

ENHANCED DIFFERENTIAL ADSORPTION DYNAMICS OF OSTEOGENESIS AND ANGIOGENESIS INFLUENCING PROTEINS FOR BONE TISSUE ENGINEERING

Submitted in partial fulfillment of the requirements for the award
of Bachelor of Technology degree in Biotechnology

By

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INSTITUTE OF SCIENCE AND TECHNOLOGY
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Accredited with Grade “A++” by NAAC

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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of Sylvia Jessiela Pearl R (40230047) who carried out the project entitled “ **ENHANCED DIFFERENTIAL ADSORPTION DYNAMICS OF OSTEOGENESIS AND ANGIOGENESIS INFLUENCING PROTEINS FOR BONE TISSUE ENGINEERING**” Under my Supervision from November 2023 - March 2024.

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ABSTRACT

This comprehensive research study offers a comparative analysis of serum adsorption dynamics onto various metallic surfaces commonly used in orthopedic implant materials. Investigated substrates include titanium, titanium aluminum alloy, stainless steel grade 304, and stainless steel grade 316. Using albumin as a model, the study delves into the intricate interplay between proteins and metal surfaces to enhance implant biocompatibility and performance. In addition to examining albumin adsorption kinetics and equilibrium behavior, in vitro experiments elucidate protein binding characteristics and conformational changes upon adsorption. Graphical representations and isotherm models comprehensively analyze experimental data, revealing distinct adsorption profiles and surface-specific variations in protein affinity. These findings highlight nuanced surface properties influencing protein binding and underscore the importance of tailored surface modifications for optimizing protein-material interactions. Such insights hold significant implications for enhancing orthopedic implant biocompatibility, osseointegration, and long-term performance, thereby advancing bone tissue regeneration and repair. Future research may focus on exploring additional protein species and elucidating molecular mechanisms governing protein adsorption onto metallic surfaces, fostering innovative biomaterial strategies in bone tissue engineering.

Keywords: Orthopedic implants, Titanium, Titanium aluminum alloy, Stainless steel grade 304, Stainless steel grade 316, Albumin, Protein adsorption kinetics, Isotherm models, Bone tissue engineering.

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LIST OF ABBREVIATIONS

BMP	Bone Morphogenetic Proteins
VEGF	Vascular Endothelial Growth Factor
FGF	Fibroblast Growth Factors
PDGF	Platelet-Derived Growth Factor
TGF- β	Transforming Growth Factor-Beta
IGF	Insulin-Like Growth Factor
MSC	Mesenchymal Stem Cells
ECM	Extracellular Matrix
TEMED	N,N,N',N' -Tetramethylethylenediamine
APS	Ammonium Persulfate
BSA	Bovine Serum Albumin
FBS	Fetal Bovine Serum
Ti	Titanium
TiAl	Titanium Aluminium
SS 304	Stainless Steel -Grade 304
SS 316	Stainless Steel -Grade 316
SDS	Sodium Dodecyl Suphate

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CHAPTER 1

INTRODUCTION

Proteins are large, complex molecules that serve a multitude of essential functions in living organisms. Proteins provide structural support to cells, tissues, and organs. For example, the protein collagen forms the structural framework of connective tissues such as skin, tendons and bones, imparting strength and elasticity. Proteins serve as carriers and transporters, facilitating the movement of molecules such as ions, nutrients and gases across cell membranes and throughout the body. (Donaldson & Jackson, 2011). Proteins play a crucial role in the behaviour of implant materials. They can either inhibit or promote metal degradation, depending on their type, concentration, and the properties of the implant material ("ReCAP: Talha Naushad," 2019).

Angiogenic and osteogenic proteins are two classes of biomolecules that play crucial roles in the processes of angiogenesis (the formation of new blood vessels) and osteogenesis (the formation of bone tissue), respectively. These proteins are involved in various physiological and pathological conditions, including wound healing, tissue repair, bone regeneration, and the growth of tumours. Angiogenesis is a complex biological process involving the formation of new blood vessels from pre-existing vasculature. Angiogenic proteins promote and regulate angiogenesis by stimulating endothelial cell proliferation and migration. These proteins act through a series of signalling pathways that orchestrate the formation of new blood vessels in response to physiological stimuli or pathological conditions, for example, VEGF-A, VEGF-B, VEGF-C, and VEGF-D. Other angiogenic proteins include FGFs, angiopoietins, PDGF, TGF- β , and IGF.

Osteogenesis is the process of bone formation, which involves the differentiation of mesenchymal stem cells (MSCs) into osteoblasts, the deposition of extracellular matrix (ECM), and the mineralization of the matrix to form mature bone tissue. Osteogenic proteins play key roles in regulating these processes by promoting

osteoblast differentiation, ECM synthesis, and mineralization, For example, BMPs, TGF- β . Other osteogenic proteins include osteopontin, osteocalcin, osteonectin, and bone sialoprotein.(Bai et al., 2021)

Albumin, a major protein found in blood plasma, is primarily known for its role in maintaining osmotic pressure, transporting various molecules such as hormones, fatty acids, and drugs, and contributing to the regulation of pH and buffering capacity in the blood. However, albumin itself does not possess intrinsic angiogenic or osteogenic properties. Albumin does not directly stimulate angiogenesis or osteogenesis like specific growth factors or signalling proteins such as vascular endothelial growth factor (VEGF) for angiogenesis or bone morphogenetic proteins (BMPs) for osteogenesis. Instead, albumin acts as a carrier molecule for various bioactive substances that influence angiogenesis or osteogenesis indirectly. Albumin has high affinity binding sites that can interact with a wide range of molecules, including growth factors, hormones, cytokines, and small molecules. These molecules can bind to specific sites on albumin, forming complexes that are transported in the bloodstream to target tissues. Albumin can bind to growth factors such as insulin-like growth factor (IGF), vascular endothelial growth factor (VEGF), and fibroblast growth factor (FGF), protecting them from degradation and enhancing their bioavailability(Basu et al., 2013). By binding to growth factors and other signalling molecules, albumin can regulate their release kinetics and tissue distribution, ensuring a sustained and controlled delivery to target cells.

Protein adsorption is the process by which proteins in a biological fluid adhere to and accumulate on the surface of a solid material, such as a biomaterial or an implant. This phenomenon occurs spontaneously when the material is exposed to a protein-containing environment, such as blood, serum, or interstitial fluid. Protein adsorption plays a critical role in mediating the interactions between biomaterials and biological systems, influencing various cellular responses and tissue integration processes. Proteins in the surrounding biological fluid are transported to the surface of the biomaterial through diffusion and convection. The rate and extent of protein

transport depend on factors such as protein concentration, flow rate, and surface properties of the material. Upon reaching the material surface, proteins undergo initial attachment facilitated by weak physical interactions, including van der Waals forces, electrostatic interactions, hydrogen bonding, and hydrophobic interactions. These interactions enable proteins to adhere to the material surface reversibly. When proteins adsorb onto the surface of implants, they can form a protective film that inhibits corrosion and decreases tribocorrosion. However, the interaction of metal ions with proteins can accelerate the corrosion of metallic implants(Jurczak et al., 2020). Recent advancements in implant surface modifications have focused on using proteins, peptides, and peptidomimetics as promising agents for titanium surface coatings. Additionally, coating implant materials with extracellular matrix proteins like fibronectin and laminin can enhance their biocompatibility by promoting cell adhesion and migration different proteins have been identified to bind to implant surfaces, including extracellular and intracellular proteins(Fu et al., 2020)Specific proteins such as fibrinogen and albumin have been shown to have significant binding differences between different implant surfaces.

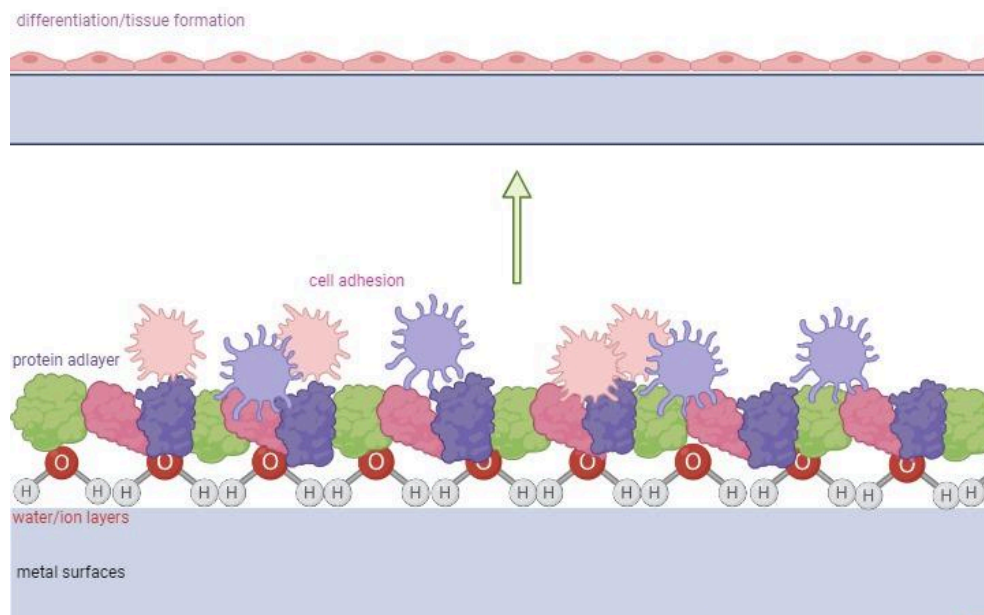


Fig 1.1 Mechanism of protein adsorption and tissue proliferation

Orthopedic implants play a pivotal role in modern medicine, facilitating the restoration of musculoskeletal function and enhancing the quality of life for millions of individuals worldwide. The success of these implants hinges not only on their structural integrity but also on their compatibility with the surrounding biological milieu. In recent years, there has been a burgeoning interest in the development of biomaterials tailored to promote tissue regeneration and integration within the host environment. Among these biomaterials, metallic alloys have garnered significant attention due to their mechanical robustness, corrosion resistance, and biocompatibility. Metallic implants utilized in orthopedic applications are exposed to the complex milieu of bodily fluids upon implantation, leading to intricate interactions with proteins present in serum and plasma. Protein adsorption onto implant surfaces serves as the initial event in the cascade of biological responses that ultimately dictate the long-term fate of the implant, including tissue integration, inflammation, and implant longevity. Understanding the dynamics of protein adsorption on metallic surfaces is thus paramount for elucidating the biocompatibility and performance of orthopedic implants.

This study aims to investigate the differential adsorption dynamics of albumin, a key protein influencing, namely, osteogenesis and angiogenesis, onto various metallic surfaces commonly employed in orthopedic implants. By elucidating the intricate interplay between proteins and metal substrates, this study seeks to provide valuable insights into the design and optimization of orthopedic implants for enhanced tissue regeneration and clinical outcomes. (Chen et al., 2020)

The metals under investigation include titanium, titanium aluminum alloy, stainless steel grade 304, and stainless steel grade 316, chosen for their widespread use in orthopedic implantation. Titanium is a widely used material in biomedical engineering and implantology due to its excellent biocompatibility, mechanical properties, and corrosion resistance. Titanium implants are commonly employed in various medical applications, including orthopedic and dental implants,

cardiovascular devices, and prosthetic components. Titanium exhibits excellent biocompatibility, meaning it can coexist with biological systems without causing adverse reactions or immune responses, titanium forms a passive oxide layer on its surface, which is chemically stable and biologically inert. This oxide layer prevents corrosion and reduces the release of metal ions into the surrounding tissues, minimizing the risk of inflammation and allergic reactions. The biocompatible nature of titanium, along with its surface properties, facilitates the adhesion, proliferation, and differentiation of osteogenic cells, leading to the formation of new bone tissue on the implant surface. Titanium-aluminium alloys, also known as Ti-Al alloys, are a class of metallic materials that are increasingly being investigated for biomedical implant applications due to their favorable combination of properties. Titanium-aluminum alloys exhibit good biocompatibility, similar to pure titanium, making them suitable for implantation in the human body. The presence of titanium in the alloy contributes to the formation of a stable oxide layer on the surface, which enhances biocompatibility by preventing corrosion and reducing the release of metal ions into the surrounding tissues. Additionally, aluminium content in the alloy does not compromise its biocompatibility, as aluminium is generally considered to be biologically inert. (Barberi & Spriano, 2021a)

Stainless steel 304, a type of austenitic stainless steel, is commonly used as an implant material in various biomedical applications due to its excellent combination of mechanical properties, corrosion resistance, and biocompatibility. (Cacciotti, 2017) The alloy can withstand mechanical stresses and loading conditions encountered in orthopedic and dental implants, providing structural integrity and support to the implanted tissue or device. Stainless steel 316, another type of austenitic stainless steel, is commonly utilized as an implant material in various biomedical applications, particularly in orthopedic and surgical implants and has enhanced corrosion resistance compared to stainless steel 304. The alloy can withstand mechanical stresses and loading conditions encountered in orthopedic implants, providing structural integrity and support to the implanted tissue or device.

Additionally, stainless steel 316 retains good formability and machinability, allowing for the fabrication of implants with complex geometries and customized designs

Through a comparative analysis of protein adsorption kinetics and equilibrium behavior across these metal substrates, this study aims to discern nuanced differences in surface properties and protein affinity, thus shedding light on the biocompatibility and performance of orthopedic implants in clinical settings.

In pursuit of this objective, experimental methodologies encompassing protein adsorption assays, surface characterization techniques, and data analysis utilizing graphical representations and isotherm models have been employed. The outcomes of this study are expected to advance our understanding of metal-protein interactions and inform the rational design of orthopedic implants tailored to promote tissue regeneration and integration, thereby addressing the evolving needs of patients and clinicians in the field of orthopedic surgery.

CHAPTER 2

LITERATURE SURVEY

2.1 BONE TISSUE ENGINEERING

Bone tissue engineering aims to address challenges associated with bone regeneration by developing functional alternatives to traditional bone scaffolds. Bone tissue engineering aims to regenerate bone using functional alternatives. Biomaterials induce osteoconduction, provide stability, and integrate or degrade. While autologous or allograft bone scaffolds can aid regeneration, they pose issues like fast degradation rates, reduced bioactivity, and donor site morbidity. To overcome these challenges, bone tissue engineering focuses on creating scaffolds that induce osteoconduction, provide mechanical stability, and integrate into the bone structure or degrade safely in the body. Various biomaterials are employed, each with distinct advantages and drawbacks. This review discusses the requirements of bone tissue engineering, including the regeneration process, scaffold manufacturing, materials selection, and incorporation of bioactive molecules, growth factors, and cells to facilitate successful bone regeneration. (Perić Kačarević et al., 2019). Bone tissue engineering involves utilizing cells, growth factors, scaffolds, and mechanical stimulation to enhance bone repair and regeneration, offering promising strategies for treating bone fractures and defects. Bone tissue engineering offers new strategies for bone repair and regeneration. Components include cells, growth factors, scaffolds, and mechanical stimulation (Lim, 2022).

Bone tissue engineering also aims to develop solutions for complex bone injuries. It involves bioactive molecules, stem cells, and biomimetic substitutes to enhance bone regeneration and repair processes effectively. The understanding of biofactors and stem cells is crucial for regenerative medicine (Manzini et al., 2021). Bone tissue engineering is an emerging field that aims to combat the limitations of conventional

treatments of bone disease by providing a firm structural support, withstand load bearing, and rapidly respond to metabolic demand. It is a field addressing limitations of traditional bone disease treatments by creating vascularized structures that provide structural support, withstand loads, and meet metabolic demands efficiently (Akter & Ibanez, 2016). In this paper, the authors classified 3D bioprinting constructs into two categories: acellular and cellular, based on the suitable materials for these constructs and the fabrication processes used to manufacture them. Bioprinting in bone tissue engineering involves layer-by-layer assembly of materials to create complex structures for biological functions, categorized as acellular or cellular constructs using polymers, metals, and hydrogels (Choe et al., 2022). In this paper, the importance of semiconductor industry-derived materials as a potential solution to combat oxidative stress and address the challenges associated with aging bone was highlighted. But, the existing clinical approaches face challenges in effectively treating complex craniofacial bone defects, including issues such as oxidative stress, inflammation, and soft tissue loss.

2.2 IMPORTANCE OF BIOMATERIALS

Biomaterials play a crucial role in revolutionising bone regeneration by stabilising and expediting the healing process, especially in complex craniofacial bone defects, addressing challenges like oxidative stress and inflammation (Awad et al., 2023). In this article, a micro-nanohybridised hydrogel based on hydroxyapatite (HAp) structure is used for bone tissue engineering, which mimics the natural structure of the extracellular matrix and represents a physiological microenvironment for normal cellular functions. Biomaterials play a crucial role in bone tissue engineering by providing scaffolds for cell activity, mimicking natural structures, and aiding in bone regeneration through osteostimulation and biocompatibility (Najman et al., 2023). In this article, a polyhedron-like scaffolds with spatial topologies were constructed via 3D printing technology to mimic the meshwork structure of cancellous bone, which greatly promoted the osteogenic differentiation of bone mesenchymal stem cells

(BMSCs) via activating PI3K-Akt signals, and exhibited satisfactory performance on angiogenesis and neurogenesis. Biomaterials, like polyhedron-like scaffolds, play a crucial role in bone regeneration by promoting osteogenic differentiation, angiogenesis, and neurogenesis, leading to innervated and vascularized bone regeneration (Zhang et al., 2023). In this article, the authors compared metallic and synthetic polymer implant biomaterials that can be employed to secure load bearing bone fractures due to their ability to withstand the mechanical stresses and strains of the body, with a focus on their classification, properties, and application. The paper compares metallic and synthetic polymer implant biomaterials used for load-bearing bone fractures, focusing on their classification, properties, and application. It discusses the use of metals like stainless steel and titanium, as well as polymers like polyethylene and polyetheretherketone (PEEK), in orthopaedic implants to achieve similar functional capacity to natural bone (Al-Shalawi et al., 2023).

2.3 MATERIALS FOR BONE TISSUE ENGINEERING

Titanium is known for its lightweight nature, high strength, corrosion resistance, and high melting point. In this article, 3 mm of stainless steel and titanium sheets were joint under the resistance spot welding method with an aluminium interlayer, and the optimised welding parameters were provided under the Taguchi method L9 orthogonal array along with the mechanical properties' investigation. Titanium is extremely lightweight, Ti6Al4V alloy is practical, SS304 has good corrosion resistance, and SS316L offers improved corrosion resistance and weldability in resistance spot welding. The main advantage of this process is creating the complicated shapes in a single process with high dimensional accuracy, reduction in wastage of high-cost materials, and reducing the secondary operation time as mentioned in this paper (Taufiqurrahman et al., 2022) (Karpagaraj et al., 2020). Titanium-aluminium alloy combines the lightweight properties of titanium with the strength and ductility of aluminium, making it suitable for various applications. This study compares the corrosion behaviour of 316L stainless steel and a titanium alloy

in a Hank solution and shows that the titanium alloy has a higher potential than 316L Stainless steel and lower corrosion current. Titanium alloy is mechanically stronger than 316L stainless steel, with higher potential and lower corrosion current in Hank's solution. Stainless steel 316L is commonly used in biomedical devices. In this article, the authors proposed a utility model for three-layer metal composite sheet, including titanium layer, aluminium lamination and stainless steel layer, and the aluminium laminations set up in between the two layers. Titanium is lightweight with high strength and corrosion resistance. Titanium-aluminium is ductile. Stainless steel 304 is corrosion-resistant, while stainless steel 316 offers improved corrosion resistance in harsh environments. (Aroussi et al., 2019) (Ying, Mingliang et al., 2019). Stainless steel 304 exhibits excellent durability, biocompatibility, and corrosion resistance, making it ideal for applications in nuclear plants, chemical processing, and oil wells. In this article, the authors measured the contact angle variation of a water drop measured on the surfaces of samples of AISI 304 and 316 stainless steel and of glass when coated with TiO_2 , TiO_x and TiN films of approximately 600nm thick. Titanium-based coatings like TiO_2 , TiO_x , and TiN enhance wettability on stainless steel (304, 316) and glass surfaces, altering contact angles from hydrophilic to hydrophobic due to surface modifications. (Estrada-Martínez et al., 2017). On the other hand, stainless steel 316 offers good corrosion resistance, biocompatibility, and durability, making it suitable for biomedical devices like stents and surgical instruments. Additionally, coatings like TiO_2 , TiO_x , and TiN enhance the properties of stainless steel electrodes by improving their conductive and inhibitory characteristics in electrochemical environments.

Studies have explored the application of stainless steel 316L in bone tissue engineering, indicating its potential for bone lesion support. In this paper, a thin film of poly(L-lactic acid) was deposited on top of the stainless-steel substrate via spin coating with PLLA solution concentrations of 2.5 wt% or 5.0 wt% (Rocha et al., 2022). Additionally, research has focused on enhancing the biological response of stainless steel 316L through porous coatings, impacting corrosion resistance and

promoting bone cell ingrowth. In this article, the authors evaluated medical 316L stainless steel (SS)-based devices covered by a polymer poly (L-lactic acid) (PLLA) coating for bone lesion mechanical and functional support. PLLA-SS316L devices showed superior in vitro and in vivo outcomes. PLLA coating provided osteo-inductive properties to the device (Branquinho et al., 2021). Furthermore, the fabrication of nano dimples on 316L stainless steel surfaces has shown promising results in improving osteoblast viability and functions while reducing bacterial colonisation, suggesting enhanced osseointegration and infection prevention. In this paper, the corrosion resistance and biocompatibility of titanium (Ti) porous coating with high and low porosity gradients on grit-blasted and ground 316L stainless steel by cold spray were studied. Porous coatings on ground substrate exhibit good corrosion properties (Wathanyu et al., 2022). High porosity gradient coatings on ground steel show good biological responses. However, specific studies on the use of titanium, titanium-aluminium alloy, and stainless steel 304 in bone tissue engineering applications were not found in the provided contexts.

2.4 PROTEIN ADSORPTION DYNAMICS ON BIOMATERIALS

Protein adsorption on metal surfaces is a crucial phenomenon influencing various industrial and biomedical processes. Studies have shown that the adsorption of proteins onto metal surfaces is influenced by factors such as protein concentration, surface roughness, charge, and hydrophilicity. In this paper, a multiscale study of protein corona on iron surfaces and nanoparticles in contact with cow milk proteins was performed, where the binding energies of proteins with the substrate were quantified by the adsorption affinity. In this paper, a multiscale study of protein corona on iron surfaces and nanoparticles in contact with cow milk proteins was performed, where the binding energies of proteins with the substrate were quantified by the adsorption affinity (Mosaddeghi Amini et al., 2023). In this paper, the authors used scanning Kelvin probe force microscopy (SKPFM) to reveal the

synergistic effect of various bovine serum albumin (BSA) concentrations and overpotential conditions on BSA protein adsorption mechanisms and its influence on the corrosion behaviour of the CoCrMo alloy in phosphate-buffered saline solution. Protein adsorption on CoCrMo alloy involves conformational arrangements, electrochemical interactions, and counterion adsorption, impacting corrosion behaviour locally due to surface potential variations and cluster formation (Rahimi et al., 2022). How the surface characteristics affect protein adsorption is investigated, aiming to provide as comprehensive a picture as possible of adsorptive mechanisms and type of chemical bonding with the surface, as well as of the characterization techniques effectively applied to model and real implant surfaces. Surface free energy, charge, microroughness, and hydroxylation degree influence protein adsorption on metal surfaces. Protein conformation is guided by protein structure, nano-scale topography, and functional groups exposed on the surface (Barberi & Spriano, 2021). The interaction between proteins and metal surfaces can impact corrosion resistance, biocompatibility, and the formation of protein layers crucial for implant integration. Results confirm that the surface roughness of the size comparable with protein molecule dimensions exerts a decisive influence on their adsorption kinetics derived from QCM measurements. Protein adsorption on metal surfaces involves hydration functions, water factors, and roughness influence, affecting adsorption kinetics, as seen with human serum albumin on a gold substrate (Adamczyk et al., 2018). Adsorption of proteins onto hydrophilic surfaces adheres to three steps, namely, biased diffusion, anchoring, and stepwise adsorption accompanied by structural adaptation, which provides insights into the development of inorganic surfaces for biomedical and therapeutic applications. Protein adsorption on metal surfaces involves biased diffusion, anchoring, and stepwise adsorption with structural adaptation, as shown in simulations with collagen-like peptides on gold nanosurfaces (Tang et al., 2019).

Research indicates that protein adsorption on metal surfaces follows a multi-step process involving biased diffusion, anchoring, and stepwise adsorption with structural adaptation .

Understanding the mechanisms of protein adsorption on metal surfaces is essential for optimizing industrial processes, enhancing implant performance, and ensuring consumer safety in the food industry and beyond.

2.5 EXISTING RESEARCH ON PROTEIN ADSORPTION

Protein adsorption onto various surfaces is a crucial area of research with implications in biomedicine, bioanalytical chemistry, materials engineering, and nanotechnology. Protein adsorption onto various surfaces is a crucial area of research with implications in biomedicine, bioanalytical chemistry, materials engineering, and nanotechnology. In this article , the authors investigated the adsorption behavior of myoglobin, haemoglobin, cytochrome-c, and lysozyme on carboxy-functionalized polystyrene microparticles using zeta potential (ζ) and absorption spectroscopy measurements. Protein adsorption on carboxy-functionalized microparticles was studied using zeta potential and absorption spectroscopy, revealing monolayer adsorption with zeta potential and multilayer adsorption with absorption spectroscopy (Miyagawa et al., 2023). In this article , Dicarboxymethyl cellulose (DCMC) was synthesized and tested for protein adsorption, and the results showed that protein adsorption onto DCMC is charge dependent. The research paper explores protein adsorption onto a novel functionalized cellulose-based polymer, DCMC, showing charge-dependent adsorption of positively charged proteins following Langmuir isotherm and pseudo second-order model (Gago et al., 2022). The use of nanoscale surfaces to remodel the conformational landscape and the aggregation pathways of amyloidogenic peptides and proteins has been proposed as a promising strategy against several severe human diseases as mentioned in this paper . Protein adsorption to surfaces can induce conformational changes, impacting structure and function. Research

explores adsorption mechanisms and structural dynamics, crucial for biotechnology and biomolecular studies (Protein Adsorption and Conformational Changes, 2022). Protein adsorption onto surfaces of diverse materials of both natural and artificial origin is of utmost relevance in many areas of research and technology: medicine, pharmaceutical sciences, analytical sciences, biotechnology, nanotechnology, and cell biology as discussed by the authors. Protein adsorption onto various material surfaces is extensively studied in medicine, biotechnology, and nanotechnology, as highlighted in the paper "Protein Adsorption and Conformational Changes." (Assfalg, 2021). How the surface characteristics affect protein adsorption is investigated, aiming to provide as comprehensive a picture as possible of adsorptive mechanisms and type of chemical bonding with the surface, as well as of the characterization techniques effectively applied to model and real implant surfaces. Protein adsorption on titanium surfaces is influenced by surface properties like free energy, charge, roughness, and hydroxylation, impacting cell response and osseointegration in implant devices (Barberi & Spriano, 2021b).

2.6 BIOLOGICAL RESPONSES TO PROTEIN ADSORPTION

Protein adsorption plays a crucial role in cell adhesion, proliferation, and differentiation on biomaterial surfaces. Various studies highlight the impact of protein adsorption on these cellular processes. For instance, the presence of distinct proteins on 3D printed titanium surfaces influences pro-inflammatory cytokine gene expressions and cell behavior. In this paper, the surface effects of the products generated by 3D printed titanium on osseointegration, six surface types of titanium discs produced by the direct metal laser sintering (DMLS) and electron beam melting (EBM) methods, with two sizes of titanium particles and post-printing acid etching, were used to examine the surface topography and to explore the protein adsorption, pro-inflammatory cytokine gene expressions, and MC3T3-E1 cell adhesion, proliferation, and differentiation. Protein adsorption on 3D printed titanium surfaces affects cell adhesion, proliferation, and differentiation, with

acid-etched surfaces favoring osteogenic differentiation based on the study's findings(Jin et al., 2022). Additionally, surface modifications affecting protein adsorption can alter cell adhesion and proliferation on engineering plastics processed with supercritical carbon dioxide. The results suggest that when supercritical treatment is performed under conditions that affect the surface properties of the material, the authors should consider that cell adhesion and proliferation may change. The results suggest that when supercritical treatment is performed under conditions that affect the surface properties of the material, the authors should consider that cell adhesion and proliferation may change. Moreover, the correlation between protein adsorption and nanometer-scale structures on biomaterial surfaces can modulate cell adhesion through changes in fibrinogen and fibronectin adsorption forces(Watanabe et al., 2020). Molecular dynamics simulations further demonstrate how protein adsorption on Sr²⁺-doped hydroxyapatite surfaces influences cell-material interactions and adhesion mechanisms. These findings collectively emphasize the critical role of protein adsorption in regulating cell responses on biomaterial surfaces.

2.7 FUTURE DIRECTION:

Protein adsorption using blood serum is a crucial aspect in various fields like biotechnology and biomolecular engineering. Different materials, such as porous silica, peptide gels, PEG brushes, carbon-based materials, and mixed-mode adsorbents, have been studied for their protein adsorption capabilities. In this article, high-dimensional data spectromicroscopy analysis in the infrared region (μ -FTIR) was employed to investigate the enzyme's conformational change when adsorbed onto solid matrices, across a ca. 20 mm² area(Mendes et al., 2023). For instance, studies have shown that materials like porous silica modified with fluoride-species exhibit favorable adsorption of human blood serum proteins, with maximum adsorption capacity near the isoelectric point(Stachiv et al., 2023). Peptide gels,

when combined with fetal bovine serum, transform into cytocompatible materials by absorbing serum proteins, thus enhancing cell viability. PEG brushes have been effective in preventing undesired protein adsorption onto material surfaces exposed to biological fluids. Carbon-based materials like expanded graphite and graphene nanoplatelets have shown high protein adsorption capacities, making them potential candidates for biomedical applications. Mixed-mode adsorbents have been specifically designed for expanded bed adsorption processes, showing promising results in adsorbing proteins like human serum albumin and immunoglobulin G.

CHAPTER 3

AIM AND SCOPE

3.1 AIM

The aim is to examine and evaluate the adsorption rates of various metal surfaces for the purpose of bone tissue engineering.

3.2 SCOPE

- To investigate the rate of protein adsorption on implant materials
- To evaluate the adsorption of protein on material by various body regulating source fluid like serum.
- Compare the adsorption of protein on chemical based body mimicking protein fluids such as BSA, Fetal bovine serum and composition of nutrient mixture with Fetal bovine serum.
- Various isotherm models, such as Langmuir and Freundlich, are studied in order to quantify the rate of adsorption and comprehend the interaction between proteins and biomaterials.
- Estimation of adsorbed protein by Instrumentation analysis and In-vitro experiments.
- To sort and quantify the adsorbed protein by SDS PAGE

CHAPTER 4

MATERIALS AND METHODOLOGY

4.1 MATERIALS

- Six well plate
- Bovine serum alum
- Fetal bovine serum
- Serum
- Stainless steel 304
- Stainless steel 316
- Titanium
- Titanium aluminium alloy
- RPMI media
- 1 X PBS
- Forceps
- Distilled water
- 75% ethanol
- Eppendorf tubes
- Weighing scale
- Spatula
- CO2 incubator
- Gel rock and shaker
- Rotary shaker
- SDS page setup
- SDS
- Acrylamide
- Bisacrylamide
- Tris base
- Glycine

- Distilled water
- Glycerol
- Bromophenol blue
- β -Mercaptoethanol
- Coomassie brilliant blue
- Methanol
- Glacial acetic acid
- TEMED
- APS

4.2 METHODOLOGY

4.2.1. SELECTION AND PREPARATION OF MATERIAL SUBSTRATES:

In the study, material substrates were carefully chosen based on their significance to the research objectives, with a particular emphasis on metals. Four specific types of metals were selected: stainless steel 304, stainless steel 316, titanium, and titanium aluminium alloy. These substrates offer distinct properties and surface characteristics that are pertinent to the investigation at hand.

Each metal substrate underwent a standardised preparation process to ensure uniformity and reliability in the experiments. Initially, the metals were precisely cut into 1x1 centimetre dimensions utilising a cutting machine. This consistent sizing facilitates comparability and ease of handling during subsequent procedures.

Subsequently, the metal substrates underwent a meticulous polishing process to attain smooth and uniform surfaces. This polishing step is crucial as it minimises surface irregularities and enhances the consistency of protein adsorption across the substrates.

This cleaning procedure serves to eliminate any potential surface contaminants or

residues that could compromise the integrity of the experiments or interfere with protein adsorption processes. By ensuring the cleanliness of the substrates, the reliability and reproducibility of the findings can be maintained, thus enabling accurate assessment of protein-material interactions and their implications within the scope of the study.

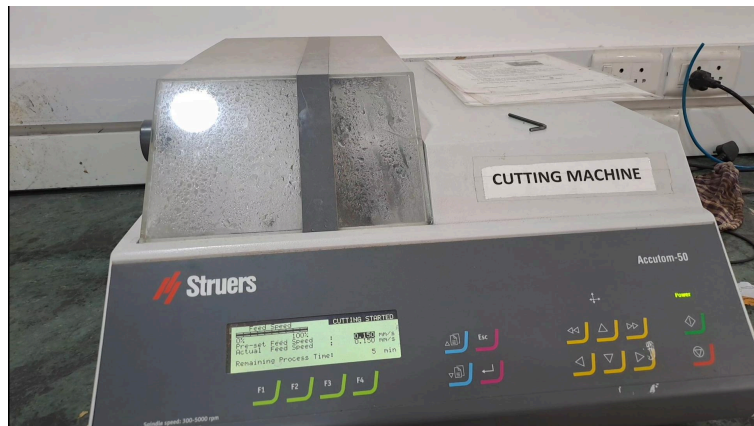


Fig 4.1 The metal samples were measured and cut in equal sizes



Fig 4.2 The metal samples were polished.

4.2.2 PREPARATION OF PROTEIN SOLUTIONS:

Protein solutions are crucial components in mimicking physiological conditions for various biological studies. These solutions typically comprise a range of proteins commonly found within biological systems, such as albumin, fibrinogen, collagen, among others relevant to the specific research focus. In a comparative analysis, three distinct protocols were employed.

Firstly, in the preparation of the BSA (Bovine Serum Albumin) solution, a meticulous measure of 400 micrograms of protein per millilitre was taken and added accordingly. This approach ensures precise control over the concentration of the solution, contributing to consistency and reproducibility in subsequent experiments.

Secondly, for the FBS (Fetal Bovine Serum) solution, a different methodology was adopted. Here, a proportionate amount, specifically 10% of the FBS solution, was mixed with 1 millilitre of RPMI media. This technique allows for the incorporation of essential growth factors and nutrients present in FBS into the culture media, facilitating optimal cell growth and function.

Lastly, in the preparation of serum, a more intricate process was undertaken. Fresh blood was obtained from a healthy volunteer and subjected to centrifugation at 3000 revolutions per minute for a duration of 15 minutes. This centrifugal force enables the separation of serum from other blood components, resulting in a distinct and purified serum fraction. Such meticulous procedures ensure the reliability and reproducibility of experimental outcomes by maintaining precise control over the concentration and composition of protein solutions across various studies



Fig 4.3 BSA solution and FBS RPMI solution



Fig 4.4 Serum separated from the blood



Fig 4.5 Serum collected after centrifuging blood

4.2.3 IMMERSION OF MATERIAL SUBSTRATES:

In the experimental setup, each metal sample was immersed in different protein solutions to facilitate the adsorption of proteins onto their surfaces. The immersion process aims to investigate the interactions between the selected metals and various protein solutions, thereby elucidating their potential effects on the material substrates.

Initially, two millilitres of the BSA (Bovine Serum Albumin) solution were added to the well containing the metal sample, ensuring complete immersion of the metal surface. Subsequently, the well was placed in a CO₂ incubator and maintained for a duration of 24 hours. During this incubation period, the metal surface interacts with the BSA solution, allowing for the adsorption of proteins onto its surface. Similarly, a two-millilitre solution of FBS (Fetal Bovine Serum) RPMI was added to another well containing the metal sample, ensuring complete immersion of the metal surface in the protein solution. The well was then placed in a CO₂ incubator and incubated for

24 hours. Throughout this incubation period, the metal surface interacts with the FBS RPMI solution, facilitating the adsorption of proteins onto its surface.

Furthermore, serum was added to additional wells containing the metal samples, and the samples were immersed in the serum solution. The immersion process was conducted for a shorter duration of three hours, followed by incubation in the CO₂ incubator. During this incubation period, the metal surfaces interact with the serum solution, allowing for the adsorption of proteins onto their surfaces.

By immersing the metal samples in different protein solutions and incubating them under controlled conditions, researchers can observe and analyze the adsorption of proteins onto the surfaces of the material substrates. This experimental approach provides valuable insights into the protein-material interactions, contributing to a better understanding of the biological responses elicited by the metal surfaces.



Fig 4.6 Metal samples incubated in serum

4.2.4 EXTRACTION OF ADSORBED PROTEINS:

Following the 24-hour incubation period in the CO₂ incubator, the metal samples immersed in BSA and FBS RPMI solutions were carefully removed from the protein solutions. To ensure the removal of unbound proteins and any residual contaminants, the metal substrates underwent a thorough washing process using phosphate-buffered saline (PBS) solution, repeated thrice.

Subsequently, the washed metal samples were subjected to elution in a rotary shaker containing a 1% SDS (Sodium Dodecyl Sulfate) solution for a duration of one hour. SDS, known for its ability to disrupt both protein-protein and protein-substrate interactions, facilitated the release of the adsorbed proteins from the metal surfaces into the solution.

Following the elution process, the metal samples were again incubated for three hours, after which they were washed with PBS solution to remove any unbound proteins that might have been released during the elution step.

Next, the elution process was repeated using a 2% SDS solution. The metal samples were eluted in a shaker, aided by a gel rock, for a prolonged duration of 24 hours. This extended incubation period is ensured through extraction of the adsorbed proteins from the metal surfaces into the SDS solution.

Finally, upon completion of the elution process, the metal samples were removed from the SDS solution, and the eluates containing the released proteins were carefully collected. These eluates were then stored at a temperature of -20 degrees Celsius to maintain their stability and integrity for subsequent analysis.

This comprehensive experimental protocol allows for the efficient extraction and collection of proteins adsorbed onto the surfaces of the material substrates, thereby facilitating further analysis and characterization of the protein-material interactions.



Fig 4.7 Elution of metal samples using a gel rock and shaker

4.2.5 QUANTIFICATION OF EXTRACTED PROTEINS:

Following the elution of proteins from the material substrates, quantification of the eluted proteins becomes essential to determine the amount of protein adsorbed onto the substrates accurately. One widely used method for protein quantification is the Lowry assay.

The Lowry assay relies on the reaction of proteins with specific reagents, such as copper ions and Folin-Ciocalteu reagent, under alkaline conditions. This reaction leads to the formation of a coloured complex, the intensity of which is directly proportional to the concentration of proteins present in the sample. Spectrophotometric measurement of the absorbance of this coloured complex allows for the quantification of protein concentration.

To perform the Lowry assay, the eluted protein samples are first diluted appropriately to ensure that their concentrations fall within the linear range of the assay. Then, aliquots of the diluted samples are mixed with the Lowry reagents, and

the reaction is allowed to proceed under controlled conditions. After incubation, the absorbance of the resulting coloured complex is measured using a spectrophotometer at a specific wavelength.

A standard curve generated using known concentrations of a protein standard allows for the determination of the protein concentration in the eluted samples. By comparing the absorbance readings of the samples to those of the standard curve, the amount of protein adsorbed onto the material substrates can be accurately quantified.

The Lowry assay offers several advantages, including high sensitivity and broad applicability to a wide range of protein types. However, it is important to note that the assay requires careful optimization and standardisation to ensure accurate and reproducible results. Additionally, alternative methods such as the Bradford assay or BCA assay may also be employed for protein quantification, depending on the specific requirements of the study.

4.2.6 ANALYSIS OF EXTRACTED PROTEINS:

In the process of characterising the composition and molecular weight distribution of the eluted proteins, SDS-PAGE (sodium dodecyl sulphate-polyacrylamide gel electrophoresis) emerges as a common technique. SDS-PAGE separates proteins based on their molecular weights, allowing for the visualisation and analysis of protein bands within a sample.

To initiate the SDS-PAGE analysis, stacking and separating gels were meticulously prepared, ensuring optimal resolution of protein bands. The gel apparatus was set up, and the gel was casted with precision to maintain uniformity and reliability in the results.

Subsequently, the eluted proteins were carefully mixed with loading dye, a solution that facilitates the loading of proteins into the gel wells, and 15 microliters of this

mixture were loaded into the SDS-PAGE wells. BSA (Bovine Serum Albumin) was incorporated as a standard marker to facilitate the estimation of molecular weights of the eluted proteins.

The SDS-PAGE apparatus was then operated to conduct the electrophoresis, allowing proteins to migrate through the gel matrix under the influence of an electric field. After completion of the electrophoresis run, the gel was stained with Coomassie Blue, a dye commonly used to visualise proteins. The staining process allows for the detection of protein bands within the gel.

Following staining, the gel was destained overnight to remove excess dye and enhance the clarity of the protein bands. The resultant protein bands were observed to be dark, indicating the presence of abundant albumin protein within the eluted samples.

This comprehensive SDS-PAGE analysis provides valuable insights into the composition and molecular weight distribution of the eluted proteins, facilitating further characterization and interpretation of the protein-material interactions under investigation.

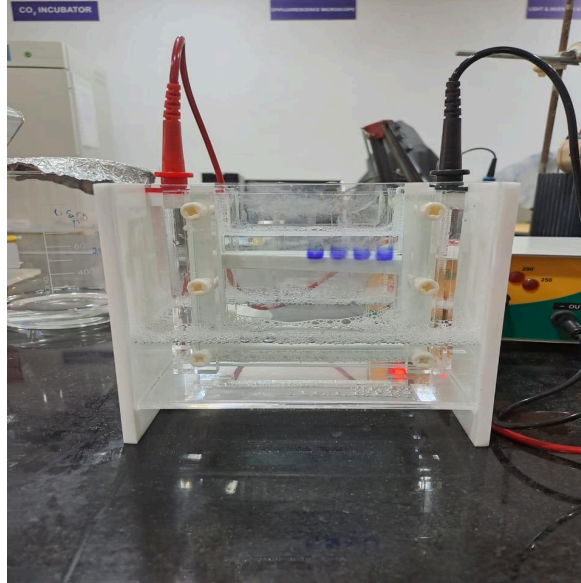


Fig 4.8 SDS - PAGE with the samples loaded in it

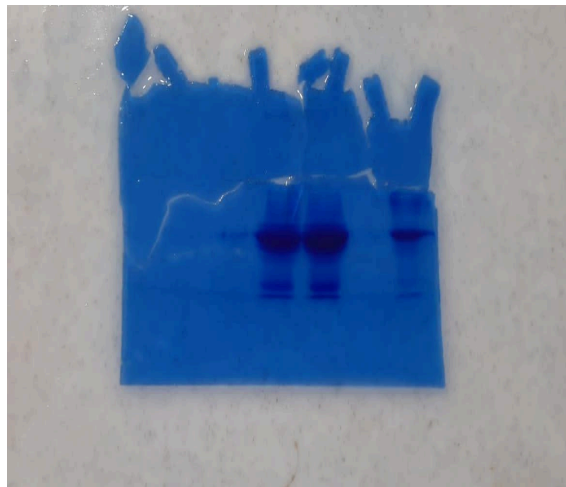


Fig 4.9 The bands were obtained

4.2.7 MEASUREMENT OF PROTEIN CONCENTRATION:

In tandem with SDS-PAGE analysis, the concentration of proteins within the eluted solutions can be directly quantified using spectrophotometry. A Nanodrop spectrophotometer is frequently utilised for this purpose due to its ability to measure

absorbance at specific wavelengths, with 280 nm being commonly employed for proteins.

The process begins by preparing the eluted protein solutions and ensuring they are adequately diluted to fall within the linear range of the spectrophotometer. Then, small volumes of the diluted solutions are pipetted onto the sample pedestal of the Nanodrop spectrophotometer.

The spectrophotometer measures the absorbance of the protein solutions at 280 nm, where proteins typically absorb light due to the presence of aromatic amino acids like tryptophan and tyrosine. Based on established calibration curves or standard protocols, the absorbance readings are correlated with the concentration of proteins in the solutions.

By comparing the absorbance values obtained from the eluted protein solutions to those of known protein standards or calibration curves, the concentration of proteins in the samples can be accurately determined. This direct spectrophotometric measurement provides a rapid and reliable method for quantifying protein concentration in the eluted solutions, complementing the information obtained from SDS-PAGE analysis.

Overall, the combination of SDS-PAGE analysis and spectrophotometric quantification offers a comprehensive approach to characterise the composition and concentration of proteins in the eluted solutions, facilitating a deeper understanding of protein-material interactions and their implications in various biological contexts.

4.2.8 DATA ANALYSIS AND INTERPRETATION:

In the analysis phase of the study, various analytical techniques and statistical methods were employed to assess the extent of protein adsorption onto the material substrates. The data obtained from protein quantification using the Lowry assay and

Nanodrop spectrophotometry were meticulously examined. Using the R programming environment, several libraries were utilised to aid in data manipulation, visualisation, and statistical analysis. These included 'ggplot2' for creating visually appealing plots, 'dplyr' for data manipulation tasks, 'reshape2' or 'tidyr' for data reshaping, 'ggpubr' or 'gridExtra' for customising and arranging plots, and the base R library 'stats' for statistical analysis.

The collected data were plotted on graphs, illustrating the relationship between protein concentration and absorbance at 280 nm. Moreover, adsorption isotherms such as Langmuir and Freundlich models were calculated and plotted to characterize the adsorption behavior of proteins onto the material substrates. These models, fitted to the experimental data, provided insights into the adsorption kinetics and mechanisms.(da Costa Marques et al., 2022)

In interpreting the results, researchers considered the study objectives and context, correlating the observed data with factors influencing protein adsorption. Material surface properties, protein characteristics, solution conditions, and experimental parameters were among the key factors taken into account. This comprehensive analysis not only shed light on the factors influencing protein adsorption onto material substrates but also contributed to a deeper understanding of protein-material interactions and their implications in various biological and biomedical applications.

4.2.9 VALIDATION AND REPRODUCIBILITY:

To ensure the credibility and replicability of the findings, experiments are routinely carried out in triplicate or more, adhering to rigorous experimental protocols. Control experiments play a crucial role in accounting for background signals and experimental variability, thus bolstering the robustness of the results.

Performing experiments in triplicate or more enables researchers to assess the consistency and reliability of their findings by observing the degree of variability across multiple iterations. This approach minimizes the influence of random fluctuations or errors, providing more confidence in the observed trends or effects.

Moreover, control experiments serve as benchmarks to distinguish between the signal of interest and background noise or artifacts. By including appropriate controls, researchers can identify and account for any spurious signals arising from experimental conditions, reagents, or equipment, ensuring that the observed effects are indeed attributable to the variables under investigation.

By adhering to these rigorous experimental practices, researchers can enhance the validity and trustworthiness of their results, facilitating meaningful interpretation and extrapolation of findings to broader contexts. This commitment to quality assurance and experimental integrity underpins the advancement of scientific knowledge and the development of reliable solutions in various fields of study.

CHAPTER 5

RESULT AND DISCUSSION

5.1 NANODROP

Table 5.1.1 Concentration of samples at 280 nm

Sample	Protein Concentration mg/ml
TiAl(BSA)	0.716
SS 304(BSA)	1.476
Ti-Al(FBS)	1.096
SS 304(FBS)	1.576
TiAl(Serum)	1.758
SS 304(Serum)	0.328
Ti(BSA)	0.444
SS 316(BSA)	0.852
Ti(FBS)	1.344
SS 316(FBS)	1.024
Ti(Serum)	0.38
SS 316(Serum)	0.304

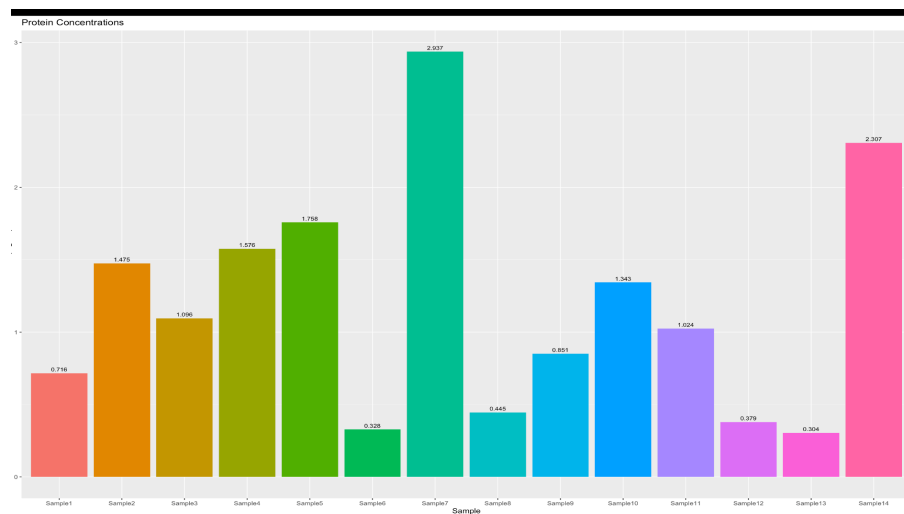
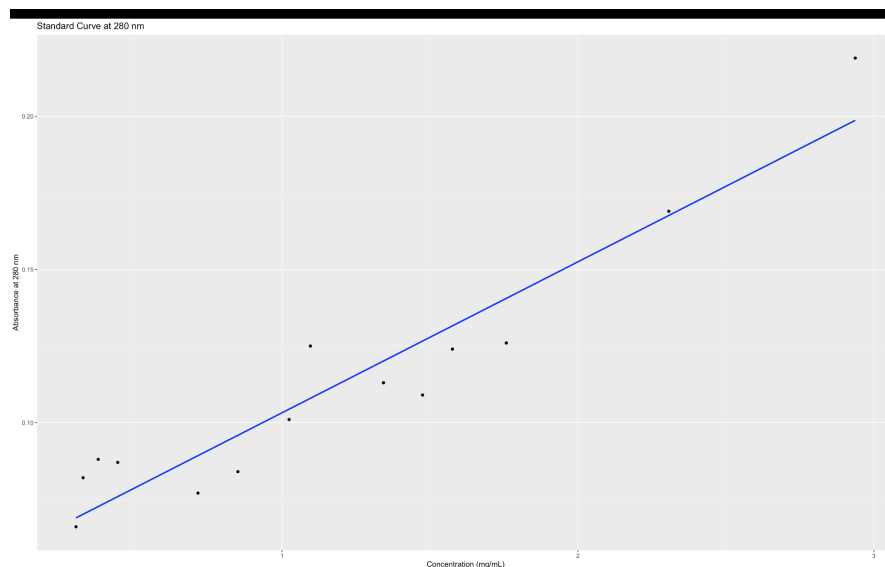


Fig 5.1 Graphical representation of concentration of samples at 280 nm

Table 5.1.2 Adsorption and Protein Concentration of samples at 280 nm

Sample	Protein Concentration mg/ml	Adsorption
TiAl(BSA)	0.716	0.077
SS 304(BSA)	1.476	0.109
Ti-Al(FBS)	1.096	0.125
SS 304(FBS)	1.576	0.124
TiAl(Serum)	1.758	0.126
SS 304(Serum)	0.328	0.082
Ti(BSA)	0.444	0.087
SS 316(BSA)	0.852	0.084
Ti(FBS)	1.344	0.113
SS 316(FBS)	1.024	0.101
Ti(Serum)	0.38	0.088
SS 316(Serum)	0.304	0.066



5.2 Scatter plot of Concentration of protein samples and adsorption at 280 nm

From the graphical representation, it is understood that in case of BSA, stainless steel 304 has adsorbed more proteins, followed by stainless steel 316 and titanium aluminium alloy and titanium in the next, respectively. In the case of FBS, stainless steel 304 has again adsorbed more protein with titanium following that and stainless steel 316 and titanium aluminium alloy in the next, respectively. In the case of serum obtained from blood, titanium aluminium alloy has adsorbed more protein with stainless steel 304 following it and titanium and stainless steel 316 being in the next, respectively. The results for these three different protein solutions were different because of serum being a naturally occurring body regulating source and BSA and FBS being chemical based body mimicking protein fluids. Therefore the interaction and adherence of the same protein albumin is different in different metals.

Each metal surface has a unique chemical composition, which can influence its interaction with proteins. Stainless steel 304, stainless steel 316, titanium, and titanium aluminium alloy have different elemental compositions, leading to variations in their surface chemistry. Certain metal surfaces may have chemical properties that promote stronger interactions with proteins, resulting in higher adsorption.

The surface charge of metal surfaces can affect protein adsorption. Proteins such as BSA, FBS, and serum proteins contain charged amino acid residues, and their adsorption onto metal surfaces is influenced by electrostatic interactions. Differences in the surface charge of metals may result in variations in the strength of electrostatic interactions with proteins, leading to differences in adsorption levels.

5.2 LOWRY'S PROTEIN ESTIMATION

Table 5.2.1 Standard Protein Concentration and Absorbance at 660 nm

Standard	Protein Concentration mg/ml	Absorbance at 660 nm
Standard 1	0.1	0.025
Standard 2	0.5	0.125
Standard 3	1	0.25
Standard 4	2	0.5
Standard 5	5	1.25

Table 5.2.2 Estimated Protein Absorbance at 660 nm

Sample	Absorbance at 660 nm	Protein Concentration mg/ml
TiAl(BSA)	0.179	0.716
SS 304(BSA)	0.369	1.476
Ti-Al(FBS)	0.274	1.096
SS 304(FBS)	0.394	1.576
TiAl(Serum)	0.434	1.758
SS 304(Serum)	0.082	0.328
Ti(BSA)	0.111	0.444
SS 316(BSA)	0.213	0.852
Ti(FBS)	0.336	1.344
SS 316(FBS)	0.256	1.024
Ti(Serum)	0.095	0.38
SS 316(Serum)	0.076	0.304

SLOPE : 0.25

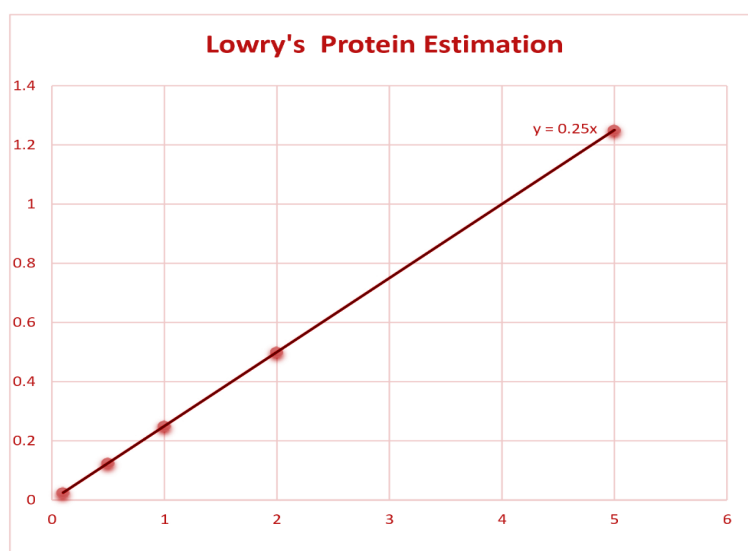


Fig 5.3 Lowry's Protein Estimation Graph

A slope of 0.25 in a Lowry protein assay indicates the relationship between absorbance and protein concentration. Specifically, it suggests that for every unit increase in protein concentration (e.g., mg/ml), the absorbance at the specified wavelength (660 nm) increases by 0.25 absorbance units. The slope represents the molar absorptivity or extinction coefficient of the protein being analysed at the specific wavelength. A higher slope indicates that the protein absorbs light more strongly at the given wavelength, meaning that for a given concentration of protein, you would expect a higher absorbance reading.

Metal surfaces can vary in their roughness, which can impact the available surface area for protein binding. Surfaces with higher roughness may provide more binding sites for protein adsorption, resulting in increased adsorption levels. Stainless steel 304, stainless steel 316, titanium, and titanium aluminium alloy may differ in their surface roughness, contributing to variations in protein adsorption.

Specific interactions between metal ions on the surface and amino acid residues in proteins can also contribute to protein adsorption. Metal ions present on the surface of metals may interact with certain amino acid residues in proteins, enhancing adsorption onto specific metal surfaces

5.3 LANGMUIR'S ADSORPTION ISOTHERM

Langmuir adsorption isotherm is a model used to describe the adsorption of molecules onto a surface. It assumes that adsorption occurs at specific sites on the surface and that once a site is occupied, no further adsorption can occur at that site.

The Langmuir isotherm equation is often expressed as:

$$\theta = (K * P) / (1 + K * P)$$

Where:

θ is the fraction of the surface covered by adsorbate molecules,

P is the pressure of the gas or vapour in equilibrium with the surface,

θ_m is the maximum coverage of the surface by adsorbate molecules,

K is the equilibrium constant.

Table 5.3.1 Adsorption and Protein Concentration of samples

Sample	Protein Concentration mg/ml	Adsorption
TiAl(BSA)	0.716	0.077
SS 304(BSA)	1.476	0.109
Ti-Al(FBS)	1.096	0.125
SS 304(FBS)	1.576	0.124
TiAl(Serum)	1.758	0.126
SS 304(Serum)	0.328	0.082
Ti(BSA)	0.444	0.087
SS 316(BSA)	0.852	0.084
Ti(FBS)	1.344	0.113
SS 316(FBS)	1.024	0.101
Ti(Serum)	0.38	0.088
SS 316(Serum)	0.304	0.066

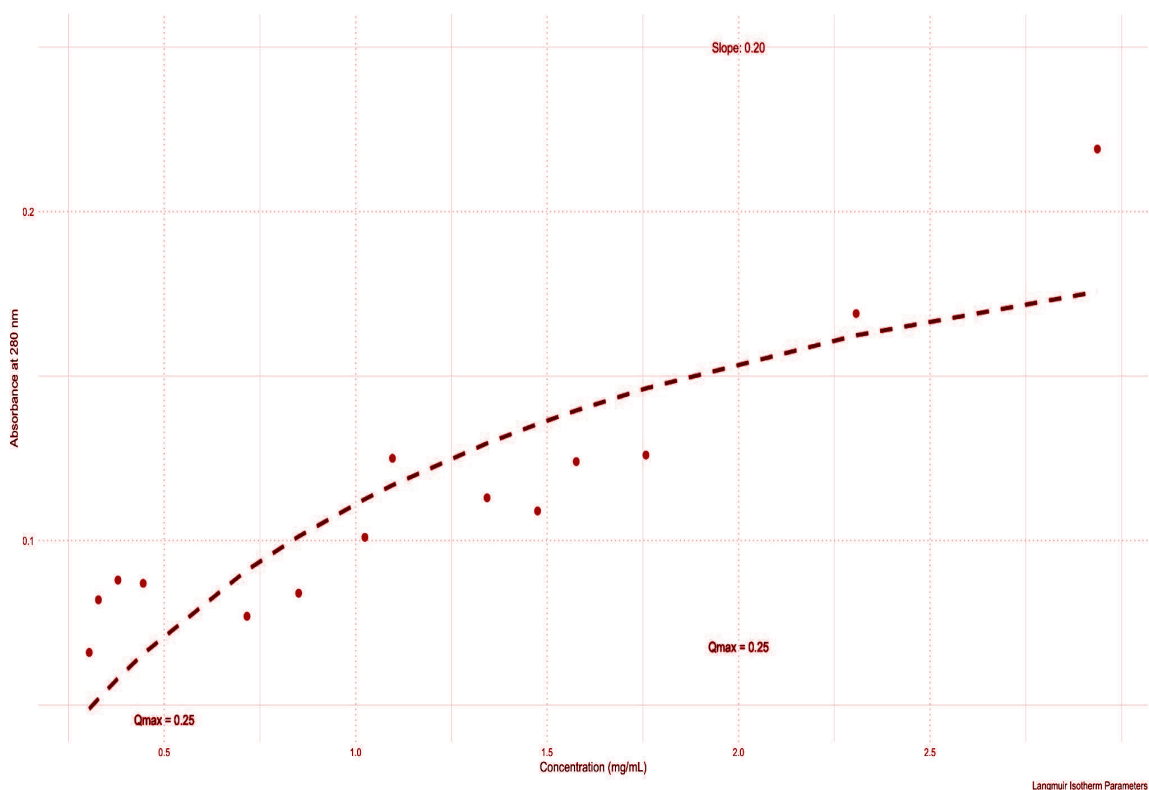


Figure 5.4 Langmuir Isotherm model for the samples at 280 nm

Langmuir Parameters:

Qmax (Maximum Adsorption Capacity):

Qmax value: 0.2398

This represents the maximum adsorption capacity of the metal surface for the protein molecules. In this case, it suggests that the maximum amount of protein adsorbed per unit mass or surface area of the metal sample is approximately 0.2398 absorbance units.

b value (Equilibrium Constant): 1.076

This parameter reflects the affinity of the protein molecules for the metal surface. A higher value of b indicates stronger interactions between the protein and the metal surface. In this case, a b value of 1.076 suggests favourable protein-metal interactions.

Slope value: 0.2579

Interpretation: The slope of the Langmuir plot provides insights into the rate of increase in absorbance with increasing protein concentration. In this case, the slope of approximately 0.2579 indicates the rate at which absorbance increases with increasing protein concentration.

The Langmuir plot visually represents the experimental data points along with the fitted Langmuir curve.

The dashed line represents the Langmuir isotherm fit to the data, showing how well the Langmuir model describes the protein adsorption behaviour on the metal surface.

Interpretation:

Based on the Langmuir parameters and the plot, we can infer that the metal surface exhibits a finite maximum adsorption capacity for the protein molecules, and there are favourable interactions between the protein and the metal surface, as indicated by the obtained values of $Q_{\max}$ and b

5.4 FREUNDLICH ADSORPTION ISOTHERM

The Freundlich adsorption isotherm is another model used to describe the adsorption of molecules onto a surface, particularly when the surface is heterogeneous or when multilayer adsorption occurs. Unlike the Langmuir isotherm, the Freundlich isotherm does not assume a monolayer adsorption and allows for a more flexible description of the adsorption process.

The Freundlich isotherm equation is typically given as:

$$q = K \cdot C^{1/n}$$

Where:

q is the amount of adsorbate adsorbed per unit mass of adsorbent (often expressed in terms of mass of adsorbate per mass of adsorbent or volume of gas adsorbed per unit mass of adsorbent),

C is the concentration of the adsorbate in the solution or the pressure of the gas phase (depending on the system),

K and n are empirical constants characteristic of the adsorbent-adsorbate system.

Table 5.4.1 Adsorption and Protein Concentration of samples

Sample	Protein Concentration mg/ml	Adsorption
TiAl(BSA)	0.716	0.077
SS 304(BSA)	1.476	0.109
Ti-Al(FBS)	1.096	0.125
SS 304(FBS)	1.576	0.124
TiAl(Serum)	1.758	0.126
SS 304(Serum)	0.328	0.082
Ti(BSA)	0.444	0.087
SS 316(BSA)	0.852	0.084
Ti(FBS)	1.344	0.113
SS 316(FBS)	1.024	0.101
Ti(Serum)	0.38	0.088
SS 316(Serum)	0.304	0.066

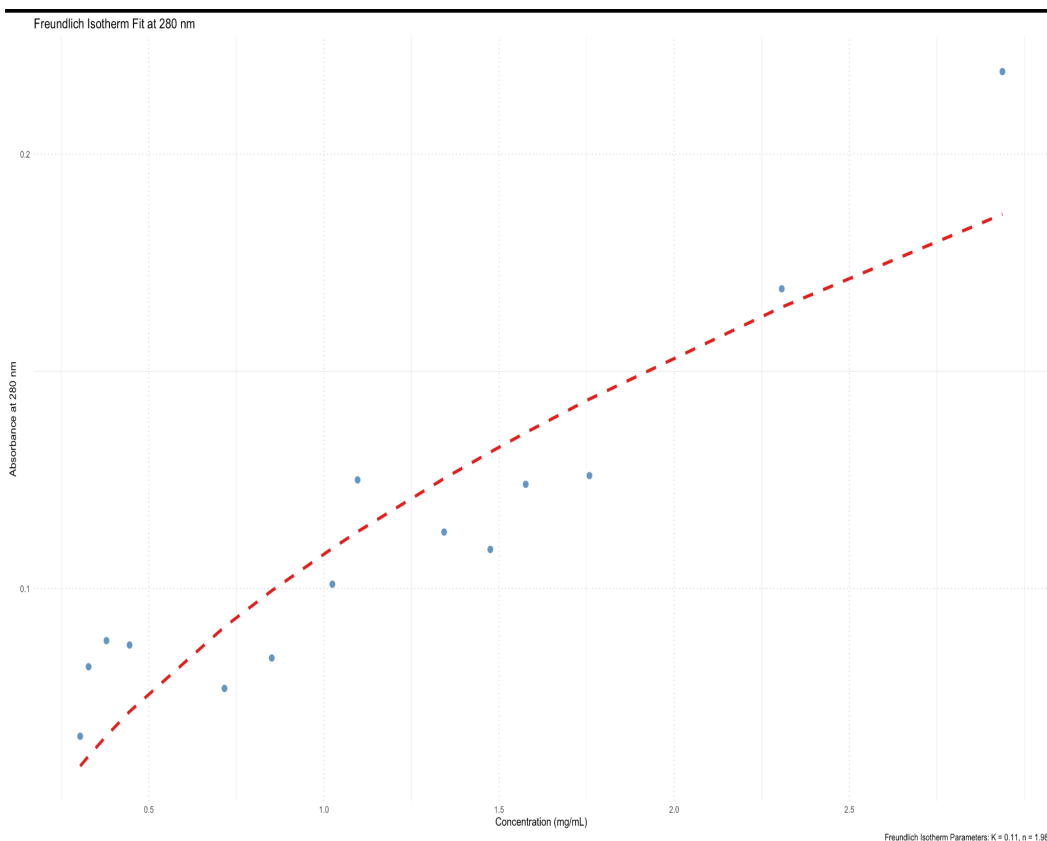


Fig 5.5 Freundlich adsorption isotherm model

Freundlich Parameters:

K value(Freundlich Constant): 0.1845

The Freundlich constant represents the adsorption capacity of the metal surface for the protein molecules. In this case, it suggests that the adsorption capacity of the metal surface is approximately 0.1845 for the given protein concentrations.

n value (Freundlich Constant): 1.622

The Freundlich constant n reflects the heterogeneity of the adsorption sites on the metal surface and the strength of adsorption.

A higher value of n indicates favourable adsorption. In this case, a value of 1.622 suggests relatively strong and heterogeneous adsorption.

The Freundlich plot visually represents the experimental data points along with the fitted Freundlich curve. The dashed line represents the Freundlich isotherm fit to the data, showing how well the Freundlich model describes the protein adsorption behaviour on the metal surface.

Based on the Freundlich parameters and the plot, we can infer that the metal surface exhibits a significant adsorption capacity for the protein molecules, and there are favourable and heterogeneous interactions between the protein and the metal surface, as indicated by the obtained values of K and n .

Pisarek et al. showed that a TiO₂ layer of larger surface roughness was formed on Ti substrates by chemical etching (soaking the samples either in NaOH or H₃PO₄/H₂O₂ solution). The albumin adsorption increased in treated Ti samples compared to untreated samples due to the roughness increment induced by the etching treatments. In recent years, there has been increasing interest in the formation of nanostructured bioactive coatings based on titanium (or other metal) by different surface treatment methods, such as acid and alkali treatment and electrochemical oxidation. The Lowry's assay and nanodrop done in my study has also confirmed this by the increase of adsorption in Titanium. Bovine serum albumin (BSA) as a standard protein for estimating protein concentration due to its availability, cost-effectiveness, and purity.

Wing-Hin Lee shows that the Langmuir isotherm is based on the assumption of a monolayer protein adsorption onto a homogeneous adsorbent surface with identical adsorption sites. In contrast, the modified Freundlich model takes into consideration the multi-layer protein adsorption and interactions on a heterogeneous surface. The same outlook has been observed on this project work as well.

An excellent study that can be used to provide a conservative estimate of k_a as well as an example of a Langmuir-looking isotherm for protein adsorption was published by Wertz and Santore in the Langmuir journal in 1999. Wertz and Santore adsorbed albumin to hydrophobic methyl-terminated self-assembled monolayers under varying flow rates and solution concentrations to study albumin adsorption behavior under mass transport-limited conditions. Their results showed that albumin adsorption followed the Leveque solution to the convection-diffusion equation for transport-limited adsorption.

The author, Ganesh Kumar Rajahmundry discusses that the Freundlich constants, K and n , provide crucial insights into the adsorption behavior of protein molecules on the metal surface. A K value of 0.1845 indicates the adsorption capacity of the metal surface for the given protein concentrations. This suggests that the metal surface has a moderate adsorption capacity, capable of adsorbing a significant amount of protein molecules.

On the other hand, the n value of 1.622 reflects the heterogeneity of the adsorption sites on the metal surface and the strength of adsorption. A higher n value indicates favorable adsorption, implying relatively strong and heterogeneous interactions between the protein and the metal surface. This suggests that the metal surface offers a diverse range of adsorption sites with varying affinities for protein molecules.

The Freundlich plot visually represents the experimental data points and the fitted Freundlich curve. The dashed line represents the Freundlich isotherm fit to the data, indicating how well the Freundlich model describes the protein adsorption behavior on the metal surface. The close agreement between the experimental data and the fitted curve suggests that the Freundlich model adequately captures the adsorption phenomenon.

In summary, the obtained Freundlich parameters and the plot collectively indicate that the metal surface exhibits a significant adsorption capacity for protein molecules. Additionally, the favorable and heterogeneous interactions between the protein and the metal surface contribute to the strong adsorption behavior observed. These findings provide valuable insights into the protein-metal surface interaction dynamics, with implications for various applications in biotechnology, materials science, and environmental remediation.

The Langmuir parameters reveal important insights into protein adsorption on the metal surface. A Q_{max} value of 0.2398 indicates the maximum adsorption capacity,

suggesting that the metal surface can adsorb up to this amount of protein per unit mass or surface area. The b value of 1.076 signifies favorable interactions between the protein and the metal surface, indicating strong affinity.

The slope value of 0.2579 reflects the rate of absorbance increase with rising protein concentration. The Langmuir plot visually represents the experimental data and the fitted Langmuir curve, with the dashed line indicating how well the Langmuir model fits the data.

Overall, these findings suggest that the metal surface has a finite maximum adsorption capacity for protein molecules, with favorable interactions facilitating adsorption. This analysis provides valuable insights into protein-metal surface interactions, crucial for various applications in biotechnology and materials science.

CHAPTER 6

SUMMARY AND CONCLUSION

These protein adsorption studies have yielded significant insights into the intricate interplay between biomaterial surfaces and proteins, offering promising avenues for advancements in tissue engineering and biomedical applications. Through a comprehensive series of experiments integrating surface modifications and elution techniques, we discerned distinct patterns of protein adsorption across various biomaterial surfaces. Notably, our observations underscored the influential roles of surface roughness, smoothness, area, and protein concentration in governing protein adsorption phenomena.

These findings deepen our comprehension of protein-biomaterial interactions, crucial for the development of biomaterials endowed with enhanced biocompatibility and tissue integration characteristics. By elucidating the nuanced mechanisms underlying protein adsorption, our research underscores the potential of tailored protein adsorption systems, paving the way for the design and implementation of biomaterials that precisely meet the demands of diverse biomedical applications. These protein adsorption studies have provided valuable insights into the interaction between biomaterial surfaces and proteins, with significant implications for tissue engineering and biomedical applications.

Through a series of experiments utilising surface modifications and elution techniques, we observed distinct patterns of protein adsorption on different biomaterial surfaces. Our findings suggest that the roughness, smoothness, surface area, and concentration of the proteins play an important role in protein adsorption. These results contribute to our understanding of protein-biomaterial interactions and have important implications for the design of biomaterials with enhanced biocompatibility and tissue integration properties. This research provides insights into the selective adsorption of proteins onto functionalized materials, shedding light on the potential for tailored protein adsorption systems.

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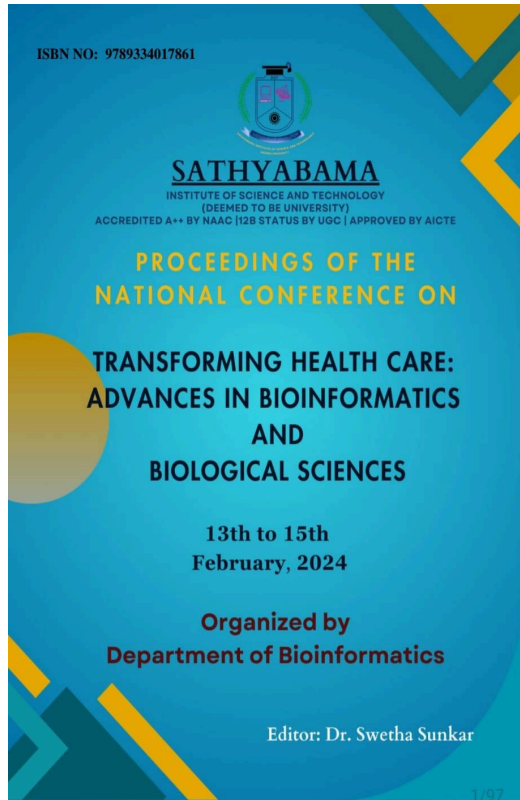
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ANNEXURE

Conference Publication



ABBS - 2024

COMPUTATIONAL MODELLING ON PROTEIN ADSORPTION DYNAMICS FOR ENHANCED BONE TISSUE ENGINEERING

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ABSTRACT

This study investigates and delves into the intricate dynamics of protein adsorption on biomaterial surfaces to advance strategies for enhancing bone tissue engineering. Protein adsorption is a critical process that influences the overall performance and efficacy of biomaterials in various tissue regeneration applications. To gain a comprehensive understanding of the adsorption capacity and intensity under different conditions, the researchers meticulously analysed experimental data collected from a diverse range of protocols. These data were then subjected to rigorous examination to discern the impacts of different conditions on the adsorption process representing experimental data and establish robust isotherm models such as the Langmuir model, representing monolayer adsorption, and the Freundlich model, describing heterogeneous systems. These models were fitted to the experimental data using advanced nonlinear regression techniques implemented in the programming language R. Furthermore, statistical analysis was conducted to detect any significant variations in adsorption behaviour across distinct experimental conditions. Molecular dynamics simulations and computational analysis techniques, we examine the interactions between proteins and biomaterial surfaces to elucidate the underlying mechanisms governing adsorption dynamics. The research focuses on elucidating how factors such as surface chemistry, and topography influence protein adsorption behaviour. By employing computational methods, the research also strives to quantify the rates at which proteins adsorb onto biomaterial surfaces, all the while endeavouring to understand the fundamental mechanisms that govern these intricate interactions. The findings from this computational analysis contribute to a deeper understanding of protein-biomaterial interactions, paving the way for the design and optimisation of innovative biomaterials tailored for enhanced bone tissue regeneration.

Keywords: Protein adsorption, Biomaterial, Bone tissue engineering, Protein – biomaterial interactions, Computational modelling

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Internal Guide Name : Dr. P. PRAKASH

External Guide Name : Dr. K. GOBI SARAVANAN

PO / PSO / PEO / SDG	Justification
PO-1	Apply knowledge of fundamentals of biological sciences, and arithmetic solution like adsorption isotherms
PO-2	To analyse the bone tissue damage problems and to find solutions using biological and engineering sciences.
PO-3	To design orthopedic implants with biocompatible materials like titanium and stainless steel, ensuring safety for implantation and optimize implant surfaces to enhance tissue regeneration and integration in the body.
PO-5	To use modern tools and resources to find the bone damage problems and to solve it
PO-6	To contribute to the society in bone regenerative applications



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PO-9	Individual work in evaluating the bone regeneration using protein adsorption studies on metal implants.
PO-12	Lifelong learning involves attending conferences, reading scientific publications, collaborating with experts in the field and pursuing further education to enhance this understanding and contribute effectively to evaluate protein adsorption and metal implants
PSO-1	Conceptualize and implement solutions for the technical problems in the healthcare domain and environment
PSO-2	Validate high throughput technologies in-vivo and in-vitro cell and tissue culture techniques thereby meet and global research standards