etc. and can lead to problems with the integrity of the equipment or severe responses .(Gilbert, 2017)

2.3.2: Leaching of metal ions :

The consistent release of metal ions over the surface of metal implants is known as metal ion release. The number of metal ions leaches out of metal implants having surface oxides has been demonstrated to be dependent on the composition and topology of the oxide layer of a metal implant (Sullivan et al., 2015). The rate of metal ion release is typically maximum after immediate implantation of an implant which gradually decreases with time. However, there are instances in literature where the oxide layer of metal implants is not protective, which results in the leaching of metal ions which remained continued for a longer period after implantation. A higher rate of metal ion release is also seen in such cases of implantation.

2.3.3: Effects of leaching of metal ions from an implant :

One of the most commonly reported severe responses with significant systemic consequences is the increase in the level of metal ions in serum/blood. The analysis of blood and serum showed that even with the successfully implanted MoM orthopedic implants, there was an increased level of Co and Cr ions.(Bitar & Parvizi, 2015; Cheung et al., 2016)

In patients with MoM hip implants, a literature review of 11 randomized controlled trials (RCTs) and 93 observational studies of 9,957 patients showed that there is an increased level of metal ions (Co, Cr, Ti, Ni, Mo) in body fluids such as blood, urine, plasma, erythrocytes, and serum, etc. (Hartmann et al., 2013).

A report on the analysis of six different studies on metal implants described that a higher level of cobalt ions is found in the serum of patients in which metal implants caused adverse local tissue response. The comparison of metal hip resurfacing implant with convention hip replacement showed the differences in the mineral density of bones and several cardiac functions. It indicates that even a small increase in the release of metal ions for a longer span may lead to systemic consequences (Prentice et al., 2013)

In the case of the metal hip implants where there is no elevation of metal ion release can lead to the condition of adverse local tissue response. (Tetreault, Jacobs, Mahmud, & Nam, 2018)

Cobaltism is a term used to describe the effects of Co on the body in reaction to metal orthopedic implants (Cheung et al., 2016; Gessner, Steck, Woelber, & Tower, 2019; Zywiel et al., 2016)

2.3.4: Hemolysis :

These implantable devices that are temporarily implanted in the body commonly cause hemolysis whereas, the prevalence and clinical importance of hemolysis in patients receiving long-term, permanent left ventricular assist device (LVAD) treatment remain unclear. The conduction of final research on the population consisted of 4,850 individuals who were monitored for an average of 11.1 months and exhibited hemolysis in 97 percent of cases. Hemolysis is a frequent condition following CF-LVAD implantation, and it is linked to an

increased risk of morbidity and mortality. Other device and implant features that may contribute to hemolytic episodes and suitable management techniques for afflicted individuals should be investigated in the future.(Katz et al., 2015)

When the implantation of a left ventricular assist device into the body of patients was analyzed, it was found that 37% of them had hemolysis. (Cowger et al., 2014)

similarly in another study the 18 % of patients with the implanted vascular devices were diagnosed with hemolysis These data show that hemolysis is linked to a high risk of death and a marker for thrombosis (Ravichandran et al., 2014).

As there are few issues associated with metal implants that need to be catered in order to develop an appropriate implant which cover up all these problem areas.

2.4 :Proposed Solution :

The most appropriate solution is the deposition of the coating layer on biomedical implants. It seems to be a potential way to improve the interaction between implants and body tissue, as well as their biocompatibility and functionality, without changing the properties of a material (Jiang, Han, Zheng, Chen, & Zhu, 2019; Priyadarshini, Rama, Chetan, & Vijayalakshmi, 2019)

Corrosion rate, degradability rate, elastic modulus, and fatigue resistance have been identified as basic features in the selection of the appropriate nontoxic surface modification approach for biomedical implants. As the surface of a biomedical implant govern the response of the implant when it encounters a working environment, so surface modification is the key factor in the fabrication of an implant.

An appropriate technique is used to modify the implant's surface topography, allowing the changed surface to enhance the mechanical stability, biocompatibility, and hemocompatibility of biomedical implants.

2.5: Techniques used in deposition of coating layer on implants :

In the realm of biomedicine, several coating methods have been utilized to coat a layer on biomedical implants. Based on the application of biomedical implants, a suitable and appropriate approach is chosen. A few of these methods are described here .

Coatings are divided into two categories.

- Dry method of coating
- Wet method of coating

The dry method includes biomimetic coating, electrophoretic coating, laser deposition, and plasma spray coatings whereas, the wet method includes spin coating, dip coating, and spray-coating deposition.(Al-Amin et al., 2020)

2.6: Chemical vapor deposition:

Chemical vapor deposition (CVD) is the coating process that uses chemical reactions with heat from a thermally induced substrate surface to provide a coating of a solid reaction product by supplying reagents in gaseous form. Vapor-phased substances are condensed to produce solid phase material .(Creighton & Ho, 2001) The main benefit of CVD deposition is that it can accommodate a large number of precursors; however, only a few elements from the periodic table, such as noble gases, halogens, and a few actinides and alkali metals, cannot be deposited using this method

The noncolored elements in table no 2.2 cannot be coated using the chemical vapor deposition technique.

IA	IIA	IIIA	IVA	VA	VIA	VIIA	VIII			IB	IIB	IIIB	IV	VA	VIB	VIIB	O
Ha																	He
Li	Be											B	C	N	O	F	Ne
Na	Mg											Al	Si	P	S	Cl	Ar
K	Ca	Sc	Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	Ga	Ge	As	Se	Br	Kr
Rb	Sr	Y	Zr	Nb	Mo	Tc	Ru	Rh	Pd	Ag	Cd	In	Sn	Sb	Te	I	Xe
Cs	Ba	La	Hf	Ta	W	Rc	Os	Ir	Pt	Au	Hg	Tl	Pb	BI	Po	At	Rn
Fr	Ra	Ac															

LANTHANIDES	La	Ce	Pr	Nd	Pm	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu
ACTINIDES	Ac	Th	Pa	U	Np	Pu	Am	Cm	Bk	cr	Es	Fm	Md	No	Lr

Table 2. 2: Periodic table ,colored boxes indicate CVD coated elements , non-colored boxes indicate elements that cannot be coated with CVD.

2.6.1:Working Principle of CVD:

The most basic type of chemical vapor deposition is a process where precursor gases move through a chamber holding one or more heated items and place a solid coating on them. The heated surfaces have a chemical reaction resulting in a thin layer deposit on the surface. other non-reacted precursor gases and chemical byproducts formed which are being ejected from the chamber via processing units to ensure that the deposited layer is uniform.

2.6.2: CVD Apparatus:

The following are the fundamental components and functions of a CVD device.

• Precursors are supplied by a gas supply system to the reactor chamber.

• deposition/ reaction Chamber — A room for deposition.

• Loading substrate system- Loading and unloading system for substrates, mandrels and other products.

• Source energy - Provide the energy/heat essential for the reaction/decomposition of the precursors.

• Vacuum system - A removal system for all unwanted gas species except for reaction or deposition.

• Exhaust system – This is a system that removes volatile by-products from the reaction chamber.

• Exhaust treatment systems - Exhaust gases may not be appropriate for discharge into the atmosphere and will need to be treated or converted to safe/harmless substances.

• Process control equipment — gauges, controllers, and other devices for monitoring process variables e.g., pressure, temperature, and time. This category would also include alarms and safety gadgets.

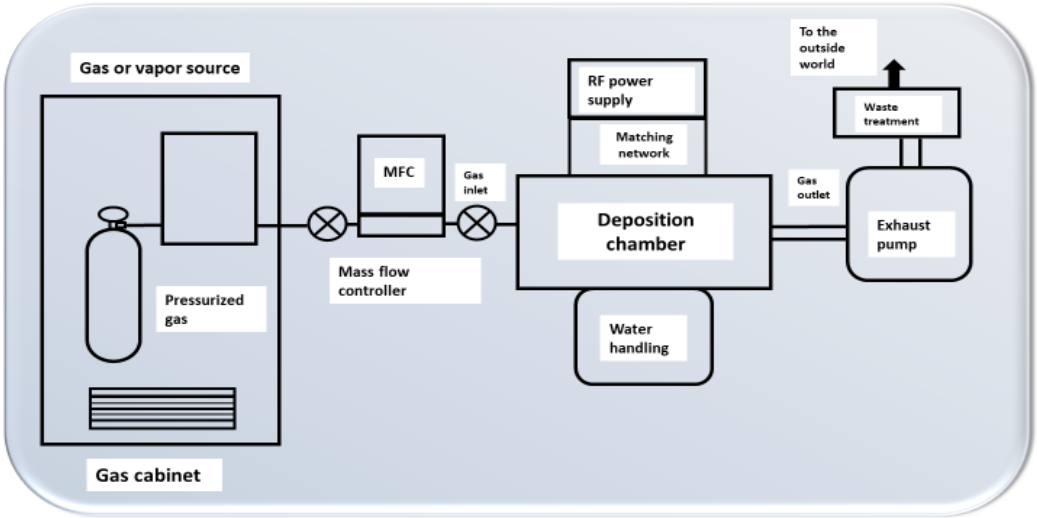


Figure 2. 1 CVD Processing Equipment

2.6.3: Precursor Material :

There are several types of CVD precursor materials, including:

Groups	Precursor Material
Halides	$TiCl_4$, $TaCl_5$, WF_6
Hydrides	SiH_4, GeH_4, $AlH_3(NMe_3)_2$, NH_3
Metal alkyls	$AlMe_3$, $Ti(CH_2tBu)_4$
Metal alkoxides	$Ti(OiPr)_4$
Metal dialylamides	$Ti(NMe_2)_4$
Metal diketones	$Cu(acac)_2$
Metal carbonyls	$Ni(CO)_4$

Table 2. 3: Precursor material used in CVD processing

• Others - it also includes a variety of metal organic compounds, complexes, and ligands.

In literature, we can come across so many examples where the material is coated by using the CVD processing method .such as one good example is the deposition of a TiN layer on Cr-Co alloy using CVD.

2.6.4: Deposition of TiN on Cr-Co Alloy :

The CVD deposition of TiN on the Co–Cr alloy led to a uniform, phase-transformed and recrystallized layer of tiny grains near the alloy surface and enhanced the hardness of the region. The PVD-coated alloy, on the other hand, showed no microstructural or phase alterations. On a Cr-Co alloy, CVD coating applied as follows: At 850°C, N2 and H2 gases travel from the reservoir to the mixing chamber, where the gases mixed and a chemical reaction occurs, creating

TIN, which then sent to the furnace, where the graphite tube deposits tin on the substrate. The graphite tube is prepared with TiN to avoid the carbon effect. The control mechanism keeps the conditions in check. (Song, Min, Hong, & Kwon, 2020)

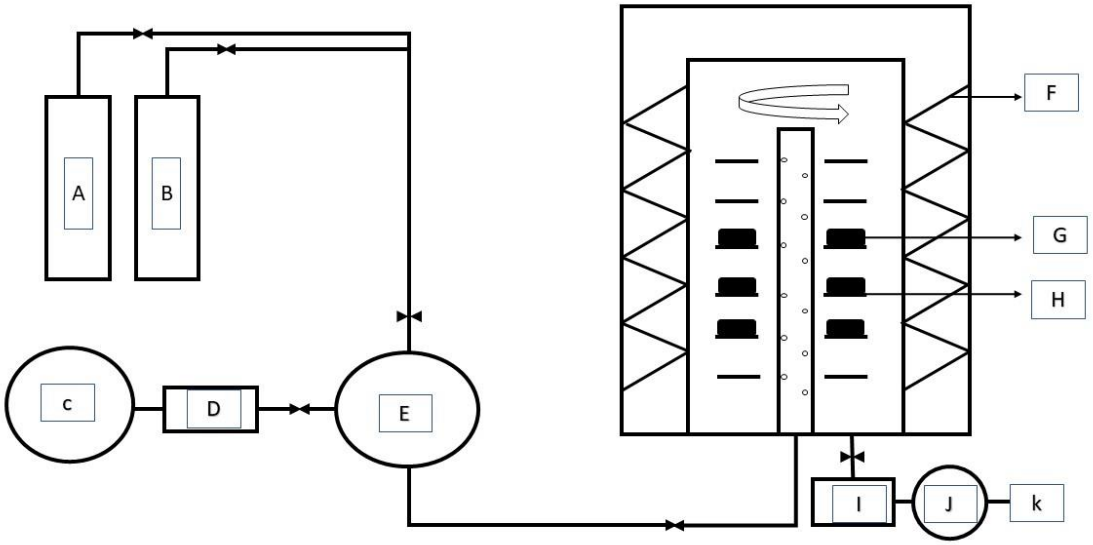


Figure 2. 2: Schematic of CVD furnace

Figure No 2.2: The schematic of the CVD furnace employed is presented in this figure A: nitrogen gas feeder; B: hydrogen feeder; C: liquid TiCl4 feeder; D: Liquid mass controller flow; E: vaporization and gas mixing equipment; F: heating element; G: specimen; H: gas rotary tube; I liquefier gas by-product, J: rotary vacuum pump (RVP); K: neutralized exhaust material.

Because of a few restrictions of CVDs such as the high temperature needed for heating a gas phase, it has two effects: higher cost and material constraints. The most preferential treatment procedure is PECVD because of toxicity and substratum constraints such as inorganic materials in particular, low temperature and chemical stability, solvent and corrosion resistance and substrate limits due to complicated geometries and composition.

2.7: Plasma Enhanced Chemical Vapor Deposition:

Thin-film deposition at considerably lower temperatures on organic or inorganic materials done by using electrical energy to form a plasma that ionize natural gas and produces free radicals, which then polymerized to form a deposition layer.

2.7.1: History :

An electron beam was used for the first time in the 1960s for the disintegration of organic material. According to scientists' When an organic molecule subjected to an electron beam, it produces free radicals that polymerize and stick to the target surface, generating a deposition layer. Later, in 1960, scientist employed a defocused, low-intensity electron beam to create solid silicon oil layers(Christy, 1960). Research showed that the formation of metallic coatings from organometallic vapors decomposed by an electron beam in 1961(Baker & Morris, 1961). researchers described the fabrication of a silicon oxide layer using an organosilicon chemical and its breakdown in a low-energy plasma(Alt, Ing Jr, & Laendle, 1963). In 1964, scientists reported that the same method has been used to manufacture silicon oxide films at ambient temperature, but that a source of plasma generation using radio frequency (RF) is also used to reduce the unwanted sputtering noted in previous work. (Mattox, 2010)

Reinberg invented the "Reinberg reactor" in 1971, which used a parallel plate, capacitively linked RF reactor to deposit semiconductor encapsulation and optical coatings at lower temperatures. The ability of this reactor to induce radial, laminar flow of reactant gases across the substrate improved the homogeneity of the desired coatings. The procedure was also known as chemical ion-plating at the time, and it was widely employed for the deposition of silicon nitride films for encapsulation and metal carbides. As a result, "plasma-assisted" or "plasma-enhanced" CVD has emerged during the last few decades. (Wertheimer, 2014)

2.7.2: PECVD Processing :

There is a deposition chamber in which precursor substrate using PECVD techniques is deposited. Temperature gauges are generated by heating the deposition chamber (usually between 50 and 100°C), which prohibit particle deposition of the substrate while processing. The gas input of gas placed in the chamber top is carried by stainless steel tubes connecting to the side of the deposition chamber. The platon is used for the loading and heating of the substrate by up to 250 degrees. If the deposition chamber is not in use, it is vacuumed. To clean it, flow N2 gas until it reaches atm pressure. The power needed to form plasma is provided through a high-voltage RF link at the top of the chamber with a frequency of 13,56 MHz. The main source gases that flow into the chamber are methane and silane, with flow rates between 0.5 and 1.5. It takes less than one minute for the stabilization of pressure and gas flow rates, with pressure ranging from 50mtorr to 5 torrs. To ionize the gas molecule, a high voltage is supplied to the electrode. When a molecule is ionized, it becomes more reactive, and it forms a thin coating of material on the substrate. The thickness of the layer is determined by the amount of time spent on it. To stop the deposition process ,the RF voltage and gas flow must be turned off. For the removal of unnecessary reactive gases, nitrogen is pumped into the chamber. Due to the dry and non-combustible nature of nitrogen, it removes all those unnecessary gases that can produce an unstable and possibly ignitable environment in the chamber. It can efficiently displace moisture and oxygen, resulting in a more stable environment. To vent the chamber to atm pressure, choose vent. Remove the substrate, Clean up the room, Run etch-back to produce plasma, which cleans the chamber and removes the material that has accumulated on the base and walls of the chamber.(Kummer et al., 2002)

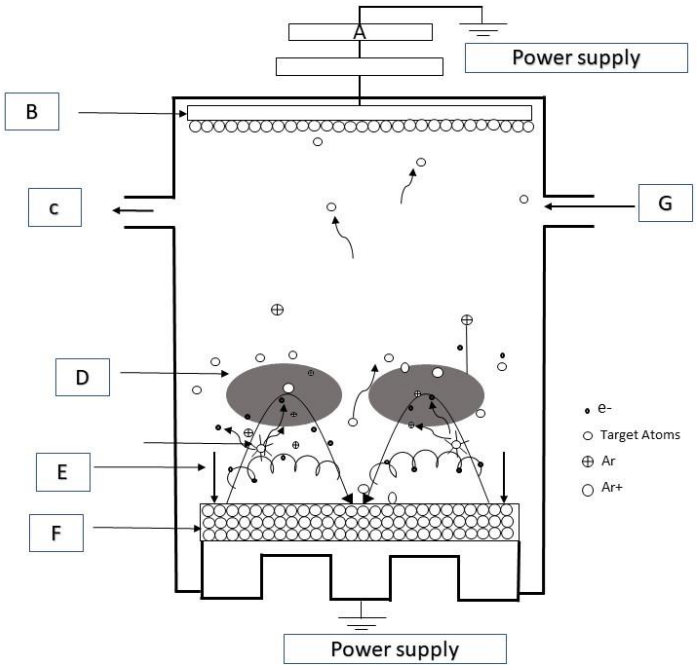


Figure 2. 3: Schematics of PECVD

A:RF generator ,B: platon on which substrate is placed (which act as an anode), C:vacuum control pump, D:plasma production ,E: electric and magnetic field lines, F: cathode , G: gas inlet

Further research from the literature describes the parameters and operating procedures of PECVD. This section discusses several PECVD coatings for a better understanding of PECVD.

2.7.3: Properties of silicon carbide layer deposited on cardiac implant using PECVD:

J Huran et al. determined that IR, AES, and RBS can be used to investigate the properties of silicon carbide layers produced by PVECD. The existence of Si-C, Si-H, and C-H bonds seen in the IR spectra. The RBS and AES data demonstrate that Si and C concentrations are almost similar. Other elements, such as oxygen and nitrogen, have relatively low quantities. Film characteristics are determined by the Si/C ratio. In a high-frequency parallel-plate plasma reactor, amorphous silicon carbide films were formed on bare silicon substrates whereas the frequency, rf power, and substrate temperature are maintained at 13.56 MHz, 0.06 Wcm, and

350°C, respectively. The electrodes were 12 cm in diameter and 4 cm apart. The upper electrode received radio frequency power, whereas the bottom electrode, which contained the substrates, was grounded. The SM and CH gas mixtures were directly pumped into the reaction chamber, with a gas flow rate of SM was 10 and that of CH was 40seems. The ERD technique was used to determine hydrogen content. The 4He+ ion beam from JINR's Van de Graaff accelerator in Dubna was used for this purpose. With the target inclined at an angle of 15” concerning the beam direction and the recoiled protons measured forward at an angle of 30', at an ion energy of 2.4 MeV . (Huran, Hrubcin, Kobzev, & Liday, 1996)

2.7.4: Amorphous silicon carbide is coated on vascular stents using PECVD:

PECVD is used to coat amorphous silicon carbide on vascular stents to improve biocompatibility and mechanical strength. Precursor gases such as Silane (SiH4), methane (CH4), and phosphine (PH3) are employed to deposit n-doped a-SiC:H. Equipment, as illustrated in Figure 3, is used for the deposition and plasma pretreatment of the substrates.

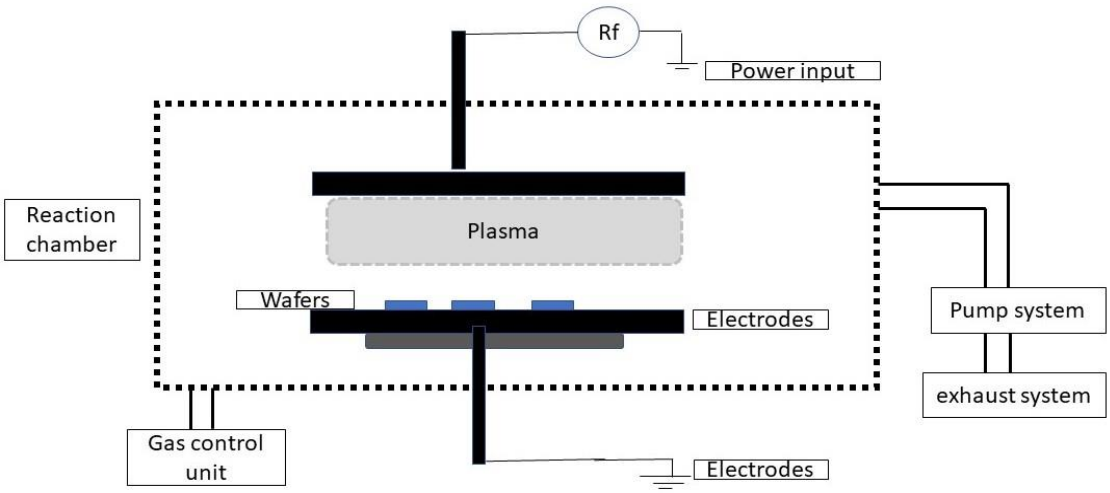


Figure 2. 4:PECVD equipment for SiC Deposition

Cleaning the substrate with grease and lubricants, ultrasonic treatment with tensidic chemicals, and lastly rinsing with distilled water and alcohol are all part of the pre-coating procedure. Plasma etching aids in the removal of adsorbed oxygen (and likely other interface pollutions) that compromise coating adherence, resulting in surface activation of the substrate. The intermediate coating can improve coating adherence even further. The soft layer that forms reduce the SiC-H mechanical stress. PECVD will be used to coat the surface, silane and methane are used as source gases, with 1% phosphene added to improve conductivity. Changing the methane content of the process gas results in the deposition of a-SixC1-x: H, with a bandgap adjustable between 1.6 eV and 2.2 eV through "x." The gas flow ratio is 1:2 because this gas flow ratio achieves the optimum bandgap. To avoid diffusion processes that result in the loss of hydrogen atoms and, as a result, a high density of states inside the bandgap at high temperature and poor corrosion behavior at low temperature, the temperature maintained was 250C. There were minor micro-fractures owing to the limited tensile strength of the ceramic material, but the layer deposited by using PECVD techniques has good mechanical properties, and it is also biologically compatible. Optical electron microscopy and scanning electron microscopy (SEM) demonstrate that the coating adheres well. (Bolz, Amon, Ozbek, Heublein, & Schaldach, 1996)

2.7.5: Deposition of SiC layer on cardiovascular implant to enhance the biocompatibility using PECVD technique :

Because of the extremely brittle nature of solid semiconductors, any single material cannot meet all the requirements of mechanical strength and biocompatibility that is why cardiovascular stents are coated with SiC to enhance hemocompatibility. As a result, a hybrid design is presented as a novel method for improving the hemocompatibility of cardiovascular stents.

Silicon carbide (a-SiC: H) is an amorphous compound with a hydrogen-rich variation. The plasma-enhanced chemical vapor deposition (PECVD) method is used to deposit this material. Figure 1 depicts the technical capabilities of the equipment, which include a-SiC: H deposition as well as several plasma pretreatments required to improve coating adherence.

As a precursor gas, a mixture of silane and methane is employed. To ionize the gas following parameters are used 250C temperature,0.1 mbar pressure and 13.56 MHz frequency.

The cytotoxicity of mice fibroblasts is assessed using a saline solution as a negative control and cuso4 as a positive control. The results showed that amorphous silicon carbide did not induce any cytotoxic reaction in mice fibroblasts L929 when incubated for 24 hours and stained with trypan blue, but the positive and negative controls showed the predicted results.

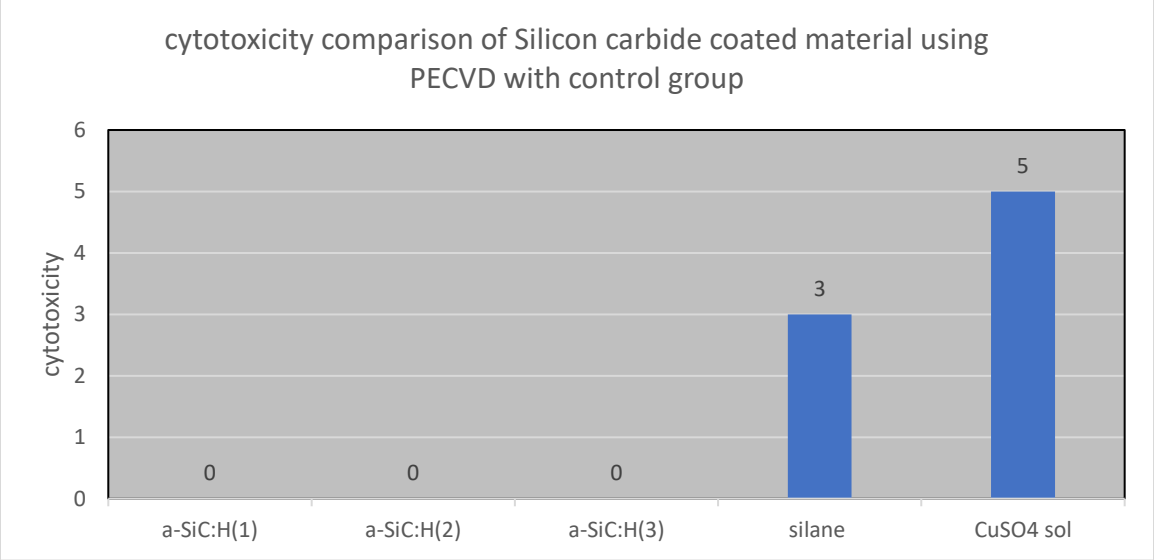


Figure 1: a-SiC:H test substrates (3 batches), indicating their cytotoxicity in comparison with the cytotoxicity of saline and CuSo4 solutions.

The hemolytic test is used to assess the hemolytic concentration in which mixing of sheep erythrocytes with a-SiC: H coated stents takes place at 37C temperature and incubates for 72 hours while using aqua destillata and saline as controls. due to damage hemoglobin releases and

concentration of hemoglobin released is assessed using potassium hexacyano ferrat and potassium cyanide. After contact of sheep erythrocytes with the a-SiC: H extract, measure the hemoglobin content that was released and compare it with the control solution that is saline solution. Because the saline solution is known to be safe for red blood cells, amorphous silicon carbide must be safe as well. (Harder, Rzany, & Schaldach, 1999).

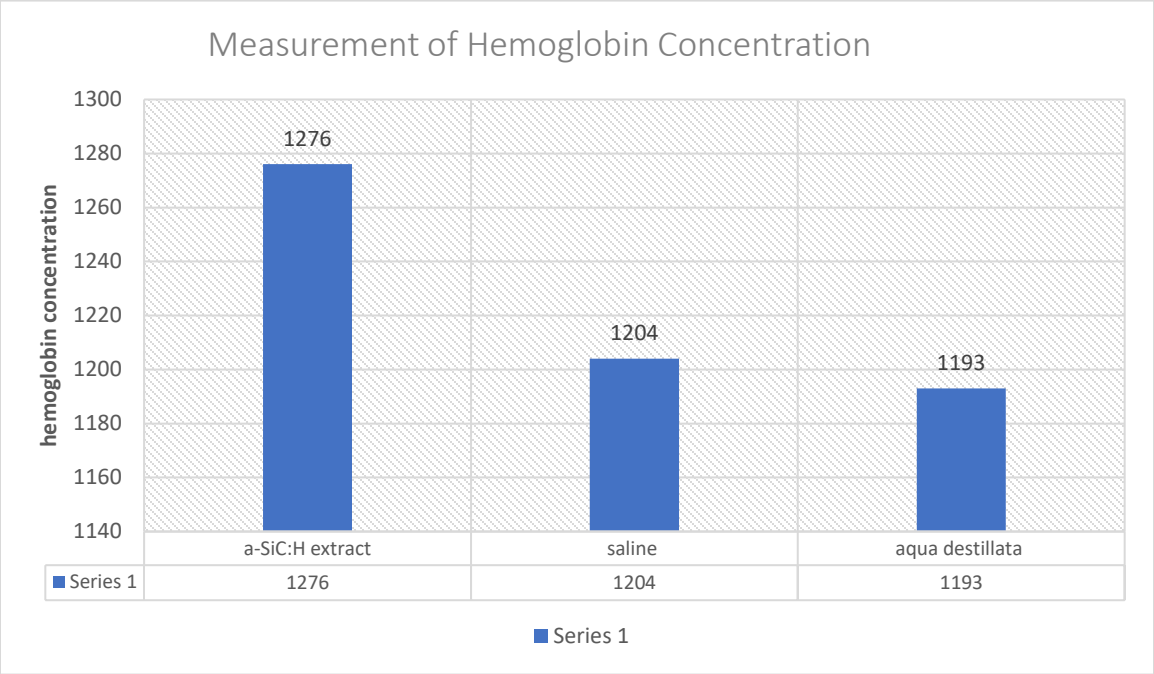


Figure 2 :Concentration of hemoglobin in the centrifugation excess of a-SiC:H, saline, and distilled water solutions, indicating the comparative damage to erythrocytes. aqua dest. = aqua destillata (distilled water

2.8:Plasma spray processing :

In this process, heat generated by plasma is utilized to melt ceramics or metal powders. The melted components are then sprayed onto the targeted surface to produce a layer which protect it.

Plasma spraying has the following advantages:

• Cost effective process

• Rapid deposition rate

• Low probability of thermal damage of the coating and substrate than other high-temperature techniques because plasma gas is used here, and it is chemically inert

• coating can be done at a relatively low temperature, so the target material remains cool.

2.8.1: Procedure:

The plasma spray gun is comprised of the water-cooled anode which is made up of copper and the cathode which is made up of tungsten .plasma gas mainly argon, nitrogen, helium, or hydrogen passes through an anode and flows across the cathode. high voltage discharge produces plasma which ionizes the gas molecule causing a conductive trajectory between the cathode and the anode for the DC arc to develop. As a free or neutral flame, the plasma comes out of the anode nozzle and it does not carry any charge. external powder port located near the anode nozzle exit is the most frequent method of feeding powder into the plasma flame. As a result, the powder is rapidly heated and accelerated, with spray distances ranging from 25 to 150 mm.

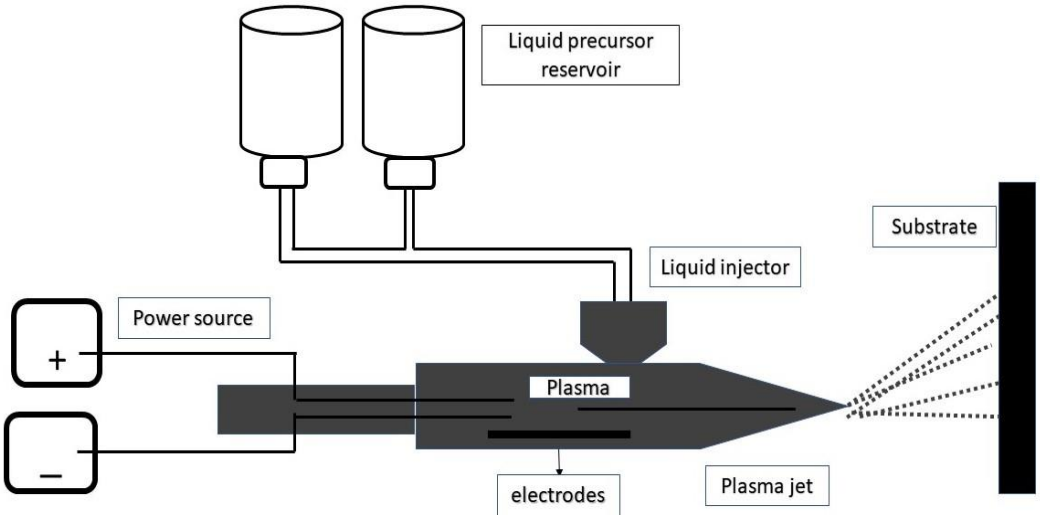


Figure 2. 5: Schematic diagram of a typical plasma spraying process.

2.8.2: Adhesion Strength:

The adhesion strength of coatings deposited by plasma spray coating techniques depends on two factors the nature of the coating material and the nature of the target material. Plasma-sprayed HA or CaP coatings have been reported to have poor adherence on surfaces of an implant in several investigations.

On Ti–6Al–4V alloy substrates, composite coatings of HA/Titanium or HA/yttrium stabilized zirconia (YSZ)/Ti–6Al–4V has been used to increase adhesion further. This is due to the inclusion of Ti or its alloy to the HA coating, which reduces residual stresses created during the spraying process. (Gu, Khor, Pan, & Cheang, 2004; Zheng, Huang, & Ding, 2000)

Yang et al. coated Ti and CoCrMo implant materials with ZrO2 coatings and discovered that ZrO2 (4 percent CeO2) had high adhesion. ZrO2 (4 percent CeO2) coatings have average adhesion strengths greater than 68 to titanium and greater than 67.7 MPa to CoCrMo, with failure occurring within the ZrO2 coating. (Y. Yang, Ong, & Tian, 2003)

2.9: Laser deposition :

Another method used to coat biomedical implants is laser deposition. In this process laser beam is used to deposit a layer on the implant because heat resistance and hard materials can also be controlled using this process.

2.9.1: Procedure :

Laser ablation is another term for pulsed laser deposition. The main concept behind this approach is that a high-powered laser beam offers enough energy to melt and vaporize the target substance. The ablated material generates a plasma plume of highly excited atoms, ions,

electrons, and molecules because the focused pulse layer has a high energy density. In a vacuum or gas environment, this plume expands and carries the material that needs to be condensed on substrate . Deposition of a layer of appropriate thickness is possible with pulsed lasers with a high repetition rate.

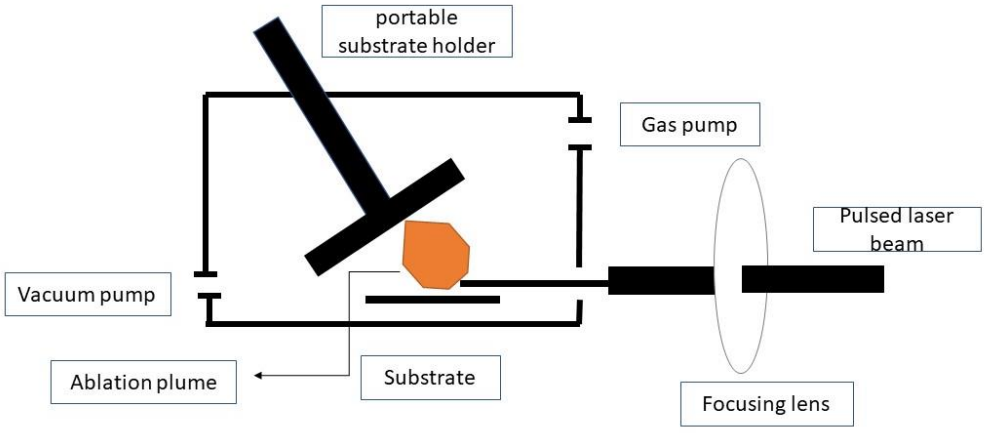


Figure 2. 6:Schematic diagram of PLD system

Arias et al. experimented to check the adhesive characteristics of coating material using amorphous and crystalline HA as a coating material, metal substrates and required thickness was 1m . they discovered that both amorphous and crystalline HA coatings stuck effectively to the metal substrates, resulting in no detachment. PLD also enhanced the roughness of the substrates. (Arias et al., 2003)

Blind et al. also performed an experiment and used HA as coating material and Ti as substrate and demonstrated that HA film produced uniform coating on the surface of a substrate with average Ra (average roughness) 36.nm and Rq (root mean square roughness) 49.25 nm. Water-aided PLD was used to deposit HA coatings on titanium substrates, and the coating adhesion strength was found to be improved. (Dailey & Jordan, 2005)

2.10:Physical vapor deposition :

To control the roughness and crystallinity of surface coatings of metals and oxides are developed on an implant by the process called sputtering or physical vapor deposition (Barranco, Borras, Gonzalez-Elipe, & Palmero, 2016; Macleod, 2018). While working sputter system produces a glow discharge which shows the deposition processing. A basic sputtering system is comprised of three things, a vacuum-filled chamber, metallic electrodes, and current supply (Macleod, 2018). In addition, film deposition may be achieved with a voltage of several keV and a pressure of more than 0.01 mbar.

The discharge is produced by the bombardment of an ion with molecules in the cathode. As a result, the kinetic energy of the molecules increases, and they come out of electrodes with maximum energy. To optimize momentum transfer, the atomic weight of the bombardment ion and target material should be equal. The thin layer is deposited on the substrate when these molecules collide with the anode or substrate while moving in a straight line .

Figure 9 shows a schematic of the sputtering system.

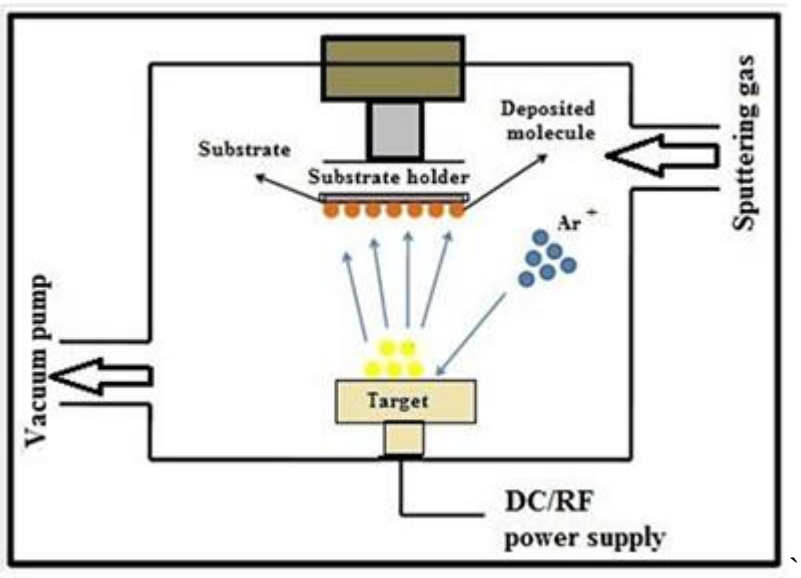


Figure 2. 7:PVD Setup Diagram.

2.10.1: Kinds of sputtering process:

Direct current (DC) and radio frequency (RF) sputtering are the two most popular kinds of sputtering procedures. For the target material, which is a good conductor of electricity. DC sputtering technique is used, which is powered by DC. It is cost-effective and simple to regulate. When the target material is dielectric, the commonly used sputtering method is RF sputtering. The structure and optical characteristics of these films were examined using both DC and RF sputtering techniques (Dumitru, Morosanu, Sandu, & Stoica, 2000; Morosanu, Dumitru, Cimpoiasu, & Nenu, 1997).

2.10.2: Advantages of PVD or sputtering process :

The sputtering technique provides several advantages.

- Sputtering is a simple way to create materials with a high melting point.
- The composition of the deposited films is identical to that of the starting components.
- For ultrahigh vacuum applications, the sputtering method can be used.
- Sputtering sources can be used with reactive gases like oxygen.
- It is environment friendly
- It enhances the biocompatibility of biomedical implants
- It enhances the hardness of the material.
- It doesn’t require the usage of special precursors.
- No toxic by-products are formed by using the PVD technique.
- It is safer to use because of the absence of precursor gases.

This table2.4 lists all the coating processes commonly utilized in biomedical implant coating. It summarizes all the benefits and drawbacks of employing various ways to deposit

layers on biomedical implants. Based on benefits and drawbacks, it is simple to determine which technique is most appropriate.

Deposition Method	Advantages	Disadvantages
Sol-gel method	• Coats 3D complex porous substrates • Low temperatures • Low cost • Very thin coatings (<1 μm)	• It needs temperature-controlled environment
Electrostatic deposition	• Uniform coating • Low cost	• Line of sight deposition • Fragile coatings
Electrophoretic Deposition	• High rate of deposition • Uniform coating	• Hight temperature required • Cracks in coatings
Thermal spraying	• Rate of deposition is high	• Coating decomposes due to high temperature • Amorphous coating • Line of sight deposition
Plasma spraying	• Cost effective • Smooth coating • Deposition rate is high	• It requires high temperature • Non uniform coating • Less adherence between film and substrate
Dip coating	• 3D coating • Cost effective • High deposition rate	• High temperature required • Non uniform coating • Fragile coating
Bio-mimetic Coating	• Coat material irrespective of substrate geometry • Don't require high temperature	• Require specific solution formation • Require maintenance of PH • time consuming process
Pulsed laser Deposition	• It gives uniform coating • Versatility	• Line of sight deposition • High cost • Pretreatment of samples
Sputter coating	• Thick coating • Good adherence between substrate and film	• Time consuming process • High cost • It gives amorphous coatings
Physical vapor deposition	• Environment friendly • Cost effective • Smooth coatings	• Thin coatings • Low deposition rate

Chemical vapor deposition	• Avoid line of sight deposition • High deposition rate	• High temperature requirement • Toxic precursor gases
Plasma enhanced chemical vapor deposition	• High deposition rate • Low temperature	• High-cost equipment • Toxic precursor gases

Table 2. 4: Advantages and Disadvantages of commonly used deposition techniques

CHAPTER NO 3: METHODOLOGY

On basis of the application and the working environment of an implant, it was concluded that an implant should have the following qualities,

- Biocompatibility
- Nontoxicity
- No leaching of metal ions
- Non-hemolytic
- High mechanical strength

3.1: Problem Area :

Few issues are associated with biomedical implants. Out of all these issues, three problems areas have been chosen to cater by using this research methodology.

The following are three areas of concern.

- Metal ion leaching
- Hemocompatibility
- Low mechanical strength

3.2: METHODOLGY

There are three key phases in the methodology that was employed.

- Coating development and optimization
- Coating development and optimization
- Biological testing

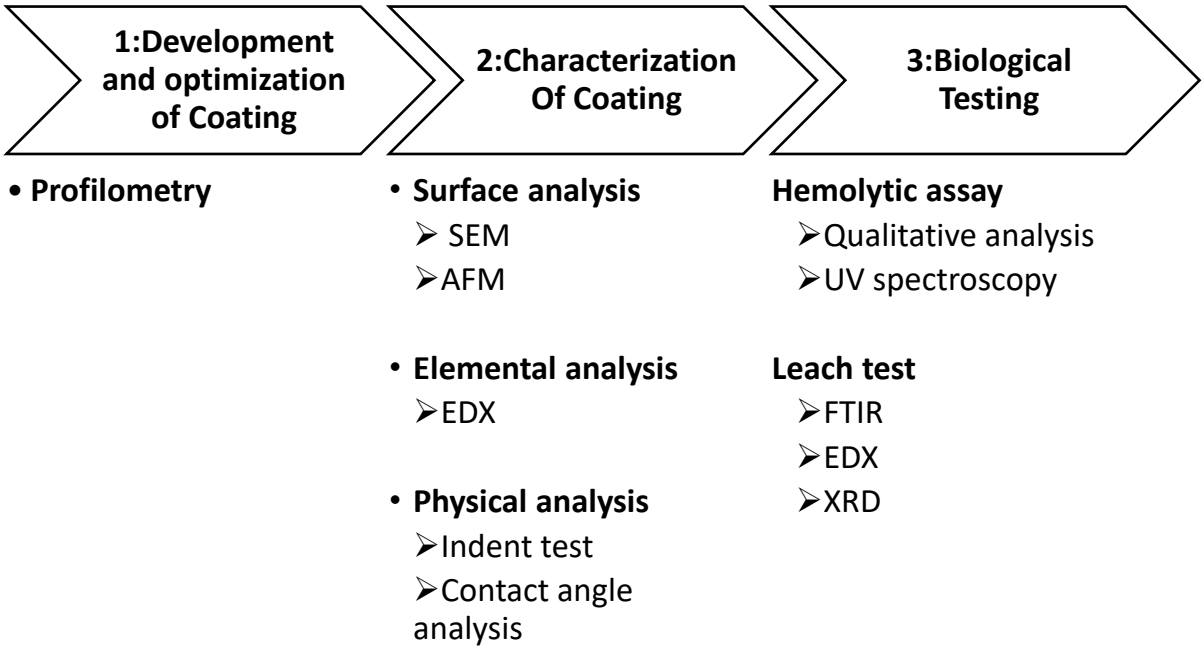


Figure 3. 1:Flowchart of Research Methodology

3.3: Substrate sample :

As a substrate, an electropolished stainless steel sample with the following characteristics was utilized

- Stainless steel has a 1/1 cm dimension.
- It was 316L medical-grade stainless steel.
- It has a 0.5 mm thickness.

Figure 3. 2:Stainless Steel

3.3.1: Sample Preparation :

The sample was cleaned before commencing the coating process to remove any contaminants or moisture that could interfere with the coating procedure.

Two easy steps make up sample preparation.

- Isopropanol was used to clean the sample.
- The material was dried for 15 minutes by heating it to 60 degrees Celsius.

3.4: Development and optimization of coating :

The deposition of graphite substrate pure carbon coating is done on the implant, which is a novel coating in the field of biomedical sciences because it was not coated on stainless steel implant using the PVD technique before, so the first step was the optimization of coating thickness.

Following parameters have been used in the deposition of the coating layer on a substrate using PVD

Parameters	**Values**
Pressure	10 ^-5 torr
Maximum Current supply	60 to 70 A
Distance between graphite rods and stage	6 to 7 cm
Sample rotation	6RPM
Time	Variable, depending on the thickness of coating layer

Table 3. 1 : PVD Deposition Parameters

PVD was used to deposit the coating layer on stainless steel implants. It is a closed setup in which the substrate is placed onto the stage and a vacuum is created. All the parameters were constant, including pressure, current supply, the distance between graphite rods and stage, and

sample rotation. Time was the sole changeable parameter. The thickness of the coating depends on the variability of time. For different time interval, different level of coating thickness is achieved.

3.4.1: Optimization of coating thickness:

Because all other factors remained constant, the thickness of the coating layer varies as time changes. The thickness of the coating layer was analyzed by using the profilometry technique.

(Li et al., 2018)used optical profilometry to analyze the thickness of the diamond coating on Cr Co alloy substrate.

(Liu et al., 2012)used optical profilometry as an analysis technique to check the thickness of the coating layer while checking the influence of coating thickness

3.5: Characterization:

Three methods of analysis are used to characterize the deposited layer.

Surface analysis

Elemental analysis

Physical analysis

3.5.1: Surface Analysis :

Two characterization approaches are used in the surface analysis of coated samples.

a) Scanning Electron Microscopy (SEM)

This method generates an image when a beam of electrons is focused on the sample surface, backscattering of electron produce signal which can be scanned to generate an image. At very high magnifications, the released electrons provide a grayscale picture of the material.

• It was used to examine the microstructure of a coated sample.

b) Atomic Force Microscopy :

It is an imaging technique used to analyze surface properties. it doesn't use an optical source and it is high-resolution techniques .AFM was used to examine the topography and roughness of the coated sample

(Das, Guha, Das, & Ghadai, 2020)used AFM and SEM for analyzing morphology, topography, and roughness of TiCN coating on Si substrate.

(Yu, Tan, Yang, & Yang, 2015)also used AFM and SEM to analyze the topography and microstructure of Ta coating on Ti6Al4V alloy by the CVD process.

(Sweitzer, Scholz, Montezuma, & Rizzo III, 2006)used AFM and SEM to analyze the smoothness and roughness of DLC and Al2O3 coated retinal implants .

3.5.2: Elemental Analysis :

• EDX (Energy Dispersive X-Ray Analysis), often known as EDS or EDAX, is an x-ray method for determining the elemental composition of a sample.

- (Yu et al., 2015)used EDX to analyze the elemental composition of Ta coating on Ti6Al4V alloy by CVD process .
- (Guo Yang, Chen, Qin, & Feng, 2014)used EDX and FTIR to analyze elemental composition and functional group of HA coating on steel .
- (Guoqiang Yang et al., 2019)used EDX and FTIR to analyze elemental composition , chemical bonds, and functional group of pyrolytic carbon coating on carbon fibers .

3.5.3: Physical Analysis :

The coated sample's physical analysis involves two types of testing: hardness testing and contact angle analysis.

a) **Micro Indentation Test :**

A micro indentation test is used to determine the hardness of the coated sample.

b) **Contact Angle Analysis :**

Contact angle analysis was used to determine the hydrophobic and hydrophilic content of the coated sample.

- (Das et al., 2020)used indentation test to investigate mechanical properties such as hardness of TICN coated Si substrate .
- (Yu et al., 2015) used contact angle analysis to investigate the wettability of Ta coating on Ti6Al4V alloy by CVD process.
- (Durdu, Korkmaz, Aktuğ, & Çakır, 2017) used contact angle analysis to check the wetability and hydrophobicity of HA coated steel .
- (Gopal et al., 2017)investigated hydrophobicity and hardness of diamond coated Ti based alloys by using indent test and contact angel analysis.

3.6: Biological Testing :

The biological analysis of the coated sample was the third and final phase of the research methodology. Biological testing further comprises of two types of tests

3.6.1: Leach Test

Leaching of metal ions from the biomedical implants when they encounter body fluids challenges the biocompatibility of the implant.

The impact of several blood mimic physiological solutions on metal ion leaching from various stainless steels was studied by Herting et al. The rate of metal ion leaching rises under accelerated conditions.

Procedure :

An accelerated aging study was carried out to assess the leaching of metal ions from the coated sample. An accelerated aging study was carried out to analyze the leaching of metal ions from the coated sample. The preparation of the PECF solution, which is a blood mimic solution, was the first step in the study procedure.

The PECF solution consisted of 1.1g KCl, 3.4g NaCl, 12.5g NaHCO3, 1.75g NaH2PO4 in 500 ml of de-ionized water(Lin, Chen, & Run-Chu, 2001; Şen & Avcı, 2005) .

- Place the sample coated sample in PECF solution
- Incubate the solution with the coated sample at 105°C for 45 days
- Take 5 ml of solution every week
- Analyze the solution using FTIR
- on the 45th day, analyze the solid sample using XRD and SEM

(Gopal et al., 2017)investigated crystal structure and microstructure of implant after leaching of metal ions of diamond coated Ti based alloys by using XRD and SEM analysis.

(Kazemi, Ahangarani, Esmailian, & Shanaghi, 2020) investigated the leaching of metal ions from HA/TiN coated Ti-6Al-4V implant by using XRD and SEM .

(Yu et al., 2015)used FTIR,XRD and SEM to investigate the corrosion and ion release of Ta coating on Ti6Al4V alloy by CVD process.

3.6.2: Hemolysis test

Hemocompatibility of the coated sample was analyzed by hemolysis test

Procedure :

(Ghafoor, Ali, & Riaz, 2020) used the following procedure for hemolysis test .

• Samples are incubated for 24 hours in PBS solutions and called S1.

• Isolation of RBCs by diluting blood with PBS solution

• Centrifugation of blood and PBS for 10 minutes at 10,000 RPM

• Washing of RBCs with PBS by centrifuging it at 10000 rpm for 5 mints

• Mixing of S1 with RBCs suspension

• S1 and RBCs suspension was incubated for 5 hours

• Centrifuge it for 3 minutes at 10,000 RPM

• UV analysis of supernatant at 550nm

Qualitative analysis and UV spectroscopy technique was used for the analysis of the hemolysis test

- (Guoqiang Yang et al., 2019) used UV spectroscopy to analyze the hemolysis of graphite-coated magnesium alloy.
- (Vignesh, Sakthinathan, Velusamy, & Ramakrishna, 2021) used UV spectrophotometry to analyze the hemolysis on hydroxyapatite-coated Mg substrate.
- (Ghafoor et al., 2020) used UV spectrophotometry to analyze the hemolysis on Drug-Eluting Coronary Stent Coatings.

CHAPTER NO 4 : RESULTS AND DISCUSSION

Graphite substrate pure carbon coating was deposited on electropolished stainless steel material of 1/1 cm utilizing the physical vapor deposition method.

4.1: Development of Coating :

The total number of samples chosen was 10, and carbon coating was deposited to them for various time intervals ranging from 1 to 10 minutes, with a one-minute gap between them. Table 4.1 displays each of the 10 samples and their coating times.

Samples	**Coating time (minutes)**
Sample 1	1
Sample 2	2
Sample 3	3
Sample 4	4
Sample 5	5
Sample 6	6
Sample 7	7
Sample 8	8
Sample 9	9
Sample 10	10

Table 4. 1: Coating Time

4.2: Coating Thickness Analysis :

Because all other factors remained constant, the only changeable parameter was time, which affected the thickness of the coating layer. Profilometry was used to determine the thickness of the coating. Table No 4.2 summarized data for coating layer thickness in nm and μm along with their respective coating time.

As it is evident from table No 7, the thickness of the coated layer increased as the time interval for coating increased. There was a gradual increase in the thickness of coating as the time increased.

Coating Time (minutes)	**Coating Thickness (nm)**	**Thickness (μm)**
1	No coating	No coating
2	No coating	No coating
3	15	0.015
4	100	0.100
5	180	0.180
6	300	0.300
7	600	0.600
8	900	0.900
9	1200	1.200
10	1500	1.500

Table 4. 2: Coating Thickness

4.3: Shortlisting of Sample :

Three of these samples were chosen to be tested and analyzed further. The following is a list of samples that have been shortlisted. Table 4.3 summarized the data for three selected samples and respective coating thickness.

Sample	**Thickness (μm)**
Sample 1	0.0150
Sample 2	0.180
Sample 3	1.50

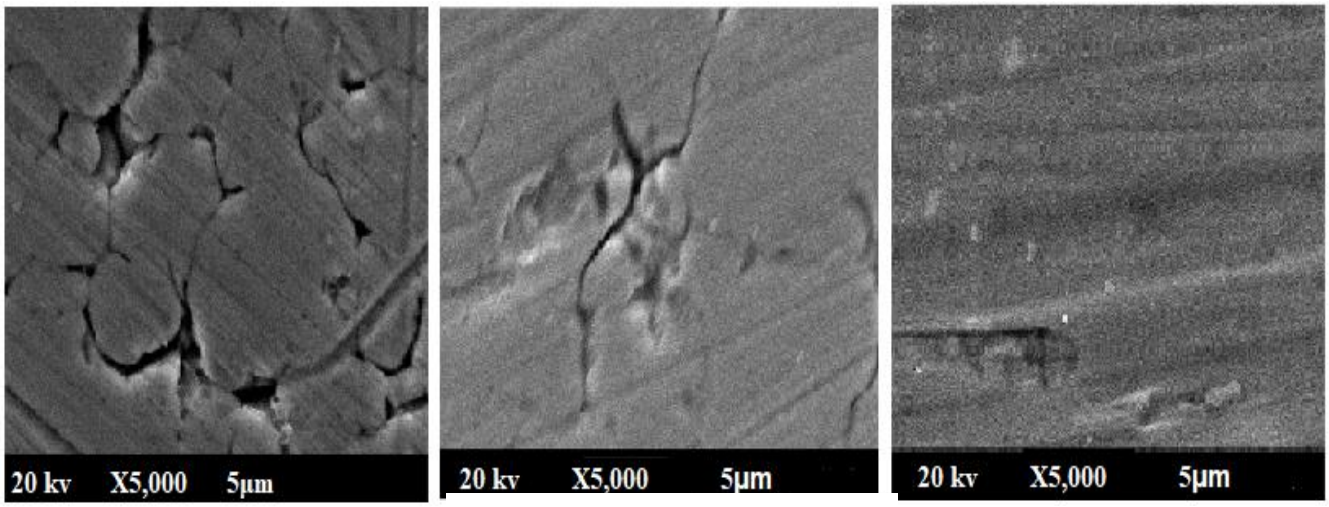
Table 4. 3: Shortlisted Sample

4.4: Surface Analysis :

Microstructure, roughness, and topography analysis are all components of the sample's surface analysis.

4.4.1: Microstructure Analysis :

The microstructure of samples having different coating thicknesses was analyzed by scanning electron microscopy.



A: Sample 1=15nm B: Sample 2=180nm C: Sample 3=1.5 µm

Figure 4. 1: SEM images of shortlisted samples

Fig4.1 (A) shows that the SEM image of Sample 1 with a coating thickness of 15 nm exhibited micro cracks. Because the coating thickness was so low, it couldn't cover the whole surface of the sample, resulting in micro cracks.

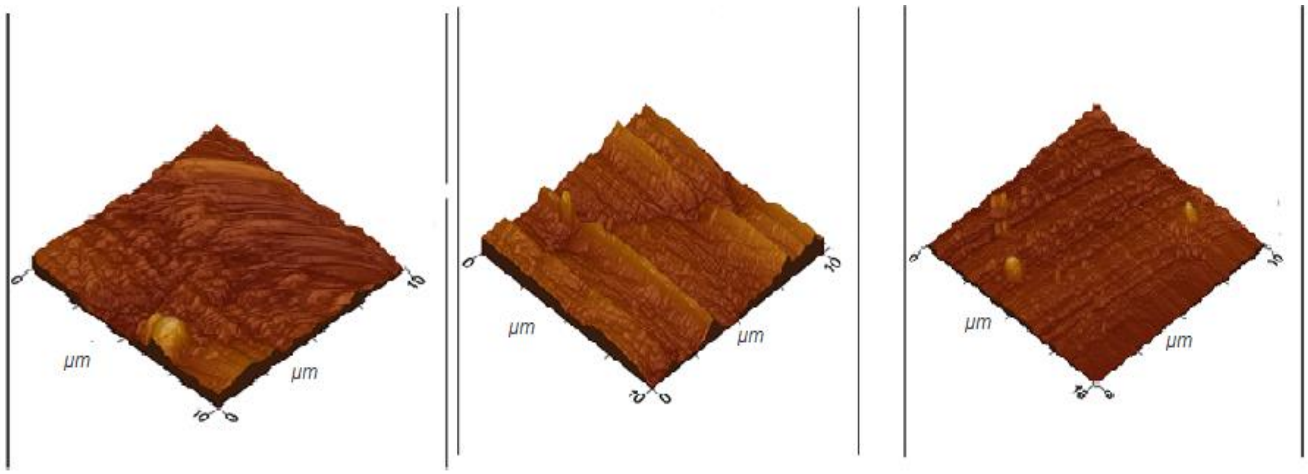
Fig 4.1(B) showed that there were fewer microcracks when the coating thickness was increased up to 180nm.

Similarly, fig 4.1(C) showed that a sample with a 1.5µm thick coating exhibited a smooth surface with no micro cracks.

It was concluded from the SEM results, that the smoothness of the coating layer increases as the thickness of the coating layer increases.

4.4.2: Topography Analysis :

AFM was used to examine the topography of the coated sample; the AFM results for topography analysis complement the SEM results. The smoothness of the surface increases as the coating thickness increases.



A: sample 1: 15 nm B:Sample 2=180nm C: :Sample 3=1.5µm

Figure 4. 2: Topography of shortlisted samples

The topography of samples 1 and 2 with coating thicknesses of 15nm and 180nm, respectively, was not smooth, as shown in figures 4.2 A and B. Both pictures displayed peaks and grooves that became smoother as the layer thickness increased by 1.5 µm. As shown in fig 4.2 C, the topography of sample 3 with a coating thickness of 1.5µm was smooth and free of peaks and grooves.

4.4.3: Roughness Analysis :

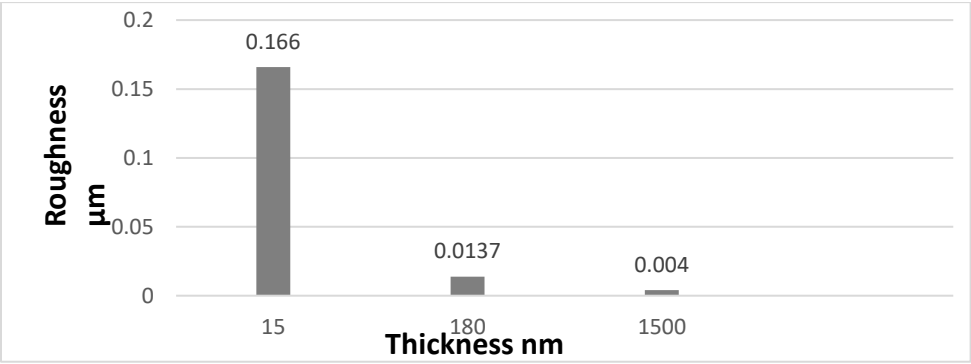
Atomic force microscopy was utilized to analyze the roughness of the coated samples. As the thickness of the surface increased, the roughness of the surface gradually decreased. Roughness was 0.166 μm for a sample with a coating thickness of 15 nm, but it drops to 0.004 μm when the thickness was increased to 1.5 μm, which is almost negligible.

The average roughness of the coated samples was summarized in table 4.4.

Roughness Analysis			
Sr.no	Sample1 (15 nm)	Sample2 (180nm)	Sample3 (1.5 μm)
1	0.167	0.0137	0.004
2	0.166	0.0137	0.005
3	0.165	0.0137	0.003
Average Roughness (μm)	0.166	0.0137	0.004

Table 4. 4: Roughness of Shortlisted Sample

The fact that there is an inverse relationship between coating thickness and surface roughness is further clarified by the graphical depiction of the data . Graph 1 shows that as the thickness of the coating layer increases, the roughness of the surface decreases.



Graph No 1:Roughness Analysis of Shortlisted Samples

4.5: Selection of Final Sample :

Based on the surface analysis of the coated sample, one sample was selected out of all these three samples. selection of the sample was based on the structural properties such as maximum smoothness, least roughness, and smooth topography

Based on the results of SEM and AFM, a sample with a thickness of 1.5 μm was selected because it has a smooth microstructure with no cracks, the least roughness that is almost negligible, and smooth topography with no peaks and grooves.

After selection of 1.5μm thick-coated sample following test and analysis have been performed

4.6: Elemental Composition Analysis :

Energy dispersive x-ray microscopy was used to determine the elemental composition of the coated material. When the elemental composition of the coated sample was compared to that of a pure stainless-steel sample, it was found that both samples had the same elemental composition except for the carbon content, which increased by 10% because the sample had been coated with graphite substrate pure carbon coating.

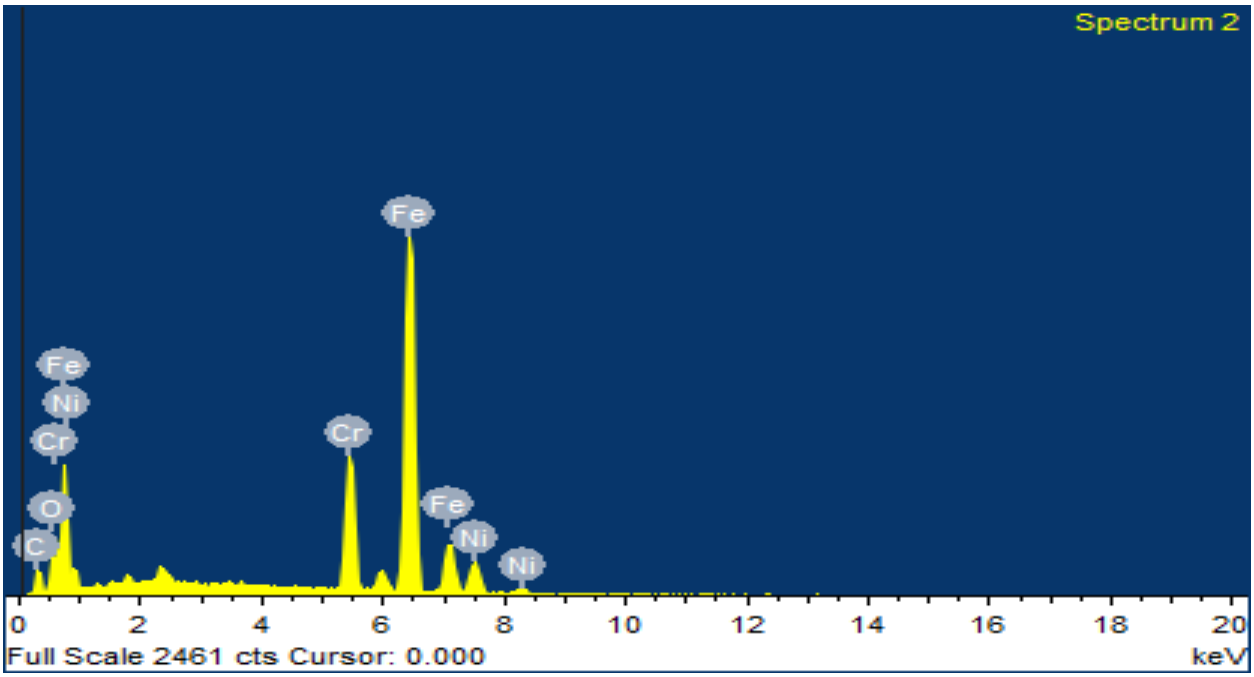


Figure 4. 3: Graphical Representation of elemental composition of coated sample

Fig 17 depicts the EDX spectrum of the coated sample that indicates the elemental composition of coated sample. The comparison of the elemental composition of the coated sample with the pure stainless-steel sample gives us information about the change in elemental composition.

Elements	**Coated sample (wt. %)**	**Pure SS (wt.%)**
C	10.06	0.026
O	3.08	4.00
Cr	14.81	16.55
Fe	63.05	69.42
Ni	9.00	10.0

Table 4. 5: Elemental Composition of Coated sample

Table 4.5 summed up the weight percentage of the elemental composition of pure stainless steel and coated stainless steel. The weight percentage of all the elements remains the same except carbon which increases from 0.026 to 10.06 %. The fact that the coating has not affected the

elemental composition is confirmed by a comparison of weight percentages of pure and coated samples.

4.7: Physical Analysis

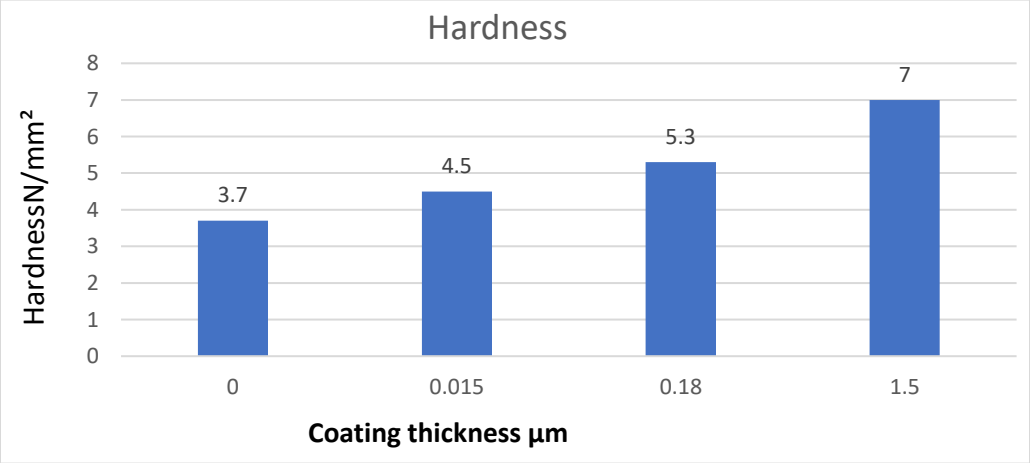
Physical analysis of the sample includes the hardness and contact angle analysis.

4.7.1: Hardness Analysis :

The sample's hardness was determined using a micro indentation test. The results of the micro indentation test revealed that hardness has a direct relationship with coating layer thickness. The hardness of the material increases as the thickness of the coated layer increases.

The uncoated sample had a harness of 3.7 N/mm² which was increased to 7N/mm² for the sample with the maximum coating thickness. The hardness of the coated sample was nearly two times that of the uncoated sample.

Graph 2 summarized the data for the sample with different coating thickness and their respective hardness.

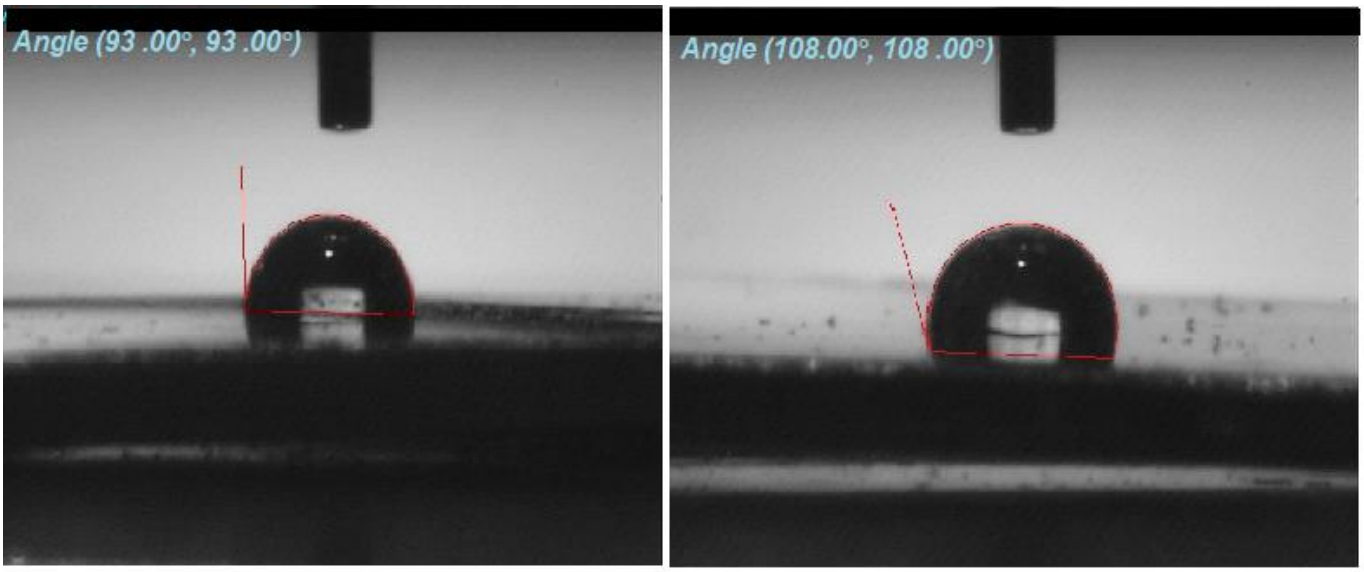


Graph No 2: Hardness analysis of samples

4.7.2: Contact Angle Analysis :

To promote normal revascularization and prevent thrombosis, biomedical implants should have a balanced hydrophobic and hydrophilic content. For biomedical implants, the ASTM D7334-08 specified balanced hydrophobic and hydrophilic content range is 85° to 94° (Strnad, Chirila, Petrovan, & Russu, 2016).

When contact angle analysis was performed on the coated sample, it was analyzed that the coating has reduced the contact angle.



A:coated sample B: uncoated sample

Figure 4. 4: contact angle of coated and uncoated sample

Fig 4.4 A showed that the contact angle for the coated sample was 93 °, whereas fig 4.4 B showed that the uncoated sample had a contact angle of 108°. As a result, we may infer that the coating has balanced the sample's hydrophobic and hydrophilic components by decreasing the hydrophobic content of the sample.

4.8: Biological Tests :

Biological testing includes hemocompatibility tests and metal ion leaching analysis.

4.9: Leach Test:

Leach test analysis was done by using three different techniques

a. FTIR

b. XRD

c. SEM EDX

4.9.1: FTIR Analysis :

The coated sample was immersed in PECF solution, the sample was analyzed after every week, and the FTIR analysis of the sample indicated that there is no leaching of metal ions from the coated substrate.

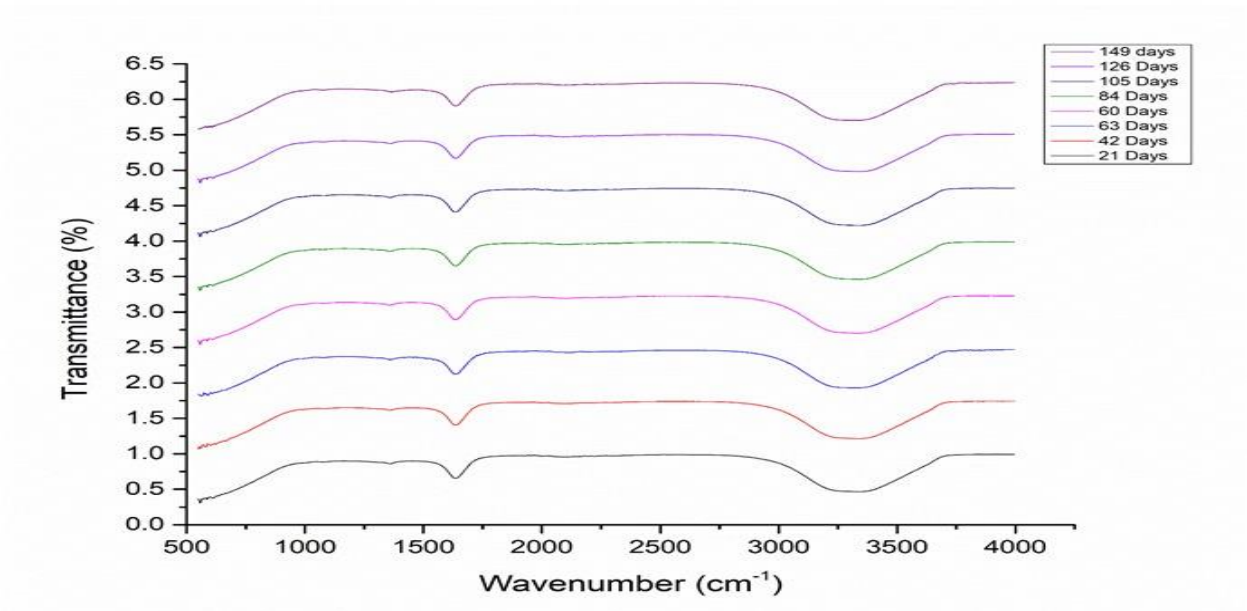


Figure 4. 5 Graphical representation of data from FTIR analysis of sample

Fig 4.5 showed the graph that summarizes the data from FTIR analysis. There was no change in the graph's pattern, and it remained the same even after 149 days so, it was indicated that there was no metal ion leaching.

4.9.2: XRD Analysis:

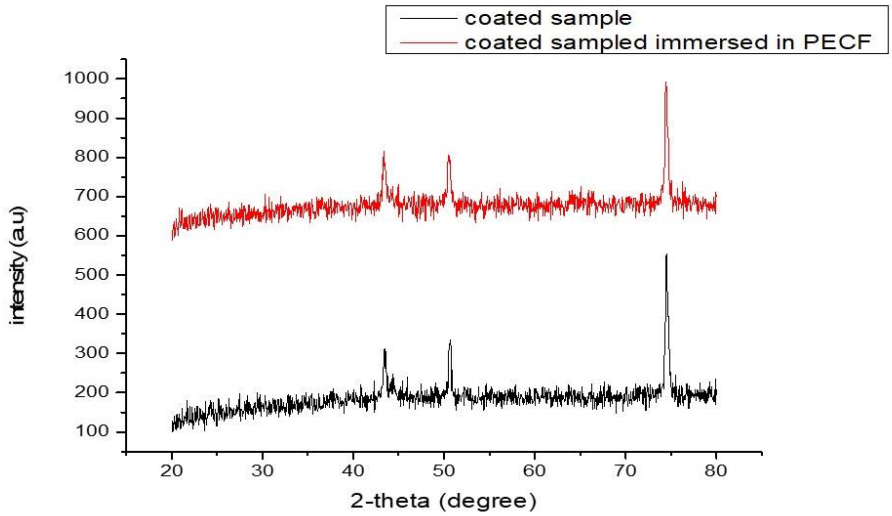


Figure 4. 6: Graphical representation of EDX data for coated sample immersed in PECF sol.

Fig 4.6 showed that the peaks for both samples i.e., coated sample immersed in PECF solution and coated sample remained same . The crystallographic structure of the sample has not changed even after 149 days so, it was concluded that there is no leaching of metal ions .

4.9.3: EDX Analysis:

Elemental composition analysis of the sample placed in PECF solution to study the accelerated aging for 149 days also confirmed that there is no leaching of metal ions.

Elements	Pure SS (wt.%)	Coated sample (wt. %)	Coated sample placed in PECF (wt. %)
C	0.026	10.06	10.18
O	4.00	3.08	3.67
Cr	16.55	14.81	14.97
Fe	69.42	63.05	62.03
Ni	10.0	9.00	9.15

Table 4. 6: EDX data for weight %age of elemental composition of samples

The results from SEM EDX were summarized in table no 4.6. When the elemental composition of a coated sample immersed in PECF solution for 149 days was examined using EDX, it was found that the elemental composition remained the same. As a result, it was concluded that there was no metal ion leaching.

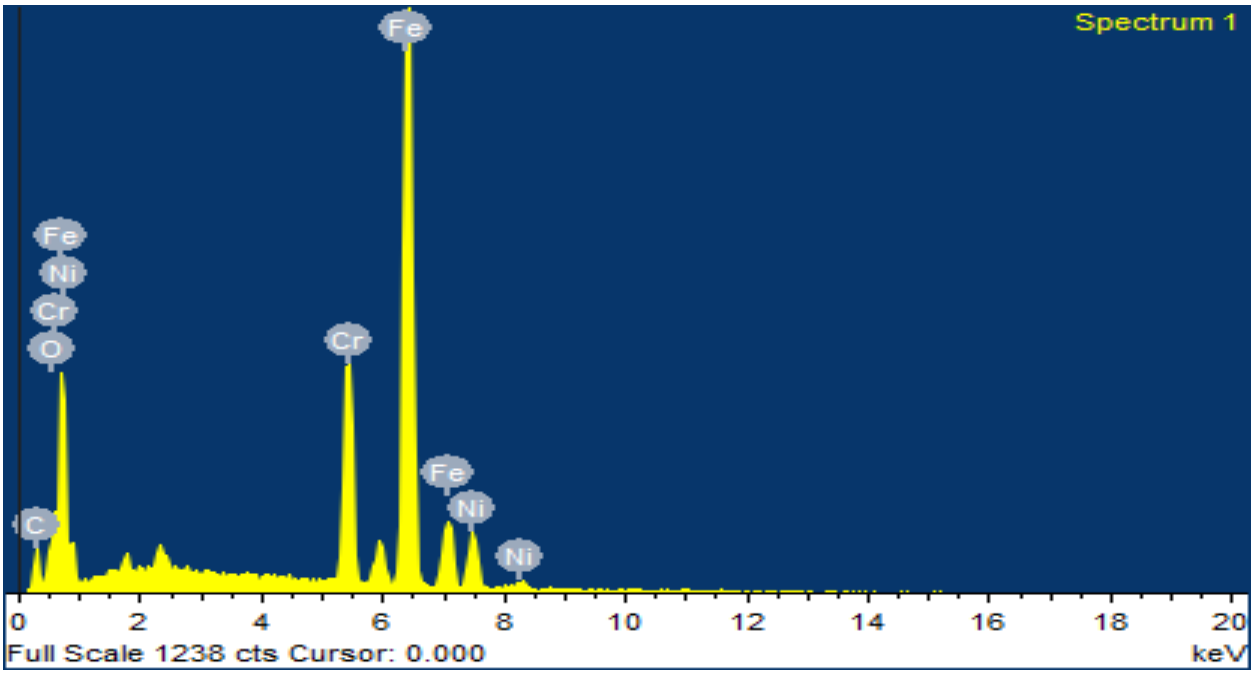


Figure 4. 7: Graphical representation of EDX data for sample immersed in PECF solution.

Fig 4.7 showed the graphical representation of elemental composition of sample placed in PECF solution for 149 days and then analyzed by using the technique of SEM EDX.

Both the graphical representation and tabular data showed that there is no leaching of metal ions because the elemental composition of coated sample , pure stainless steel and coated sample placed PECF solution remained same .

4.10: Hemolytic Assay :

The hemocompatibility of biomedical implants was tested using a hemolytic assay. Because a biomedical implant interacts with bodily fluids and blood regularly, it must be hemo-compatible.

There are two approaches to analyze hemolytic properties.

a. qualitative analysis

b. UV spectroscopy method

4.10.1: Qualitative Analysis:

The color of the supernatant indicates the hemolysis .Color of the supernatant was analyzed and compared for the positive control, negative control, coated sample, and uncoated sample.

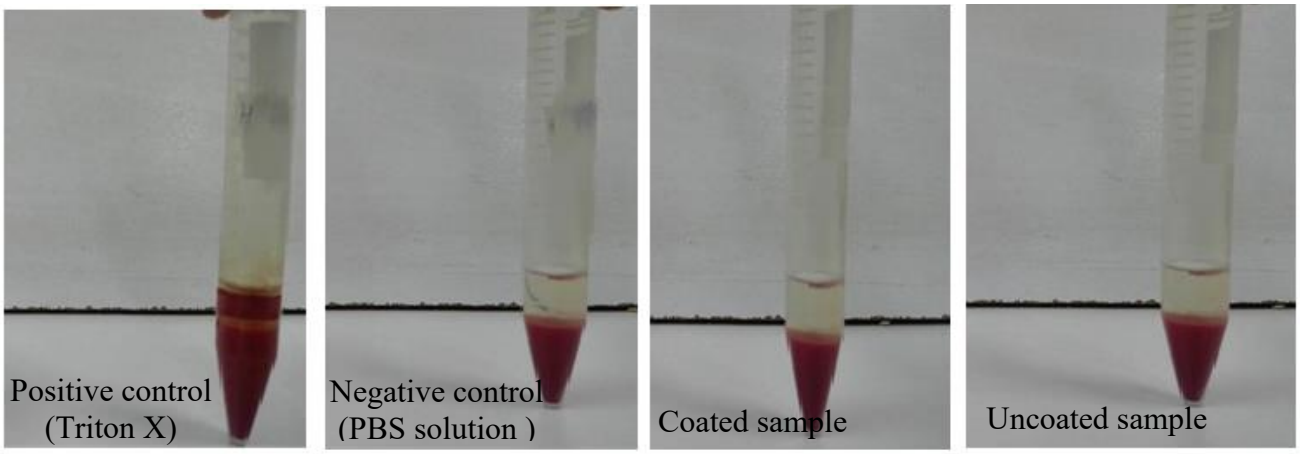


Figure 4. 8:Qualitative Analysis of Hemolysis in Samples

The pictures for the positive control, negative control, coated, and the uncoated sample was shown in Fig 4.8. By quantitatively analyzing the results, it was found out that the color of the positive control which is triton X is red, whereas the supernatant for negative control was colorless. Both the coated and uncoated samples had the same color, indicating that they had the same effects as the negative control. According to the quantitative study, the positive control exhibited 100% hemolysis, whereas the negative control, coated sample, and uncoated sample showed no hemolytic activity.

4.10.2: UV Spectroscopy Method

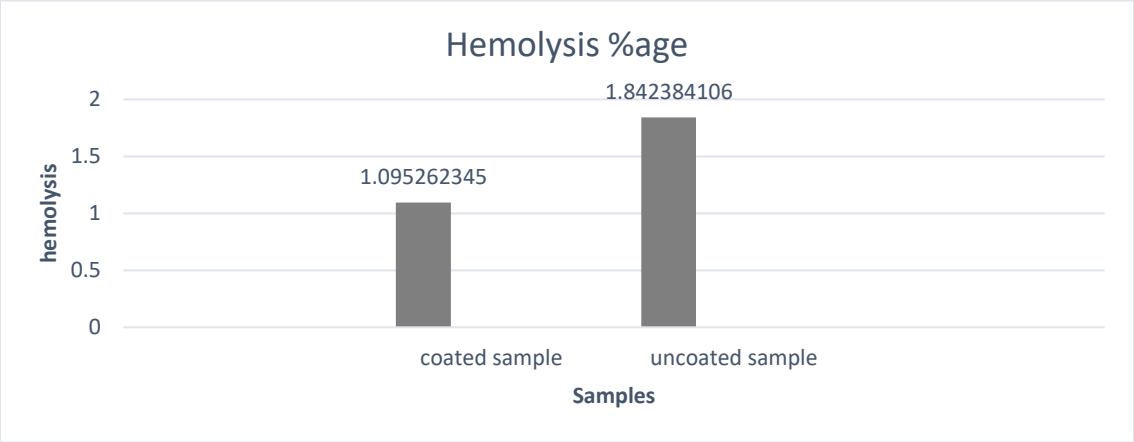
Finding out the hemolysis % is another form of hemolytic analysis. This was done by utilizing UV spectroscopic techniques at 540 nm wavelength to determine the absorbance of all samples, including coated, uncoated, positive, and negative controls. The percentage of hemolysis was determined using the following formula.

percent hemolysis= [(absorbance of sample – absorbance of PBS) / (absorbance of positive control- absorbance of negative control)] * 100 (Guo et al., 2021)

Sample	Absorbance	Hemolysis %age
positive control	7.98	100
negative control	0.128	0
coated sample	0.214	1.095262354
uncoated sample	0.274	1.842384106

Table 4. 7: Hemolytic percentage of samples

Table 4.7 summarized the hemolysis % data for all samples, including the positive, negative, coated, and uncoated samples.



Graph No 3: exhibited a graphical depiction of the data in Table 4.7.

The experiment indicated that the positive control had 100 percent hemolysis while the negative control had zero percent hemolysis.

The coated sample has a hemolysis percentage of 1.09%, whereas the uncoated sample has a hemolysis percentage of 1.84%, both of which fall within the non-hemolytic material range defined by ASTM F 756-00.

As a result, it was determined that coating had further lowered the hemolytic percentage. Table 4.8 summed up data for the ranges of hemolytic material by ASTM F 756-00 ranges (Mesdaghinia et al., 2019)

Type	**Percentage**
Non-Hemolytic	0-2 %
Slightly hemolytic	2-5%
Hemolytic	>5%

Table 4. 8: Hemolysis Standard Range

CHAPTER NO 5 : CONCLUSION AND FUTURE PRESPECTIVES

5.1: Conclusion :

The goal of this research was to develop a coating that can enhance the properties of the pure substrate. Physical vapor deposition method was used to design and fabricate graphite substrate pure carbon coatings on electropolished stainless steel sample. By using the appropriate parameters, several coating thicknesses were achieved. Further analysis was done by using various characterization techniques, and it was determined that the sample with a coating thickness of 1.5 μm improved the properties of stainless steel. The sample with a 1.5 μm thick coating layer has a smooth surface with no micro cracks, the least roughness, the smoothest topography, as well as the maximum hardness which is almost twice of pure stainless steel.

When the studies were carried out on the sample with a 1.5 μm thick-coated layer, there was no leaching of metal ions or hemolysis. As a result, the coated material was determined to be hemo-compatible. It was concluded that a material fulfilling these criteria would be utilized in biomedical applications such as the fabrication of coronary stents.

5.2: Future perspectives :

Before employing it in an application, it should undergo additional mechanical testing. Compression testing, tensile strength testing, and fracture strength testing are examples of this type of testing. Platelet adhesion tests will also be useful in demonstrating that this covering is biocompatible. Biocompatibility and hemocompatibility of biomedical implants will also be tested on animals before taking it to human trials.

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