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Review Article

The Role of Proteins in Modulating Biocompatibility: A Comprehensive Overview of Implant Advanced Ceramic Coatings

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ABSTRACT

The biocompatibility of coatings applied to implants is a critical factor in ensuring optimal implant performance and patient safety. Recent studies have highlighted the significant impact of protein interactions on the biocompatibility of these coatings. This review aims to provide a comprehensive overview of the current understanding of how proteins influence the biocompatibility of coatings applied to implants. The biocompatibility of these coatings is affected by various factors, including the type and concentration of proteins present in the surrounding environment. Proteins can interact with the coating material, altering its surface properties—such as hydrophilicity, roughness, and charge—and subsequently affecting the host response,including inflammation, fibrosis, and osseointegration.Protein adsorption onto the surface forms a layer that mediates blood cell adhesion and cellular responses, significantly influencing the surface's biocompatibility. This review emphasizes the dual nature of proteins: while some enhance biocompatibility by promoting cell adhesion and proliferation, others may induce adverse effects. The article explores the mechanisms through which proteins interact with coatings and discusses how these interactions can be optimized to improve biocompatibility. Finally, the review highlights the potential of protein-modified coatings to enhance both biocompatibility and functionality in various implant applications, including orthopedic and cardiovascular implants. Such coatings demonstrate the ability to improve cell adhesion, promote tissue integration, and reduce inflammatory responses.

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1. INTRODUCTION

Implant coatings have revolutionized the field of biomedical engineering by enhancing the biocompatibility and functionality of implantable devices (Virk et al., 2019). The interaction between implants and surrounding biological tissues is crucial for the success of these devices, and coatings play a vital role in facilitating this interaction (Millet, 2021). Implantable devices, such as joint replacements, pacemakers, and artificial organs, have significantly improved the quality of life for millions of people worldwide. However, the success of these devices is often limited by the body's response to the implant, which can lead to complications such as inflammation, tissue damage, and device failure. To overcome these challenges, researchers have turned to implant coatings

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designed to interact with the surrounding biological tissues and promote a favorable healing response (Matter et al., 2021,Sasaki et al., 2022, Al-Mugheed et al., 2022).

Implant coatings can be broadly classified into two categories: passive and active coatings. Passive coatings, such as titanium dioxide and hydroxyapatite, are designed to reduce the risk of infection and promote tissue integration by inhibiting bacterial growth and enhancing cell adhesion. Active coatings, on the other hand, are designed to stimulate specific biological responses, such as bone growth or tissue regeneration, by releasing bioactive molecules or growth factors. Both types of coatings are essential for enhancing the biocompatibility and functionality of implantable devices, ultimately leading to improved patient outcomes. Passive coatings inhibit bacterial growth and enhance cell adhesion, thereby promoting tissue integration, while active coatings stimulate specific biological responses to facilitate healing and regeneration(Han et al, 2021).

Active coatings can also be tailored to release different bioactive molecules or growth factors to ensure optimal healing and tissue regeneration. Advances in material science and manufacturing techniques have enabled the customization of coatings to meet specific patient needs (Cahn, 2003). Additionally, active coatings have the potential to revolutionize personalized medicine by enabling targeted and tailored treatment options for individual patients (Sugandh et al., 2023). The incorporation of advanced technologies, such as nanotechnology and the addition of proteins to coatings, allows for precise control over the release of bioactive molecules, thereby improving the effectiveness of implant coatings (Malik et al., 2023&Huang et al., 2017). These coatings can improve cell adhesion, reduce bacterial adhesion, enhance the mechanical properties of implants, and increase their longevity and durability. Furthermore, they can create barriers against harmful bacteria, reducing the risk of infection and improving patient safety (Arciola et al., 2018&Pecoraro et al., 2023).

One promising bioactive material is hydroxyapatite, a calcium phosphate compound that closely resembles the mineral component of bone tissue. Zhang et al. (2014)highlighted recent trends in the development of implant coatings, emphasizing the potential benefits of hydroxyapatite-based coatings for orthopedic implants. Additionally, Arcos and Vallet-Regí (2020) discussed the use of substituted hydroxyapatite coatings for bone implants, further underscoring the potential of this material to enhance orthopedic implant performance. In recent years, there has been growing interest in using proteins, such as whey protein and collagen, in the development of bioactive coatings. The interaction between implant coatings and proteins is a critical aspect of their functionality. Proteins play a vital role in the body's response to implants, and a coating's ability to interact with these proteins can significantly influence outcomes. This interaction impacts the biocompatibility of implants, affecting inflammation levels, tissue integration, and ultimately, implantation success (Chen et al., 2006). Whey protein, in particular, has been explored for creating edible films and coatings with bioactive properties. Ramos et al. (2012) provided a comprehensive review of the formulation, mechanical, and bioactive properties of edible films and coatings derived from whey proteins. They highlighted the potential of whey protein-based coatings to serve as carriers of bioactive compounds, further enhancing their functionality for various applications.

Furthermore, the combination of hydroxyapatite with collagen has garnered attention in the development of bioactive coatings. Dullius et al. (2018) discussed the use of whey protein hydrolysates as a source of bioactive peptides for functional foods, shedding light on the potential of these peptides to contribute to coating bioactivity. This suggests that combining hydroxyapatite and collagen with bioactive peptides derived from whey protein could offer a multifaceted approach to enhancing the bioactivity of coatings for orthopedic implants.

Although research on active biofilms containing proteins derived from milk serum (whey protein) or hydroxyapatite-collagen has yielded promising results, many unanswered questions remain. Therefore, in this article, we call for an in-depth study to evaluate the long-term performance and biocompatibility of these bioactive substances, focusing on identifying the mechanisms underlying their bioactive properties, particularly in improving osseointegration and biocompatibility.

2. Overview of Implant Coatings based on Hydroxyapatite

The use of implant coatings containing proteins such as hydroxyapatite (HA), collagen, or whey protein has garnered significant interest in the field of biomedical engineering. Various studies have explored the incorporation of growth factors and antibacterial agents into HA coatings to enhance osteoinductivity and antibacterial properties. Xie et al. (2014) investigated the incorporation of both growth factors and silver (Ag) into hydroxyapatite coatings on metallic implant surfaces. The study highlighted the challenges associated with this process, particularly the potential agglomeration of Ag nanoparticles and the need for mild processing conditions to maintain the activity of the growth factors.

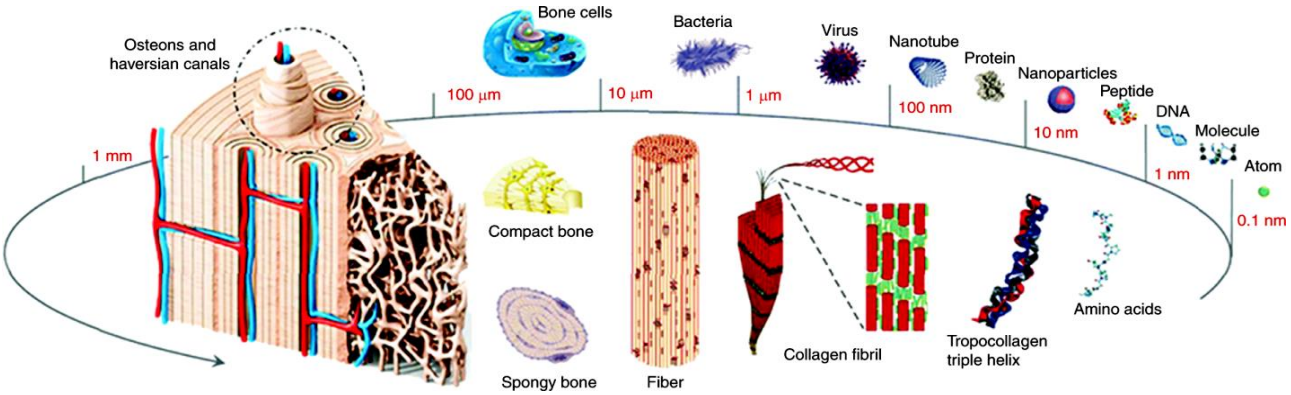

Figure 1. Hierarchical structure of bone tissue with various dimensions (Zhu&Liu, 2020).

Xie et al. (2014) utilized electrochemical deposition (ED) of Ag and electrostatic immobilization of bone morphology protein-2 (BMP-2) to prepare hydroxyapatite (HA) coatings on titanium (Ti) surfaces. The researchers employed chitosan (CS) as a stabilizing agent to chelate Ag ions and generate uniformly distributed Ag nanoparticles in the coatings. Additionally, BMP-2 was immobilized on the coatings through electrostatic attraction with CS and heparin. The study demonstrated the sustained release of BMP-2 and Ag ions from HA coatings, highlighting the potential of this coating for modifying metallic implant surfaces and enhancing biocompatibility. Another relevant study by Abdulkareem et al. (2015) explored the anti-biofilm activity of zinc oxide and hydroxyapatite nanoparticles as dental implant coating materials. While this study did not directly address the incorporation of proteins into coatings, it provided insights into the antibacterial properties of HA-based coatings, which are a crucial aspect of implant materials.

Furthermore,ceramic coatings, especially bioactive materials like hydroxyapatite and zirconia, play a vital role in improving the performance of implants by enhancing their compatibility with the body and promoting osseointegration. These ceramics create strong chemical bonds with surrounding tissues, which help the implant integrate seamlessly into its new environment and encourage bone growth and healing. Hydroxyapatite is particularly noteworthy because it closely mimics the mineral structure of bone, making it effective at supporting the attachment and growth of osteoblasts—cells that are essential for bone formation. The high porosity of these ceramic coatings is also significant, as it allows for better absorption of proteins, which is crucial for enhancing biocompatibility. Moreover, these coatings are highly resistant to corrosion, wear, and chemical reactions in various bodily environments, whether acidic or basic. This resistance helps minimize the risk of infection and bacterial growth, which are major concerns during implant surgeries. Recent innovations have made it possible for these coatings to release bioactive molecules in a controlled manner, further promoting tissue regeneration. In summary, incorporating ceramic coatings not only boosts the mechanical stability of implants but also encourages positive biological responses, ultimately leading to better patient outcomes and fewer complications related to implant procedures (Furko & Balázsi, 2020).

Despite significant advancements in implant coatings containing proteins like hydroxyapatite, there are still important knowledge gaps that warrant further research. Enhancing biocompatibility through effective protein interactions is vital, as it can lead to lower inflammation and infection rates, faster healing times, and higher success rates for implants. This is especially critical in clinical settings focused on patient safety and recovery. In high-risk populations, the stakes are even higher, as complications can greatly impact overall health (Kandasamy et al., 2018). Additionally, optimizing the immobilization of growth factors and antibacterial agents is necessary to ensure their sustained effectiveness. Advancing these areas will be crucial for improving biomedical applications of implant coatings.

3. Importance of Biocompatibility in Implant Design

Recent advancements in material science and cell biology have led to the development of novel orthopedic implant coatings to address various issues, such as promoting osteointegration, deterring bacterial adhesion, and minimizing prosthetic infection. Natural bone (Figure 1) consists of organic and inorganic components, such as hydroxyapatite (HA) nanocrystals, which are sometimes deposited in different collagen domains during bone biomineralization. Therefore, coatings composed of bioceramics, extracellular matrix proteins, biological peptides, or growth factors have been found to impart bioactivity and biocompatibility to the metallic surface of conventional orthopedic prostheses. This promotes bone ingrowth and the differentiation of stem cells into osteoblasts, ultimately

enhancing the osteointegration of the implant (Zhu&Liu, 2020, Tan et al., 2017, Xia et al., 2013, and Ma et al., 2014).

One of the most widely used techniques for coating implants is the application of a layer of hydroxyapatite (HAp) due to its excellent biocompatibility and osteoconductive behavior. Substituted HAp coatings have been successfully deposited on orthopedic prostheses and dental implants, expanding their applications to bone regeneration therapies (Tan et al., 2017).This demonstrates the significant role of hydroxyapatite in enhancing the biocompatibility of orthopedic implants. Moreover, research has presented convenient and effective methods for incorporating growth factors and antibacterial agents into HA coatings, which exhibit high antibacterial properties and good biocompatibility (Zhang et al., 2014). Mussel-inspired heparin-mimicking coatings on membranes have shown promising results, including increased hydrophilicity and electronegativity, decreased plasma protein adsorption, and suppressed platelet adhesion, thereby highlighting their excellent blood and cell compatibilities (Arcos &Vallet-Regí, 2020). Additionally, nano-HA/PCL scaffolds have demonstrated excellent biocompatibility and bioactivity, along with enhanced bone formation capacity in vitro and in vivo (Xia et al., 2013). Furthermore, the development of a micro-architected hip implant with mechanical properties biocompatible with those of the femoral bone has shown potential in reducing bone loss and preventing implant micromotion, thus decreasing the risk of periprosthetic fracture and the likelihood of revision surgery (Xie et al., 2014). Moreover, hydroxyapatite (HA)-collagen scaffolds have been found to support consistent bone formation while demonstrating goodbiocompatibility and osteogenesis (Ma et al., 2014). Synthetic BMP-2-related peptide (P24) introduced into a introduced into a bioactive scaffold based on nano-hydroxyapatite/collagen/poly(L-lactic acid) (nHAC/PLLA) has significantly stimulated bone growth, confirming the enhanced bone healing rate of these compounds (Wang et al., 2018). The functionalization of hydroxyapatite-collagen scaffolds for the sustained delivery of angiogenic growth factors has been found to enhance vessel formation and bone regeneration, offering an ideal platform to promote angiogenesis and tissue regeneration (Villa et al., 2015). Moreover, the CaP–MNP composite has demonstrated good biocompatibility, the ability to significantly promote cell proliferation and differentiation, and the capacity to accelerate BMP-2 expression and new bone-like tissue formation (Niu et al., 2015). However, the literature (Zhu et al., 2020;Tan et al., 2017;Xia et al., 2013;Ma et al., 2014;Wang et al., 2018;Villa et al., 2015;Niu et al., 2019) highlights the significance of biocompatibility in orthopedic implant design, particularly focusing on coatings and materials such as hydroxyapatite, collagen, growth factors, and synthetic peptides. While existing research has provided valuable insights, further exploration of these materials and coatings is still needed to enhance the biocompatibility and osteointegration of orthopedic implants.

3.1.Understanding the Importance of Proteins in Biocompatibility

Proteins play a significant role in enhancing the biocompatibility of biomaterials coatings, particularly in relation to their capacity to immobilize proteins and growth factors via non-covalent interactions. Arcos and Vallet-Regí (2020)discussed the significance of proteins in the biocompatibility of hydroxyapatite coatings. They emphasized the capability of proteins to immobilize growth factors through non-covalent interactions, thereby opening new possibilities for preparing hybrid coatings that promote bone healing processes. Similarly, Wypij et al.(Ma et al., 2014;Wang et al., 2018;Villa et al., 2015;Niu et al., 2019) highlighted the importance of proteins such as collagen (Col) and hydroxyapatite (HA) in bone tissue regeneration. They indicated the critical role of these proteins in enhancing the new bone-regenerating capability of tissue engineering scaffolds. Furthermore, Khan et al. (2020) synthesized silver-coated bioactive nanocomposite scaffolds based on grafted beta-glucan/hydroxyapatite via a freeze-drying method, underscoring the importance of proteins in improving the biocompatibility of the coating and its potential applications in bone tissue engineering.

Taken as a whole, these findings (Ma et al., 2014;Wang et al., 2018;Villa et al., 2015;Niu et al., 2019;Khan et al., 2020) underscore the critical importance of proteins in the biocompatibility of hydroxyapatite coatings. Future research in this area could focus on elucidating the specific mechanisms by which proteins enhance biocompatibility and holds promise for advancing the development of innovative biomaterials for bone tissue engineering and regeneration.

3.2. Protein Structure and Function

Collagen is a protein known for its biodegradability, biocompatibility, availability, and versatility, making it suitable for various tissue engineering applications (Parenteau-Bareil& Berthod, 2010).Collagen scaffolds have been widely used in tissue engineering due to their excellent properties; however, their poor mechanical properties limit their applications to some extent (Parenteau-Bareil et al., 2010). Collagen plays a crucial role in maintaining the biological and structural integrity of the extracellular matrix (ECM) and provides physical support to tissues (Dong et al., 2016;Giannandrea et al., 2014). Even though collagen exists in many different forms, they all share one common characteristic: they are composed of three chains twisted together in a unique manner (Figure 2) (Gelse & Aigner, 2003).

One of the significant structural components of the extracellular matrix (ECM) is collagen, whose resistance and maintenance are involved in several biological processes (Gelse&Aigner, 2003).Alterations in the quantity and quality of the ECM, including the collagen network, have been found to play a central role in the pathogenesis of heart failure (HF) associated with cardiac diseases (Nair et al., 2013). However, there are identified gaps in understanding the function of collagen protein in the context of tissue engineering, ECM maintenance, and its role in various biological processes. These gaps indicate promising avenues for future investigation in this field.

Whey protein, a byproduct of cheese production, is a versatile biomaterial with potential applications in regenerative medicine, biofabrication, and tissue engineering. The structure of whey protein is characterized by its solubility, molecular weight, and amino acid composition (Figure 3). Whey proteinsare globular proteins containing a considerable number of α-helix patterns with evenly distributed hydrophilic, hydrophobic, acidic, and basic amino acids along their polypeptide chains. The major constituents of whey proteins include α-lactalbumin (α-LA), β-lactoglobulin (β-LG), bovine serum albumin (BSA), immunoglobulins (IG), bovine lactoferrin (BLF), bovine lactoperoxidase (LP), and minor amounts of glycomacropeptide (GMP) (Minj& Anand, 2020). The composition of each constituent is shown in Table 1.

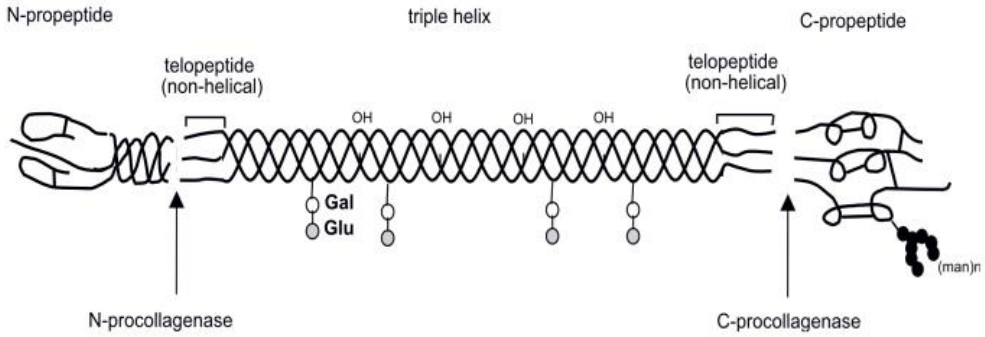

Figure2. Molecular structure of collagens with different subdomains as well as cleavage by N- and C-procollagenase (indicated by the type of collagen molecule) (Gelse & Aigner, 2003).

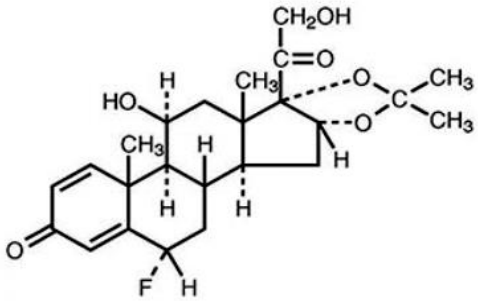

Figure 3. Chemical structure formula of Whey protein (Gaoyuanbio, 2024).

TABLE 1. Whey protein constituents and its composition (Minj& Anand, 2020).

Whey Protein constituent	Concentration (g/L)	Molecular Weight in kDa	Number of Amino Acid Residues
α – Lactalbumin	1.2	14,175	123
β – Lactoglobulin	1.3	18,277	162
Bovine serum albumin	0.4	66,267	582
Immunoglobulins (A, M, and C)	0.7	25,000(light chain) and 50,000-70,000(heavy chain)	-
Bovine lactoferrin	0.1	80,000	700
Glycomacropeptide	1.2	6700	64
Bovine Lactoperoxidase	0.03	70,000	612

The properties of whey protein, including mechanical strength, biocompatibility, and degradation behavior, are essential for its application as a biomaterial (Minj& Anand, 2020). These properties have been explored for various applications, such as bone tissue engineering scaffolds and regenerative medicine. Qu et al. (2019) provided a comprehensive review of biomaterials for bone tissue engineering scaffolds, emphasizing the potential of protein-based constructs in this field. Similarly,Sun and Tan (2013) highlighted the use of alginate-based biomaterials in regenerative medicine applications, suggesting the need for comparative studies with whey protein-based biomaterials. Despite progress in understanding whey protein structure and properties for biomaterials (Minj & Anand, 2020;Gaoyuanbio, 2024;Qu et al., 2019), several knowledge gaps and potential directions for future research remain.

3.3. Role of Proteins in Cellular Responses

The role of proteins in cellular responses in bioactive coatings is a multifaceted and complex area of research that encompasses various aspects of cellular physiology. Proteins are involved in a wide array of cellular processes, including signaling, cellular homeostasis, and oxidative stress regulation. The findings by Guo et al. (Guo et al., 2020) emphasize the role of mitogen-activated protein kinases (MAPKs) as key signaling molecules that regulate processes such as proliferation, differentiation, and apoptosis. This highlights the critical role of proteins in regulating fundamental cellular behaviors. Moreover, autophagy, a process involving proteins, plays a significant role in maintaining cellular homeostasis and can be influenced by oncoproteins, impacting tumor progression (Galluzzi et al., 2015). These findings underscore the importance of proteins in cellular responses, particularly in the context of disease progression. Proteins such as glutathione peroxidase-1 (GPx-1) play a crucial role in modulating cellular oxidant stress and redox-mediated responses through their enzymatic activity (Lubos et al., 2011). This highlights the importance of proteins in regulating cellular redox physiology and oxidative stress, which are critical factors in cellular responses to various stimuli.

The role of scaffold proteins in regulating selectivity in pathways and shaping cellular responses is also an important aspect of cellular physiology (Good et al., 2011). Scaffold proteins play a crucial role in controlling the flow of cellular information, highlighting their significance in orchestrating cellular responses. Despite the comprehensive understanding of the role of proteins in cellular responses, several knowledge gaps exist in this area of research. For instance, the specific mechanisms by which proteins modulate cellular responses in the context of bioactive coatings remain to be fully elucidated. Additionally, the interplay between different types of proteins and their collective impact on cellular responses in bioactive coatings requires further investigation.

3.4. Protein Interactions with Implant Surfaces

Proteins play a crucial role in the interaction between implant surfaces and biological systems, affecting the biocompatibility and performance of implants. Specifically, whey protein and collagen have been studied for their interactions with implant surfaces (Tavares et al., 2019;Feng et al.,2019;Davidenko et al., 2016). Protein adsorption is considered to be the most important factor in the interaction between polymeric biomaterials and body fluids or tissues (Wei et al., 2014). Understanding the dynamics of protein interactions with implant surfaces is crucial for the development of biocompatible and bioactive surface modifications for prolonged in vivo efficacy (Meyers & Grinstaff,2012).

Feng et al. (Feng et al., 2019) investigated the effect of an antioxidant and antimicrobial coating based on whey protein nanofibrils with TiO_2 nanotubes on the quality and shelf life of chilled meat. The findings suggested that the whey protein-based coating had a positive impact on the quality and shelf life of the meat and demonstrated its ability to interact with implant surfaces and improve functionality. Tavares et al. (Tavares et al., 2019) studied the microencapsulation of garlic extract using whey protein isolate/chitosan and gum arabic/chitosan as wall materials. They highlighted the influence of anionic biopolymers on the physicochemical and structural properties of the microparticles, providing insights into the behavior of whey protein in complex coacervation and its potential for use in implant surface modifications.

Wei et al. (Wei et al., 2014) shed light on the nature of protein adsorption on various biomaterial surfaces and coatings, providing insights into the mechanisms underlying protein-surface interactions. Protein adsorption is influenced by nonpolar interactions, such as van der Waals forces, hydrophobic attraction, and hydration forces, as well as polar interactions, including electrostatic and ionic screening effects, salt bridges, and hydrogen bonds. These interactions play a crucial role in determining the biocompatibility of biomaterials by affecting protein-induced adhesion of organisms and the biological responses triggered by protein adsorption. Understanding and controlling these interactions are essential for improving the biocompatibility of biomaterials. Additionally, Trindade et al. (Trindade et al., 2016) explored the foreign body reaction to biomaterials and the mechanisms involved in the buildup and breakdown of osseointegration. The findings contribute to understanding the host response to implanted biomaterials. Davidenko et al. (Davidenko et al., 2016)evaluated cell binding to collagen and gelatin, focusing on the effect of 2D and 3D architecture

and surface chemistry. Their research highlighted the significance of surface architecture and chemistry in mediating cell interactions with implant materials. Furthermore, Chen et al. (Chen et al., 2013) emphasized the importance of interfacial characteristics in governing protein binding affinity, which has implications for understanding protein interactions with implant surfaces. As the buried surface area increases, the binding affinity also increases, leading to enhanced biocompatibility of implants.

Coatings that include active materials such as proteins achieve attachment strengths through the use of multiple attachment domains, allowing them to establish seemingly irreversible bonds with strengths between ideal covalent and noncovalent bonds. While the force required to break covalent bonds is reported to be a few nanonewtons and for hydrogen bonds only a few piconewtons, adsorptive coatings leverage a cooperative multivalent attachment strategy to enhance their attachment strengths (Meyers et al., 2012). By creating surfaces that mimic the properties of cell membranes, these coatings facilitate improved interactions with surrounding tissues, promoting integration and regeneration. This selective surface modification allows for optimal performance of implants by minimizing fibrous capsule formation and promoting tissue regeneration, ultimately enhancing biocompatibility and reducing the foreign body reaction (Meyers & Grinstaff, 2012). Despite the extensive research on protein interactions with implant surfaces, there are still knowledge gaps that warrant further investigation. Future research should focus on elucidating the specific molecular mechanisms underlying protein adsorption on different implant materials and coatings. A deeper understanding of the interplay between surface characteristics and protein adsorption dynamics is essential for the rational design of implant materials with tailored biocompatibility and bioactivity.

3.5 Adsorption of Proteins onto Implant Surfaces

The adsorption of proteins onto implant surfaces is a critical process that influences the subsequent biological response at the implant-tissue interface. Hanawa (2019) discusses the titanium-tissue interface reaction and its control through surface treatment, indicating that immobilizing biomolecules like proteins on the titanium surface can improve cell spreading, bone formation, and antibacterial properties, thereby promoting osseointegration. This process involves creating surfaces that interact favorably with living tissues, leading to better host responses, enhanced cell adhesion, and ultimately, improved biocompatibility of titanium implants. The biocompatibility of a material is influenced by various reactions that occur between the material and the host body. These reactions include the adsorption of molecules, protein adsorption, cell adhesion, bacterial adhesion, activation of macrophages, tissue formation, and inflammation. Furthermore, these reactions occur hierarchically, both temporally and spatially, as depicted in Figure 4 (Hanawa, 2019).

Lv et al. (2018) highlight the role of surface hydrophilicity in modulating macrophage polarization, elucidating that surface hydrophilicity controls the adsorption and conformation of specific proteins, such as fibronectin and fibrinogen. This finding is consistent with the work of Boyan et al. (2017), who demonstrate the influence of surface roughness and hydrophilicity as osteogenic biomimetic surface properties. The mechanism of the relationship between biocompatibility and protein adsorption involves understanding the thermodynamic interactions upon adsorption of non-specific binding proteins and biomacromolecules onto surfaces.

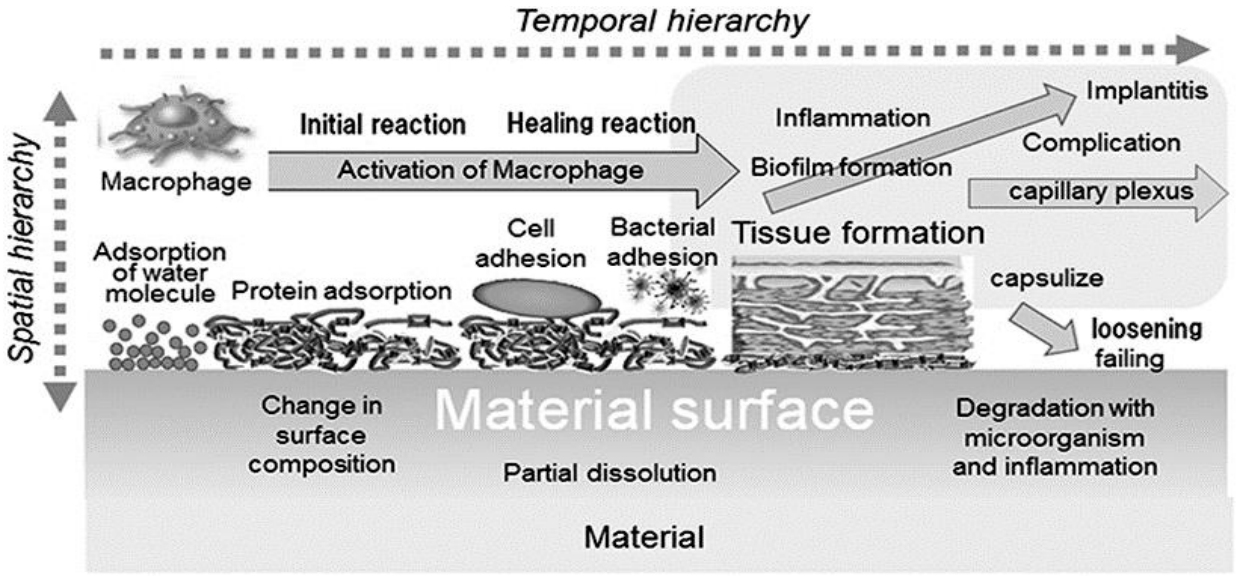

Figure 4. Interfacial reactions of materials and the host body (Hanawa, 2019).

For biocompatible applications like drug delivery carriers or tissue engineering, it is crucial to consider thermodynamic parameters of interaction, such as enthalpy and entropy changes, to ensure favorable and controlled protein adsorption onto surfaces. This understanding aids in designing materials that promote specific protein interactions while maintaining biocompatibility for various in vivo applications. The thermodynamics of adsorption on nanocellulose surfaces have been investigated by Lombardo and Thielemans (2019), who indicate that the driving force for the irreversible binding of non-specific proteins onto nanocellulose surfaces is entropy. Although the thermodynamic interaction parameters of protein adsorption are affected by pH variations, the enthalpy and entropy changes of adsorption describe the interaction between cellulose surfaces and the cellulose-binding module as exothermic and enthalpy-driven. This highlights the significant contribution of specific carbohydrate-protein interactions, often associated with aromatic residues or specific peptide-carbohydrate interactions. This interaction is characterized by the formation of new bonds, such as electrostatic, aromatic, hydrogen bonds, or van der Waals forces. Understanding the thermodynamic principles underlying protein adsorption offerscrucial information for designing implant surfaces with enhanced protein adsorption capabilities.

While these studies (Hanawa, 2019;Lv et al., 2018;Boyan et al., 2017; Lombardo& Thielemans, 2019) provide valuable insights into the adsorption of proteins onto implant surfaces, knowledge gaps remain that warrant further investigation. For instance, the specific molecular mechanisms by which surface hydrophilicity influences protein adsorption and conformation need to be elucidated. Additionally, the long-term effects of protein adsorption on cell behavior and tissue integration remain to be fully understood. Further research in this field holds the potential to advance the development of biomaterials with improved biocompatibility and tissue integration properties.

3.6. Protein-Based Strategies for Enhancing Biocompatibility

The use of medical implants has become increasingly common for various clinical applications, such as tissue regeneration, drug delivery, and surgical sealants. However, a key challenge in the development of medical implants is ensuring their biocompatibility, biodegradability, and biodistribution. Protein-based strategies have emerged as a promising approach to address these challenges. Croissant et al. (2018) underscore the potential of protein-based bioactive drugs and cells in enhancing the biocompatibility of medical implants, indicating that the protein corona is a layer of proteins that forms almost instantaneously on the surface of nanoparticles when they are dispersed in protein-containing media mimicking biological environments. This corona consists of a mixture of various proteins that adsorb onto the nanoparticle surface, influencing their biological behavior, biodistribution, and interactions with cells. The protein corona is dynamic, involving reversible (soft corona) and irreversible (hard corona) protein adsorption, and it can significantly impact the fate and performance of nanoparticles in biological systems. Protein-based strategies for enhancing the biocompatibility of implants focus on controlling the protein corona formation on the surface of nanoparticles. By using specific surface engineering techniques, such as cationic or anionic functionalization or pegylation, the adsorption of proteins onto nanoparticles can be regulated. This controlled protein corona formation improves nanoparticles' biocompatibility, blood circulation time, and targeting abilities, ultimately enhancing their performance in biological systems (Croissant et al., 2018).

Annabi et al. (Annabi et al., 2017)discuss the biocompatibility of MeTro hydrogels through in-vitro and in-vivo experiments. They note that the MeTro gel is a photocrosslinked, elastic, biocompatible, and slowly biodegradable sealant material made from a modified human protein called tropoelastin. In-vivo studies demonstrated minimal inflammatory host responses and tunable degradation rates after implantation in rats, indicating excellent biocompatibility. In-vitro cytocompatibility tests showed that the MeTro gel supported the growth and proliferation of mesenchymal stem cells and endothelial progenitor cells, with cell viability exceeding 95% over seven days, confirming its non-cytotoxic nature. The engineered MeTro gel displayed strong adhesive properties and interacted positively with tissue collagen fibers, enhancing its biocompatibility as a protein-based sealant.

The way protein interactions, especially with whey protein and collagen, affect the biocompatibility of implants and their ability to integrate with bone is fascinating and involves several important processes. When an implant is placed in the body, proteins quickly attach to its surface, forming a bioactive layer that encourages cells to adhere and multiply. Collagen, a vital part of the extracellular matrix, plays a crucial role in helping osteoblasts—cells responsible for bone formation—attach and differentiate, leading to new bone growth around the implant. Whey protein also contributes significantly by enhancing cellular metabolic activity and promoting better integration due to its favorable binding characteristics. Additionally, these protein interactions initiate important signaling pathways through integrin receptors, which influence how cells behave and respond to their environment. Over time, the stability of these protein layers is essential for ensuring successful osseointegration because factors like protein breakdown or competition

for binding sites can affect the implant's long-term performance (Feng et al., 2019;Dullius et al., 2018;Davidenko et al., 2016;Tavares et al., 2019).
Despite these advancements, several knowledge gaps exist in the field of protein-based strategies for enhancing biocompatibility. Future research is needed to address these gaps and explore novel protein-based approaches for improving the biocompatibility of medical implants.

3.7. Surface Modification Techniques using Proteins

The success of medical implants heavily relies on their integration with the surrounding tissues, which necessitates surface modification techniques (Adipurnama et al., 2017, Amani et al., 2019, Liu& Eschweiler, 2020, Besinis&Hadi, 2017). Surface modification techniques using proteins have been investigated for vascular tissue engineering applications by Adipurnama (Adipurnama et al., 2017), which emphasizes the versatility of protein-based surface modifications in different biomedical contexts, as well as the use of Polysaccharides, which contain free amino, carboxyl, and hydroxyl groups that allow for altering their surface properties. In fact, surface modification with hydrophilic molecules improves hemocompatibility by reducing protein adsorption and platelet adhesion. Hydrophilic surfaces create a structured water layer that repels proteins and platelets, thus controlling the coagulation cascade and immune inflammation. This enhancement in hemocompatibility is achieved by creating a surface that minimizes blood-material interactions, ultimately improving the performance of biomaterials in contact with blood. The biocompatibility of surfaces involves the interaction of proteins with the material. Initially, proteins adsorb onto the surface, forming a protein layer. Thislayer affectsthe adhesion of blood cells, especially platelets, and guides cellular responses like migration, coagulation, and proliferation. The protein layer acts as a mediator between the material and host cells, affecting the overall biocompatibility of the surface (Adipurnama & Butruk-Raszeja, 2017).

According to Figure 5, the first step of this reaction is the plasma protein adsorption/desorption process, commonly known as the Vroman effect, followed by platelet adhesion and eventually endothelial and smooth muscle cell migration. As the plasma proteins settle in, platelets begin to adhere to the surface. This adhesion triggers a chain reaction, leading to the migration of endothelial and smooth muscle cells. These cells play a crucial role in the repair and regeneration of the damaged area (Adipurnama&Butruk-Raszeja, 2017). This activation leads to the release of several molecules that act as a signal, alerting the body to the presence of a potential threat. As these signals are sent out, a series of complex and successive reactions takes place. During this stage, a crucial event occurs: thrombin, an enzyme, converts the soluble fibrinogen into insoluble fibrin strands. The fibrin strands, once formed, are then crosslinked, creating a sturdy mesh known as a thrombus. This thrombus serves as a barrier, preventing further blood loss and initiating the healing process. This usually happens in the lungs, brain, gastrointestinal tract, kidneys, or legs. It is a significant cause of patient morbidity and mortality (Adipurnama & Butruk-Raszeja, 2017).

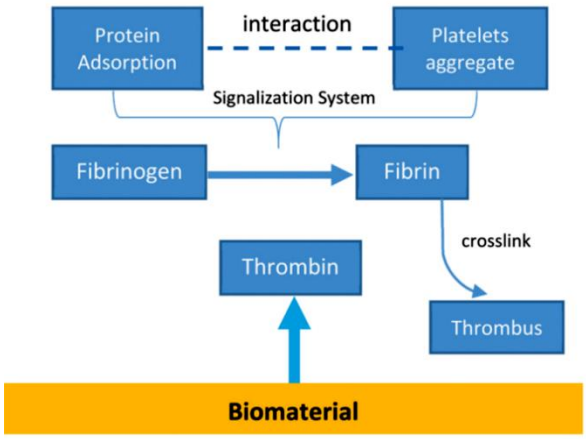

Figure 5. Representation of blood–material interface showing thromboembolic development, which begins with protein adsorption onto the surface, followed by the interaction with blood cells (platelets) and ending up with the development of thromboemboli (Adipurnama & Butruk-Raszeja, 2017).

Although researchers have made progress in using proteins to modify medical implant surfaces, more work is still required(Adipurnama et al., 2017, Meyer, 2019, Liu et al., 2020, Sheikh et al., 2015, Meng et al., 2020). We must study how these coatings hold up long-term and interact with the body. Additionally, we need to understand the mechanical effects of these modifications to ensure implant safety and performance. Addressing these knowledge gaps will lead to better, more reliable medical devices for patients.

3.8. Biofunctionalization for Improved Cellular Response

The foreign body reaction, which occurs in the first few weeks following implantation, plays a significant role in determining the biocompatibility of these materials. Therefore, the biofunctionalization of biomaterials to enhance cellular response and biocompatibility is an important issue. Sheikh et al. (2015) discussed the critical role of biomaterial surface properties in modulating the foreign body reaction following implantation (Figure 6). They indicated that the biofunctionalization process involves enhancing the cellular response to implants by incorporating proteins into the coating. Proteins can be immobilized on the implant surface to promote cell adhesion, proliferation,

and differentiation, thereby improving integration with the surrounding tissue.

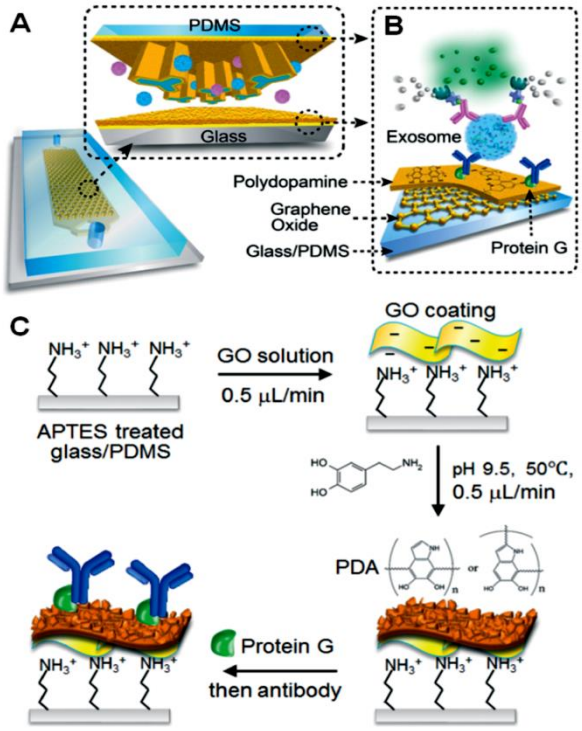

Figure 6. (a) Visualize a single-channel PDMS/glass device, with an exploded view showcasing the coated PDMS chip packed with an array of Y-shaped microposts, (b) The channel surface and microposts are meticulously coated with graphene oxide (GO) and polydopamine (PDA), creating a nanostructured interface for the sandwich ELISA of exosomes, complete with enzymatic fluorescence signal amplification, and (c) Discover the intricate procedure for surface functionalization of the microfluidic chips (Sheikh et al.,2015).

This protein coating can be achieved through methods like covalent coupling or adsorption, creating a bioactive interface that enhances biocompatibility and reduces biofouling. The use of proteins in implant coatings can significantly improve the overall performance and biocompatibility of the implant, making it more suitable for biomedical applications. Indeed, biofunctionalization for improved cellular response through the use of proteins in implant coatings involves several key mechanisms. Firstly, proteins in the coating facilitate cell adhesion by providing specific binding sites for cell surface receptors, thereby promoting cell attachment to the implant surface. Secondly, these proteins can modulate cell signaling pathways, influencing cell behavior, proliferation, and differentiation to enhance tissue integration and regeneration. Lastly, the presence of proteins in the coating can create a bioactive microenvironment that mimics the natural extracellular matrix, promoting a favorable cellular response whilereducing inflammation or immune rejection. Overall, protein-based biofunctionalization of implants plays a crucial role in improving cellular interactions and tissue integration for enhanced biomedical applications (Sheikh et al.,2015). Additionally, Meyer (2019), provides insights into the potential use of proteins in the coating of biomaterials to enhance biocompatibility and, in Figure 4 provides a visual representation of the hierarchical assembly of collagen molecules into fibrillar structures (Meyer,2019).

Figure 7 illustrates the process of collagen formation, whereMonomeric protocollagen chains trimerize, propeptides are cleaved off, and collagen molecules self-assemble into microfibrils and fibrils. The oxidation of lysine and hydroxylysine by lysyl oxidase initiates the formation of natural enzyme-derived crosslinks, which contributesto the structural stability of collagen. However, collagen in biofunctionalization for improved cellular response acts as a scaffold that mimics the extracellular matrix, enhancing biocompatibility and promoting cell adhesion and growth when used in coating implants with proteins. This mechanism creates an environment that supports cellular interactions, leading to better integration of the implant with surrounding tissues and improved overall performance of the implant (Meyer,2019).

3.9. Challenges and Future Directions in Protein-Modulated Biocompatibility

Despite significant strides in protein engineering, substantial knowledge gaps and hurdles remain to be addressed. For example, effectively integrating protein-based coatings into biomedical and industrial settings requires a more comprehensive understanding of protein interactions, structural stability, and long-term performance. Moreover, the scalability and cost-effectiveness of these coatings pose significant challenges that necessitate further research and development to ensure practical implementation (Meng et al.,2020;Annabi et al., 2017;Yi et al., 2022). These challenges include minimizing drawbacks, enhancing functionality, and improving implant protection. Protein-based coatings face limitations in achieving optimal biocompatibility and effectiveness in safeguarding biomaterial implants.It is also worth noting that synthetic biology is revolutionizing protein engineering by enabling the creation of proteins with specific functions, such as improved adhesion and controlled release of bioactive molecules. Recent research has introduceda robust cell-free transcription and translation system that facilitates the design of proteins with tailored properties. This system not only enhances protein synthesis but also provides insights into messenger RNA and protein degradation rates, which are crucial for manipulating gene expression and protein stability. Breakthroughs in cell-free transcription

and translation systems have improved protein synthesis and stabilization, making it easier to develop advanced biomaterials. Furthermore, Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) technology enables precise modifications to protein sequences, helping craft customized proteins to fulfill specific needs. By integrating these innovative techniques into coating designs, researchers can develop multilayered implant coatings that enhance tissue repair and integration. Additionally, advancements like engineered riboswitches allow for the regulation of gene expression based on the presence of certain metabolites, helping improve resistance to bacterial colonization (Garamella et al., 2016).

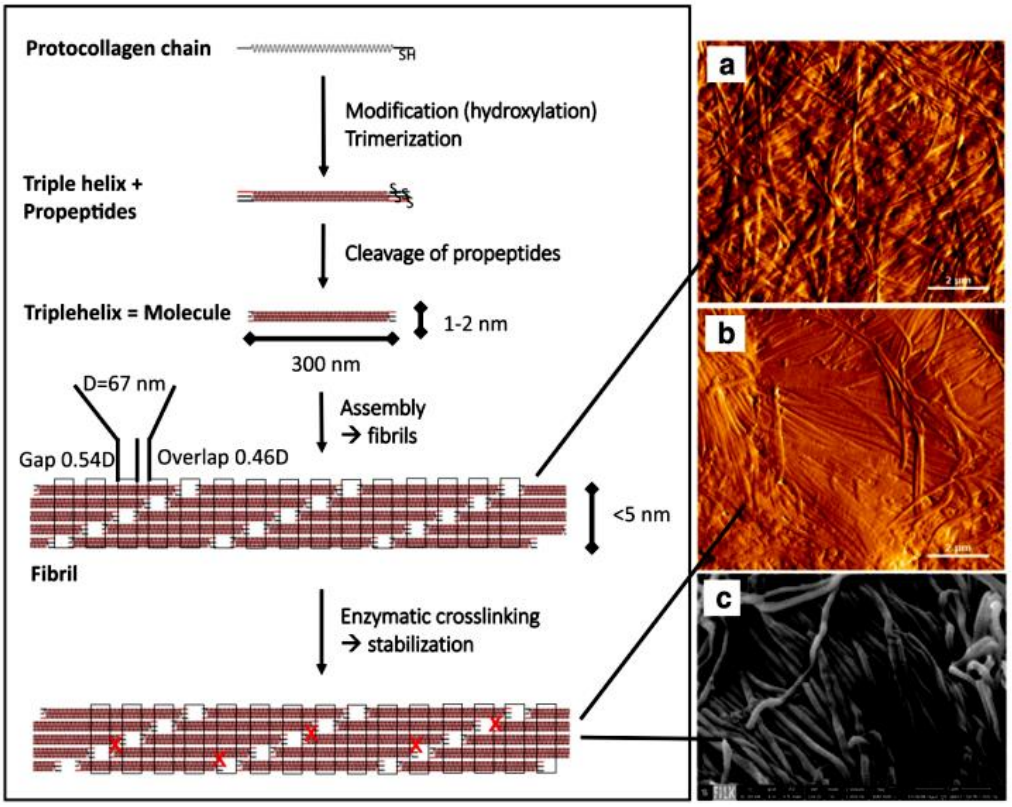

Figure 7. Collagen Assembly: Trimerization, Propeptide Cleavage, and Fibril Formation for Structural Stability, a) Atomic force microscopic image (AFM) of reassembled collagen (dried); b) AFM image and c) scanning electron microscopic image of dried porcine skin splits (dried). (Meyer, 2019)

The development of innovative protein engineering techniques, inspired by advances in extracellular vesicle-based drug delivery systems (Meng et al.,2020) and highly elastic human protein-based sealants for surgical applications (Annabi et al., 2017), opens new avenues for enhancing the biocompatibility of coatings. Exploring combination strategies with protein-modulated biocompatibility—similar to the progress in PD-1/PD-L1 blockade in cancer therapy (Yi et al., 2022)can offer valuable insights for overcoming current limitations and optimizing future directions in protein-based coatings. Future directions aim to address these challenges by reducing chemical components, maximizing benefits, and designing protein-based coatings with specific bioactive properties to enhance tissue integration and healing.

Future research should focus on understanding the specific interactions between collagen, whey protein, and their environments, particularly in biomaterials. Investigating optimal processing techniques, such as cross-linking or blending these proteins with other biomaterials, could significantly enhance their mechanical properties and stability. Additionally, it is essential to explore the degradation kinetics of collagen and whey protein in vivo. Studies should aim to characterize how these proteins degrade over time and how their degradation products affect surrounding tissues. Furthermore, examining the impact of various surface modifications on protein adsorption and bioactivity could provide valuable insights for improving the performance of coatings derived from these proteins.

When considering the limitations and long-term challenges associated with active coatings that incorporate hydroxyapatite, collagen, and whey protein, several critical issues arise. A primary concern is ensuring adequate adhesion between the coatings and substrate materials, as poor bonding can lead to

delamination or failure during use. While hydroxyapatite is advantageous for bioactivity, its brittleness may compromise the mechanical integrity of coatings under physiological stress. Additionally, both collagen and whey protein are prone to enzymatic degradation, diminishing their effectiveness over time in clinical applications. This degradation may result in a loss of functionality in promoting tissue integration and healing. To address these challenges, innovative design strategies are necessary, such as enhancing cross-linking methods or incorporating synthetic alternatives to improve durability while preserving biocompatibility.
Furthermore, research is needed to evaluate the long-term performance and biocompatibility of these advanced biomaterials in relevant clinical settings. Trends in protein engineering emphasize improving human health outcomes by enhancing the biocompatibility and functionality of biomaterial implants, paving the way for more effective solutions in regenerative medicine.

4. Conclusion

This article highlights the potential of biomimetic coatings and protein-based surface modification techniques for medical implants. These coatings mimic the cell membrane and extracellular matrix, thereby enhancing biological communication and integration. Hydroxyapatite coatings, combined with proteins such as collagen, improve biocompatibility, cell adhesion, and tissue integration. This approach supports tissue regeneration, reduces inflammation, and enhances implant performance. Future research should focus on developing advanced coatings with anti-inflammatory, mechanical, and immunomodulatory functions. Translating these techniques from in vitro studies to in vivo applications and clinical trials is crucial for validating their efficacy and safety. The integration of these coatings has demonstrated significant promise in improving implant performance and biocompatibility. However, further work is required to fully understand the mechanisms underlying protein interactions with coatings and to develop novel surface modifications that enhance biocompatibility. The future of medical implants lies in integrating cutting-edge technology and biomimicry principles to create functional and adaptive implants

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