Mg doped ZnO NPs for biomimetic titanium Implant coatings

By

Ayesha Qureshi

(Registration No: 00000360611)

Department of Biomedical Engineering and Sciences

School of Mechanical and Manufacturing Engineering (SMME)

National University of Sciences & Technology (NUST)

Islamabad, Pakistan

(2024)

Mg doped ZnO NPs for biomimetic titanium Implant coatings

By

Ayesha Qureshi

(Registration No: 00000360611)

A thesis submitted to the National University of Sciences and Technology, Islamabad,

in partial fulfillment of the requirements for the degree of

Masters in Biomedical Sciences

Supervisor: Dr. Nosheen Nosheen Fatima Rana

Co Supervisor: Dr. Mehak Rafiq,

Dr. Aneeqa Noor, Dr. Muhammad Asim Waris

School of Chemical and Materials Engineering

National University of Sciences & Technology (NUST)

Islamabad, Pakistan

(2024)

THESIS ACCEPTANCE CERTIFICATE

Certified that final copy of MS/MPhil thesis written by **Regn No. 00000360611 Ayesha Qureshi** of **School of Mechanical & Manufacturing Engineering (SMME)** has been vetted by undersigned, found complete in all respects as per NUST Statues/Regulations, is free of plagiarism, errors, and mistakes and is accepted as partial fulfillment for award of MS/MPhil degree. It is further certified that necessary amendments as pointed out by GEC members of the scholar have also been incorporated in the said thesis titled. **Mg doped ZnO NPs for biomimetic titanium Implant coatings**

Signature: 

Name (Supervisor): Nosheen Fatima Rana

Date: 03 - Sep - 2024

Signature (HOD): 

Date: 03 - Sep - 2024

Signature (DEAN): 

Date: 03 - Sep - 2024

Form TH-4

National University of Sciences & Technology (NUST)

MASTER'S THESIS WORK

We hereby recommend that the dissertation prepared under our supervision by: Ayesha Qureshi (00000360611)
Titled: Mg doped ZnO NPs for biomimetic titanium Implant coatings be accepted in partial fulfillment of the requirements for the award of MS in Biomedical Sciences degree.

Examination Committee Members

1.	Name: Aneeqa Noor	Signature:
2.	Name: Mehak Rafiq	Signature:
3.	Name: Muhammad Asim Waris	Signature:
Supervisor: Nosheen Fatima Rana	Signature:	
	Date: 03 - Sep - 2024	

Head of Department — 03 - Sep - 2024 Date

COUNTERSINGED

03 - Sep - 2024
Date

Dean/Principal

CERTIFICATE OF APPROVAL

This is to certify that the research work presented in this thesis, entitled "Mg doped ZnO NPs for biomimetic titanium Implant coatings" was conducted by Mr./Ms Ayesha Qureshi under the supervision of Dr. Nosheen Fatima Rana. No part of this thesis has been submitted anywhere else for any other degree. This thesis is submitted to the School of Mechanical and Manufacturing Engineering in partial fulfillment of the requirements for the degree of Master of Science in Field of Biomedical Sciences. Department of National University of Sciences and Technology, Islamabad.

Student Name: Ayesha Qureshi Signature 

Supervisor Name: Dr. Nosheen Fatima Rana Signature: 

Name of Dean/HOD: Dr.Muhammad Asim Waris Signature: 

AUTHOR'S DECLARATION

I Ayesha Qureshi hereby state that my MS thesis titled "Mg doped ZnO NPs for biomimetic titanium Implant coatings" is my own work and has not been submitted previously by me for taking any degree from National University of Sciences and Technology, Islamabad or anywhere else in the country/ world.

At any time if my statement is found to be incorrect even after I graduate, the university has the right to withdraw my MS degree.

Name of Student: Ayesha Qureshi

Date: 3-08-2024

PLAGIARISM UNDERTAKING

I solemnly declare that research work presented in the thesis titled " **Mg doped ZnO NPs for biomimetic titanium Implant coatings** " is solely my research work with no significant contribution from any other person. Small contribution/ help wherever taken has been duly acknowledged and that complete thesis has been written by me.

I understand the zero tolerance policy of the HEC and National University of Sciences and Technology (NUST), Islamabad towards plagiarism. Therefore, I as an author of the above titled thesis declare that no portion of my thesis has been plagiarized and any material used as reference is properly referred/cited.

I undertake that if I am found guilty of any formal plagiarism in the above titled thesis even after award of MS degree, the University reserves the rights to withdraw/revoke my MS degree and that HEC and NUST, Islamabad has the right to publish my name on the HEC/University website on which names of students are placed who submitted plagiarized thesis.

Student Signature: Ayesha

Name: Ayesha Qureshi

Dedicated to my beloved parents, for your endless support and unwavering belief in me. To my dear husband, for your constant encouragement. To my precious daughter, who inspires me every day. And to my wonderful sister, for always standing by my side. This achievement is as much yours as it is mine!

ACKNOWLEDGEMENTS

In the name of Allah, the Beneficent, the Merciful First of all

I have no words to express my deepest and infinite sense of gratitude to ALLAH, the Almighty, who knows all the hidden and exposed things in the entire Universe, Who has bestowed all the courage and opportunities upon us to complete this work.

I want to express sincere acknowledgment to my Supervisor, Dr. Nosheen Fatima, for her queen supervision and practical advice during this research work. She always guided me in a time of need. A sincere thanks to Dr. Aneeqa Noor, Dr. Asim Waris, and Dr. Mehak Rafiq for being a part of my thesis guidance and evaluation committee.

I would like to take this moment to especially thank my senior Tehreem Tanveer and Afrah Nawaz for their throughout support and guidance at every step of the research.

A special thanks to all my friends for their continuous support and encouragement and for always being there for me. I cannot thank enough to my parents, husband, sister and daughter the most important people in my life, for putting their trust in me and for always having my back.

TABLE OF CONTENTS

LIST OF TABLES

Page No.

LIST OF FIGURES

Page No.

ABBREVIATIONS

NPs	Nanoparticles
SBF	Stimulated Body Fluid
Mg	Magnesium
Ti	Titanium
ZnO	Zinc Oxide

ABSTRACT

Hard tissue implants are highly preferred to support, replace and enhance the activity of different parts of the body. The major problem faced by the implant is the growth of bacteria which develops biofilm which causes inflammation. The immune system recognizes it as a foreign object resulting in implant rejection. This study aimed to synthesize antimicrobial implant mimicking natural composition of bone. Bare zin oxide (ZnO) and magnesium (Mg) doped zinc oxide (ZnO) nanoparticles (NPs) were synthesized by coprecipitation method and were characterized by Ultraviolet Visible spectroscopy (UV-Vis), Scanning Electron Microscopy (SEM) spectroscopy, X-Ray Diffraction (XRD), Energy Dispersive X- Ray (EDX) and Fourier transform infrared spectroscopy (FTIR) and zeta potential. NPs were coated on titanium disc (Ti) by biomimetic method in Stimulated Body Fluid (SBF). Discs were priorly alkaline treated with 5 M NaOH for 3 days then heat treated at 600°C for 1 hour in electric furnace. Antibacterial activity was tested against *Staphylococcus aureus*. Four groups were assigned to discs on the basis of treatments and NPs. Group I discs were alkaline and heat treated followed by dipping in SBF containing bare ZnO for 7 days, group II discs were alkaline and heat treated followed by dipping in SBF containing Mg doped ZnO for 7 days, group III untreated discs were dipped in SBF for 7 days, group IV discs were alkaline and heat treated followed by dipping in SBF with Mg doped ZnO for 14 days. The surfaces were characterized using SEM. Their antibacterial activity was then evaluated. Group IV showed enhanced antibacterial activity. Therefore, Mg doped ZnO NPs coating on Ti Disc with alkaline and heat treatment is highly recommended as an hard tissue implant. This coating replicates the inherent makeup of bone and was not identified as an extraneous entity. This reduced the likelihood of rejection and showed enhanced antibacterial activity, osseointegration, and reduced processing cost.

Keywords: Doping, ZnO NPs, Mg doped ZnO NPs, Titanium Implants, SBF.

CHAPTER 1: INTRODUCTION

1.1 Nanotechnology

Nanotechnology is employed to create novel restorative materials that possess improved properties and antibacterial capabilities. Nanomaterials possess significant role in mitigating biofilm formation, impeding demineralization, re-mineralizing the structure, and battling bacteria that cause caries. Nanostructures have recently gained significant attention due to their unique features that vary depending on their size. Nanotechnology refers to the ability to make, modify, and utilize matter at the scale of nanometers. Particle size plays a critical role in nanotechnology because the dimensions of a material have a substantial impact on its features at the nanoscale. Nanotechnology refers to the application of advanced techniques in designing and producing products that make human life easy, eliminating discomfort (Abaszadeh et al., 2023). Nanotechnology possesses a broad spectrum of applications that are revolutionizing several fields. Within the field of medicine, the use of nanoparticles to specifically target cells is causing a significant transformation in drug delivery systems. It is being used to improve treatment methods and advance our technology to decrease the adverse effects produced as a result. Such as to treat cancer and eradicating the harmful cells without effecting healthy cells. It is playing very important role in development and progress of our society to tackle issues faced by humans nowadays. It is being used in solar cells to capture larger amount of sunlight and utilize most of it to make energy. Nanomaterials are facilitating the development of smaller, quicker, and more efficient technologies, such as improved transistors and memory chips, in the electronics sector. Additionally, it plays a crucial part in the advancement of flexible electronics, such as wearable technology and screens that can be folded, in high-density hydrogen fuel cells, which exhibit enhanced environmental sustainability. Nanotechnology has various environmental uses, such as using nanoparticles for water purification to eliminate contaminants and for pollution control to trap and neutralize pollutants in both air and water. Nanotechnology enhances the construction sector by producing more stronger and durable products. In addition, nanocoating has the ability to render surfaces self-cleaning, hence decreasing

maintenance expenses and enhancing hygiene. Nanotechnology is utilized in consumer items to produce sophisticated textiles that possess stain-resistant, water-repellent, and antibacterial properties. Additionally, it is employed in cosmetics to improve the delivery of active chemicals and enhance the overall performance of the products. Nanotechnology has revolutionized biomedical science by providing cutting-edge solutions for the diagnosis, treatment, and prevention of diseases. One of the primary functions of nanoparticles is to facilitate medication administration by engineering them to transport drugs directly to specific cells. This approach improves the effectiveness of the drugs and reduces the occurrence of adverse effects, particularly in the treatment of cancer. Nanotechnology enhances diagnostic imaging methods, facilitating earlier and more precise illness identification using instruments such as quantum dots. Theranostics is a medical technique that blends therapy and diagnostics by utilizing nanoparticles to concurrently diagnose and cure diseases, resulting in a more integrated healthcare strategy. In addition, nanoparticles play a vital role in tissue engineering by constructing scaffolds that facilitate the development of new tissues, so assisting in the restoration or substitution of impaired tissues and organs. Nanoparticles provide novel approaches to address infections, surpassing the constraints of traditional antiviral and antibacterial therapies. In addition, nanotechnology plays an important role in the advancement of wearable instruments that monitor the health condition in real time, allowing continuous monitoring and early warning systems for different health conditions as in figure 1.1. Nanotechnology enables the manipulation of materials at the molecular level, offering a wide range of opportunities for advancing biomedical science and enhancing the effectiveness and personalization of treatments. The wide range of applications showcased here exemplify the immense capacity of nanotechnology to enhance several facets of our existence, with continuous research offering even more groundbreaking possibilities in the future.

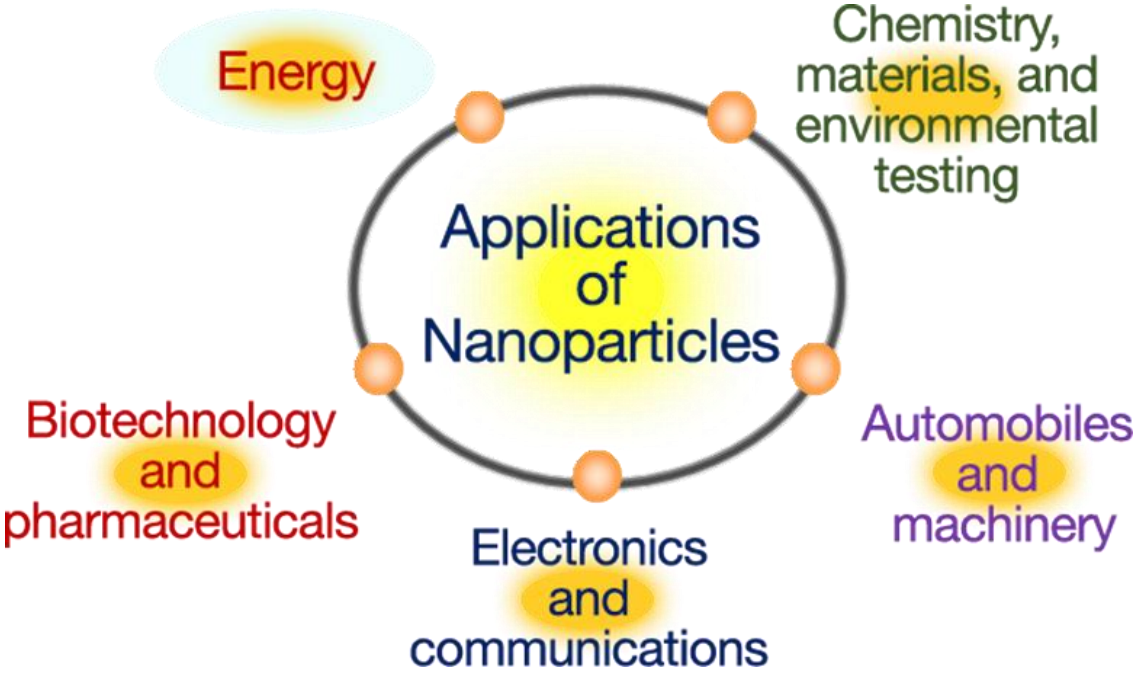


Figure 1.1: Applications of nanoparticles (Fukuda et al., 2017).

Nanotechnology has fundamentally transformed several fields of research, providing groundbreaking answers and progressive developments. Physicochemically, nanoparticles function as very effective catalysts, expediting chemical reactions and facilitating the production of novel materials with improved characteristics such as heightened strength and conductivity. Nanotechnology has revolutionized biology by enabling the development of customized medication delivery systems, which enhance treatment effectiveness and reduce adverse effects. Additionally, nanosensors have facilitated the identification of biological molecules for diagnostic purposes and environmental monitoring. Physical sciences derive advantages from the distinctive optical and electrical characteristics of quantum dots, which find application in sophisticated displays and solar cells, as well as from nanomagnetics, which augment data storage technologies. Engineering include the advancement of nanocomposites, which integrate nanoparticles with bulk materials to enhance mechanical, thermal, and electrical characteristics. It also involves the fabrication of nanoelectronics, which result in the production of smaller, faster, and more efficient electronic components. Nanotechnology is utilized in environmental science for the purpose of water purification

and air quality enhancement. This involves the use of nanofilters and air purification machinery to eliminate chemicals and pollutants. Advanced materials science utilizes nanotechnology to develop intelligent materials that react to environmental stimuli and nanocoating that offer improved resistance to corrosion and microbial proliferation. Nanosensors and nanomaterials find application in geology for the purposes of mineral prospecting and soil remediation activities. Nanotechnology plays a crucial role in the field of medicine by facilitating the development of cancer therapies that selectively target cancer cells, therefore reducing harm to healthy cells. Additionally, it augments imaging techniques for early detection and monitoring of diseases. The capacity to precisely control matter at the atomic and molecular scales provides limitless opportunities in several scientific domains, so stimulating innovation and advancement (Fukuda et al., 2017).

1.2 Hard Tissue Implants

The human body contains four hard tissues bone, cementum, dentin and enamel. These four hard tissues are present in oral environment, and only one of them is present inside and outside of mouth, namely bone. Bone, cementum, and dentin share some similarities. Firstly, all three are connective tissues. Additionally, they are composed of the same principal ingredients. Furthermore, their composition consists of an approximately same volume of organic and inorganic material (Chen et al., 2023). In recent times, Titanium and its alloys have gained significant appeal as biomaterials owing to its lightweight nature, exceptional resistance to biocorrosion, compatibility with living organisms, and impressive mechanical characteristics (Lin et al., 2021). Currently, commercially pure titanium, which has relatively low strength, is utilized in dentistry. On the other hand, the Ti alloy, which has better strength, is employed in many orthopedic applications that include bearing stress (Zhang et al., 2020). Although titanium and titanium-based alloys possess exceptional qualities, they are considered bioinert. This means after implanting them in the body, they are typically surrounded by fibrous tissue and are unable to chemically bind with bone. Nevertheless, it is widely acknowledged that the application of calcium phosphate coatings has resulted in increases their clinical stability and longevity compared to untreated Ti implants (Sidhu et al., 2021). Various techniques

have been devised for applying coatings to metal implants, such as sol-gel coating, sputter-deposition, electrophoretic deposition, plasma-spraying, and biomimetic precipitation. These different techniques are being used to increase the binding of implant with the neighboring bone and tissues so that it is recognized as foreign entity and becomes the part of the body supporting and enhancing the functioning of the biological organ. For this purpose surfaces are treated that promote optimal primary stability, even distribution of load, and the preservation of osseointegration (Lopez-Valverde et al., 2021). Surface treatment can be used to modify several characteristics of the Ti implant, such as its chemical composition, shape, topography, and roughness (W. Nicholson, 2020). These modified and more stable implants are then used across multiple medical disciplines, such as orthopedics for replacement of joints and bone plates. In dental care such as in dental implants and maxillofacial surgery to reconstruct face bone as in figure 1.2 (Chen et al., 2023).

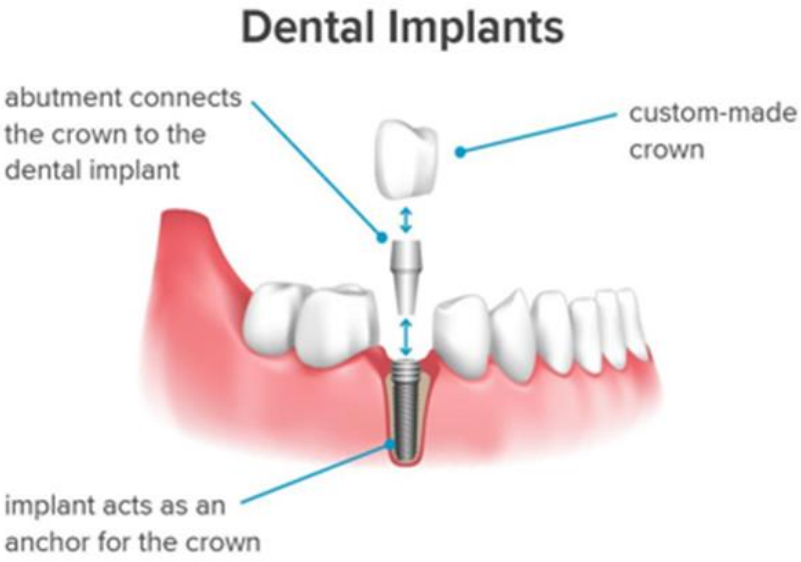


Figure 1.2: Abutment connecting modified dental implants to the crown (Leong et al., 1996).

1.3 Biomimetic Coatings

Biomimetic coatings are a category of substances that replicate the structure, content, and functionality of natural systems. These coatings offer a range of advantages, including as increased resilience, self-cleansing characteristics, and enhanced biological compatibility. Biomimetic coatings have gained significant interest in recent years as a highly promising technology for several uses in medical devices. Nature frequently serves as the source of inspiration for biomimetic coatings. Implants face the problem of rejection due to material used in them causing inflammation leading to rejection. By mimicking the natural composition of bone integration of implant and bone healing can be improved. These coverings generally comprise materials that imitate the composition and structure of genuine bone. CaP coatings, closely resemble the natural structure of bone with the same mineral composition. These coatings have the potential to greatly enhance the bioactivity of titanium implants, thereby promoting osseointegration. Mostly titanium is used as an implant but lack of reactivity of titanium can result in poor osseointegration. To overcome this problem biomimetic approach is used by offering a surface that promotes cellular adhesion, proliferation, and differentiation, which are crucial steps for achieving successful bone integration. Different methods are used for this purpose, such as sol-gel processes and dip-coating procedures. These alterations have the potential to enhance tissue adhesion and vascularization, which plays an important role in the stability and effectiveness of implants in the long run. The addition of Mg and Zn to biomimetic coatings has been demonstrated to further augment their characteristics. Mg is essential for bone metabolism, while Zn has antibacterial properties that can aid in the prevention of infections related to implants. Research has indicated that the application of magnesium-doped coatings on titanium implants can enhance their mechanical strength and biological activity, resulting in improved clinical outcomes. The biomimetic approach is gaining interest due to enhanced patient results in orthopedic and dental applications. Current research is actively improving these coatings by finding out novel materials and techniques to enhance their performance and biocompatibility (Matter et al., 2021). Biomimetic coating can be seen in figure 1.3.

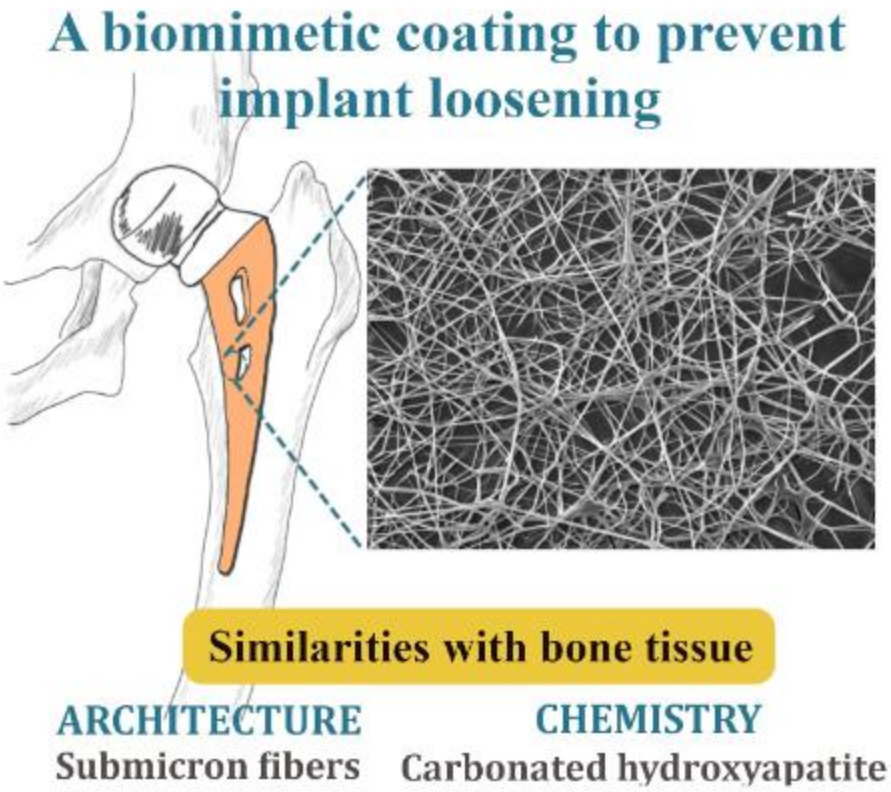


Figure 1.3: Biomimetic coating with it architectural and compositional similarity with natural bone tissue (Mignon et al., 2023).

A biomimetic coating, specifically developed to prevent implant loosening, replicates the structural and compositional properties of genuine bone tissue. Typically, this coating incorporates submicron fibers that mimic the intricate and interconnected structure of bone, therefore improving the mechanical stability and integration of the implant with the adjacent bone. Furthermore, the composition of the coating frequently include carbonated hydroxyapatite, a mineral constituent that closely resembles that present in genuine bone. This resemblance in composition enhances biological compatibility and stimulates the attachment and proliferation of bone cells, resulting in enhanced osseological integration. Biomimetic coatings can greatly improve the durability and effectiveness of implants by closely mimicking the structure and composition of natural bone tissue, therefore minimizing the likelihood of loosening and eventual failure. The proposed methodology

signifies a notable progression in the field of biomedical engineering, with the objective of enhancing patient results in orthopedic and dental implant therapeutic interventions (Mignon et al., 2023).

CHAPTER 2: LITERATURE REVIEW

2.1 Implant rejection

Hard tissue implant rejection refers to the response of immune system to a hard tissue implant, like a dental implant, which hinders its appropriate integration with the nearby bone tissue. This can result in the implant's failure to operate as intended. Osseointegration is essential for the successful integration of a hard tissue implant. It is a biological process to fuse implant with the bone and surrounding tissues without causing any inflammation and discomfort. However, if the body rejects the implant, this disrupts the process and is marked by inflammation, the gradual breakdown and absorption of the implant, and the loss of the bone tissue that had previously developed around the section of the implant inside the bone. Dental implant rejection is a pathological disease characterized by inflammation and infection. As harmful germs reproduce, mucositis first causes purulent inflammation without any bone resorption. If not treated within time, it can advance to peri-implantitis, a condition characterized by infectious inflammation that leads to the exposure, damage, and loss of hard structures. It is worth mentioning that implant rejection is a rather uncommon occurrence, with a prevalence ranging from 3% to 10%. The rejection rate for zirconium implants is exceptionally low, often ranging from 0.5% to 2%. Prompt identification and therapy are crucial for controlling this disease and guaranteeing the durability of the implant (Alcaraz et al., 2018). Effect of drug release from titanium implant can be seen in figure 2.1.

Figure

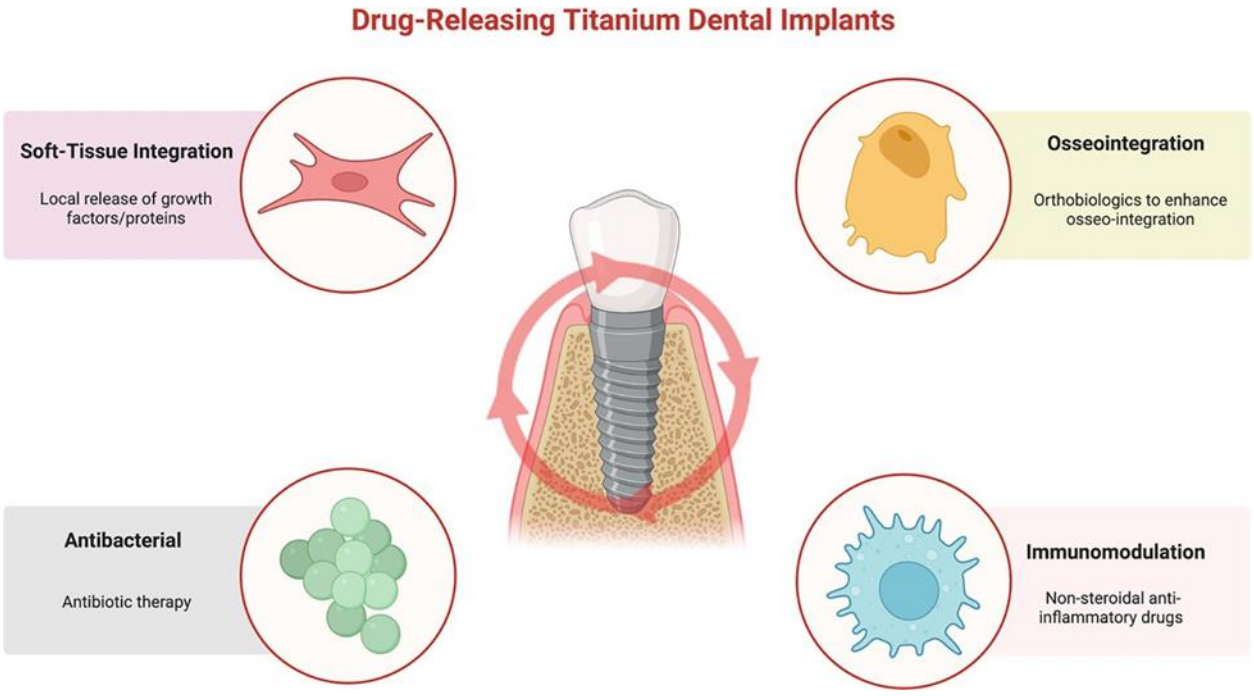


Figure 2.1: Drug releasing titanium dental implant and its effect (Gulati et al., 2023).

2.2 Pathophysiology

Implant rejection is caused by a complicated interaction between the immune system and the material that is used. Steps are as follows:

2.2.1. Initial Immune Response

When an implant is inserted into the body, the immune system identifies it as an exogenous entity. This initiates an inflammatory reaction, that is the the body's primary defense mechanism. The main participants in this response are macrophages. These cells endeavor to engulf and digest foreign material. Cytokines are also secreted to attract additional immune cells to the location.

2.2.2. Chronic Inflammation

If the initial response of the body’s immune system fails to reject the foreign object, it might lead to the development of chronic inflammation. This stage is marked by the

ongoing activity of macrophages, which persistently strive to break down the implant. Fibroblasts are also stimulated. These cells generate fibrous tissue to separate the implant from the remainder of the body.

2.2.3. Fibrosis and Encapsulation

Over the period of time, the human body has the tendency to develop a fibrous capsule around the implanted object. This is an effort to reject foreign objects. However, this might result in diminished functionality that hampers the operation of the implant and also induces pain and discomfort at the site of the implant.

2.2.4. Osseointegration Failure

This step is essential for dental or orthopedic implants. As osseointegration is very delicate and critical step. Improper bone development and micro-movements can lead to failure of the implant with urge to remove it.

2.2.5. Infection

Growth of bacteria at the implant site can worsen the process. As it can form biofilm on the surface of implants, inhibiting the effect of antibiotics.

2.2.6. Systemic Factors

Specific systemic factors can lead to implant rejection so for this reason it is very important to understand body's immune system and possible responses to the implant that might increases rejection rate. Such as in diabetic patient inadequate blood sugar control, can hinder the healing process and elevate the risk of infection. Similarly, a person with autoimmune diseases, which involve an already hyperactive immune system, can result in a greater susceptibility to rejection. Employing biocompatible materials, enhancing surgical methods, and addressing patient-specific risk factors and dealing with the person accordingly can greatly reduce rejection rate (Baseri et al., 2020).

2.3 Prevalence

2.3.1. Worldwide

The success rate of dental implants is 90-95% while rejection rate is about 5-10%. The reasons of rejection is infection, inadequate osseointegration, and mechanical complications. Patents who face problems due to implant malfunction were 4.6% and second operation was done. The rates of failure for other implants such as orthopedic, hip and knee replacements, can differ. The incidence of infections generally ranges from 1-2%, although the overall failure rate can be increased due to mechanical malfunction (Khan et al., 2007; Kim et al., 2020).

2.3.2. Pakistan

The cochlear implant program in Lahore, Pakistan, reported an implant failure rate of 3.8%. The combined incidence of significant and mild problems was 11.5%. We do not have much data on the frequency of various implant rejection and their causes. Nevertheless, variables such as restricted healthcare accessibility, socioeconomic circumstances, and absence of specialist medical infrastructure can impact the efficacy of implants. So, we need to have more surveys and arrange seminars to educate our people and make them aware of the possibilities to get over their lost parts (Khan et al., 2007).

2.4 Levels of rejection

Hard tissue implant rejection is categorized in three stages depending on the rejection they face. First is the initial stage where the implant is little mobile but only in two directions with an amplitude of less than 0.5 mm. This is the initial sign of rejection, but it can be controlled with suitable therapies. Next is the moderate stage where implant exhibits triaxial motion with a displacement ranging from 0.5 to 1 mm. Here it is a more complicated process of rejection, sometimes accompanied by inflammation and discomfort. If untreated it can lead to more severe stage where the implant shows substantial movement in three directions, with an amplitude greater than 1.5-2 mm. Such a high percentage of rejection usually requires the removal of the implant and may need

an additional and more complex treatment to resolve the underlying concerns (Ahmed et al., 2021).

2.5 Etiology

The aging population, rising infection rates, and less physical activity resulting from improved living standards are significant and unavoidable variables contributing to the decline in bone mineral density, bone quantity, and muscular strength in the population. These outcomes contribute to a higher prevalence of bone fractures over the course of individuals' lifetimes. While bone has a higher ability to regenerate compared to other tissues or organs, the healing process requires the shattered fragments to be properly aligned and fixed. Various procedures and instruments can be employed to furnish bone replacements with such characteristics. The majority of the fixation tools currently available are constructed from non-eroding metals because of their inherent rigidity and durability, which are necessary for supporting the weight-bearing function of the skeletal system (Erkin & Vasif, 2010). The utilization of implants in medical contexts, including catheters, prosthetics, and other devices, has significantly transformed the field of medicine, particularly in recent years. Nevertheless, these implants are significantly linked to an elevated risk of infection. In fact, implant-associated infections are the most prevalent and serious problems that occur while using biomaterials. e. Approximately 26% of healthcare-associated illnesses in the USA are attributed to infections caused by devices (Shahid et al., 2021).

2.6 Biofilm Formation

The presence of biofilm on implant surfaces leads to the development of a micro-environment, where different strains and species of microorganisms thrive within a slimy environment (Nosheen et al., 2022). Implant-associated biofilms are the most problematic in humans. They can occur in various medical devices such as artificial hearts, catheters for urine contact lenses, joint prosthetics vascular grafts, intravenous and, devices used in internal fixation , vocal prosthesis, and (Kandi & Vadakedath, 2020). The prevalence of patients requiring orthopedic surgery with internal fixation devices or prosthetic joints

has shown significant growth. Every biofilm begins with the same series of events, as depicted in Figure 5. For almost all medical applications of biomaterials, a layer known as “conditioning film" is applied to the surfaces of the biomaterials (Ferrando-Magraner et al., 2020). This refers to the presence of large molecules that have been absorbed by the implant from the environment where the it is located Figure 2.2 (a). Dental restorative materials can attract and bind salivary proteins, contact lenses have the ability to attract and bind proteins and lipid components present in tear, and blood present in contact with the biomaterials have the ability to attract and bind different plasma proteins even before the first bacterium is present (Arif et al., 2022). The phenomenon of film development through conditioning takes place within a matter of seconds as soon as it is exposed to an implantation site. Orthopedic biomaterials, such as Polymethylmethacrylate (PMMA), primarily interact with bone and then has direct contact with blood (Mehmood et al., 2014). Thus, plasma proteins have the ability to create a conditioning film. This indicates that germs attach to a deposited conditioning film and rarely to a clean biomaterial surface. The surface can be accessed through other means of transportation, including diffusion, convection, or sedimentation Figure 2.2 (b). Microorganisms are conveyed to teeth or biomaterial surfaces in the oral cavity through various mechanisms. Orthopedic implants may be contaminated through direct contamination during surgery, however the majority of infections are not related to surgical procedures. Furthermore, as the duration of patient follow-up extended, there was a simultaneous increase in the occurrence of infections originating from the bloodstream. The initial attachment of microbes is temporary and influenced by its cell physicochemical properties, the biomaterial surface, and the immersed fluid. The adhesion process of microorganisms can transit from being reversible to irreversible due to the synthesis of exopolymers, resulting in a strong anchoring effect Figure 2.2 (c). The exopolymers that surround the microorganisms that adhere to a surface are responsible for embedding the biofilm, creating a structure known as a "glycocalix" Figure 2.2 (d). Glycocalyx is the term used to describe the buildup of glycoproteins on the external surface of the biomaterial. In addition to its role in anchoring the biofilm, it also provides protection against environmental assaults and antibiotics (Tammaro et al., 2020). The primary process by which a biofilm multiplies is through the growth of the organisms that adhere to it, eventually resulting in the creation

of a thick film (Belt et al., 2001). Orthopedic biofilm-centered infection can result from a wide range of pathogens. Standard hospital laboratory cultures typically identify a single causative organism on implant which is infected. However, when more extensive culture techniques are employed, it appears that infections are actually caused by many microorganisms.

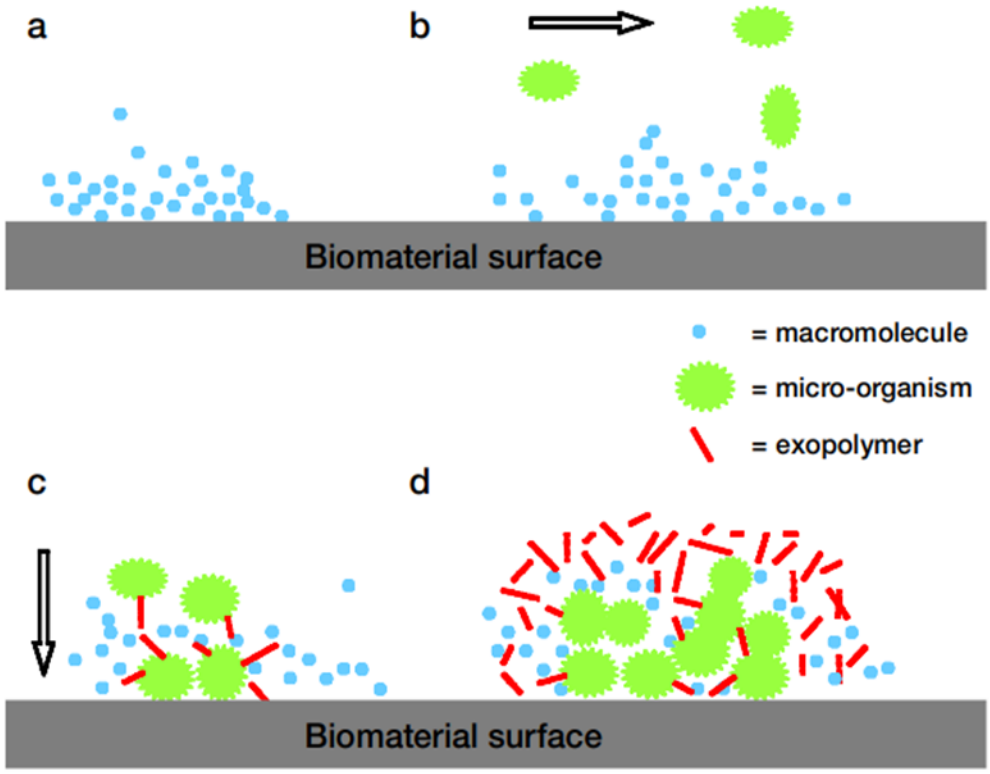


Figure 2.2: Steps followed in the formation of biofilms on an implant surface. a. Formation of a conditioning film. b. Transport of microbes to the site. c. Initial adhesion and anchoring of microbes through exopolymer production. d. Growth of microorganisms at adhesion site (Maathuis et al., 2007).

2.7 Calcium Phosphate Coatings

Calcium phosphate coatings are applied on metal implants to improve the integration of hard tissues and boost mechanical stability in the body. Hydroxyapatite (Ca10(PO4)6OH) when applied to metallic implants improves their ability to interact with living tissue by imitating the structure of natural bone. This promotes the integration of the implant with the surrounding bone tissue, a process known as osseointegration. This minimizes the likelihood of rejection or negative responses from the body. Hydroxyapatite coatings can decrease wear and friction between the implant and nearby tissues, improving the durability of the implant and reducing the likelihood of problems such implant-related inflammation or tissue harm. (Kirthana et al., 2020). Currently metallic implants are being coated with (HA) by plasma deposition method. However, this process results in uneven coatings surface on the implants and increasing temperature in this method impact the structure and appearance of the formed film. The biomimetic method, which imitates the natural structure and composition of bone, is a highly promising way for creating calcium phosphate coatings. The biomimetic method involves using highly concentrated solution as of human plasma. It is highly concentrated and aqueous solution. This technology enables the application of apatite crystals onto metal implants (Escada et al., 2011).

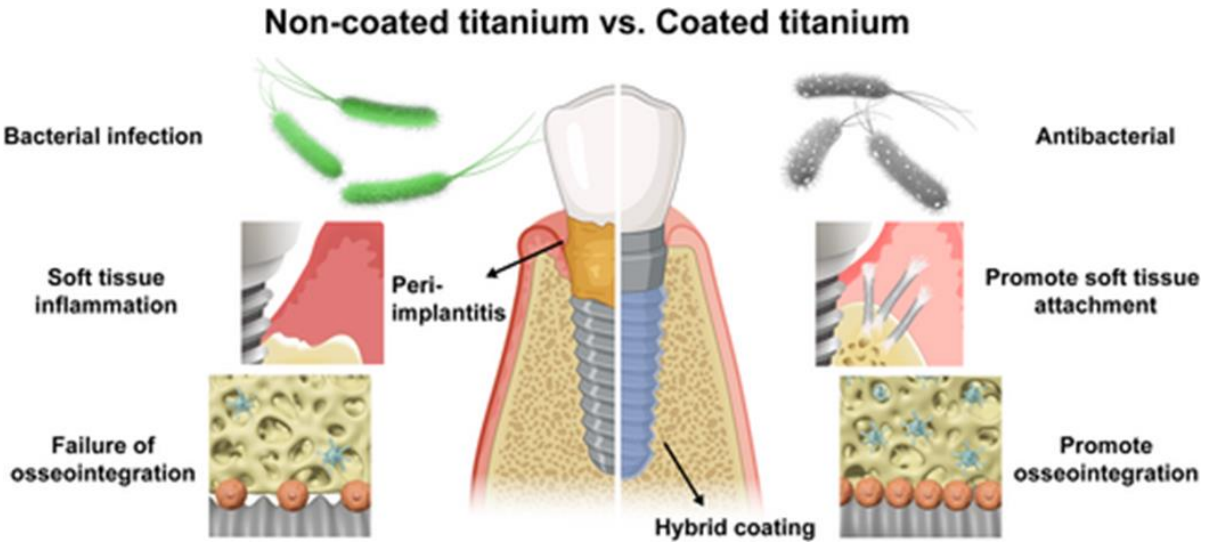


Figure2.3: Non-coated and Coated Titanium Implants (Tang et al., 2024).

2.8 ZnO NPs Advantages over other NPs

Additionally, various other nanoparticles have been utilized previously, including silver. Silver, in the forms of metallic silver, silver nitrate, and silver sulfadiazine, has been employed since ancient times for the treatment of certain bacterial illnesses. The silver ion (Ag+) is a potent antibacterial agent that exhibits good stability and has a wide range of antimicrobial actions on both gram-positive and gram-negative bacteria. Antibacterial activity of Ag NPs is due to their interaction with disulfide or sulfhydryl groups found in enzymes, leading to the disruption of metabolic pathways and ultimately causing cell death. In other case they induce holes in the bacterial membrane, leading to fragmentation of the cell. These NPs do not harm human cells neither causes any toxicity while killing microorganisms. However, silver nanoparticles have raised safety concerns due to their possible toxicity at greater doses. Although silver nanoparticles exhibit efficacy against bacteria at low concentrations, there is a potential for systemic exposure and subsequent accumulation in the body, which can result in deleterious effects. Scientists are highly interested in Cu nanoparticles because to their distinct biological, chemical, and physical features, as well as their antimicrobial activities. Additionally, their inexpensive preparation cost adds to their appeal. Researchers have shown that these nanoparticles exhibit antibacterial and antifungal properties against several

microbes. The research findings demonstrated the significant efficacy of Cu nanoparticles as antibacterial agents. Nevertheless, Cu NPs when exposed air are oxidized quickly which restrict their use. Zinc oxide nanoparticles are commonly considered to be safer alternatives as they are less toxic. Furthermore, they have been examined for stability and durability when applied as coatings on surfaces of implants. It is essential to guarantee the enduring stability of the nanoparticle coating to preserve its ability to kill microorganisms and its compatibility with living tissues over the whole lifespan of the implant. Magnesium (Mg) is a rare earth metal that has a low density and appears bright and silvery-white. It also has an elastic modulus similar to that of cortical bone, which is 45 gigapascals (GPa). It demonstrates notable antibacterial efficacy and hinders bacterial attachment and the production of biofilms, rendering it more resistant to unfavorable circumstances. Within the field of dentistry, it has a notable impact on the prevention of tooth decay and gum disease by effectively reducing inflammation induced by toxins produced by bacteria. Moreover, it has been documented to increase the acidity level to a more optimal physiological pH that is why Mg-doped ZnO NPs are attracting interest of most of the researchers specifically as coatings for implants. They increase the production of ROS which disturbs the bacterial cell membranes. Furthermore, these NPs exhibit exceptional biocompatibility and minimal cytotoxicity rendering them more secure for utilization in medical applications as well as in coatings to improve their stability, mechanical properties and osteogenesis.

2.9 Titanium Implants

These implants are widely used in dentistry and orthopedic operations as they have high biocompatibility, strength, and ability to resist corrosion. The implants are predominantly composed of titanium and its alloys, which effectively fuse with bone tissue, facilitating osseointegration. This procedure is essential for ensuring the stability and durability of the implant (Wang et al., 2021).

2.10 Shortcomings of Ti implants

Titanium on the other hand is found to emit particles in bone which causes an increase in its concentration in the human body. Generally, they do not stimulate any immunological

response but rise in these particles can lead to disruption of the normal balance within the epithelial cells, causing inflammation in the nearby tissues, degradation of bone tissue, and separation of the implant. Additionally, they travel alongside the bloodstream and gather in the distant organ. The release of Ti particles is influenced by factors such as the surface structure of the implant, corrosion in the microenvironment, and wear during medical operations. However, the exact mechanism behind this phenomenon remains unclear. Therefore, it is challenging to entirely prevent the leak. Releasing titanium particles can cause an inflammatory reaction and facilitate the absorption of bone tissue around the implant, either throughout the body or in a specific area. By preventing the release of IL-1β, IL-6, and TNF-α around titanium implants, the bone loss caused by titanium particles can be reduced (W. Nicholson, 2020).

2.11 Enhancing antibacterial activity of ZnO NPs

Magnesium doping significantly improves the antibacterial characteristics of the disc, since it enhances the effectiveness of ZnO nanoparticles against different bacterial strains. ZnO is particularly noteworthy because of its well-established application in healthcare items, its ability to resist UV rays, its compatibility with living organisms, and its reasonable cost (Abutalib & Rajeh, 2020). ZnO is a highly desirable semiconductor due to its significant characteristics, including a wide band gap of 3.37 eV, a substantial exciton binding energy of 60 meV, and excellent chemical and thermal stability. These NPs are efficient against both Gram-positive and Gram-negative bacteria (Nigam & Pawar, 2020). Mg is the best dopant for ZnO because they both show similarity in the ionic radii. Mg when doped on ZnO modifies its bandgaps resulting in an increased empty space (Tanweer, Rana, Khan, et al., 2022). When these bandgaps are exposed to light, they produce ROS that damages bacterial cell walls (Pradeev Raj et al., 2018). In this study, titanium disc were alkaline and heat treated and were dipped in SBF with simple ZnO and Mg doped ZnO to induce the formation of calcium deposition on the titanium plate, mimicking the bone structure with NPs to enhance antibacterial properties. The purpose of this experiment was to investigate the antibacterial capabilities of nanoparticles. This would enhance the potency of our implant and reduce the likelihood of rejection.

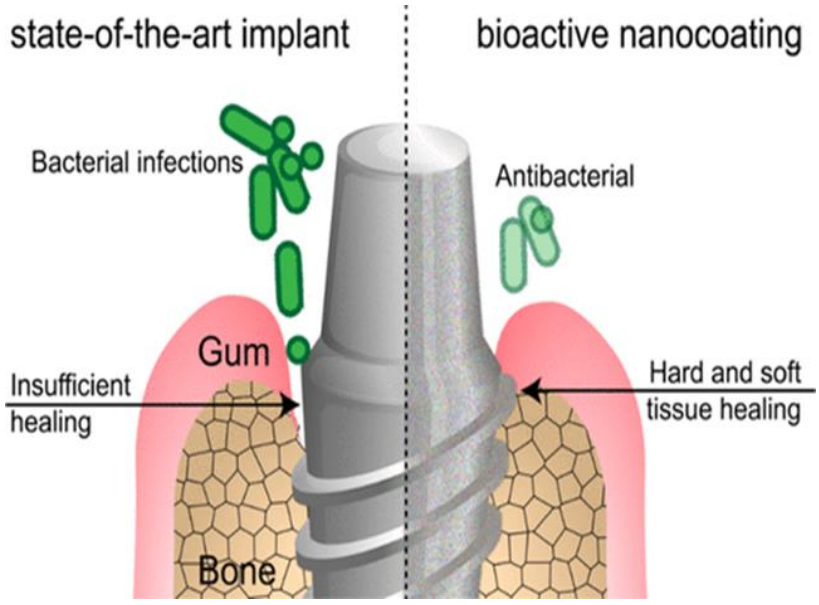


Figure 2.4: The antibacterial effect with and without nanocoating on implant (Matter et al., 2021).

2.12 Mechanism of Action of Mg doped ZnO NPs

Mg has a large effect on the antibacterial activity of ZnO. These both are positively charged ions which interact with negatively charged bacterial cell membranes, through electrostatic interaction. This interaction leads to the formation of a transmembrane pore in the cell membrane. Which alters membrane permeability. These ions enter the cell membrane, bind with the sulfhydryl (-SH) group, and inhibit enzymes. Which directly effect respiratory chain and cell division, leading to an elevation in ROS such as hydrogen peroxide (H_2O_2), superoxide anions (O_2^-), and hydroxyl radicals (OH^-). These ROS oxidizes lipids, proteins, and nucleic acids present in bacterial cells. These oxidative damage causes a disturbance in the regular functioning of these chemicals, ultimately resulting in the bacterial death. The synthesis of Mg[OH]2 and Zn[OH]2 results in a pH buffering effect, which restricts the growth of biofilm and helps prevent the development of caries. SEM results have shown that these nanoparticles enter and damage cell membranes, resulting in leakage of the cytoplasm and shrinkage of the cell. NPs were

seen close to cell membrane which shows their interaction and the reason of cell damage. The changes in the molecular biochemical makeup of bacteria were examined using FTIR. Which showed the impact of NPs on the vibrations of phosphate and sugar in the cell wall, and the distortions in the α-helix and β-sheets of the proteins within the cell (Chen et al., 2023).

2.13 Objective

The objective of this research includes:

- To enhance antibacterial properties of Ti implant as Mg-doped ZnO NPs are known for their antibacterial properties. Incorporating these nanoparticles into the coating can help prevent infections at the implant site enhancing antibacterial properties.
- To mimic the natural composition of bone that is well-tolerated by the body by using biomimetic coating.
- To improve the integration of the titanium implant with the surrounding bone.

CHAPTER 3: MATERIAL AND METHODS

3.1 Material

All Chemicals zinc acetate, sodium carbonate, magnesium acetate, Tryptic Soy Agar (TSA), sucrose and distilled water were all purchased from Sigma-Aldrich for use in the experiments.

3.2 Methods

3.2.1. Synthesis of ZnO NPs

The Bare ZnO NPs were synthesized by dissolving 0.01 mol of zinc acetate dihydrate in approximately 50 mL of deionized water with continuous stirring for 30 minutes, leading to the creation of precursor solution I. Subsequently, solution II was prepared by dissolving 0.01 mol of sodium carbonate in 50 milliliters of deionized water while continuously stirring for a duration of 30 minutes. ZnO nanoparticles were synthesized by gradually adding solution I to solution II while constantly stirring the mixture for a period of 30 minutes. Subsequently, the material obtained was isolated using filtration and underwent several washing cycles using deionized water. In the end, the gathered precipitation was dried at a temperature of 80◦C and then subjected to a calcination process at 300◦C for a period of 2 hours.

3.2.2. Synthesis of Mg-Doped ZnO NPs

To synthesize Mg-doped ZnO nanoparticles, a solution of magnesium acetate which was 5% of zinc acetate dihydrate was prepared by dissolving it in deionized water while stirring continuously. Subsequently, the solution was gradually introduced into a zinc acetate solution with a concentration of 0.01 M. The fully prepared solution was slowly added to a sodium carbonate solution with a concentration of 0.01 M, while rapidly stirring for a period of 30 minutes. The reaction produced white precipitates, which were allowed to settle and then washed using ultrasonic waves. Finally, the mixture was put onto glass plates. The plates were kept at ambient temperature for 24 hours and

subsequently dehydrated overnight in an 80°C hot air oven to remove any residual moisture. Afterwards, the material was ground and then exposed to calcination at a temperature of 300 ◦C for a period of 2 hours.

3.2.3. Characterizations of Bare and Mg-Doped ZnO NPs

To confirm the size, net charge and aggregation of NPs for coating and antibacterial properties these were assessed by the following characterization techniques:

3.2.3.1. UV Spectroscopy:

UV-Vis absorption spectroscopy is commonly used in laboratories. A light beam is sent through a sample contained in a cuvette. The constituent molecules in the sample selectively absorb light of a specific wavelength. The amount of absorption is governed by the wavelengths that can pass through the samples. First, a cuvette filled with only reference solvent is placed in the hood, and the absorption is measured. The absorption spectrum is acquired by introducing a second cuvette containing the sample material into the hood. The laser beam bifurcates, with one segment being precisely directed towards the Reference. The second cuvette, which contained the sample, was targeted by the second one. A spectrum of absorbance is generated over the entire range of wavelengths. For a specific wavelength, lambda max represents the highest level of absorption. The Beer-Lambert Law says that the absorbance of a sample is directly proportional to the concentration of the sample present in cuvette measured in mole. This absorbance shows molar absorptivity and is utilized to compare the spectra of different compounds.

3.2.3.2. SEM analysis:

Then SEM was performed using SEM VEG 3 LMU (Tescan, Czech Republic). The size and morphology of our fabricated nanoparticles was determined by the SEM analysis. This analysis was conducted by preparing the glass slides containing the fabricated samples of nanoparticles and coating them with gold (30nm) to induce the conduction into the sample under examination. The Sem images show the physical dispersion of the nanoparticles and their different sizes in nano scale.

3.2.3.3. FTIR analysis Fourier Transform Infrared (FTIR):

This approach of infrared spectroscopy is highly efficient. This approach involves the transmission of infrared (IR) radiation through a specimen, where the sample absorbs a portion of the IR radiation and transmits the rest. Consequently, the resulting spectrum exhibits the absorption and transmission of molecules, resulting into a unique pattern that can be used to identify unfamiliar substances and assess the amount and quality of the material present.

3.2.3.4. Zeta potential:

The Zeta sizer is used to measure the surface potential of a material. The zeta potential value is typically used to determine the net charge on nanoparticles and to assess their amount of force of attraction and stability. The technique of dynamic light scattering was employed to quantify the size of magnesium-doped zinc oxide nanoparticles.

3.2.3.5. EDX analysis:

Then EDX was performed with SEM VEG 3 LMU (Tescan, Czech Republic). This analysis presents a visual depiction as graphical representation of the synthesized nanoparticles.

3.2.3.6. XRD analysis:

X-Ray Diffraction analysis (XRD) provides information regarding the molecular crystalline structure and the impact of doping on this structure. This validates the architecture of the synthesized molecule.

3.2.4. Preparation of SBF solution

To prepare a 5-fold concentrated SBF solution, the reagents were dissolved in 1,000 ml of distilled water. Reagents include NaCl (40 g), MgCl2.6H2O (1.52 g), CaCl2.2H2O (1.84 g), Na2HPO4.2H2O (0.89 g), and NaHCO3 (1.76 g). Subsequently, the solution's pH was precisely modified to 7.4 by employing tris-hydroxymethyl aminomethane and

hydrochloric acid, while maintaining a temperature of 36.7°C. The SBF was periodically refreshed every 48 hours for maintaining its ion concentration.

3.2.5. Coating of Mg doped NPs on Titanium discs

The titanium discs were immersed in a 5 M NaOH solution at a temperature of 80°C for a period of 72 hours. The temperature was controlled by using a water bath. After undergoing alkaline treatment, the samples were subjected to heat treatment at a temperature of 600°C in an electric furnace. Subsequently, these discs were cooled for a duration of 1 hour. Subsequently, they were immersed in 30 ml of SBF×5 solutions with different concentrations of ZnO and Mg-doped ZnO for 7 and 14 days as in fig 3.1. The purpose of this procedure was to induce the formation of a calcium phosphate layer on the surface of the titanium implant, imitating the natural composition of bone also providing powerful antibacterial properties. The discs were divided into four groups based on the treatment they received, which involved dipping them in SBF with different types of nanoparticles. After immersing them in the solution, the samples were removed, rinsed with distilled water, and allowed to air dry for a period of 24 hours. Then these discs were placed in S. aureus culture for 24 hours in incubator as shown in fig 3.2.

Group 1	NaOH and heat-treated Disc in SBF with ZnO for 7 days
Group 2	NaOH and heat-treated Disc dipped in SBF with Mg doped ZnO NPs for 7 days
Group 3	Untreated Disc and dipped in SBF with Mg doped ZnO NPs for 7 days
Group 4	NaOH and heat-treated Disc and dipped in SBF with Mg doped ZnO NPs for 14 days

Table 1: Treated groups

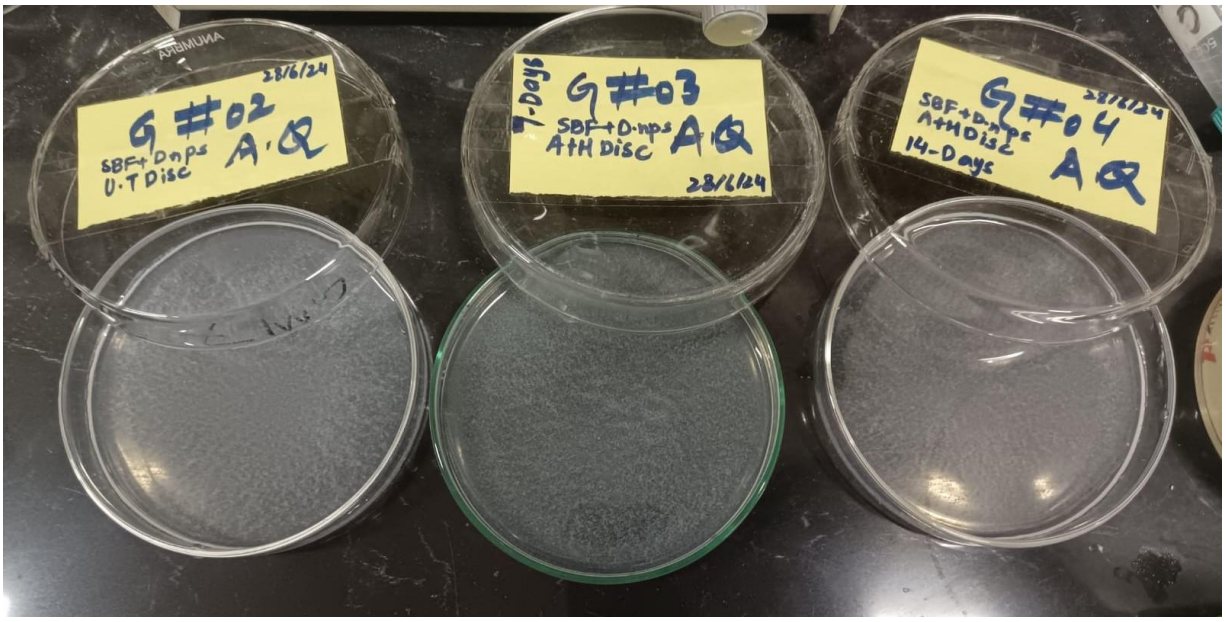


Figure 3.1: Four assigned groups discs with prepared SBF and NPs.

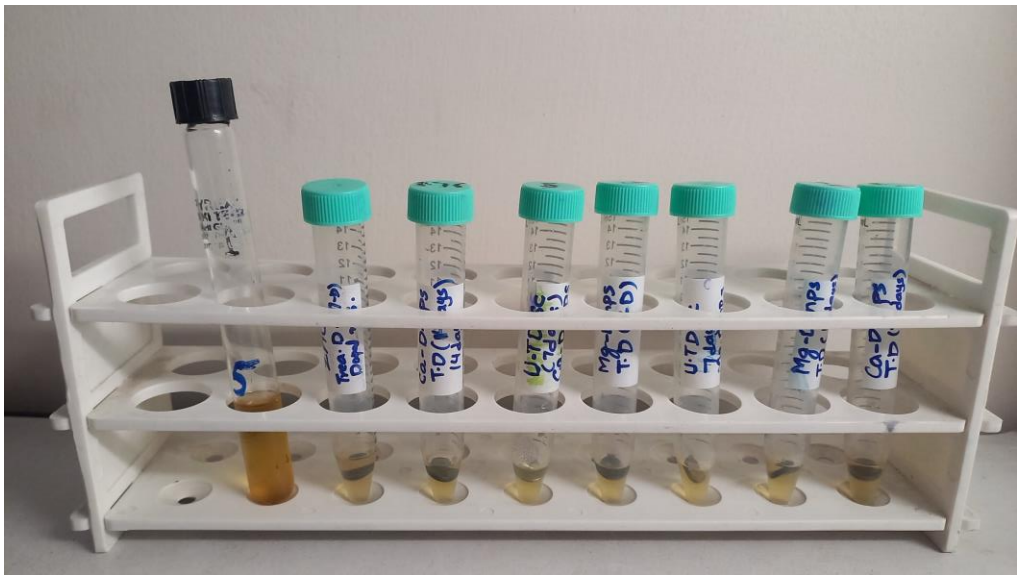


Figure 3.2: Four assigned groups with discs in *S. aureus* culture.

3.2.6. Characterization of Discs

The surfaces and coatings of four designated groups were evaluated using a SEM.

3.2.7. Bacterial isolation

10 individuals were chosen as participants to provide saliva samples. Streptococcus mutans was obtained from the samples. A homogeneous suspension of saliva was produced and subsequently diluted tenfold before being streaked on Tryptic Soy Agar as in fig 3.3. The plate was cultured at 37°C for 48 hours. The colonies obtained were identified morphologically. A pure culture was preserved in glycerol with a concentration of 80% and a temperature of -80°C (Azhar et al., 2022).

Figure 3.3: *S. aureus* colonies

3.2.8. Antibacterial in Vitro Assay

A volume of 5 mL of TSB was contaminated with 200 microliters of S.mutans (containing 1% sucrose) and let to grow overnight at T=37 ◦C. The optical density (OD) at 600nm was measured after a duration of 3.5 hours and found to be 1. Subsequently, the sample was diluted using a series of dilutions. Following dilution, a volume of 50 µL was evenly distributed on a TSA plate in order to quantify the number of first colonies. Next, 500 µL of the diluted culture was placed into sterile microcentrifuge tubes and then incubated with the Ti-Disc specimens in the incubator for a period of 6 hours. After a period of 6 hours, the discs were removed from the culture and discarded. Subsequently, 50 µL of the culture were evenly distributed on the TSA plates from each

microcentrifuge tube, and the number of colony-forming units (CFU) were assessed using the Miles and Misra technique.

3.2.9. *Colony Forming Unit (CFU)*

Colonies on the plates for each model were visually recorded, and the log CFU/ml was then calculated by the data collected. To decreases the chances of any error test was repeated three times.

CFU= (Number of colonies*dilution factor) / volume of culture plate

CHAPTER 4: RESULTS AND DISCUSSION

4.1 Characterizations of simple and Mg doped ZnO NPs

To confirm the synthesis of bare ZnO and ZnO doped with Mg was achieved by characterizations. Novel nanoparticles possessing the necessary characteristics and morphological attributes for antibacterial testing were effectively verified. Thereafter, further characterizations were performed to confirm the existence of an appropriate coating on the titanium implant. Characterizations were used to verify the synthesis of pure ZnO and to provide confirmation of the doping of ZnO with Mg. Novel nanoparticles possessing the necessary characteristics and morphological attributes for antibacterial testing were effectively verified. Therefore, further characterizations were performed to confirm the existence of an appropriate coating on the titanium implant.

4.2 UV-Vis Spectroscopy

Bare ZnO and Mg doped ZnO NPs have been prepared by the coprecipitation technique. UV spectroscopy was employed to verify the synthesis of nanoparticles. Zinc oxide nanoparticles were confirmed by the absorption peak at 362nm. The absence of any color change in Mg doped ZnO indicates that the doping process did not alter the physical properties of the nanoparticles. However, peak shift from 362 nm to 358 nm shows incorporation of Mg onto ZnO NPs in fig 4.1. Magnesium doping induces a modification in the electrical configuration of the ZnO nanoparticles.

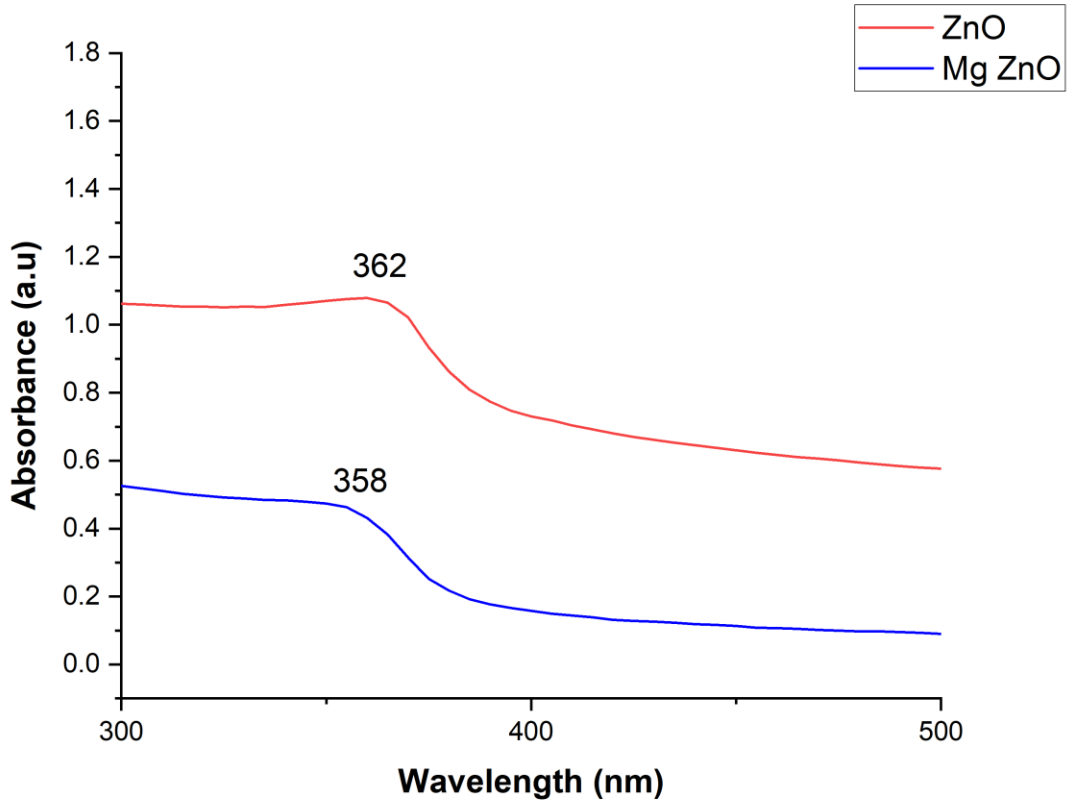


Figure 4.1: UV-vis analysis

4.3 SEM analysis of NPs

The objective of Scanning Electron Microscopy (SEM) was to examine the morphology and particle size of both original ZnO and ZnO nanoparticles doped with Mg in order to investigate the impact of Mg doping. The particle size was subsequently calculated using Image J software. Figure 4.2 (a) verifies the hexagonal geometries of ZnO nanoparticles. The majority of the particles are observed to be inside the nanoscale range. Not perceptible to the unaided human eye. These nanoparticles exhibit a tendency to aggregate and create agglomerates, suggesting an elevation in the surface charge and energy of the particles that are generated. 5% doping concentration of Mg results in the formation of hexagonal crystal-shaped and uniformly distributed nanoparticles on the ZnO surface, showing the influence of higher Mg doping. As the quantity of magnesium ions in the zinc oxide matrix was increased, the size of the final particles increased from

30 to 110 nm. In Figure 4.2 (b), the average particle size of the uncoated ZnO nanoparticles varied between 28.87 nm and 41 nm. However, the ZnO nanoparticles doped with magnesium exhibited a considerably reduced average particle size, ranging from 28.37 nm to 34 nm. The modification in particle size caused by the introduction of Mg doping illustrates the successful control of particle size in ZnO nanoparticles, even if it reduces the particles' capacity to aggregate. The EDX analysis of Mg-doped ZnO nanoparticles revealed prominent peaks of Mg, Zn, and O, providing confirmation of the effective introduction of Mg into the crystal lattice of ZnO Figure 4.3.

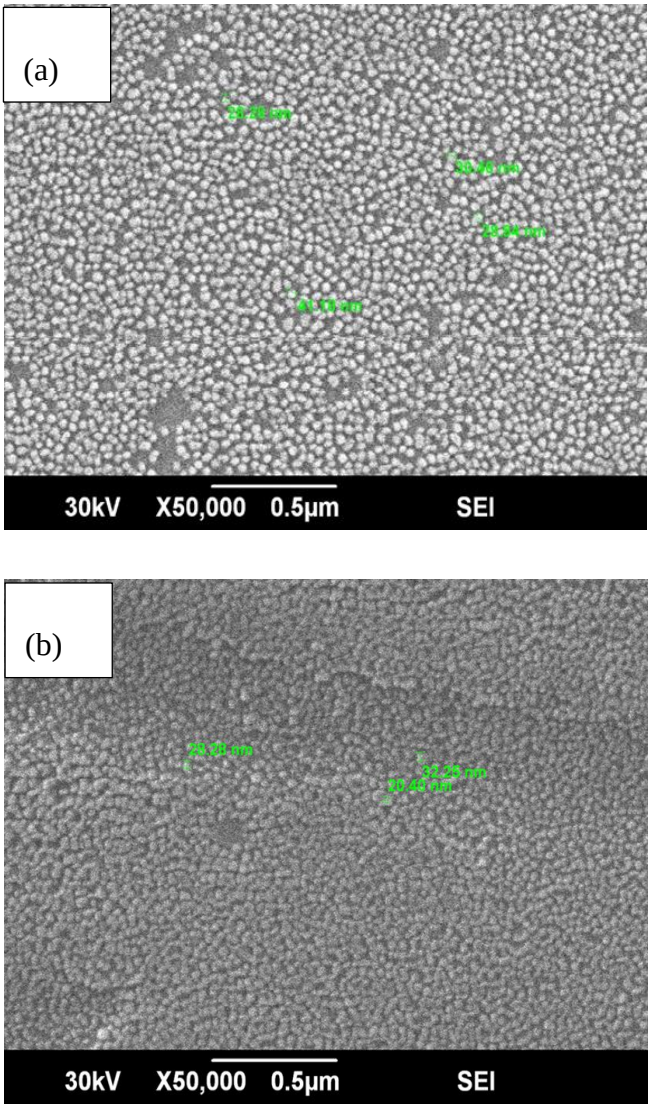


Figure 4.2: SEM Images of (a) Bare ZnO (b) Mg doped ZnO NPs

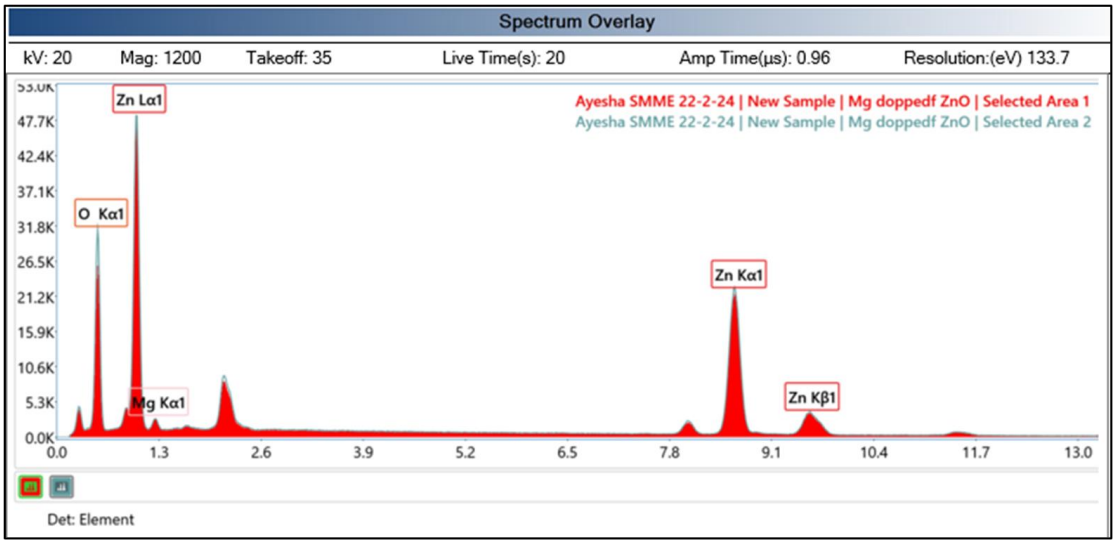


Figure 4: EDX analysis of Mg doped ZnO NPs

4.4 FTIR Analysis of Bare and Mg-Doped ZnO NPs

A broad absorption band representing OH stretching groups in water, Zn–OH, and Mg–Zn–OH, was seen within the wavelength range of 3600–3000 cm−1. Both the unmodified and magnesium-doped zinc oxide nanoparticles exhibited asymmetric carbon-hydrogen bonds, which were seen as peaks at 2971 and 2974. Undoped and doped ZnO nanoparticles exhibited C=C stretching at wavenumbers of 1620 and 1606 cm−1, respectively. The C=O bond exhibited a symmetric stretching vibration at 1459 cm−1 in the unmodified sample and at 1438 cm−1 in the sample doped with Mg. Both undoped and doped ZnO nanoparticle samples exhibited stretching frequencies of 890 and 874 cm−1 for Zn–O species as shown in figure 4.4.

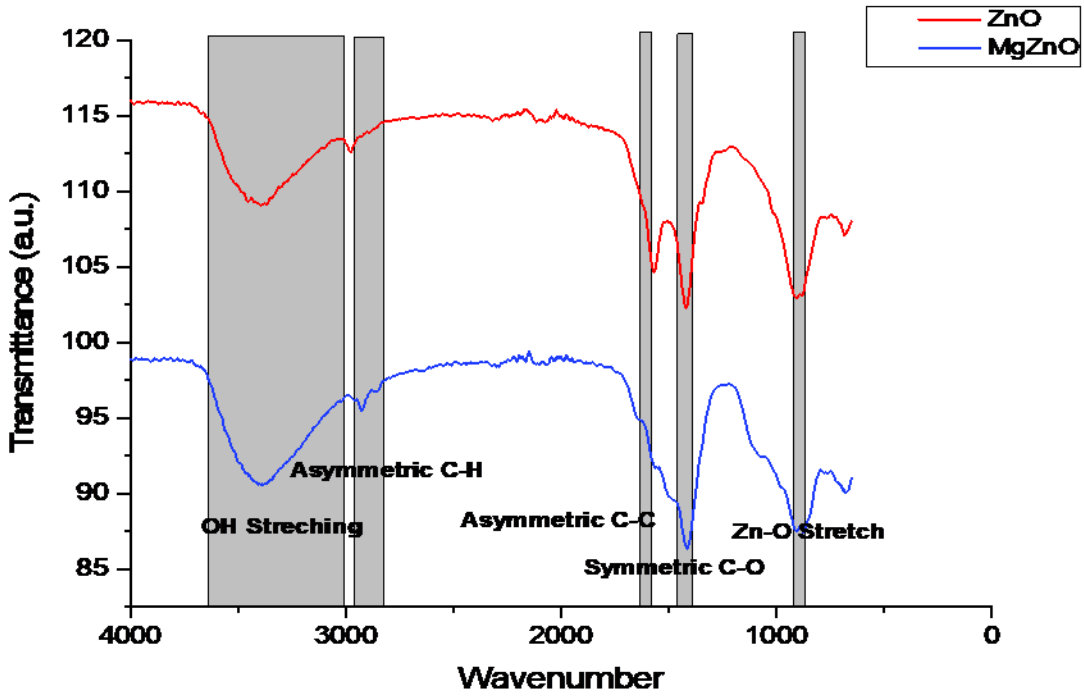


Figure 4.4: FTIR of bare ZnO and Mg doped ZnO

4.5 XRD Analysis of Bare and Mg-Doped ZnO NPs

In Figure 10, the X-ray diffraction (XRD) results of both undoped and magnesium-doped zinc oxide are shown. Major peaks were identified at the subsequent values 31.07, 33.84, 35.70, 46.84, 56.07, 62.35, and 68.02 in figure 4.5. The pattern shows that both the untreated and Mg doped ZnO nanoparticles share a wurtzite-like hexagonal shape. The observation of wider peaks and reduced intensities in the patterns of ZnO nanoparticles doped with magnesium, in comparison to unenriched ZnO, confirms the effective integration of magnesium into the crystal lattice of ZnO. The choice of doping was based on the reduction in ionic radius of Mg2+ compared to pure Zn2+. A determination of the crystal size of Mg-doped ZnO nanoparticles was made by applying the Scherer equation to the XRD patterns. D is defined by the equation $D = k\lambda \beta hkl \cos \theta$, where k is a constant of 0.90. Let κ represent the wavelength of the incident X-ray, which is precisely

0.154 nm. The word βhkl denotes the quantification of the width of a peak at half of its maximum intensity, whereas cos θ denotes the angular location of the peak.

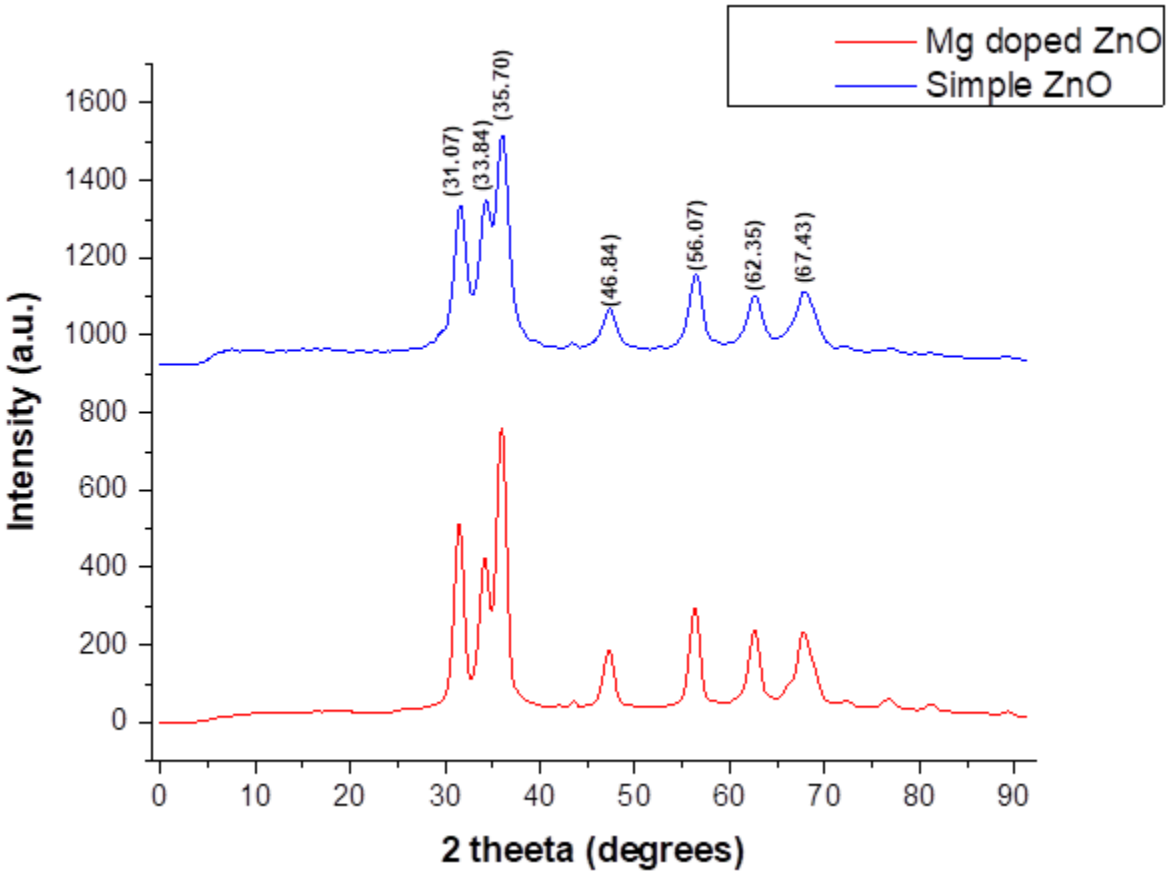
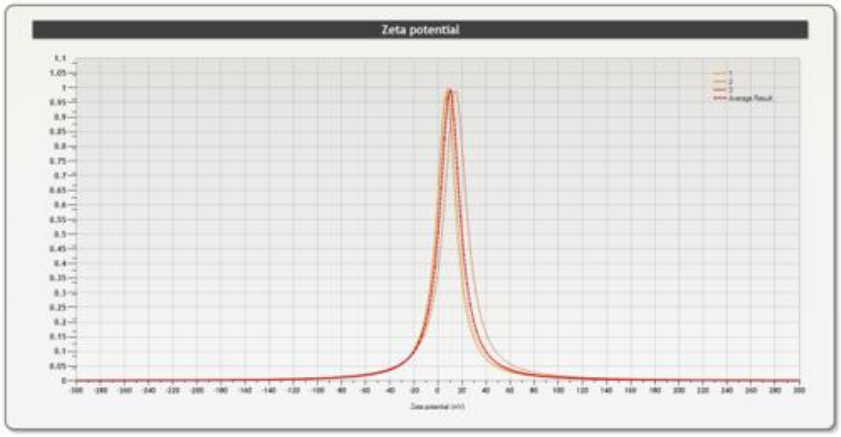


Figure 5: XRD of bare ZnO and Mg doped ZnO NPs.

4.6 Zeta Potential

Zeta of ZnO was 9.85mV in figure 4.6 (a) which was shifted to -8.55mV figure 4.6 (b) after doping which confirmed the doping of Mg on ZnO and stability of nanoparticles.

(a)



(b)

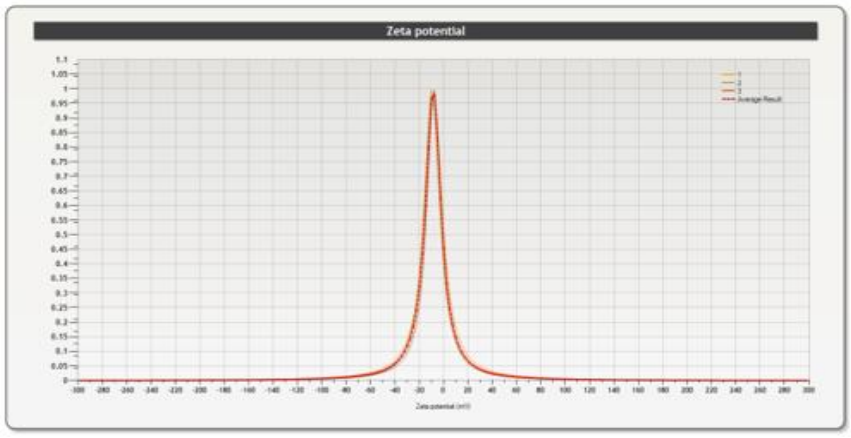


Figure 6: Zeta potential of (a) Bare ZnO and (b) Mg doped ZnO NPs

4.7 Atomic Force Microscopy

Average roughness (Ra) bare ZnO surface was analyzed 0.31nm which shows moderate rough surface. In Mg doped ZnO value increased to 3.93 nm, an increase in surface roughness. Results showed uniform granular morphology. Height distribution was also analyzed Rq of ZnO was 2.2 nm and Mg doped ZnO was 44 nm whichh confirmed increse in roughness.

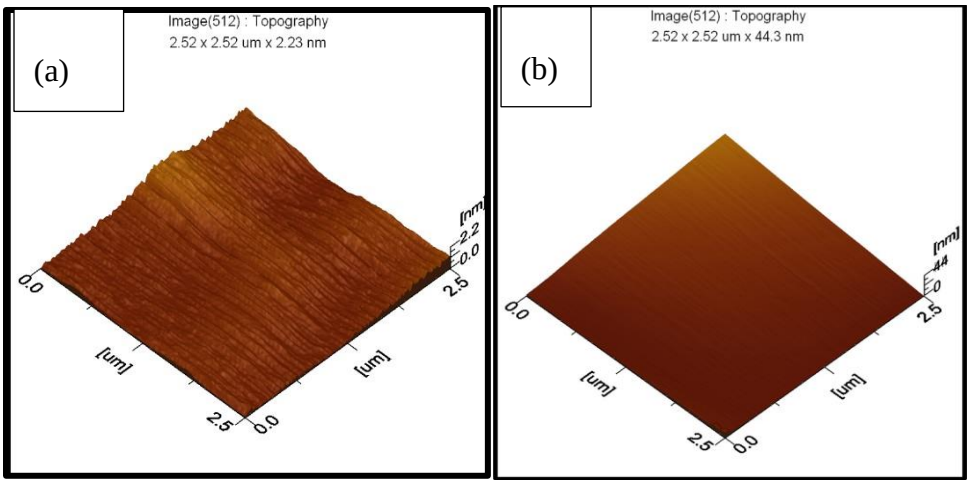


Figure 4.7: AFM of (a) Bare ZnO and (b) Mg doped ZnO

4.8 Optical Microscopy

Images showed no significant effect in figure 4.8(d) which was not given any of the treatment and was dipped directly into SBF with Mg doped ZnO NPs for 7 days. Highest effect was seen in figure 4.8(e) which was given alkaline and heat treatment and was dipped in SBF with Mg doped ZnO for 14 days.

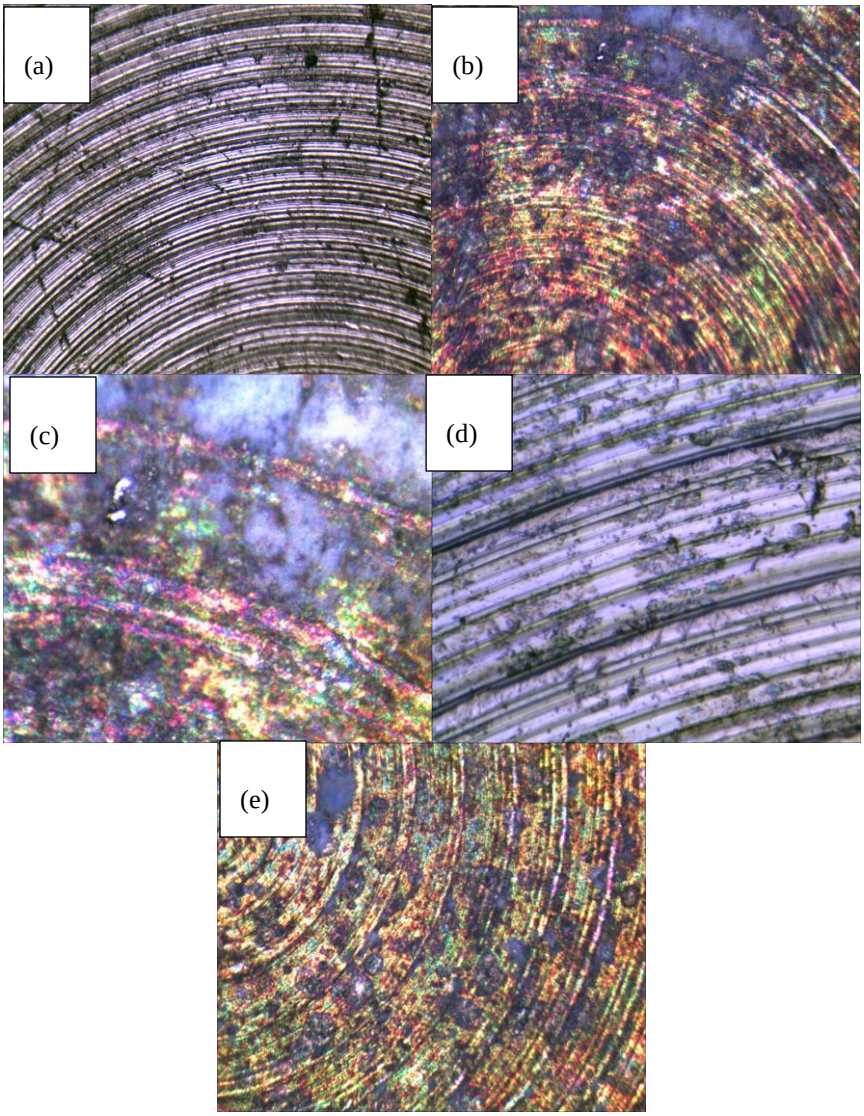


Figure 4.87: Optical Microscopy of (a) Bare disc (b) alkaline and heat-treated disc soaked in SBF×5 with ZnO NPs for 7 days (c) alkaline and heat treated disc soaked in SBF×5 with Mg doped ZnO NPs for 7 days (d) untreated disc and soaked in SBF×5 with Mg doped ZnO NPs for 7 days (e) alkaline and heat treated disc soaked in SBF×5 with Mg doped ZnO NPs for 14 days.

4.9 SEM analysis of Ti-Discs

In Figure 4.9, scanning electron micrographs of machined Ti alloy substrates are shown. A 5 M NaOH solution was applied to the surfaces of these substrates at 80°C for a period of 72 hours. Further, the surfaces have been activated using NaOH and then heated at 600°C for 1 hour. The specimens had numerous machine marks on their surfaces in figure 4.9(a). Microporous structures were observed in the substrates after being immersed in a 5 M NaOH solution at 80°C for 72 hours. The dimensions of the pores in this structure vary between 50 and 100 nanometers. The NaOH was used as an activator and then the disc underwent a heat treatment at 600°C for 1 hour. Consequently, we observed a densely packed structure with interior voids, as well as some fissures that probably formed during the heat treatment. The detection of cracking was attributed to differences in the thermal coefficients of expansion between the substrate and the surrounding surface layer. It was shown that the whole surface of the substrate will undergo drying cracks when overlaid with such a coating. Electrochemical treatment of metal surfaces leads to the formation of microporous structures, suggesting the probable existence of a sodium titanate hydrogel (Na2Ti5O11 or Na2Ti6O13). There was observed deposition of calcium phosphate on the surfaces of samples after immersion in simulated bodily fluid (SBF) containing either pure ZnO nanoparticles (NPs) or magnesium-doped ZnO NPs for different durations. Specimens in Group I underwent alkaline treatment, followed by heat treatment, and finally immersed in the SBF containing ZnO for 7 days. Surface analysis of the specimens revealed the existence of nanosized, rod-shaped calcium phosphate particles containing ZnO nanoparticles figure 4.9(b). By comparison, the samples from group II, which were subjected to identical treatment and immersion for the same period, showed an equivalent quantity of spheroid particles without any precipitation of calcium phosphate on their surfaces fig 4.9(c). Group III comprised an untreated specimen that was then submerged in SBF solution for a period of 7 days with magnesium-doped zinc oxide nanoparticles. The disc exhibited a thin layer and a decreased level of particle buildup figure 4.9(d). Furthermore, following a 14-day immersion in the simulated bodily fluid (SBF) that included magnesium-doped zinc oxide nanoparticles (NPs), the samples subjected to heat treatment at 600°C mostly developed a fresh layer of calcium phosphate. This stratum consisted of spherical

formations giving the appearance of islands, as depicted in figure 4.9(e). Following a 14-day immersion in SBF, the Ti disc exhibited an increased presence of bigger, aggregated island-like spheroids, as depicted in figure. It was the last layer of coating.

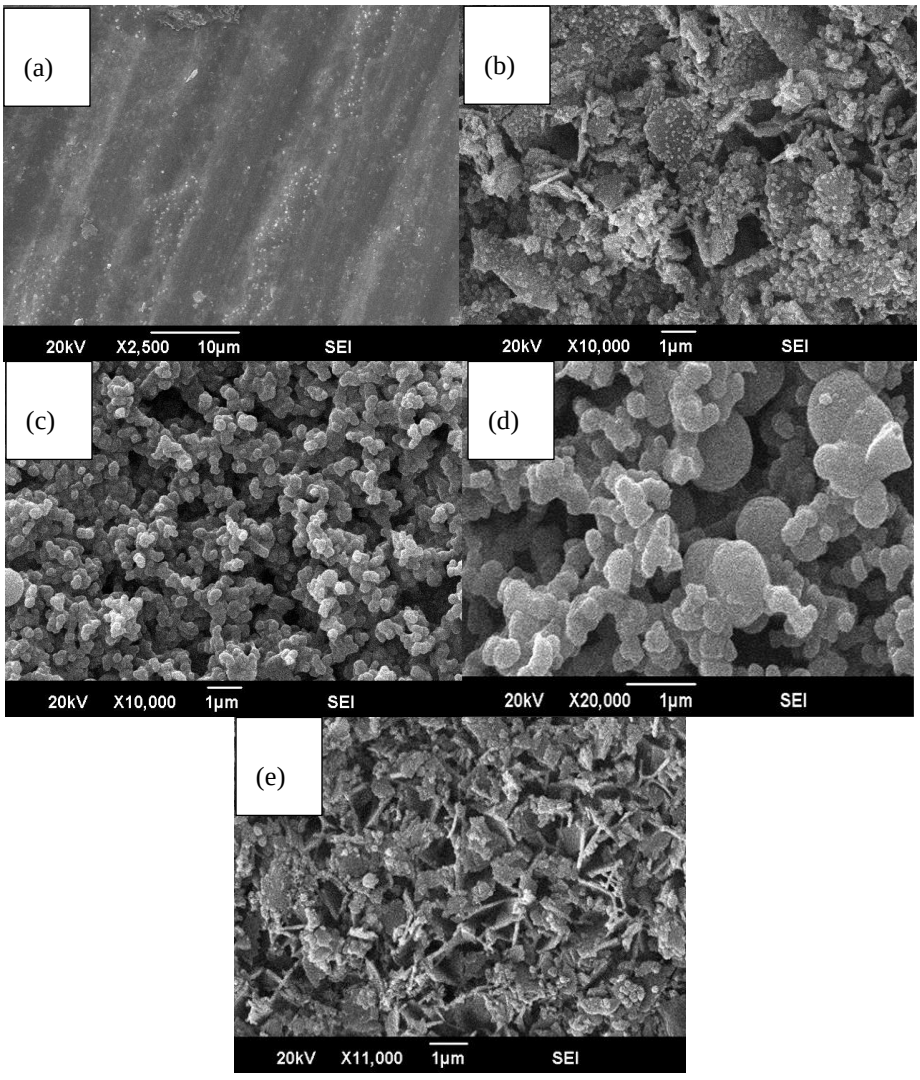


Figure 8: SEM of Discs (a) Bare disc (b) alkaline and heat-treated disc soaked in SBF×5 with ZnO NPs for 7 days (c) alkaline and heat treated disc soaked in SBF×5 with Mg doped ZnO NPs for 7 days (d) untreated disc and soaked in SBF×5 with Mg doped ZnO NPs for 7 days (e) alkaline and heat treated disc soaked in SBF×5 with Mg doped ZnO NPs for 14 days.

4.10 Antibacterial Activity of Bare and Mg-Doped ZnO NPs

The NaOH and heat-treated disc dipped in SBF with Mg doped ZnO NPs for 14 days showed the highest antibacterial activity as compared to other three groups. Statistical analysis was performed using the GraphPad Prism software. Data includes the mean and standard deviations. Moreover, group comparisons were conducted by a t-test and one-way ANOVA analysis. Results showed enhanced antibacterial activity against *S.aureus* as a result of incorporation of Mg in ZnO matrix ($p<0.0001$).

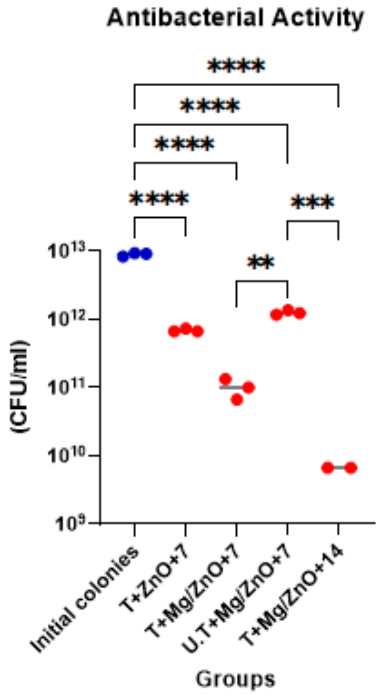


Figure 4.10: Antibacterial effect of Bare ZnO and Mg doped ZnO on group I, II, III, IV. Single star (*) means $p<0.05$, (**) means $p<0.01$, (***) means $p<0.001$, (***) means $p<0.0001$ and ns means statistically non-significant.

CHAPTER 5: CONCLUSION

The present work investigated the efficacy of applying a biomimetic technique to apply a magnesium-doped zinc oxide coating onto a titanium implant. The implant was subjected to an alkaline treatment with sodium hydroxide (NaOH) for 72 hours, with subsequent heat treatment at 600°C for 1 hour. Thorough immersion in simulated body fluid (SBF) for 14 days resulted in a uniform layer of calcium phosphate with magnesium-doped zinc oxide nanoparticles (ZnO NPs). This was verified using scanning electron microscopy (SEM), energy dispersive X-ray spectroscopy (EDS), and atomic force microscopy (AFM). This coating replicates the inherent makeup of bone and was not identified as an extraneous entity. This reduced the likelihood of rejection and showed enhanced antibacterial activity, osseointegration, and also reduce processing cost.

REFERENCES

Abaszadeh, F., Ashoub, M. H., Khajouie, G., & Amiri, M. (2023). Nanotechnology development in surgical applications: recent trends and developments. European journal of medical research, 28(1), 537.

Abutalib, M. M., & Rajeh, A. (2020). Influence of ZnO/Ag nanoparticles doping on the structural, thermal, optical and electrical properties of PAM/PEO composite. Physica B: Condensed Matter, 578, 411796.

Alcaraz, J. P., Cinquin, P., & Martin, D. K. (2018). Tackling the concept of symbiotic implantable medical devices with nanobiotechnologies. Biotechnology Journal, 13(12), 1800102.

Arif, W., Rana, N. F., Saleem, I., Tanweer, T., Khan, M. J., Alshareef, S. A., Sheikh, H. M., Alaryani, F. S., Al-Kattan, M. O., & Alatawi, H. A. (2022). Antibacterial activity of dental composite with ciprofloxacin loaded silver nanoparticles. Molecules, 27(21), 7182.

Baseri, M., Radmand, F., Hamedi, R., Yousefi, M., & Kafil, H. S. (2020). Immunological aspects of dental implant rejection. BioMed Research International, 2020(1), 7279509.

Belt, H. v. d., Neut, D., Schenk, W., Horn, J. R. v., Mei, H. C. v. d., & Busscher, H. J. (2001). Infection of orthopedic implants and the use of antibiotic-loaded bone cements: a review. Acta Orthopaedica Scandinavica, 72(6), 557-571.

Chen, C., Huang, B., Liu, Y., Liu, F., & Lee, I.-S. (2023). Functional engineering strategies of 3D printed implants for hard tissue replacement. Regenerative Biomaterials, 10, rbac094.

Erkin, A., & Vasif, H. (2010). Biodegradable hard tissue implants. Журнал Сибирского федерального университета. Биология, 3(1), 3-17.

Escada, A. L. d. A., Machado, J. P. B., Schneider, S. G., Rezende, M. A., & Claro, A. A. (2011). Biomimetic calcium phosphate coating on Ti-7.5 Mo alloy for dental application. Journal of Materials Science: Materials in Medicine, 22, 2457-2465.

Ferrando-Magraner, E., Bellot-Arcís, C., Paredes-Gallardo, V., Almerich-Silla, J. M., García-Sanz, V., Fernández-Alonso, M., & Montiel-Company, J. M. (2020).

Antibacterial properties of nanoparticles in dental restorative materials. A systematic review and meta-analysis. Medicina, 56(2), 55.

Iqbal, J., Jan, T., Ismail, M., Ahmad, N., Arif, A., Khan, M., Adil, M., & Arshad, A. (2014). Influence of Mg doping level on morphology, optical, electrical properties and antibacterial activity of ZnO nanostructures. Ceramics International, 40(5), 7487-7493.

Kandi, V., & Vadakedath, S. (2020). Implant-associated infections: A review of the safety of cardiac implants. Cureus, 12(12).

Kasi, G., Viswanathan, K., Sadeghi, K., & Seo, J. (2019). Optical, thermal, and structural properties of polyurethane in Mg-doped zinc oxide nanoparticles for antibacterial activity. Progress in Organic Coatings, 133, 309-315.

Khan, M., Mukhtar, N., Saeed, S., & Ramsden, R. (2007). The Pakistan (Lahore) cochlear implant programme: issues relating to implantation in a developing country. The Journal of Laryngology & Otology, 121(8), 745-750.

Kim, A., Abdelhay, N., Levin, L., Walters, J. D., & Gibson, M. P. (2020). Antibiotic prophylaxis for implant placement: a systematic review of effects on reduction of implant failure. British dental journal, 228(12), 943-951.

Kirthana, S., Nagamallika, J., & Nizamuddin, M. (2020). A FEA on assembled fractured human femur bone with and without HA coated prosthetic plate material. Materials Today: Proceedings, 22, 2890-2898.

Lin, Z., Song, K., & Yu, X. (2021). A review on wire and arc additive manufacturing of titanium alloy. Journal of Manufacturing Processes, 70, 24-45.

Lopez-Valverde, N., Macedo-de-Sousa, B., Lopez-Valverde, A., & Ramirez, J. M. (2021). Effectiveness of antibacterial surfaces in osseointegration of titanium dental implants: A systematic review. Antibiotics, 10(4), 360.

Matter, M. T., Maliqi, L., Keevend, K., Guimond, S., Ng, J., Armagan, E., Rottmar, M., & Herrmann, I. K. (2021). One-step synthesis of versatile antimicrobial nano-architected implant coatings for hard and soft tissue healing. ACS Applied Materials & Interfaces, 13(28), 33300-33310.

Mehmood, S., Ansari, U., Ali, M. N., & Rana, N. F. (2014). Internal fixation: An evolutionary appraisal of methods used for long bone fractures. International Journal of Biomedical and Advance Research, 5(3), 142-149.

Nigam, A., & Pawar, S. (2020). Structural, magnetic, and antimicrobial properties of zinc doped magnesium ferrite for drug delivery applications. Ceramics International, 46(4), 4058-4064.

Nosheen, A., Hussain, M. T., Ashraf, M., & Iqbal, K. (2022). A novel approach to modify and functionalize acid black 1 dye for antimicrobial and UV protective textiles. Dyes and Pigments, 205, 110486.

Pradeev Raj, K., Sadaiyandi, K., Kennedy, A., Sagadevan, S., Chowdhury, Z. Z., Johan, M. R. B., Aziz, F. A., Rafique, R. F., Thamiz Selvi, R., & Rathina Bala, R. (2018). Influence of Mg doping on ZnO nanoparticles for enhanced photocatalytic evaluation and antibacterial analysis. Nanoscale research letters, 13, 1-13.

Sauer, K., Stoodley, P., Goeres, D. M., Hall-Stoodley, L., Burmølle, M., Stewart, P. S., & Bjarnsholt, T. (2022). The biofilm life cycle: Expanding the conceptual model of biofilm formation. Nature Reviews Microbiology, 20(10), 608-620.

Shahid, A., Aslam, B., Muzammil, S., Aslam, N., Shahid, M., Almatroudi, A., Allemailem, K. S., Saqalein, M., Nisar, M. A., & Rasool, M. H. (2021). The prospects of antimicrobial coated medical implants. Journal of applied biomaterials & functional materials, 19, 22808000211040304.

Sidhu, S. S., Singh, H., & Gepreel, M. A.-H. (2021). A review on alloy design, biological response, and strengthening of β-titanium alloys as biomaterials. Materials Science and Engineering: C, 121, 111661.

Tammaro, L., Di Salle, A., Calarco, A., De Luca, I., Riccitiello, F., Peluso, G., Vittoria, V., & Sorrentino, A. (2020). Multifunctional bioactive resin for dental restorative materials. Polymers, 12(2), 332.

Tang, W., Fischer, N. G., Kong, X., Sang, T., & Ye, Z. (2024). Hybrid coatings on dental and orthopedic titanium implants: Current advances and challenges. BMEMat, e12105.

Tanweer, T., Rana, N. F., Khan, M. J., Saleem, I., Azhar, S., Shafique, I., & Menaa, F. (2022). Transition Metal Doped Zinc Oxide Nanoparticles Ameliorates

Antibacterial Potential of Dental Resin Composite in a Closed System In-vitro Biofilm Model. 2022 E-Health and Bioengineering Conference (EHB),

Tanweer, T., Rana, N. F., Saleem, I., Shafique, I., Alshahrani, S. M., Almukhlifi, H. A., Alotaibi, A. S., Alshareef, S. A., & Menaa, F. (2022). Dental composites with magnesium doped zinc oxide nanoparticles prevent secondary caries in the alloxan-induced diabetic model. International Journal of Molecular Sciences, 23(24), 15926.

W. Nicholson, J. (2020). Titanium alloys for dental implants: A review. Prosthesis, 2(2), 11.

Zhang, L.-C., Chen, L.-Y., & Wang, L. (2020). Surface modification of titanium and titanium alloys: technologies, developments, and future interests. Advanced Engineering Materials, 22(5), 1901258.

Parnia, F., Yazdani, J., Javaherzadeh, V., & Dizaj, S. M. (2017). Overview of nanoparticle coating of dental implants for enhanced osseointegration and antimicrobial purposes. Journal of Pharmacy & Pharmaceutical Sciences, 20, 148-160.

De Giglio, E., Cafagna, D., Cometa, S., Allegretta, A., Pedico, A., Giannossa, L. C., ... & Iatta, R. (2013). An innovative, easily fabricated, silver nanoparticle-based titanium implant coating: development and analytical characterization. Analytical and bioanalytical chemistry, 405, 805-816.

Secinti, K. D., Özalp, H., Attar, A., & Sargon, M. F. (2011). Nanoparticle silver ion coatings inhibit biofilm formation on titanium implants. Journal of Clinical Neuroscience, 18(3), 391-395.REFERENCES

Ahmed, N., Abbasi, M. S., Mariam, Q., William, H., Iftikhar, H., Badar, H., & Irfan, A. B. (2021). Analysis of Dental Practitioners Perception Towards Dental Implants. *Journal of the Pakistan Dental Association*, *30*(1).

Azhar, S., Rana, N. F., Kashif, A. S., Tanweer, T., Shafique, I., & Menaa, F. (2022). DEAE-Dextran Coated AgNPs: A Highly Blendable Nanofiller Enhances Compressive Strength of Dental Resin Composites. *Polymers*, *14*(15), 3143.

Gulati, K., Chopra, D., Kocak-Oztug, N. A., & Verron, E. (2023). Fit and forget: the future of dental implant therapy via nanotechnology. *Advanced Drug Delivery Reviews*, *199*, 114900.

Leong, D., Yap, A., Tay, J., & Tan, W. (1996). Dental implants.

Maathuis, P., Bulstra, S., Van der Mei, H., van Horn, J., & Busscher, H. (2007). Biomaterial-associated surgery and infection a review of literature. *Detection, prevention and direct post-operative intervention in orthopaedic implant infection*, 17.

Mignon, P., Allouche, A.-R., Innis, N. R., & Bousige, C. (2023). Neural network approach for a rapid prediction of metal-supported borophene properties. *Journal of the American Chemical Society*, *145*(50), 27857-27866.

W. Nicholson, J. (2020). Titanium alloys for dental implants: A review. *Prosthesis*, *2*(2), 11.

Wang, Z., Wang, X., Wang, Y., Zhu, Y., Liu, X., & Zhou, Q. (2021). NanoZnO-modified titanium implants for enhanced anti-bacterial activity, osteogenesis and corrosion resistance. *Journal of nanobiotechnology*, *19*, 1-23.