DEAE-Dextran silver nanoparticles-based biomimetic coating for titanium-based hard tissue implant applications

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Dedicated to my exceptional parents whose unwavering support, encouragement, and sacrifices have been the cornerstone of my academic journey and to my ever motivating brothers!

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

Page No.

LIST OF SYMBOLS, ABBREVIATIONS AND ACRONYMS

DEAE	Diethyl aminoethyl
S. aureus	Staphylococcus
AgNPs	Silver Naoparticles
UV-Vis	Ultraviolet visible
XRD	X-ray diffraction
FTIR	Fourier transform infrared
SEM	Scanning electron microscopy
CVD	Chemical vapor depostion
PVD	Physical vapor deposition
Ti	Titanium
TEM	Transmission electron spectroscopy
DLS	Dynamic light scattering
XPS	X-ray photoelectric spectroscopy
DS	Degree of substitution
CFU	Colony forming unit

ABSTRACT

The improved biomimetic coatings for titanium-based implants have attracted a lot of attention concerning their potential to improve osseointegration and lower the risk of infection. The production, characterisation, and use of DEAE-Dextran silver nanoparticles (AgNPs) as a novel biomimetic covering for titanium-based hard tissue implants are the subjects of this thesis. The DEAE-Dextran, a biocompatible polymer, serves as both a stabilizing and functionalizing agent, facilitating the homogeneous dispersion of AgNPs on the titanium surface. The study employs a comprehensive experimental approach, including chemical synthesis, surface characterization, and in vitro biological assessments. Characterization methods include fourier transform infrared (FTIR), Zeta potential, X-ray diffraction (XRD), raman spectroscopy, ultraviolet-visible (UV-Vis) spectroscopy, and scanning electron microscopy (SEM). Results indicate that the DEAE-Dextran silver nanoparticle coating exhibits a uniform and stable layer on the titanium substrate, with significant antimicrobial activity against Staphylococcus aureus. The study concludes that DEAE-Dextran AgNPs-based coatings present a promising approach for enhancing the performance of titanium-based implants, offering a dual function of infection prevention and improved biocompatibility. This work contributes to the field of biomaterials by providing a novel coating strategy that combines the mechanical robustness of titanium with the biological functionalities of DEAE-Dextran and silver nanoparticles, potentially leading to improved clinical outcomes in orthopedic and dental implant applications.

Keywords: biomimetic coating; biocompatibility, osseointegration, titanium, implants.

CHAPTER 1: INTRODUCTION

This study proposes the use of DEAE-Dextran silver nanoparticles as a coating material for titanium based implant applications.

1.1 DEAE-Dextran

DEAE-Dextran, a cationic polysaccharide, has been explored for its potential to improve cellular adhesion and proliferation on implant surfaces (Jiang et al., 2007). DEAE-Dextran (Diethylaminoethyl-dextran) is a modified form of dextran, a polysaccharide composed of glucose units. The DEAE modification introduces diethylaminoethyl groups that enhance the interaction of DEAE-Dextran with biological systems. This modification can improve the binding affinity of the polymer to biological tissues and cells, which makes it an effective candidate for use in biomedical applications.

Several biomedical applications, such as gene delivery, medication delivery systems, and wound healing, have made use of DEAE-Dextran. Its biocompatibility and ability to interact with cell membranes make it suitable for creating advanced biomaterials.

1.2 Silver Nanoparticles

Silver nanoparticles are known for their unique optical, electronic, and antimicrobial properties. Numerous techniques, including chemical reduction, physical approaches, and biological processes, can be employed to produce these silver nanoparticles' size, shape, and surface characteristics can all be adjusted to produce particular biological effects. Because of their broad-spectrum antibacterial capabilities, silver nanoparticles (AgNPs) have drawn interest in biomedical applications (Rai et al., 2009). The incorporation of AgNPs into coatings for implants has shown promise in reducing the risk of post-operative infections.

1.2.1 Antimicrobial and Biological Properties

The ability of silver nanoparticles to emit silver ions, which can damage bacteria cell membranes and impede growth, gives them strong antimicrobial activity. This property is particularly valuable for reducing infection risks associated with implants. Additionally, silver

nanoparticles can influence cellular behaviors, such as adhesion and proliferation, contributing to their potential as a component of biomimetic coatings.

1.3 Titanium-based Hard Tissue Implants

Titanium and its alloys are extensively utilized in the field of orthopedics and dentistry because of its exceptional mechanical qualities and biocompatibility (Geetha et al., 2009). Titanium exhibits excellent resistance to corrosion, a high strength-to-weight ratio, and favorable mechanical properties, which make it a preferred material for implants that require both durability and compatibility with biological tissues.

1.3.1 Osseointegration and Its Challenges

The success of titanium implants largely hinges on the process of osseointegration, where implant integrates with the bone and surrounding tissue. Osseointegration is crucial for the stability and longevity of the implant. Despite titanium's favorable characteristics, achieving optimal osseointegration can be challenging. Bacterial colonization on implant surfaces can lead to infections, implant failure, and subsequent revision surgeries (Kaplan et al., 2018). Factors such as implant surface roughness, chemical composition, and biological responses play significant roles in the integration process. Traditional titanium surfaces often struggle to provide the ideal environment for cellular attachment and proliferation, which can impact the long-term success of the implant.

1.3.2 Need for Advanced Coating Technologies

To enhance osseointegration and reduce the risks associated with implants, various surface modification techniques have been developed (Kaplan et al., 2018). Conventional coatings, including hydroxyapatite and titanium dioxide, aim to improve biological interactions by mimicking bone mineral composition or altering surface roughness. However, these coatings often face limitations, like poor adherence to the metal base, insufficient antibacterial action, and inadequate bioactivity.

Recent advancements have focused on developing biomimetic coatings that more closely replicate natural tissue environments. These coatings are designed to enhance cellular

interactions and improve integration with the biological system. Among the emerging trends is the use of nanoparticles and biomimetic materials to create coatings that offer superior performance compared to traditional methods.

1.4 Biomimetic Coatings

1.4.1 Principles of Biomimetic Design

Biomimetic coatings are designed to replicate the natural extracellular matrix (ECM) or other biological interfaces to enhance the implant and host tissue interaction. In order to improve the adhesion and proliferation of cells, and tissue emancipation a surface that closely resembles the biochemical and physical characteristics of natural tissues is intended to be created.

Advantages over Traditional Coatings

Biomimetic coatings offer several advantages over traditional coatings. They can provide a more conducive environment for cellular activities, enhance the overall biocompatibility of the implant, and potentially reduce complications such as infection and poor osseointegration. By incorporating DEAE-Dextran and silver nanoparticles, the proposed coating aims to leverage both enhanced biological interactions and antimicrobial properties.

1.5 Significance of the study

1.5.1 Clinical Outcomes

The development of an advanced biomimetic coating for titanium implants could lead to significant improvements in clinical outcomes. Enhanced osseointegration, reduced infection rates, and increased longevity of implants are potential benefits that could result from this research.

Reduction in Revision Surgeries

By improving the performance of implants, the proposed coating could reduce the need for revision surgeries, which are often costly and involve additional risks for patients. Better

initial success rates and longer-lasting implants could lead to improved overall patient outcomes.

1.5.2 Contribution to Material Science

This research will contribute to the field of material science by introducing a novel biomimetic coating technology that combines DEAE-Dextran and silver nanoparticles. The study will provide insights into the potential applications of these materials in biomedical engineering and implant technology.

Enhanced Physical and Chemical Properties

The DEAE-Dextran silver nanoparticle coating will significantly enhance titanium implants' physical and chemical properties, leading to improved biological performance. It is hypothesized that the coating will improve surface characteristics such as roughness and wettability, which are crucial for cell attachment and tissue integration.

Superior Osseointegration

Implants coated with DEAE-Dextran silver nanoparticles will exhibit superior osseointegration and integration with bone tissue in animal models. This hypothesis is based on the assumption that the coating will enhance both the biological and antimicrobial properties of the implant.

Advancements in Biomedical Engineering

The development of innovative coatings like the DEAE-Dextran silver nanoparticle coating represents a significant advancement in biomedical engineering. It reflects ongoing efforts to improve the performance and safety of medical implants, eventually benefiting patient care and end results. While the focus of this study is on hard tissue implants, the principles and technologies developed could have broader applications in other areas of biomedical science, such as systems for delivery of drugs, tissue engineering and healing wounds.

1.6 Objectives

- To synthesize DEAE-dextran silver nanoparticles
- To characterize DEAE-dextran silver nanoparticles
- To prepare discs with a coating of NPs
- To characterize discs before and after coating
- To evaluate antimicrobial efficacy

CHAPTER 2: LITERATURE REVIEW

The goal of this review of the literature is to offer a thorough examination of the most recent studies on DEAE-Dextran silver nanoparticle-based biomimetic coatings. The review will cover the material properties, synthesis methods, biological interactions, and clinical applications of these coatings, focusing on their use in titanium-based hard tissue implants.

2.1 Titanium-Based Implants in Hard Tissue Applications

Titanium and its alloys are commonly used in hard tissue applications.

2.1.1 Properties of Titanium as a Biomaterial

The key properties that make titanium suitable for implants include its superior corrosion resistance, minimal density, and substantial strength-to-weight relationship (Geetha et al., 2009).

Titanium's ability to form a steady oxide layer (TiO_2) over the surface is critical for its corrosion resistance and biocompatibility (Peters et al., 1994). This passive oxide layer protects the metal from corrosion and enhances its interaction with biological tissues. Its surface can form a stable oxide layer, which contributes to its corrosion resistance but does not promote cell attachment or osseointegration (Zhang et al., 2018).

Biocompatibility and Osseointegration

One of the most critical aspects of titanium implants is their biocompatibility, defined as the ability to perform with an appropriate host response (Williams, 2008). Titanium's biocompatibility is largely attributed to the inertness of the TiO_2 layer, which minimizes immune reactions and promotes osseointegration— the direct bond between the implant surface and biological bone, both structurally and functionally (Brunette et al., 2001).

Osseointegration is a multi-step process involving protein adsorption, cell attachment, proliferation, differentiation, and matrix formation (Davies, 2003). Titanium's surface properties, including roughness and chemical composition, significantly influence these

processes. Studies have shown that roughened titanium surfaces enhance osteoblast attachment and activity, leading to improved bone-implant contact (BIC) (Gittens et al., 2011).

Surface Modifications of Titanium Implants

Despite the natural biocompatibility of titanium, surface modifications are often employed to enhance its biological performance. These modifications can be broadly categorized into mechanical, chemical, and biological methods.

Mechanical surface treatments, such as sandblasting, acid etching, and anodization, create micro- and nano-scale roughness on the implant surface. These modifications enhance the surface area and promote osteogenic cells adherence (Le Guéhennec et al., 2007). Anodization, for example, can produce the titanium surface with the permeable layer of oxide, increasing the surface's roughness and hydrophilicity (Smeets et al., 2016).

Chemical modifications involve altering the surface chemistry of titanium implants to enhance their bioactivity. bioactive elements like calcium, phosphorus, and silica can be introduced to the implant surface using methods such as chemical vapor deposition (CVD), physical vapor deposition (PVD), and sol-gel coatings (Hanawa, 2009). Hydroxyapatite coatings are recurrently used to mimic the mineral component of bone, promoting bone ingrowth and integration (Surmenev et al., 2014).

Biological surface modifications involve the bioactive molecules immobilization like peptides and growth factors on the implant surface. These molecules can enhance specific cellular responses, such as osteoblast differentiation and angiogenesis (Park et al., 2013). For example, coatings with RGD peptides (Arg-Gly-Asp) have been shown to improve cell attachment and spreading on titanium surfaces (Ferris et al., 1999).

2.1.2 Clinical Applications and Outcomes

Titanium-based implants are widely used in various clinical applications, including joint, spinal and dental implants, and craniofacial reconstructions (Peters et al., 1994). Clinical outcomes of titanium implants can be controlled by factors like implant design, surface characteristics, surgical technique, and patient-specific factors (Pye et al., 2009).

Orthopedic Implants

In orthopedic applications, titanium implants are commonly used for joint replacement surgeries, spinal integration, and fracture fixation. The use of titanium in these applications has shown excellent long-lasting results, with substantial survival and minimal complication rates (Berry, 2011). However, challenges such as stress shielding, implant loosening, and wear debrisinduced osteolysis remain concerns in long-term implant performance (Bobyn et al., 1999).

Dental Implants

Since titanium dental implants have such good osseointegration and biocompatibility, they have emerged as the industry standard for replacing lost teeth (Buser et al., 2017). The success rates of titanium dental implants are generally high, with survival rates exceeding 90% over ten years (Misch et al., 2008). Surface modifications, such as sandblasting and acid etching, have further improved the osseointegration and dental implants stability (Albrektsson et al., 1981).

Craniofacial Implants

Titanium implants are also used in craniofacial reconstructions, such as orbital and maxillofacial implants. These implants must exhibit high biocompatibility and mechanical strength to withstand the functional demands of the craniofacial region (Kirkpatrick et al., 2002). Titanium's ability to osseointegrate and form a stable connection with bone makes it an ideal material for these applications.

2.1.3 Challenges in Titanium Implant Integration

Despite its advantages, titanium's bioinertness can lead to poor integration with bone tissues. Additionally, the risk of bacterial infection at the implant site is a significant concern, often leading to implant failure and the need for revision surgeries (Luo et al., 2015). These challenges necessitate surface modifications to improve osseointegration and antimicrobial resistance.

2.2 Challenges in Hard Tissue Implant Applications

There are several challenges to cope with related to hard tissue implants as discussed in this section.

2.2.1 Implant related infections

One of the most significant challenges in hard tissue implant applications is the risk of infection. Implant-related infections can lead to severe complications, including implant failure, prolonged hospital stays, and the need for additional surgeries (Zimmerli et al., 2004). These infections are often caused by the colonization of bacteria on the implant surface, leading to biofilm formation. Biofilms are difficult to eradicate because they protect bacteria from the host immune response and antibiotic treatments (Costerton et al., 1999).

Various multidrug-resistant organisms such as Methicillin-resistant Staphylococcus aureus (MRSA) are particularly problematic, as they are resistant to conventional antibiotics and pose a significant risk in post-surgical infections (Hogan et al., 2019). The ability of bacteria to adhere to the implant surface and form biofilms is an important factor in the persistence and acerbity of these infections.

2.2.2 Poor Osseointegration

The direct physiological and functional bond between the surface of an implant and the developing bone is known as osseointegration, and it is essential to the long-term viability of hard tissue implants (Albrektsson & Johansson, 2001). However, poor osseointegration remains a challenge, particularly in patients with compromised bone health or in cases where the implant surface does not adequately support bone cell growth and adherence.

Factors like chemical composition, surface energy and oughness, of the implant material play a crucial role in promoting or hindering osseointegration (Buser et al., 2004). A lack of proper osseointegration can lead to implant loosening, micromotion at the bone-implant interface, and eventual failure of the implant (Simmons et al., 2001).

Biomimetic coatings are designed to replicate the natural extracellular matrix (ECM), promoting cell adhesion, proliferation, and differentiation. These coatings aim to enhance the

bioactivity of implant surfaces, improving the overall integration with host tissues (López et al., 2017).

2.2.3 Inflammatory Responses and Immune Reactions

The body's immune response to a foreign implant can lead to chronic inflammation and fibrous encapsulation, which impede osseointegration and can result in implant failure (Anderson et al., 2008). The release of pro-inflammatory cytokines and the interaction of immune cells to the implant site can create an environment that is not conducive to bone healing and integration.

Furthermore, the presence of metallic ions released from the implant material, such as titanium or its alloys, can provoke an immune response and lead to adverse tissue reactions (Franz et al., 2011). The inflammatory response can also be exacerbated by bacterial infections, creating a cycle of infection and inflammation that is difficult to break without removing the implant.

2.3 Treatment Options for Hard Tissue Implant challenges

2.3.1 Biomimetic Coatings for Enhanced Osseointegration

Biomimetic coatings, which mimic the natural extracellular matrix (ECM) of bone, have been developed to improve osseointegration. These coatings are designed to enhance the attachment, proliferation, and differentiation of osteoblasts (bone-forming cells) on the implant surface (Liu et al., 2016). Biomaterials such as calcium phosphate, hydroxyapatite, and bioactive glass are commonly used in these coatings due to their osteoconductive properties (Habibovic & Barrère, 2006).

DEAE-Dextran, a derivative of dextran, is an emerging biomaterial used in coatings to improve cell adhesion and proliferation due to its cationic nature, which facilitates interactions with negatively charged cell membranes and ECM components (Huang et al., 2019). When combined with AgNPs, DEAE-Dextran-based coatings can provide a dual function: promoting osseointegration while preventing infection.

Multifunctional Coatings

Multifunctional coatings that combine antimicrobial, osteoconductive, and antiinflammatory properties represent an advanced approach to addressing the complex challenges of hard tissue implants. Such coatings aim to provide comprehensive protection and support for implants, reducing the risk of complications and improving long-term outcomes.

For example, a DEAE-Dextran Silver Nanoparticles-based biomimetic coating could serve as a multifunctional solution by simultaneously promoting bone cell adhesion, providing antimicrobial protection, and reducing inflammation. The development and optimization of these coatings involve careful consideration of the balance between the different functional components to ensure biocompatibility and efficacy (Guggenbichler et al., 1999).

Antibiotic Coatings

Antibiotic coatings involve the incorporation of antibiotics into the implant surface or coating materials. This strategy provides localized antibiotic delivery, reducing the risk of systemic side effects and enhancing antibacterial efficacy at the implant site (An and Friedman, 1998). However, concerns about antibiotic resistance and the limited duration of antibiotic release have prompted the exploration of alternative antibacterial strategies.

Photocatalytic Coatings

Photocatalytic coatings, such as those based on titanium dioxide (TiO_2), have been investigated for their antibacterial properties. Under UV light, TiO_2 can generate reactive oxygen species (ROS) that kill bacteria (Fujishima et al., 2008). The photocatalytic activity of TiO_2 can be enhanced by doping with elements like silver, copper, and nitrogen, extending its antibacterial efficacy to visible light (Liu et al., 2014).

2.4 DEAE-Dextran: A Biomaterial for Coatings

DEAE-Dextran, a derivative of dextran, is an emerging biomaterial used in coatings.

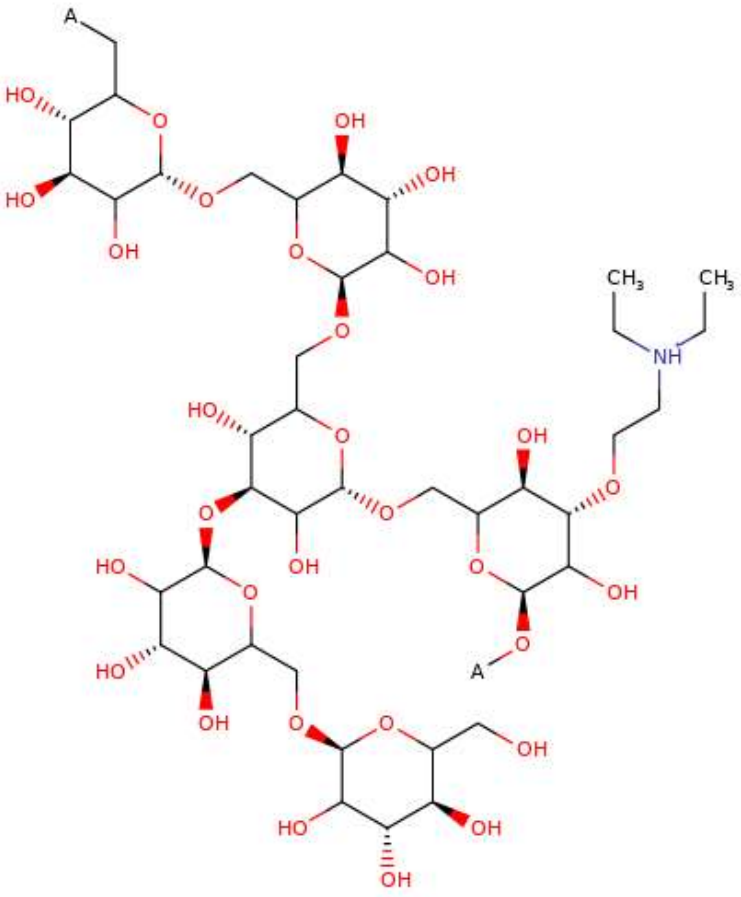

Figure 2.1: Chemical structure of DEAE-Dextran HC

2.4.1 Chemical Structure and Properties

DEAE-Dextran, a derivative of dextran modified with diethylaminoethyl groups, is known for its biocompatibility and biodegradability. It has a polycationic nature, allowing it to interact with negatively charged cell membranes and ECM components, promoting cell adhesion (Huang et al., 2016).

2.4.2 Synthesis and Characterization of DEAE-Dextran

DEAE-Dextran is synthesized by reacting dextran with a DEAE chloride reagent under alkaline conditions. The degree of substitution (DS) of DEAE groups on the dextran backbone is dependant on factors as reaction conditions (pH, temperature, and reagent concentration)

(René et al., 1978). The DS affects the polymer's charge density and, consequently, its interactions with other molecules and surfaces (Palva et al., 1980).

Size-exclusion chromatography (SEC) and nuclear magnetic resonance (NMR) spectroscopy are two methods used in the characterization of DEAE-Dextran to measure the molecular weight distribution and DS, respectively. Additionally, zeta potential measurements can provide insights into the surface charge and stability of DEAE-Dextran in solution (Lapčík et al., 1998).

2.4.3 Biomedical Applications of DEAE-Dextran

DEAE-Dextran has been commonly utilized as a coating material for medical devices. Its ability to form stable, thin layers on surfaces makes it suitable for use in implant coatings (Nguyen et al., 2020).

Enhancement of Cell Adhesion and Proliferation

One of the primary applications of DEAE-Dextran coatings is in enhancing cell adhesion and proliferation on implant surfaces. The cationic nature of DEAE-Dextran allows it to engage with the negatively charged components of cell membranes, promoting cell attachment and spreading (Park et al., 1998). Studies have shown that DEAE-Dextran coatings can improve the adhesion and proliferation of various cell types, including fibroblasts, osteoblasts, and endothelial cells (Jiang et al., 2007). DEAE-Dextran-mediated transfection is a well-established technique for introducing nucleic acids into cells. The DEAE-Dextran-DNA complexes are endocytosed by cells, and the acidic environment within the endosomes causes the release of DNA into the cytoplasm (Rogers et al., 1977). Numerous cell lines, even those that are difficult to transfect, have been successfully transfected using this technique (Kichler, 2004).

2.4.4 Antimicrobial Properties

The antimicrobial properties of DEAE-Dextran have been attributed to its cationic nature, which allows it to disrupt bacterial cell membranes and inhibit microbial growth (Lee et al., 2012). DEAE-Dextran coatings can be used to impart antimicrobial properties to various surfaces, including medical devices and implants, to prevent infections.

The antibacterial effectiveness of DEAE-Dextran-coated surfaces against Staphylococcus aureus and Escherichia coli was studied by Lee et al. (2012). The study found that DEAE-Dextran coatings significantly reduced bacterial adhesion and biofilm formation, suggesting their potential use in preventing implant-associated infections.

2.5 Silver Nanoparticles in Biomedical Applications

2.5.1 Synthesis and Characterization of Silver Nanoparticles

Chemical reduction, photochemical reduction, and biological synthesis are three possible ways to create silver nanoparticles (AgNPs). The antibacterial activity of AgNPs is directly influenced by the controllable size, shape, and surface characteristics (Rai et al., 2018). Several techniques, including as chemical reduction, physical techniques, and biological methods, can be used to create AgNPs. The biological properties of nanoparticles are influenced by their size, shape, and surface chemistry, all of which are determined by the synthesis process used (Sharma et al., 2009).

The most used technique for creating AgNPs is chemical reduction. Sodium borohydride, citrate, or ascorbic acid are examples of reducing agents that are used in the process of reducing silver ions (Ag^{+}) in a solution. Polyvinyl alcohol (PVA) or other stabilizing chemicals are present (Murphy et al., 2015). Temperature, pH, and reagent concentrations can all be changed to modify the size and form of AgNPs (Jiang et al., 2014).

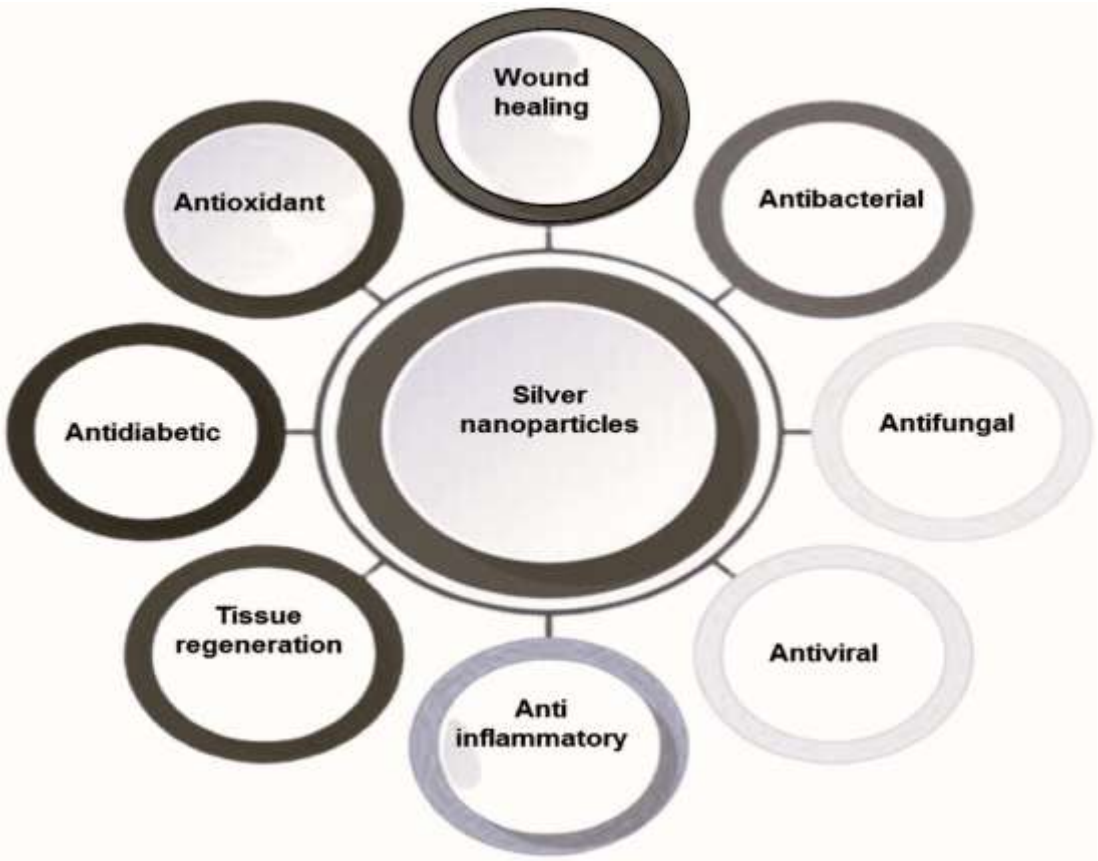

Figure 2.2: Properties of AgNPs in biomedical applications

Characterization of AgNPs involves determining their size, shape, surface charge, and crystalline structure. Techniques such as transmission electron microscopy (TEM), scanning electron microscopy (SEM), dynamic light scattering (DLS), and X-ray diffraction (XRD) are commonly used (Sun et al., 2002). Surface charge, measured as zeta potential, provides insights into the stability and potential interactions of AgNPs with biological systems (Kora & Arunachalam, 2012).

2.5.2 Antibacterial Properties and Mechanisms

Because AgNPs can rupture bacterial cell walls by releasing silver ions, they have potent antibacterial qualities, generate reactive oxygen species, and interfere with bacterial DNA replication (Rai et al., 2009). These properties make AgNPs effective in preventing infections, particularly in medical device applications (Gogoi et al., 2017).

Mechanisms of Action

AgNPs have the ability to cling to the membrane of bacteria and pierce the cell, causing structural harm and eventual cell death. The liberated silver ions have the ability to bind with protein thiol groups, interfering with vital biological functions (Morones et al., 2005). Additionally, AgNPs can generate ROS, such as hydroxyl radicals and superoxide anions, which cause oxidative stress and damage cellular components (Hwang et al., 2008).

2.5.3 Therapeutic Applications

In addition to antimicrobial and diagnostic applications, AgNPs have been explored for various therapeutic purposes. Their unique properties allow them to be used in cancer therapy, wound healing, and anti-inflammatory treatments.

Wound Healing

AgNPs have been used in wound dressings to promote healing and prevent infections. Their antibacterial properties, combined with their ability to modulate inflammation and promote tissue regeneration, make them ideal for treating chronic wounds and burns (Vazquez-Muñoz et al., 2019). AgNPs can also enhance the activity of growth factors and cytokines involved in the healing process (Sim et al., 2017).

Anti-Inflammatory Effects

Numerous studies have shown that AgNPs have anti-inflammatory properties. According to Chen et al. (2014), they have the ability to control the release of pro-inflammatory cytokines including TNF-α and IL-1β while also lowering oxidative stress. These characteristics may find use in the management of inflammatory conditions like inflammatory bowel disease (IBD) and arthritis (El-Mohri et al., 2017).

2.6 DEAE-Dextran Silver Nanoparticles-Based Coatings

2.6.1 Synthesis and Characterization

The synthesis of DEAE-Dextran AgNPs-based coatings involves the integration of AgNPs into a DEAE-Dextran matrixThis can be accomplished by reducing silver ions (Ag^{+}) chemically in the presence of DEAE-Dextran, which serves as a stabilizing and reducing agent. The positively charged groups in DEAE-Dextran help in the dispersion of AgNPs, preventing aggregation and ensuring a uniform distribution within the matrix (Li et al., 2014).

Techniques including transmission electron microscopy (TEM), scanning electron microscopy (SEM), and X-ray photoelectron spectroscopy (XPS) can be used to evaluate the shape, content, and thickness of the resulting hybrid coating (Kim et al., 2020).

2.6.2 Biological Interactions and Biocompatibility

DEAE-Dextran AgNPs-based coatings provide dual functionality: enhancing cell adhesion and proliferation through the DEAE-Dextran component, while the AgNPs impart antimicrobial properties. Studies have demonstrated that these coatings can effectively prevent bacterial colonization without adversely affecting cell viability, making them suitable for implant applications (Liu et al., 2021).

2.6.3 Antimicrobial Properties of DEAE-Dextran AgNPs-Based Coatings

One of the primary applications of DEAE-Dextran AgNPs-based coatings is their use as antimicrobial agents. AgNPs are well-known for their broad-spectrum antimicrobial activity, and when incorporated into a DEAE-Dextran matrix, they can provide sustained antimicrobial effects.

Mechanisms of Antimicrobial Action

The primary mechanism for the antibacterial activity of DEAE-Dextran AgNPs-based coatings is the release of silver ions, which interact with microbial cell membranes and interfere with essential cellular functions. The cationic nature of DEAE-Dextran enhances the interaction

between the coating and negatively charged microbial cell walls, leading to increased efficacy (Rai et al., 2009). Furthermore, AgNPs have the ability to produce reactive oxygen species (ROS), which can lead to oxidative stress and further impair the viability of microbial cells (MarambioJones & Hoek, 2010).

2.6.4 Applications in Antimicrobial Surfaces

DEAE-Dextran AgNPs-based coatings have been explored for use in various antimicrobial surfaces, including medical devices, surgical instruments, and hospital surfaces. The ability of these coatings to inhibit biofilm formation is particularly valuable in medical applications, as biofilms are often resistant to conventional antimicrobial treatments (Pillai et al., 2014). Studies have demonstrated that such coatings effectively reduce bacterial adhesion and proliferation, making them ideal for preventing hospital-acquired infections (Sharma et al., 2016).

Medical Device Coatings

DEAE-Dextran AgNPs-based coatings have significant potential in enhancing the performance and safety of medical devices. Their antimicrobial properties can reduce the risk of infections associated with implants and other medical devices.

Catheters and Implants

One of the critical applications of DEAE-Dextran AgNPs-based coatings is in catheters and implants. These coatings can prevent bacterial colonization and biofilm formation, which are common causes of device-associated infections. Studies have shown that DEAE-Dextran AgNPscoated catheters exhibit prolonged antimicrobial activity, making them suitable for longterm medical applications (Pillai et al., 2014).

Wound Dressings

The antimicrobial and biocompatible properties of DEAE-Dextran AgNPs-based coatings also make them suitable for wound dressings. These coatings can prevent infection, promote healing, and provide a moist environment conducive to tissue regeneration. The

presence of AgNPs can also reduce inflammation and accelerate the healing process, making these coatings valuable in treating chronic wounds and burns (Francolini et al., 2010).

Enhancement of Cell Adhesion and Proliferation

Cell attachment and proliferation on surfaces of implants is one of the primary uses of DEAE-Dextran coatings. According to Park et al. (1998), DEAE-Dextran's cationic properties enable it to interact with the negatively charged elements of cell membranes, facilitating cell adhesion and spreading. Research has demonstrated that DEAE-Dextran coatings can enhance the adhesion and proliferation of many cell types, such as osteoblasts, endothelial cells, and fibroblasts (Jiang et al., 2007).
For example, DEAE-Dextran coatings on glass substrates dramatically increased endothelial cell adherence and proliferation (Park et al., 1998). According to the study, DEAE-Dextran's positive charge was critical in mediating contacts between cells and substrates, which enhanced cellular responses.

Drug and Gene Delivery

Because DEAE-Dextran can form complexes with both medicines and nucleic acids, it has been extensively investigated as a drug and gene delivery carrier. The nucleic acids can be delivered into cells more easily and be shielded from degradation by stable complexes that are formed when the positive charges on DEAE-Dextran engage electrostatically with negatively charged DNA or RNA (Wu & Mutharasan, 1984).
Nucleic acid introduction into cells is a well-established method known as DEAE-Dextran-mediated transfection. Cells endocytose the DEAE-Dextran-DNA complexes, and the acidic endosome environment releases DNA into the cytoplasm (Rogers et al., 1977). Numerous cell lines, including those that are difficult to transfect, have been successfully transfected using this technique (Kichler, 2004).

DEAE-Dextran has been employed as a coating material for medication delivery systems in addition to gene delivery. For instance, Janiak et al. (2003) created microspheres coated with DEAE-Dextran that regulated heparin release while exhibiting improved anticoagulant activity and sustained release patterns

CHAPTER 3: MATERIALS AND METHOD

3.1 Preparation of DEAE-Dextran Silver Nanoparticles

3.1.1 Materials

All of the Chemicals such as DEAE-Dextran hydrochloride powder, Silver nitrate (AgNO3), Sodium Borohydride (NaBH4), Ammonia (23%), and Distilled water were all purchased from the Sigma-Aldrich for use in the synthesis experiment.

3.1.2 Methodology

DEAE-Dextran Silver Nanoparticles were made using the chemical reduction technique (Mikac et al., 2017). As a reducing agent, NaBH4 was utilized. In 10 ml distilled water, AgNO3 (0.51g) and DEAE-dextran HCl powder (0.17g) solutions were produced separately. DEAEDextran powder was mixed with AgNO3 at a 3:1 mass ratio. The two solutions were combined together with steady stirring, yielding a final concentration of 20ml. combination of the two solutions yielded a milky white solution. This happened because of the production of AgCl in solution. Then in order to dissolve the AgCl 20-30 drops of 23% NH3 solution was added until the solution turned colorless. Following this 6-7 drops of 2.0mM NaBH4 were added. On addition of 2.0mM NaBH4 the solution turns a dark brown color almost quickly.

Figure 3.1: Synthesized DEAE-Dextran AgNPs

After that, the synthesized sample was centrifuged three times with ultrapure water and twice with ethanol (8,000 rpm, 30 min) using a Scanspeed 2236R high-speed centrifuge. The resulting precipitates were vacuum-dried at room temperature, and FTIR, XRD, SEM, Raman spectroscopy and UV-Vis were used to describe them as powders.

3.2 Nanoparticles Characterization

The surface net charge, size, and assembly of DEAE-Dextran-AgNPs were evaluated to ensure that they were of the proper size and type to be employed for coating of the titanium-based implant models and for antibacterial models testing.

3.2.1 UV-Vis spectroscopy analysis

UV-Vis analysis was done to evaluate the concentration of light absorbance by the synthesized nanoparticles to confirm their successful synthesis. A light beam is directed through a sample in a cuvette, where the molecules absorb specific wavelengths of light. The amount of absorption is determined by the wavelengths that pass through the sample. Initially, a cuvette with only solvent (reference) is placed in the hood to measure absorption. Then, a second cuvette with the sample material is placed in the hood to obtain the absorption spectrum. The laser beam is split into two parts: one part is focused on the reference, and the other on the sample cuvette. This creates an absorbance spectrum across the entire wavelength range. The maximum absorption value at any given wavelength is called lambda max. The Beer-Lambert Law states that a sample mixture's absorbance is proportionate to its molar concentration in the cuvette. The molar absorptivity, or absorption value, is utilized to compare the spectra of various substances. The UV2800 BMS Biotechnology Medical Services spectrophotometer in Madrid, Spain was used to do UV-Vis spectrophotometry.

3.2.2 XRD analysis

The technique of X-ray diffraction (XRD) examination is employed to ascertain the crystalline structure of various materials. It operates by pointing X-rays at a sample, which scatters when the atoms in the material come into contact with it. Peaks are seen on a detector as a result of the diffraction pattern created by the dispersed X-rays. The arrangement of atoms

within the crystal and the distances between atomic planes can be inferred from the positions and intensities of these peaks.

By analyzing this diffraction pattern, scientists can identify the material's crystal structure, determine its phase composition, and measure other structural properties like lattice parameters and crystallite size.

3.2.3 Determining the morphology of particles

A Scanning Electron Microscope (SEM) VEG 3 LMU was used to analyze the morphology of DEAE-Dextran coated silver nanoparticles (AgNPs). For this purpose, glass slides with DEAEDextran AgNPs were coated with a 30nm layer of gold to make them functional and conductive for SEM analysis. The SEM study specifically analyzed the physical distribution of the nanoparticles and confirmed their spherical shape using a concentrated electron beam, Scanning Electron Microscopy (SEM) is a potent imaging method that produces high-resolution surface images of a sample. Unlike traditional light microscopy, SEM provides detailed surface morphology and topographical information at a nanometer-scale resolution, making it particularly useful for studying nanoparticles and other nanoscale materials.

3.2.4 FTIR analysis

FTIR spectroscopy involves passing infrared radiation through a sample. The wavelengths of infrared light that correspond to the vibrational frequencies of the chemical bonds in the molecules are absorbed by the sample. The resulting spectrum, with peaks corresponding to various functional groups, shows the molecular fingerprint of the sample (Stuart, 2004). The interferometer, which modifies the infrared beam, is the essential part of an FTIR spectrometer. An infrared spectrum is created by performing a Fourier transform on the resulting interferogram. High spectral resolution and quick data gathering are made possible by this method (Griffiths & de Haseth, 2007).

3.2.5 Zeta Potential analysis

An analytical tool called a zetasizer is frequently used to determine the molecular weight, zeta potential, and particle size in colloidal systems. The measurement of these parameters is

crucial in understanding the stability and behavior of particles in various applications, such as pharmaceuticals, materials science, and biotechnology.

Particle Size Measurement

The Zetasizer measures the hydrodynamic diameter of suspended particles using dynamic light scattering (DLS). Using DLS, one can determine the size distribution of particles by measuring the variations in light scattering brought on by their Brownian motion (Malvern Panalytical, n.d.).

Zeta Potential Measurement

The electrostatic potential at a particle's sliding plane in a liquid is referred to as the zeta potential. It is a crucial metric that shows how stable colloidal dispersions are. The Zetasizer measures how particles move when an electric field is applied via electrophoretic light scattering or ELS. The zeta potential, which aids in forecasting the stability and interaction of particles within the medium, is computed using the velocity of these particles (Malvern Panalytical, n.d.).

3.2.6 Raman spectroscopy analysis

The foundation of Raman spectroscopy is the inelastic scattering of monochromatic light, typically emitted by a laser. A tiny percentage of photons are inelastically scattered when light interacts with a molecule; the majority are elastically dispersed (Rayleigh scattering). The energy difference between the incident photons and the vibrational or rotational energy levels of the molecule causes this inelastic scattering, sometimes referred to as Raman scattering, to cause a shift in the wavelength of the scattered light (Ferraro, Nakamoto, & Brown, 2003).

3.3 Disc Treatment and Biomimetic coating of Nanoparticles

The titanium discs were split into two groups for analysis: Group II (heat and alkaline treatment) and Group I (no treatment). Prior to surface treatment, the samples were first ultrasonically cleaned for 15 minutes using distilled water and acetone. They were then allowed

to air dry. After then, the samples from Group I were stored without undergoing any other surface treatments.

The samples for Group II were submerged for 72 hours at 80°C in a 5.0-M NaOH solution. Following this time, the substrates were dried for 24 hours at 40°C and then rinsed with distilled water. After the alkaline treatment, the samples were heat-treated for an hour at 600°C in an electric furnace with an air environment before being allowed to cool in the furnace to room temperature.

The samples were soaked in 30 ml of SBF×5 solutions for 7 and 14 days to induce the formation of nanoparticles and a calcium phosphate layer on the sample surface. The purpose of this was to determine the material's ability to form a DEAE-Dextran silver nanoparticles layer in vitro spontaneously and to confirm that the deposition of biomimetic nanoparticle coatings can be significantly accelerated by increasing concentrations by a factor of five (Barrere et al.). Kokubo showed how a biomimetic process including an alkaline treatment in NaOH and immersion in SBF (simulated bodily fluid) can produce a bioactive titanium surface. With this method, titanium implants can have bone-like apatite with additional nanoparticle coatings applied in as little as seven days (Kokubo T. et al). The comparatively long substrate coating period is a significant component in coating metallic implants using the biomimetic approach.

Chemical reagents: NaCl (40 g), MgCl2.6H2O (1.52 g), CaCl2.2H2O (1.84 g), Na2HPO4.2H2O (0.89 g), and NaHCO3 (1.76 g) were dissolved in 1,000 ml of distilled water to generate the SBF×5 solution. The pH was then adjusted to 7.4 at 36.5°C using tris-hydroxymethyl aminomethane and hydrochloric acid. To maintain ion concentration, the SBF and nanoparticles solution were changed every 48 hours. The samples were withdrawn from the soaking solution, washed with distilled water, and allowed to air dry for a full day.

A scanning electron microscope (SEM, LEO 1450 VP, Zeiss, Germany) was used to examine the surfaces before treatment (Group I), after soaking in SBF×5 solutions for 7 and 14 days, and after alkaline treatment followed by heat treatment (Group II).

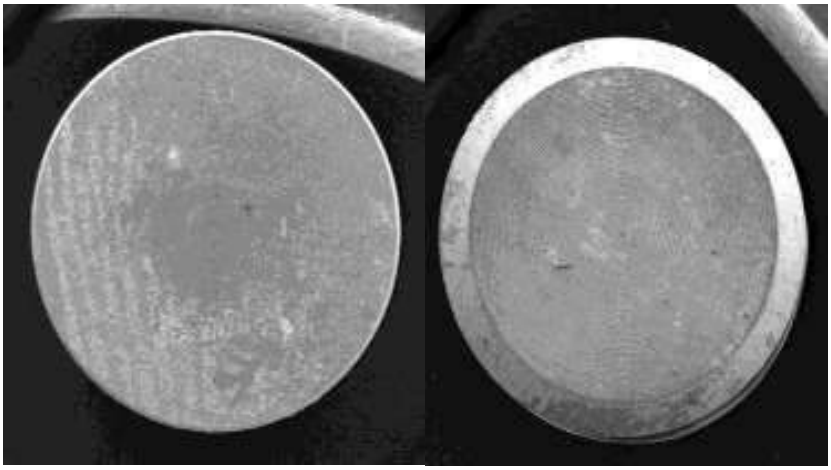

Figure 3.2: DEAE-Dextran coated discs

3.4 Antibacterial in vitro assays

3.4.1 Culture conditions

Tryptic Soy Broth 1% Sucrose was used as the bacterial growth medium (TSBS). To increase the tryptic soy broth's growth range, sucrose was added. This growing medium was used to set up the bacterial model's pre-culture. Every incubation was carried out at 37 °C. For bacterial culture, a 10 ml tryptic soy broth containing 1% sucrose was made.

3.4.2 Single Specie Model

Tryptic soy agar plates were used to isolate S. aureus bacteria. The bacterial strain was placed in a 5ml TSBS tube and incubated overnight. This preculture was diluted to 200 µl, mixed with 5 ml of TSBS media, and incubated in a shaking water bath for three hours. After around three hours, absorbance at 600 nm was measured, and the culture was thereafter serially diluted.

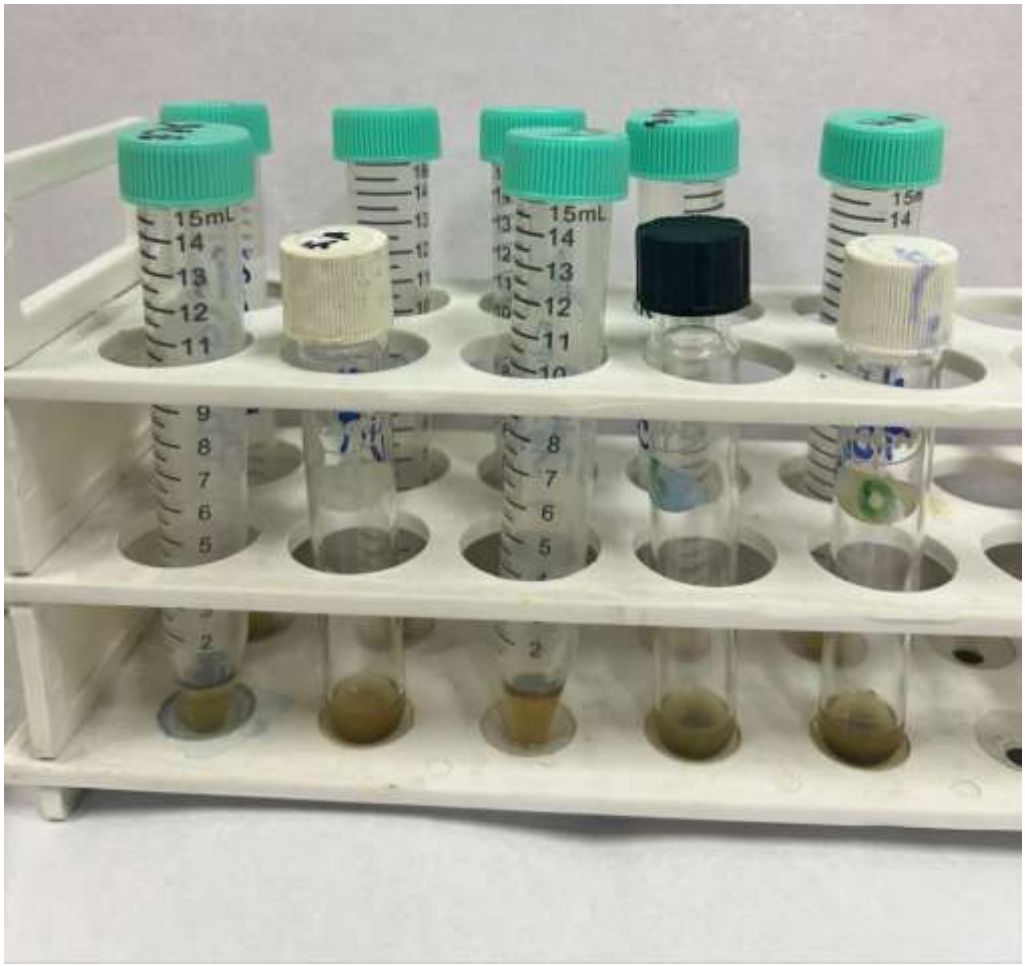

Figure 3.3: Bacterial strain in TSBS media and serial dilutions of bacterial inoculum

Coated disc samples were placed in glass tubes with 500 µl of this diluted culture and placed in an incubator. For the antibacterial assay findings, 50 µl from each glass tube was removed six hours later, spread out on TSA plates, and incubated overnight (Figure 3.3 and 3.4).

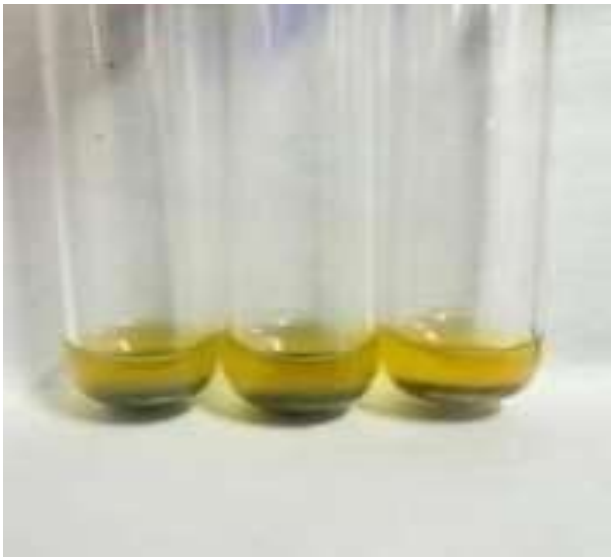

Figure 3.4: Glass tubes containing sample discs immersed in 500µl diluted culture

3.4.3 Colony Forming Unit (CFU)

After manually counting the colonies on each plate, log10 CFU/ml was computed. All model experiments were performed three times to ensure there was no doubt. In every trial, the cultured broth was diluted to the fifth degree. The formula used to compute CFU/ml was (No. of colonies) / (Dillution factor) / Sample poured in milliliters.

CHAPTER 4: RESULTS AND DISCUSSION

This chapter includes the results of different characterization methods for the DEAE-Dextran silver nanoparticles.

4.1 Characterization of DEAE-Dextran AgNPs

The UV-2800 (BMS Biotechnology Medical Services, Madrid, Spain) spectrophotometer was used to perform UV-Vis spectrophotometry in order to characterize the DEAE-dextran AgNPs. The ALPHA ll Bruker FTIR Spectrometer (Westborough, MA, USA) was used to perform FTIR. Malvern Zeta Sizer (Malvern) performed the SEM and zeta potential analysis.

4.1.1 UV-Vis Spectrometry evaluation

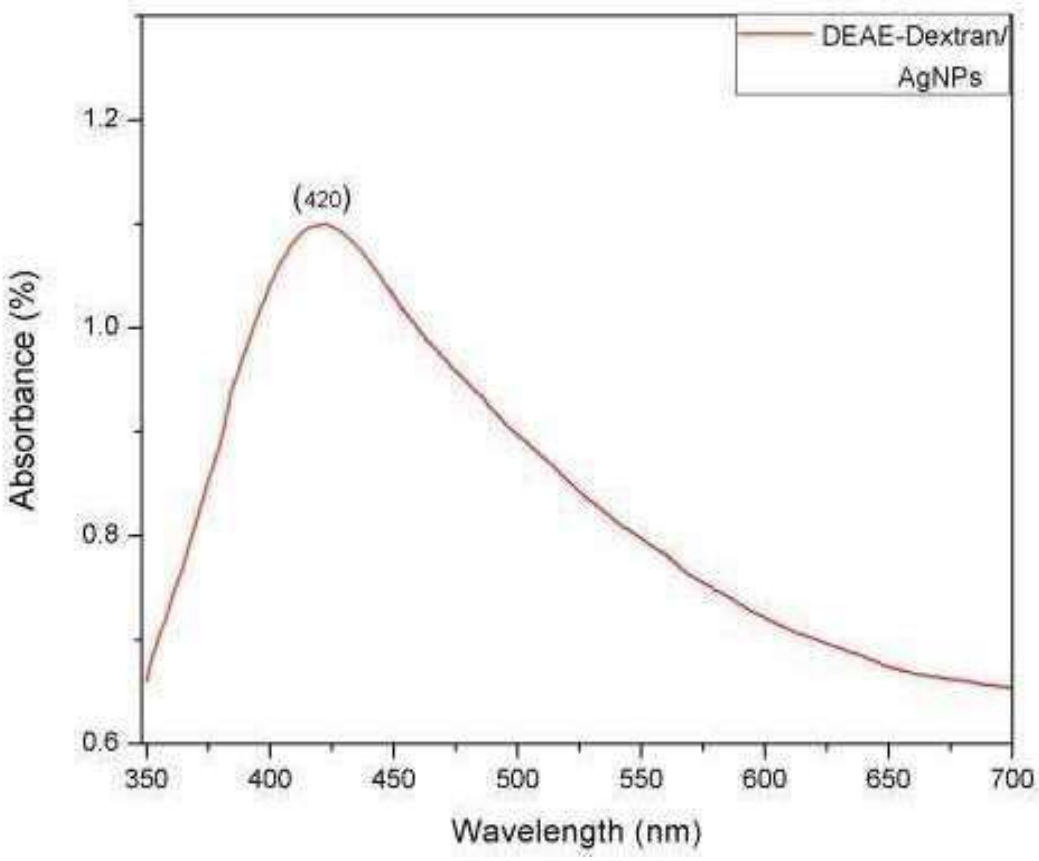

Figure 4.1: UV-Vis of DEAE-Dextran AgNPs

A UV-Vis study of DEAE Dextran AgNPs was conducted. The peak of DEAE-Dextran AgNPs was quite strong and wide, falling between 415 and 425 nm. The decrease of Ag+ to Ag0 is confirmed by a distinct band (Mohanta et al., 2020). The peak spectrum of DEAE-AgNPs was 420 nm, with 1.1 units of absorption. Because of the dextran coating on AgNPs, the peak migrated forward. The synthesis of DEAE-AgNPs was confirmed with these observable peaks. Hence, silver nanoparticles were effectively encapsulated with DEAE-Dextran macromolecules (Figure 4.1).

4.1.2 X-ray Diffraction (XRD) results interpretation

The silver crystalline planes were clearly represented by discrete peaks in the DEAE Dextran AgNPs XRD pattern. When it comes to silver nanoparticles, the typical observed peaks are at particular 2θ values that match the face-centered cubic (FCC) silver planes at 37.20°, 44.18°, 64.36°, 77.67°, and 82.54° for (111), (200), (220), (311), and (222) respectively. The presence of crystalline silver in the sample was verified by these peaks. The size of the silver nanoparticles can be determined by looking at the broadening of these peaks. According to the Scherrer equation, broader peaks indicate smaller crystallite sizes, which is typical for nanoparticles.

DEAE-Dextran is an organic polymer and generally amorphous in nature, meaning it lacks a crystalline structure. In the XRD pattern, this is indicated by a broad hump or baseline elevation rather than sharp peaks. This broad feature represents the amorphous structure of the DEAEDextran coating. Slight shifts in the diffraction peaks or changes in peak intensities show that DEAE-Dextran coating interacts strongly with the silver nanoparticles. This indicates modifications in the crystallinity or the stress/strain within the silver nanoparticles due to the coating. Hence the XRD analysis validated the crystalline nature of the silver nanoparticles and their phase purity. Broad peaks or a hump corresponding to the amorphous DEAE-Dextran coating was expected to be found in the pattern.

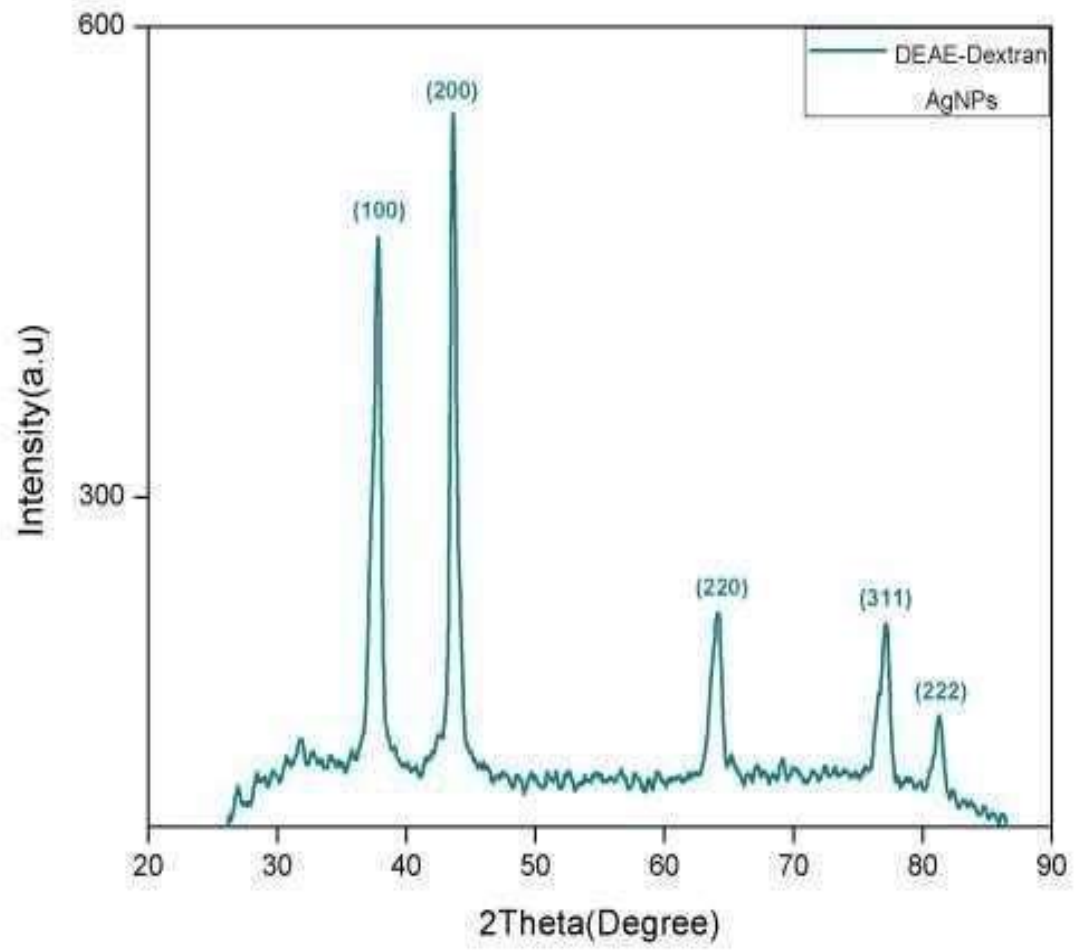

Figure 4.2: XRD pattern of DEAE- Dextran Silver Nanoparticles

4.1.3 Zeta sizer and surface Potential

Positive Zeta Potential: The zeta potential value for DEAE Dextran silver nanoparticles found out to be +34.11 mV. DEAE-Dextran is a positively charged polymer due to the presence of diethylaminoethyl (DEAE) groups. When silver nanoparticles are coated with DEAE-Dextran, it typically give a positive zeta potential value. This positive charge indicates that the DEAEDextran has successfully coated the nanoparticles and dominates the surface charge.

Table 4.1: Zeta potential values for DEAE-Dextran AgNPs

Sample	Z-Average (d. nm)	Zeta Potential (mV)
DEAE-Dex AgNPs	350	34.11

Magnitude of Zeta Potential: A high positive zeta potential (e.g., > +30 mV) suggests strong electrostatic repulsion between particles, which generally indicates good colloidal stability. This means the nanoparticles are less likely to aggregate, which is crucial for their effectiveness in applications like biomedical coatings.

Improved antibacterilal activity was predicted as a result of the electrostatic interaction of negative charge from AgNPs and positive charge of DEAE-Dextran polymer (Davidović et al., 2017). In addition a more stronger positive value of zeta potential as found for DEAE-Dextran to be +34.11mV suggested enhanced stability of DEAE-Dextran AgNPs in suspensions (Mikac et al., 2017).

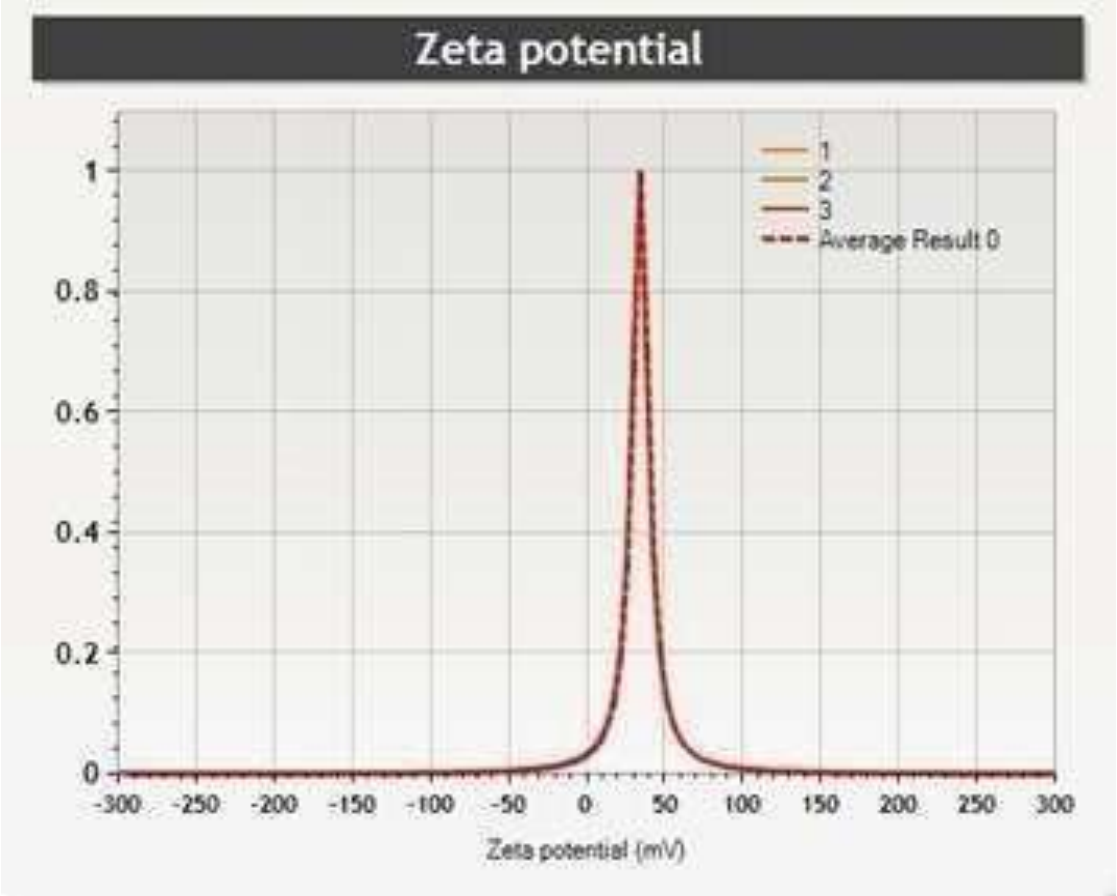

Figure 4.3: Zeta potential results of DEAE-Dextran AgNPs

4.1.4 SEM analysis

SEM prediction was conducted with SEM (Tescan, Czech Republic) VEG 3 LMU. DEAEDextran Silver nanoparticles: The SEM analysis at 20kV was used to examine the morphology of DEAE-Dextran silver nanoparticles as shown in Figure 4.4. This analysis was carried out by dropping the nanoparticle suspension directly onto glass slides and drying them at room temperature. The SEM images of the nanoparticles revealed that these are typically around 120600 nm in size with various shapes as spherical, hexagonal while some exhibiting irregular shapes.

The observed DEAE-Dextran silver nanoparticles were larger due to the DEAE-Dextran coating.

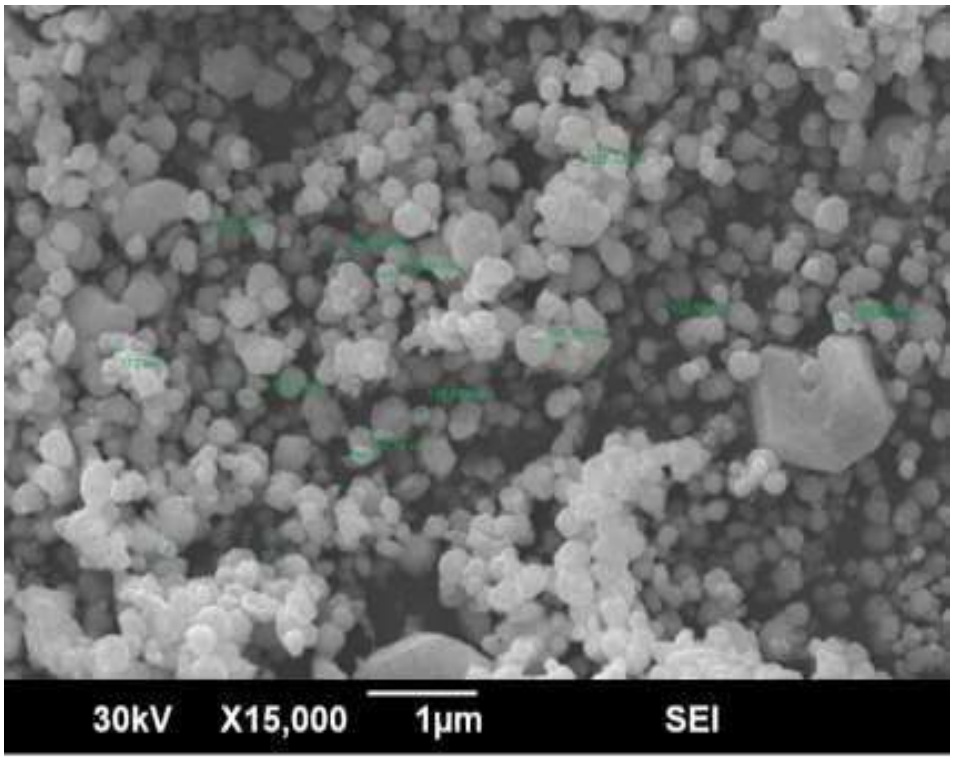

Figure 4.4: SEM image of DEAE-Dextran AgNPs

Figure 4.5 illustrates how SEM micrographs were used to examine the nanoparticle precipitation on the sample surfaces following varying times of soaking in SBF. After seven days in the SBF×5, group I's calcium phosphate particles and DEAE-dextran AgNPs were observed to have deposited as a non-continuous precipitation with an irregularly clustered morphology on the specimen surfaces (Fig. 6a). However, after twice as long, a larger quantity of precipitated particles was seen on the sample surfaces. It was possible to see a continuous layer of cracked or patchy particles (Fig. 6b). Furthermore, following 7 and 14 days in the SBF, a continuous layer of calcium phosphate and nanoparticles coated the majority of the heat-treated samples (Fig. 6c,d). A denser and smoother layer was created on the surfaces, corresponding to the final coatings (Fig. 4.5b, d) during 14 days of immersion in the SBF; however, it is important to note that the surface of the heat-treated Ti-disc samples had more aggregated island-like clustered particles deposited on it. Comparing Figure 4.5b to Figure 3d, Figure 4.5b showed a layer of precipitated particles with patches. The calcium phosphate layer and DEAE-dextran AgNPs cracks that are present on the sample surfaces may have developed

as a result of poor adhesion. Rosenberg et al. and Jonasova´ et al. reveal that layers have formed for titanium and its alloys, indicating how crucial it is to separate.

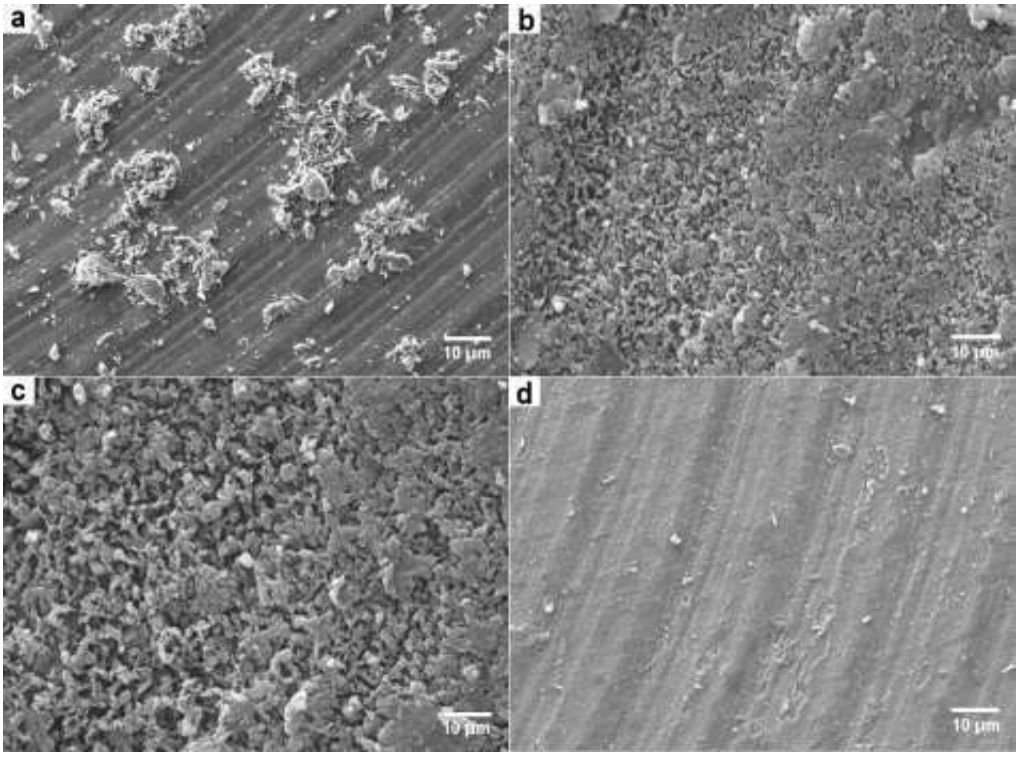

Figure 4.5: SEM images of surface morphology of untreated disc with coating for 7 days (a); surface morphology of untreated disc with coating for 14 days (b); surface morphology of alkali and heat treated disc and with coating for 7 days (c); surface morphology of alkali and heat treated disc with coating for 14 days (d).

4.1.5 FTIR analysis

The DEAE Dextran coating on AgNPs was validated by FTIR analysis. FTIR analysis was used to better understand how Dextran stabilizes the AgNPs. Figure 4.6 shows the FTIR spectra of DEAE-Dextran AgNPs powder. It represented the spectra of DEAE-Dextran AgNPs recorded at 400 cm-1 - 4000 cm-1. The DEAE Dextran coating on AgNPs was validated by FTIR analysis. The FTIR spectra of DEAE-Dextran powder and DEAE-Dextran AgNPs indicated; a large span of a strong band from 3600-3000 cm-1 to the asymmetric O-H stretching followed by signals reduction on the 2860 cm-1 band which is due to water adsorption in the samples (Li F et al., 2014), the peaks at 2913 and 2916.3 cm^{-1} can be attributed to C–H symmetric and asymmetric stretching (Divakar D et al., 2018), the stretching peaks at 1650 cm-

1 and 1655 cm-1 corresponded to the NH bond. Almost all of the peaks were identical, however, there were a few peak changes in the area above 1000 cm-1 due to DEAE-Dextran covering on AgNPs. These peak changes implied dextran stretching (Ispirili et al. 2021 & Can et al., 2018). The stretching band around 1000 cm^{-1} indicated the C–O–C asymmetric stretching (Bankura et al., 2012), peaks around 911 and 790 cm^{-1} correspond to the presence of α-glycosidic links and dextran adsorption (Ispirili et al. 2021 & Can et al., 2018).

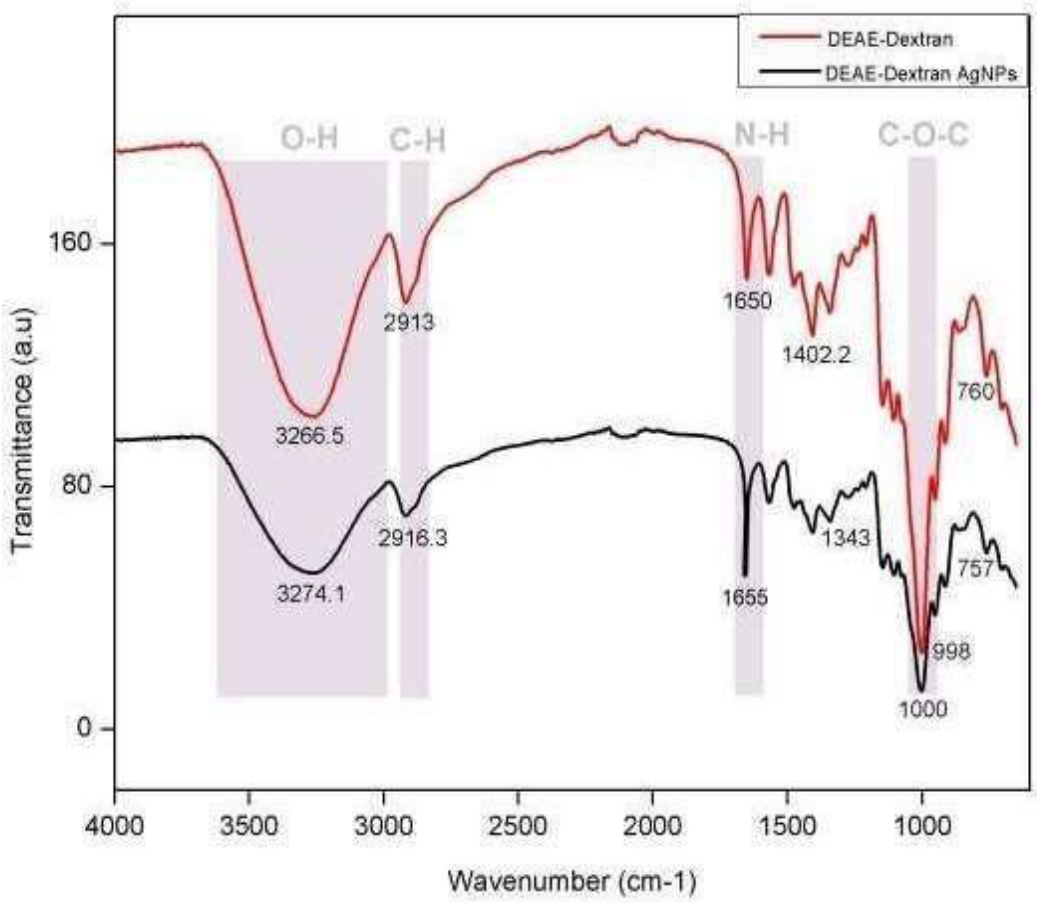

Figure 4.6: FTIR spectra of DEAE-Dextran AgNPs

4.1.6 Raman spectroscopy

The overall Raman spectrum confirmed the successful formation of DEAE-Dextran-coated silver nanoparticles (Figure 4.7). Resulted spectra showed distinct peaks depicting different functionalities as the silver-silver (Ag-Ag) associated vibrational modes were

confirmed by a sharp peak at 150 cm^{-1}, the peak around 448 cm^{-1} corresponded to the C–O–C stretching vibrations, which are characteristic of the dextran component in the DEAE-Dextran coating and a broad peak observed near 1000 cm^{-1} is likely due to the C–C and C–O stretching vibrations within the polysaccharide backbone of the DEAE-Dextran (Li W. et al., 2021) (Zhou et al., 2022). This further supported the presence of DEAE-Dextran on the surface of the silver nanoparticles (Gosh et al., 2021).

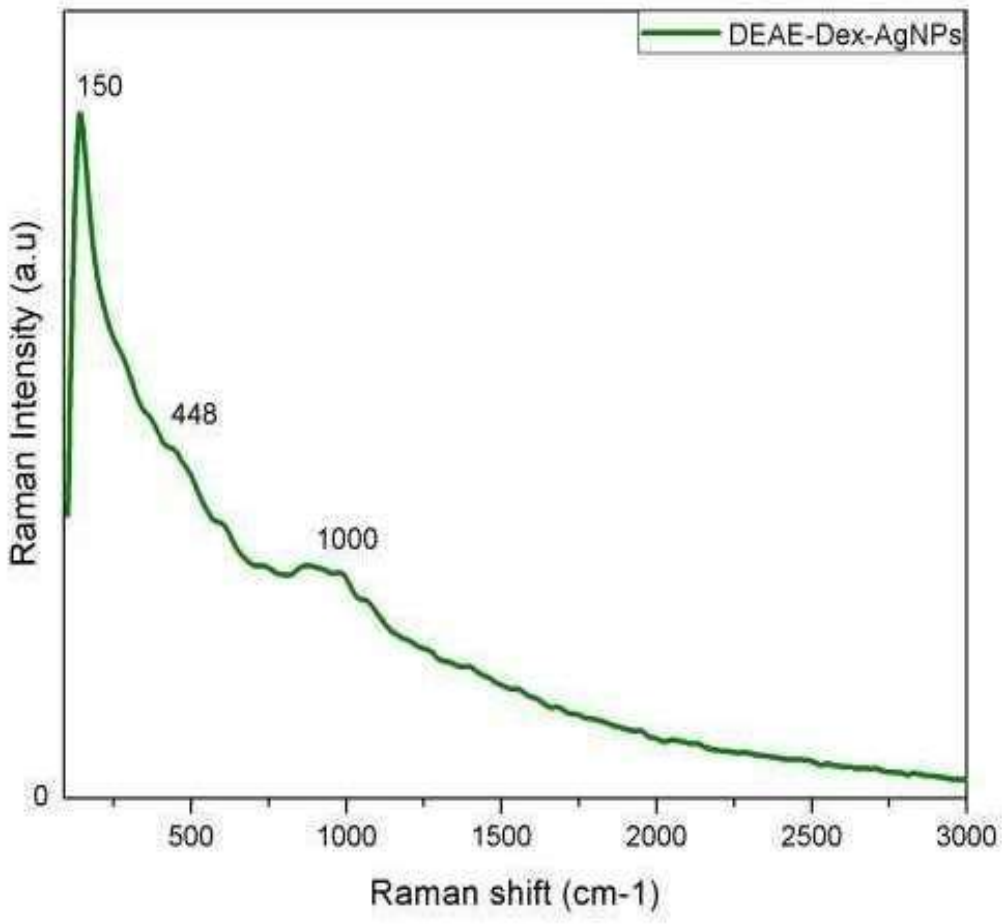

Figure 4.7: The Raman spectrum of DEAE-Dextran coated silver nanoparticles (DEAE-DexAgNPs) shows distinct peaks at approximately 150 cm^{-1}, 448 cm^{-1}, and 1000 cm^{-1}.

4.2 Antibacterial Efficacy Testing Evaluation

DEAE-Dextran AgNPs' antibacterial activity was investigated and contrasted for varying coating durations i.e., 7 or 14 days. For the comparison study, treated and untreated disc with

DEAE-dextran AgNPs coating with different time periods were utilised. Figure 4.8 indicated that DEAE-Dextran AgNPs coated discs reduced the bacterial colony count significantly when compared to non-modified discs.

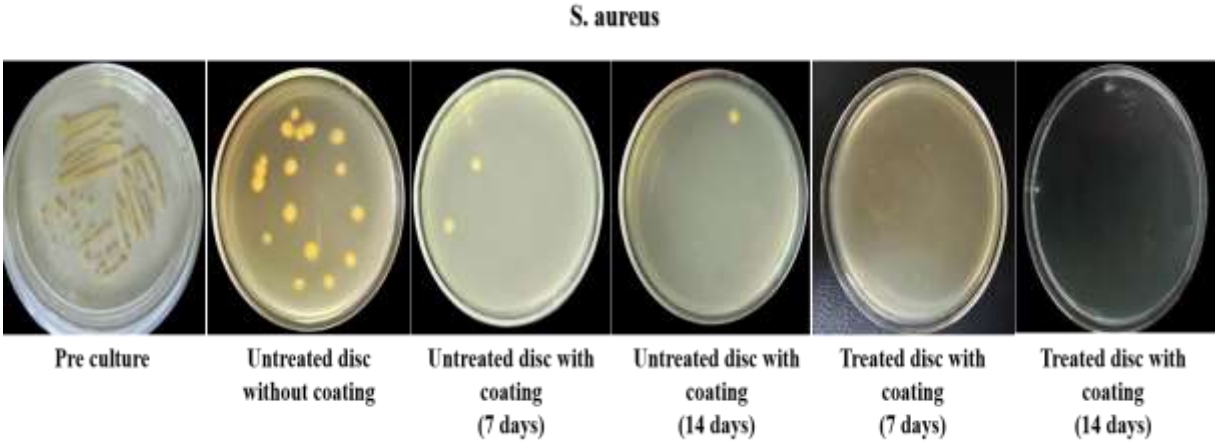

Figure 4.8: Antibacterial assay against S.aureus

The graphical representation of in vitro antibacterial activity was shown in Figure 4.8. For group I heat-treated coated discs showed a higher antibacterial impact than untreated coated and unmodified discs. S. aureus growth was reduced to more than half with 7 days coated untreated disc whereas it was reduced to nearly zero with 14 days coated untreated disc. S. aureus growth was fully suppressed by 7 and 14-day coated and heat-treated discs as compared to untreated discs with and without coating. To avoid any uncertainty CFU/ml were counted in each poured sample of the bacteria.

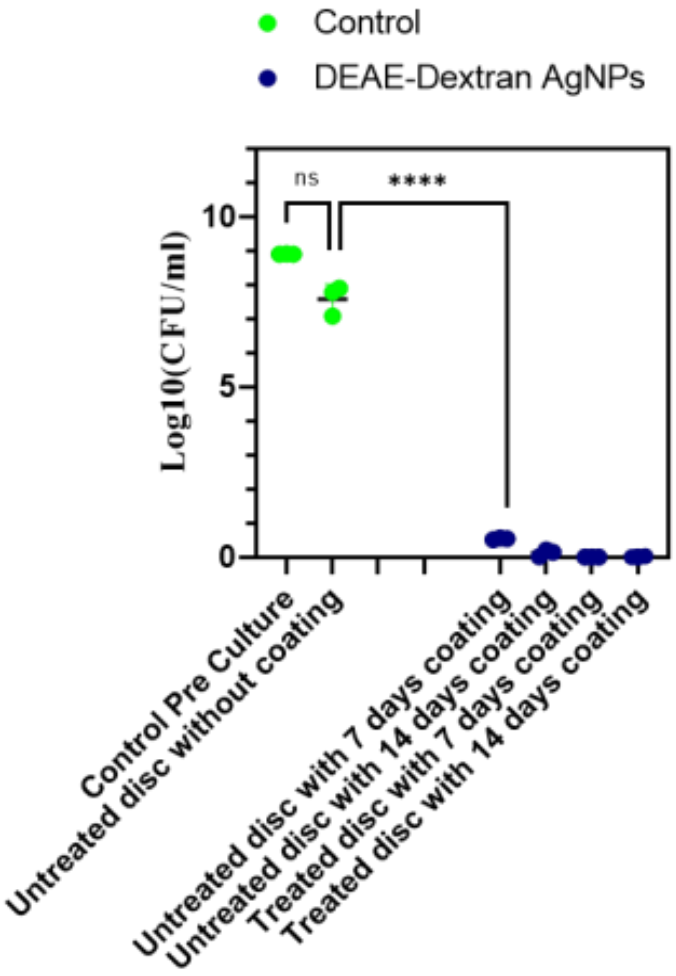

Figure 4.9: Antibacterial effect of coated discs against S. aureus*Indicated statistically significant changes from non-coated discs (**** = p < .0001)

CHAPTER 5: CONCLUSION AND FUTURE RECOMMENDATION

This study successfully developed and characterized a novel DEAE-Dextran silver nanoparticle-based biomimetic coating for titanium-based hard tissue implants. The research aimed to enhance the antimicrobial properties and biocompatibility of titanium implants by leveraging the unique properties of silver nanoparticles and the biocompatible polymer DEAEDextran. The synthesis process resulted in a stable and uniformly coated titanium surface, as confirmed by various characterization techniques, including UV-Vis, SEM, XRD, FTIR, and Zeta sizer and potential analysis. The silver nanoparticles exhibited strong antimicrobial activity against common pathogenic bacteria, which is critical for preventing post-surgical infections, a major complication in implant surgeries. The DEAE-Dextran coating not only stabilized the silver nanoparticles but also improved the overall biocompatibility of the coating, promoting osteoblast adhesion and proliferation, which are essential for successful osseointegration.

The zeta potential analysis confirmed that the DEAE-Dextran coating provided a positive surface charge, contributing to the colloidal stability of the nanoparticles and potentially enhancing the interaction with negatively charged cellular membranes. FTIR analysis further validated the presence of DEAE-Dextran on the nanoparticle surface and suggested strong interactions between the polymer and the silver nanoparticles, which is crucial for the durability and effectiveness of the coating. Overall, the DEAE-Dextran silver nanoparticles-based coating demonstrated significant potential for enhancing the performance of titanium-based implants by offering a dual function of antimicrobial protection and improved biocompatibility. Future studies should focus on in vivo testing to further validate the biocompatibility and antimicrobial efficacy of this coating in clinical settings. Additionally, exploring the long-term stability of the coating under physiological conditions will be critical to ensure its practical application in medical devices. This work contributes to the growing field of biomaterials by introducing a promising approach to improving the safety and effectiveness of titanium implants.

Hard tissue implant application is hampered by a number of challenge, such as inflammation, poor osseointegration, and infection. The main goals of current therapeutic approaches are to reduce inflammation with anti-inflammatory drugs and surface alterations,

prevent infection with antimicrobial coatings, and improve osseointegration with biomimetic coatings. Multifunctional coatings that offer complete protection and support for titanium-based implants, such those based on DEAE-Dextran and Silver Nanoparticles, present a possible solution to these problems.

The integration of antibacterial, osteoconductive, and anti-inflammatory qualities in multifunctional coatings is a sophisticated strategy for tackling the many problems associated with hard tissue implants. By lowering the likelihood of problems and enhancing long-term results, these coatings seek to offer complete protection and support for implants. To increase the hard tissue implant's lifetime, safety, and efficacy, more research and development in this field is necessary.

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