

BIOCOMPATIBILITY EVALUATION OF POLYMER COATED MAGNESIUM/GRAPHENE OXIDE IMPLANTS FOR BONE REGENERATION

Submitted in partial fulfilment for the award of
Bachelor of Technology degree in Biotechnology

By

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DEPARTMENT OF BIOTECHNOLOGY

SCHOOL OF BIO & CHEMICAL ENGINEERING

SATHYABAMA INSTITUTE OF SCIENCE AND TECHNOLOGY

(DEEMED TO BE UNIVERSITY)

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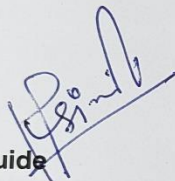
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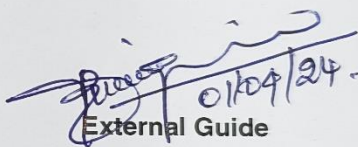
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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of **Karthik S (40230019)**, who carried out the project entitled "**Biocompatibility Evaluation of Polymer Coated Magnesium/Graphene Oxide Implants for Bone Regeneration**" under our supervision from **September-2023 to April-2024**.


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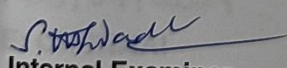

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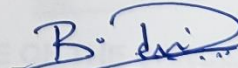

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I, **Karthik S (40230019)**, hereby declare that the Project Report entitled **“Biocompatibility evaluation of polymer coated Magnesium/Graphen Oxide implants for bone regeneration”** done by me under the guidance of **Dr. Prabhakar Singh** (Internal) and **Dr. T. Stalin Dhas** (External) at **Sathyabama Institute of Science and Technology, Chennai, Tamil Nadu, India**, is submitted in partial fulfilment of the requirements for the award of **Bachelor of Technology degree in Biotechnology**.

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ABSTRACT

In the search for suitable alternatives to titanium implants due to their frequent failure, allergic reactions, and non-degradability, magnesium-based bone implants have gained popularity for reasons such as biodegradability, biocompatibility, ability to reduce stress-shielding, and so on. Magnesium is a naturally produced element within our body that has excellent osteopromotive and regenerative characteristics. However, it is also known to have high corrosive properties in its unadulterated form, necessitating alloying. Because of their adaptability and strength, magnesium-based alloys are exceptionally effective as biodegradable materials. The research topic addressed here is the lack of durability of magnesium-based implants, which leads to local hydrogen accumulation and, ultimately, disintegration before the complete healing of the wounds. The current study is founded on the premise that manufacturing magnesium implants with graphene oxide, zinc, and hydroxyapatite and coating the implant with pectin can improve the durability, antibacterial characteristics, and hemocompatibility of such implants and thereby allow them to be evaluated for the same.

Keywords: *Biocompatibility, Cytocompatibility, Cytotoxicity, Hemocompatibility, Bone implants, stress shielding*

TABLE OF CONTENTS

CHAPTER NO.	TITLE	PAGE NO.
	ABSTRACT	v
	CONTENTS	vi
	LIST OF FIGURES	viii
	LIST OF ABBREVIATIONS	ix
1	INTRODUCTION	1
2	REVIEW OF LITERATURE	5
	2.1 MAGNESIUM ALLOYS AND THEIR PROPERTIES	5
	2.2 ZINC-BASED ALLOYS	5
	2.3 CHARACTERISTICS OF REDUCED GRAPHENE OXIDE	6
	2.4 PECTIN AND ITS PROPERTIES	6
	2.5 HAp AND ITS PROPERTIES	7
	2.6 <i>INVITRO</i> BIOCOMPATIBILITY OF MAGNESIUM IMPLANTS INCORPORATED WITH REDUCED GRAPHENE OXIDE	8
	2.6.1 ANTIBACTERIAL PROPERTIES	8
	2.6.2 HEMOCOMPATIBILITY	10
	2.6.3 <i>INVITRO</i> LEACHING	11
3	AIM AND OBJECTIVE	12
4	MATERIALS AND METHODS	13
	4.1 MATERIALS AND PROCESSING	13
	4.2 GRAPHENE OXIDE PREPARATION	13
	4.3 HYDROXYAPATITE PREPARATION	13
	4.4 FABRICATION	14
	4.5 BIOCOMPATIBILITY	15
	4.5.1. ANTIBACTERIAL ACTIVITY	15
	4.5.2. BACTERIAL ADHESION STUDIES	15
	4.5.3. CORROSION STUDIES	16
	4.5.4. HEMOLYSIS ASSAY	16

5	RESULTS AND DISCUSSION	17
	5.1. MATERIAL FABRICATION	17
	5.1.1. GRAPHENE OXIDE	17
	5.1.2. HYDROXYAPATITE	18
	5.1.3. FABRICATED IMPLANTS	18
	5.2. CHARACTERIZATION	19
	5.2.1. OPTICAL MICROSCOPIC IMAGING	19
	5.2.1.1. UNCOATED IMPLANT	19
	5.2.1.2. COATED IMPLANT	20
	5.2.3. SEM ANALYSIS	21
	5.3. LEACHING ASSAY	22
	5.3.1. LEACHING ASSAY WITH VARIABLE PH	23
	5.4. ANTIBACTERIAL ACTIVITY	25
	5.5. BACTERIAL ADHESION STUDIES	27
	5.5.1. <i>S. aureus</i> ADHESION	28
	5.5.2. <i>P. aeruginosa</i> ADHESION	29
	5.6. HEMOLYSIS ASSAY	30
	5.6.1. OBSERVATIONS AFTER 24 HOURS	31
6	SUMMARY AND CONCLUSION	33
7	REFERENCES	
8	ANNEXTURE	

LIST OF FIGURES

FIGURE		PAGE
Nos.	TITLE	NOs.
1.1	Natural bone healing process	1
1.2	Stress induced corrosion caused by metal implants	2
4.1	Pelletizer machine	14
4.2	Microplate reader	15
5.1	Synthesized Graphene Oxide Nanosheets	17
5.2	Prepared Hydroxyapatite nanoparticles	18
5.3	Fabricated Implants	18
5.4	Digital optical microscope	19
5.5	Optical microscopic image of pure Mg	19
5.6	Optical microscopic image of Mg with 1 wt % GO	20
5.7	Optical microscopic image of Mg coated with pectin	20
5.8	SEM images of uncoated sample	21
5.9	SEM images of coated sample	21
5.10	Implants immersed into HBSS for Leaching assay	22
5.11	Leaching assay (plot between wet weight and absorbance)	23
5.12	Leaching assay (pH 4)	24
5.13	Leaching assay (pH 7)	24
5.14	Implants suspended in <i>P. a</i> and <i>S. a</i> bacterial medium	25
5.15	CV-Stained bacteria suspended in 96 well plate	25
5.16	Antibacterial activity on <i>P.aeruginosa</i>	26
5.17	Antibacterial activity on <i>S.aureus</i>	26
5.18	High resolution scanning electron microscope	27
5.19	<i>P. a</i> and <i>S. a</i> bacterial adhesion on the implants	27
5.20	SEM images of adhered <i>S. aureus</i> bacteria on implants	28
5.21	SEM images of adhered <i>P. aeruginosa</i> bacteria on implants	29
5.22	Implants suspended on blood agar for hemolysis assay	30
5.23	Hemolysis assay observations after 24 hours	31

LIST OF ABBREVIATIONS

ACRONYM	EXPANSION
Ca	Calcium
Ca (NO₃)₂.4H₂O	Calcium Nitrate Tetrahydrate
Ca₁₀((PO₄)₆(OH)₂)	Calcium phosphate dihydrate
Co-Cr	Cobalt-Chromium
<i>E. coli</i>	<i>Escherichia coli</i>
FESEM	Field emission scanning electron Microscopy
g/cm³	gram per centimetre cube
GO	Graphene Oxide
GPa	Giga pascal
H₂O₂	Hydrogen Peroxide
HAp	Hydroxyapatite
HBSS	Hank's Balanced salt solution
Hrs	Hours
HR-SEM	High Resolution-Scanning Electron Microscopy
K₂HPO₄	Dipotassium Hydrogen Phosphate
Mg	Magnesium
mm	millimetre
MPa.m^{1/2}	Mega pascal square root meter
NaNO₃	Sodium Nitrate
OH	Hydroxide
PO₄	Phosphate
<i>P. aeruginosa</i>	<i>Pseudomonas aeruginosa</i>
<i>S. aureus</i>	<i>Staphylococcus aureus</i>

CHAPTER-1

1. INTRODUCTION:

Bone repair is regulated by chemical, cellular, biomechanical, and pathological variables. In general, bone healing takes six to twelve weeks, which is approximately three months. However, depending on the patient's age, underlying medical conditions, and the severity of the damage, it can occasionally take longer—up to a year. If bone damage exceeds a particular threshold, the bone tissue loses its ability to self-heal (Camarero-Espinosa et al., 2016).

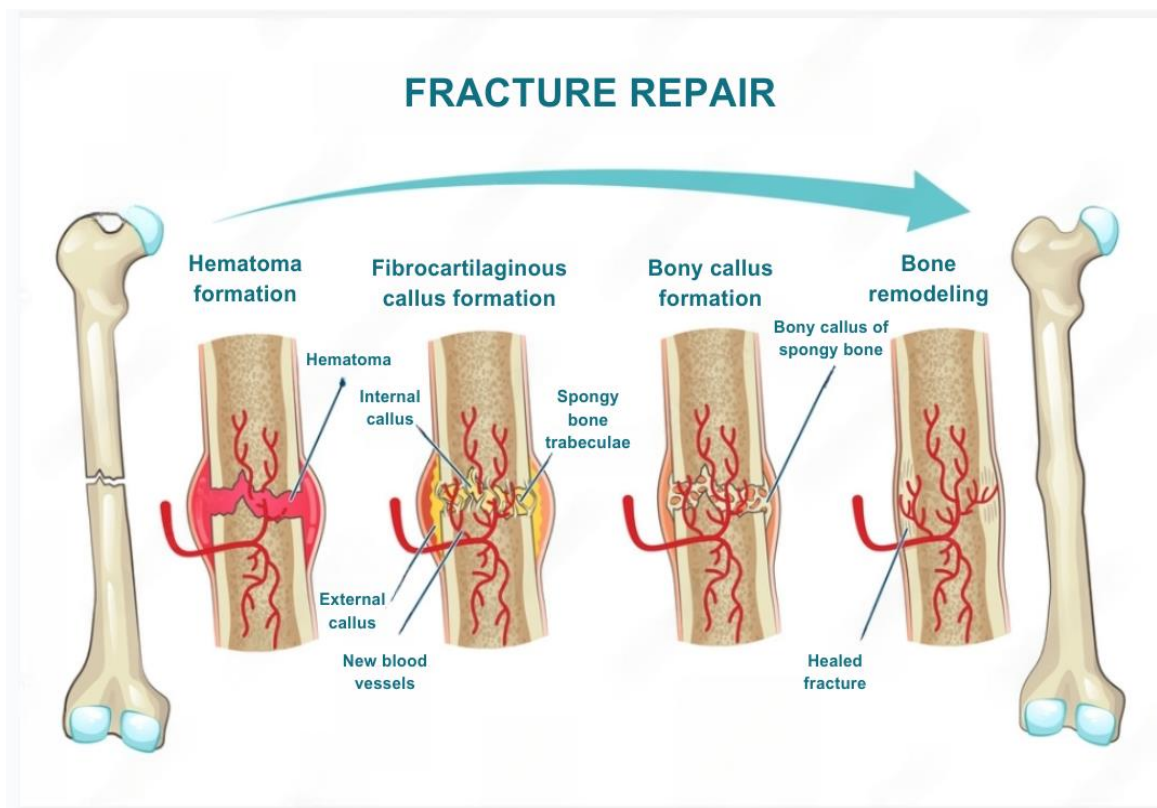


Fig 1.1: Natural bone healing process

The bone tissue can no longer heal itself if the length of the bone damage exceeds twice the diameter of the fractured bone (Amin Bigham-Sadegh et al., 2014). To repair defects like tumour formation, traumatic fractures, infections, and osteoporosis, autografts, allografts, or artificial bone substitutes, are used. Autografts have higher failure rates, though they are being used widely. Metal implants may cause discomfort at the site of injury, the necessity for repeated procedures, the occurrence of infections, and so on. Titanium is non-biodegradable, however, implants made in the purest form of magnesium break down before the fracture heals fully.

Conventionally, titanium and its alloys, stainless steel, gold, cobalt-chromium alloys, magnesium, silver, palladium, iron, zinc, tantalum, and platinum electrodes were used to make metal implants. The polymers that are utilised are hydrogels, amides, propylenes, methyl-methacrylate esters, and polyethylenes. Alumina, Zirconia, HAp, bioactive glasses, porceins, and phosphates are among the ceramics that are used. The metals can either be used as an implant material or alloyed, and the ceramics and polymers are used as coatings. Titanium, silver, palladium, gold, and tantalum are unsuitable for bone implants due to their inability to degrade (Quizhi chen et al., 2015). Stainless steel is less desired because of stress-induced corrosion and fissures, and iron can cause brain cell degeneration. Zinc and magnesium are advantageous because of their degradability (Prasad et al., 2017).

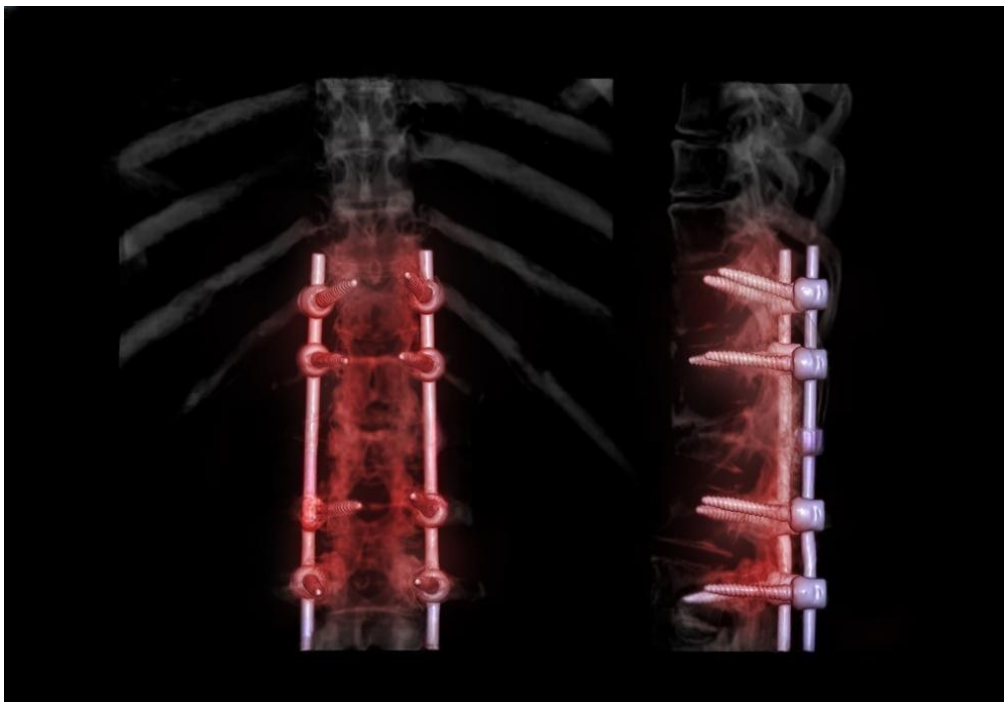


Fig 1.2: Stress induced corrosion caused by metal implants

While stainless steel and titanium alloy fracture fixation devices are helpful in achieving effective therapy, they may also cause a stress shielding effect that causes bone resorption and loosening (Junlei Li et al., 2020). This issue typically arises when there is a significant modulus difference between the implant and the bone, which interferes with fracture repair. An ideal bone implant is supposed to feature a stronger

and durable osseointegration property, a good in vivo timespan, the ability to promote osteoconduction, vascularization, and osteoinduction, the ability to prevent the stress-shielding effect, a good storage stability, etc. To create a bone scaffold, pores must have high porosity and an ideal size because they promote cell proliferation, enter blood vessels, and help create bone tissue—all of which are very beneficial processes (Frank Schulze et al., 2023). Implants made of pure Magnesium deteriorate before the fracture heals entirely.

Although pure magnesium-based implants are biocompatible, naturally biodegradable, and have favourable mechanical qualities, their lack of durability hinders wound healing and is the main factor contributing to local hydrogen build-up. It is also well known that magnesium has weak antimicrobial properties. Due to their higher specific strength, Young's modulus, which is almost identical to that of human bone, naturally degradable nature, biocompatibility, osteopromotive property, plasticity, stiffness, etc., magnesium alloys are said to be beneficial (Meysam Nasr Azadani et al., 2022). There's no need for more surgery because magnesium alloys melt, absorb, ingest, or are eventually expelled. They don't cause the patient any toxicity or adverse effects. Graphene, the fundamental constituent of graphene quantum dots, nanofibers, 3D graphite, and numerous other carbon allotropes, is the strongest and thinnest 2-D nanostructured allotrope (Jodati et al., 2021). Strong oxidizers are used to extract graphene oxide, which is a highly oxidised form of graphene. Reduced graphene oxide can be generated using methods such as microwave, thermal, chemical, and bacterial reduction (Vishali Gupta et al., 2017). Scaffolds based on graphene oxide have demonstrated encouraging outcomes in tackling the prevalent research issue of insufficient bone regeneration. Because graphene and graphene oxide are two-dimensional materials, they can lessen the harmful consequences of implants. Furthermore, they promote osteogenesis and show improved osteogenic performance, which is excellent. Their exceptional mechanical strength, excellent osteoconductive efficiency, large surface area, and exceptional adaptability are further attributes (Shadjou et al., 2018). Zinc has the potential to mitigate the primary drawback of magnesium, which is its low strength (less than 350 MPa). By strengthening osteogenesis and integration, alloying zinc with magnesium can increase its mechanical integrity and biocompatibility.

Hydroxyapatite is a key component of bone's inorganic matrix, which gives tissues rigidity and durability. Hydroxyapatite can replicate the structure of natural

bones when utilised in bone healing applications, encouraging cell adhesion, proliferation, and eventually bone regeneration. Because of its osteoconductivity and biocompatibility, it's a great substance for encouraging bone mending and integration with surrounding tissues. Furthermore, as healing advances, hydroxyapatite can progressively break down, allowing new bone tissue to grow in its place and speeding up the process of bone regeneration (Kinga pielichowska et al., 2010).

Pectin is an innate polysaccharide derived from plant cell walls that has a chemical structure similar to alginate. It artificially acts as an extracellular matrix to immobilise cells for bone tissue regeneration and can promote enhanced adhesion and proliferation of cells within microspheres (Munarin et al., 2011). It can both stay intact throughout the gastrointestinal tract and be broken down by the bacteria in the colon. Because natural polymers aren't very strong mechanically, filler like graphene oxide can be added (Claudia Lara Espinoza et al., 2018). The above-mentioned features of these materials can greatly assist in meeting the appropriate bone implant criteria.

Biocompatibility testing is conducted to ensure that orthopaedic implants meet criteria and are a viable alternative in clinical settings. The fabricated bone implants were evaluated for antibiofilm qualities, antibacterial capabilities, platelet adhesion, cellular adhesion, corrosion resistance, and hemocompatibility.

CHAPTER-2

2. REVIEW OF LITERATURE:

2.1 MAGNESIUM ALLOYS AND THEIR PROPERTIES:

Magnesium alloys have sparked increased attention in the construction of bone implants since their young's modulus is strikingly close to that of human bones. The Young's modulus of human bone ranges between 15 and 30 GPa, whereas that of 0comparable to human bone, which has a density of 1.75 g/cm³ (Yasamin Zamani et al., 2021). Magnesium alloys have an elasticity limit of 100-200 MPa, whereas bone has a limit of 130-180 MPa and a fracture toughness of 3-6 MPa/m^{0.5}. The elastic modulus of bone is 3-20 GPa, that of magnesium is 41-45 GPa, while titanium alloys have a greater elastic modulus (Pishgahi Fard Zahra et al., 2018). Magnesium-based alloys deteriorate more rapidly in acidic or neutral environments due to electrochemical reactions with water that produce hydrogen and magnesium oxide but do not protect the magnesium matrix from further corrosion (Liangyu Wei et al., 2023). The rate of degradation of magnesium implants can be reduced by alloying, heating, or surface treatment. Moreover, by applying coatings or other surface treatments, magnesium alloys can be designed to encourage osseointegration. One significant advantage of alloying is its ability to improve the mechanical strength and corrosion resistance of magnesium implants. Alloying enables the control of grain size, phase distribution, and alloy composition, resulting in stronger, ductile, and fatigue-resistant implants. Furthermore, alloying magnesium can reduce the rapid degradation rate seen in pure magnesium implants, increasing their lifespan and providing a more regulated degradation profile that is consistent with the bone healing process.

2.2 ZINC-BASED ALLOYS:

Zinc-based alloys exhibit cathodic reactions when oxygen is reduced and anodic reactions when exposed to a solution. When chlorine ions are present, the insoluble zinc hydroxide that is released functions as a passive layer that dissolves into a salt. This decreases the layer's protective effect, reopening the matrix to the solution and initiating the electrochemical reactions (Calderon et al., 2022). The structure and composition of alloys ultimately determine the rate of degradation. In comparison to other alloys, zinc-based alloys degrade at a modest rate. Zinc when

alloyed, showed a greater reduction in corrosion rates. Zinc can decrease magnesium's corrosion performance by refining the grain, which results in a uniform corrosion surface (Makhlof et al., 2018).

2.3 CHARACTERISTICS OF REDUCED GRAPHENE OXIDE:

Modified Hummer's method is a widely used method for synthesising Graphene Oxide. At the end of the process, we have reduced graphene oxide, which has several applications. Coating magnesium with graphene oxide doped hydroxyapatite significantly enhanced the implant's hydrophilic and biomineralization capabilities, opening the door for a faster rate of deterioration (Safavi et al., 2018). The mechanical characteristics of polymeric scaffolds improved dramatically after introducing graphene oxide at lower concentrations. In general, carbonaceous nanomaterials have larger aspect ratios, higher Young's moduli, and higher thermal activity. One TPa is the intrinsic strength, and the intrinsic strength is about equivalent to 130 GPa. It has a density of only 1 g/cm³. There is an exact surface area of 2630 m²/g. When peroxidase catalyses an enzymatic oxidation reaction, graphene nanoplatelets spontaneously disintegrate. This type of oxygen-supported natural degradation is seen in biological reactions (Dervishi et al., 2013). When combined with magnesium to produce a composite, graphene oxide may be able to prevent corrosion assaults and encourage the production of apatite (Wu et al., 2019). Flexural strength and roughness can both be increased using graphene oxide. Higher antibacterial activity is obtained when graphene oxide, with a sheet size ranging from 0.01 to 0.65 micrometres, is employed in bone implants (Mazinani et al., 2021).

2.4 PECTIN AND ITS PROPERTIES:

Pectin is a polysaccharide that is hydrophilic, meaning it possesses both carboxyl and hydroxyl groups. This property allows it to be highly biocompatible, easily degraded, and least harmful. The hydrophilic groups increase the physical and chemical characteristics of pectin through esterification, transesterification, coordination, and electrophilic addition. Pectin can be used to change the surfaces of implants and other medical equipment. Pectin does, however, also have undesirable corrosive and swelling qualities. For biomedical applications, pectin is highly recommended since it contains Rhamnogalacturonan II, a necessary ingredient to improve biocompatibility (Noreen et al., 2017). Because pectin's polysaccharides are

non-toxic, reasonably priced, and have anti-inflammatory and antibacterial qualities, they are suggested for use in biomedical applications. Research has demonstrated that a higher concentration of pectin in a bone scaffold composed of gelatin and pectin enhances the vitality of the cells (Nguyen et al., 2015). Pectin can be utilized to transport medications to the colon because it can be broken down by microbes there. Increased quantities of graphene oxide demonstrated high support bearing for a nanocomposite including pectin and nanohydroxyapatite (Al-Arjan et al., 2020).

2.5 HYDROXYAPATITE AND ITS PROPERTIES:

Hydroxyapatite (HAp) is a bioceramic material that closely resembles the mineral component of human bone, making it an excellent candidate for bone healing and regeneration applications. Its chemical composition, primarily calcium phosphate, provides a natural affinity for integration into the bone structure (Ressler et al., 2021). When used in orthopedic implants or scaffolds, HA promotes osseointegration, the process by which the implant fuses with surrounding bone tissue, leading to improved mechanical stability and long-term success. It covers around 65% of the inorganic phase of native bone. The density is around 3.22g/cm^3 . It is a crystalline compound made up of closely packed Ca, PO_4 and OH ions that form an appatite structure. Hydroxyapatite has the same calcium-to-phosphate ratio as humans, which is 1.67. The large pH range (4–12) and the 1.67 molar ratio of calcium to phosphorus contribute to their remarkable stability (Shi et al., 2021). One of the key properties of hydroxyapatite is its bioactivity, meaning it can actively participate in biological processes within the body. HAp's surface reacts with bodily fluids, facilitating the formation of a biologically active layer of calcium phosphate, which stimulates osteoblast activity and encourages new bone formation. This bioactive behavior accelerates the healing process and enhances bone regeneration at the implant site. Due to their greater surface energy, which results in superior mechanical qualities, a greater capacity for resorption, and increased activity, HAp nanoparticles are more stable than their bulk counterpart. Moreover, the incorporation of HA into magnesium-based implants can also modulate the release kinetics of degradation by-products, such as magnesium ions, which may have cytotoxic effects at elevated concentrations (Tsakiris et al., 2021). By controlling the degradation rate and altering the local microenvironment, HAp contributes to mitigating potential inflammatory responses and improving overall biocompatibility. Hydroxyapatite is a highly promising material

for bone implantation due to its biocompatibility, bioactivity, osteoconductivity, and corrosion resistance. One notable drawback is the intrinsic brittleness of HA, which might impair the mechanical strength of implants. This brittleness raises the possibility of implant fracture, especially in load-bearing settings when implants are subjected to significant stress. Evidence suggests that HAp nanocoatings on magnesium implants play an important role in cell adhesion and biocompatibility.

2.6 INVITRO BIOCOMPATIBILITY OF MAGNESIUM IMPLANTS INCORPORATED WITH GRAPHENE OXIDE:

2.6.1. ANTIBACTERIAL PROPERTIES:

The incorporation of graphene oxide (GO) into magnesium implants is a viable technique for improving their antibacterial capabilities, notably against common pathogens such as *Staphylococcus aureus* and *Pseudomonas aeruginosa*, which are known to cause implant-related illnesses. Graphene oxide, with its distinctive two-dimensional structure and abundance of oxygen-containing functional groups, has extraordinary antibacterial activity. It disrupts bacterial cell membranes, inhibits adhesion, and induces oxidative stress-mediated bacterial death (Mohammed et al., 2020). When applied to magnesium implants, graphene oxide coatings provide a comprehensive defence mechanism against these bacteria. Studies have shown that graphene oxide-modified magnesium surfaces efficiently limit the adhesion and colonisation of *Staphylococcus aureus* and *Pseudomonas aeruginosa*, lowering the chance of biofilm development, which is a major contributor to chronic infection (Kulshrestha et al., 2015).

Somayeh *et al.*, tested the antibacterial activity of ZM31-xRGO composite (where x represents various graphene oxide concentrations, ranging from 0 to 1.5 weight percent and ZM31 stands for Mg-3Zn-1Mn), using *Staphylococcus aureus* and *Escherichia coli* by agar diffusion method, where the controls were inoculated with 1×10^4 CFU/mL of both the bacteria. The inhibition zones formed around the composites placed in an agar medium inoculated with the bacteria in an incubator at room temperature for one day were checked with their respective controls. The results showed significant antibacterial activity after the encapsulation of reduced graphene oxide at a concentration of 1.5 wt % (Abazari et al., 2021). Safari N et al.; studied the antibacterial activity of Magnesium- 1 Aluminium- Copper based implant against bacteria like *Escherichia coli*, *Staphylococcus aureus*, etc., and found that addition of

Graphene oxide at a weight% of 0.18 significantly increased the diameter of the inhibition zone (Safari *et al.*, 2020). *Staphylococcus aureus* has been reported as the most common bacteria in wounds that require surgical intervention and hence has been used in various studies (Zhang *et al.*, 2020). The other pathogens are *Escherichia coli* and *Pseudomonas aeruginosa*. Hydroxyapatite when doped with Zinc showed considerable antibacterial activity MSCs, MRC-5 and rat primary osteoprogenitor cell lines (Szurkowska *et al.*, 2018). Sinulingga *et al.*, have proven that 2.5 Mg-2.5 Ag-HAp and 2.5 Zn-2.5 Ag- HAp showed significant antimicrobial effectiveness against *Escherichia coli* (Sinulingga *et al.*, 2021). Gopi *et al.*, have reported improved antibacterial performance, when tested with strains of *S.aureus*, *C.albicans*, *E. coli*, etc., when pectin-doped HAp nanocomposite is used to implant bones (Gopi *et al.*, 2015). Zhang C *et al.*, demonstrated that 2 Mg-0.5 Zn-Ca-based bone implant greatly reduced surface bacterial adhesion more efficiently than pure magnesium (Zhang *et al.*, 2019). Qu X *et al.*, have established and proven that Zn-2Ag alloy can greatly minimize bacterial adhesion and the biofilm formation significantly (Qu *et al.*, 2021).

Furthermore, graphene oxide coatings allow for the regulated release of antibacterial chemicals, which increases their potency against certain infections (Aunkor *et al.*, 2020). The synergistic effects of magnesium and graphene oxide lead to increased antibacterial activity through processes such as metal ion release and reactive oxygen species production, which target bacterial cells via various pathways (Kumar *et al.*, 2019). However, optimising the graphene oxide loading while also assuring the composite implant's long-term durability and biocompatibility remain critical issues. Nonetheless, incorporating graphene oxide into magnesium implants shows significant promise for generating new biomaterials with improved antibacterial characteristics, which is critical for reducing the risk of implant-associated infections caused by *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Sahoo *et al.*, 2022).

2.6.2. HEMOCOMPATIBILITY:

The hemocompatibility of magnesium implants containing graphene oxide (GO) is crucial for their successful use in biomedical devices, notably in orthopaedic and cardiovascular applications. Magnesium alloys, recognised for their biodegradability and mechanical qualities similar to natural bone, have sparked substantial attention as implant materials. However, due to their high corrosion rate and probable negative effects on blood compatibility, changes are required to improve hemocompatibility (Liu et al., 2018). Graphene oxide, with its distinct physicochemical features, represents a possible option. GO coatings on magnesium implants not only improve corrosion resistance but also provide good hemocompatibility properties (Tong et al., 2022). Graphene oxide improves hemocompatibility by decreasing platelet adhesion and activation, which reduces thrombogenicity and the risk of thrombus formation (Singh et al., 2011).

ASTM standards suggest that the samples with a hemolysis rate below 2 % are preferable and a Graphene Oxide-Titanium-Silk Fibroin-Hydroxyapatite-based nanocomposite was found to have a hemolysis rate ranging from 0 to 0.3% hence it is safe to use (Li M et al., 2016). Yuan et al., have reported that Zn-1Mg-based alloys can provide excellent hemocompatibility, where the rate of hemolysis is below 0.2% (Chen YW et al., 2021). Escudero et al., tested the hemocompatibility of Graphene oxide on a cobalt and chromium-based alloy using J774A (mouse macrophage) and found that graphene oxide that has been synthesized by electrochemical reduction can increase the biocompatibility of the alloy (Escudero et al., 2020). Zinc in its purest form as well as in combination with Graphene-based nanosheets showed hemolysis less than 2 % and hence are biocompatible (Fan et al., 2022).

Graphene oxide's antifouling properties inhibit protein adsorption and blood coagulation, favouring the creation of a persistent, non-thrombogenic blood-mimicking layer on the implant surface (Staneva et al., 2021). Moreover, the biocompatibility of graphene oxide ensures little cytotoxicity to blood components, allowing them to interact with the implant surface without causing unfavourable reactions. Furthermore, the regulated release of bioactive molecules enabled by GO coatings improves hemocompatibility by encouraging endothelialization and decreasing inflammation. Despite the promising results, challenges such as optimising graphene oxide loading and ensuring long-term stability and biocompatibility of composite implants necessitate additional research to fully realise the potential of magnesium implants

incorporating graphene oxide for improved hemocompatibility in biomedical applications.

2.6.3. IN VITRO LEACHING:

Slow degradation preserves the structural integrity of implants, whereas rapid degradation harms bone and tissue cells due to non-uniform disintegration and gas release (Hassan et al., 2022). The simulated bodily fluids are generated using the methodology established by Kokubo et al. Chen K et al. investigated the deterioration rates of 2Sr-Mg, 2Sr-Mg-Ca, and 2Sr-Mg-Zn alloys and discovered that alloys containing Magnesium provided excellent corrosion resistance (Chen K et al. 2020). Yuan et al. demonstrated that the Zn-1Mg alloy can boost corrosion and leaching resistance (Chen YW et al., 2021). Bavya Devi K et al. evaluated the degradation of MgS in vitro in an alloy of 0.5Zn-Mg in SBF and discovered improved degradation capacity (Devi et al. 2018). Bodhak et al. discovered that when HAp is electrically polarised, cell attachment increases and tissue in-growth accelerates, demonstrating its osteogenic nature (Bodhak et al., 2011). Rhamnogalactouronan II in pectin can enhance cell adherence (Gurzawska et al., 2014). Yuan et al. showed that using Zn-1Mg alloys can jointly promote cell adhesion and proliferation (Yuan et al., 2022). Chen D et al. found that when MC3T3-E1 cells were grown on Magnesium and Zinc alloy implants, they attached more easily than to Poly-L lactic acid-based implants. After 30 days of immersion, SBF showed a 15% reduction in weight loss when 5 wt. % HAp was combined with Magnesium and Zinc. After 24 days of immersion, SBF showed a 27% reduction in weight loss when 1 wt% HAp was combined with 3 wt. % Zinc and 0.5 wt. % Zirconium. After 4 days of immersion in PBS with 5 wt% HAp and Magnesium, weight loss was reduced by 75% (Jaiswal et al., 2019).

CHAPTER-3

3. AIM AND OBJECTIVE:

- To increase the durability of Magnesium implants that are being used for bone regeneration.
- Utilizing Graphene Oxide, Zinc, Hydroxyapatite and Pectin in the fabrication process of implants.
- To study the Biocompatibility of the prepared implants materials.

CHAPTER-4

4. MATERIALS AND METHODS:

4.1. MATERIALS AND PROCESSING:

Sigma-Aldrich, St. Louis, MO, USA, provided magnesium powder with a purity of $\geq 99\%$ (assay $\geq 99\%$, Fe ≤ 500 mg/kg) as the foundation material. Zinc (particle size $< 45\ \mu\text{m}$) acquired from Sigma-Aldrich, Canada, Oakville, Ontario is employed as one of the components. The Modified Hummer's method was used to synthesize graphene oxide nanosheets. The precursors used were graphite (Assay $\geq 99.50\%$) from HiMedia, Mumbai, India, and NaNO_3 (Assay $\geq 99\%$) from Merck Lifes cience Pvt. Ltd., Mumbai, India. Hydroxyapatite was synthesized utilising the Low molecular weight Heparin technique, with K_2HPO_4 (assay $> 99.5\%$) acquired from Sisco Research Laboratories Pvt.Ltd., Mumbai, India, and $\text{CaNO}_3.4\text{H}_2\text{O}$ (assay $>99\%$, pH (5% aqueous solution) - 5 to 7) as precursors.

4.2. GRAPHENE OXIDE PREPARATION BY MODIFIED HUMMER'S METHOD:

In a beaker, 0.5 g of graphite and 0.25 g of NaNO_3 were combined with 11.5 mL of sulfuric acid. The mixture was then placed in an ice bath and agitated constantly for an hour. The temperature is raised to 35°C and maintained for 30 minutes. After adding 23 mL of distilled water, the temperature is maintained at 70°C for 2 hours, with no stirring. The created graphene oxide nanosheets were washed five times with diluted HCl and distilled water before being placed in a vacuum oven for 24 hours. The cleaning procedure comprised adding 70 mL of distilled water and 5 mL of 10% v/v H_2O_2 (Bansal et al., 2022).

4.3. HYDROXYAPATITE PREPARATION BY LOW MOLECULAR WEIGHT HEPARIN METHOD:

The mixture of 1.67 g of $\text{CaNO}_3.4\text{H}_2\text{O}$ and 10 g of K_2HPO_4 were added to 100 mL of distilled H_2O and was agitated for six hours at room temperature. The mixture was mixed with approximately 69 mg of heparin and swirled until a gel was formed. The solution is dried in an oven at 80°C , and the dried gel is then placed in a silica crucible and calcined for 6 hours at 400°C in a muffle furnace (Rajeshwari et al., 2013).

4.4. FABRICATION BY POWDER METALLURGY METHOD:

Different concentrations of the synthesized Graphene oxide (0.1, 0.5 and 1 wt %), HAp, Zinc and Magnesium were ground together in a mortar and pestle and 0.05 g of the mixture is taken and pressed in a pelletizer machine (Figure 4.1) to create implant implants of 8 mm diameter. The prepared implants were then sintered at 350°C for 4 hrs in a muffle furnace and then coated with 10 wt % pectin using dip coating method, where the implants were dipped in 10 wt % pectin for 1 hr, followed by drying in an oven for 1 hr until completely dry (Sabeti et al., 2021).



Fig 4.1: Pelletizer machine

The powder metallurgy procedure aids in the fabrication of the implants. This was attributed to better sintering mechanisms, such as tight packing of irregular particles and the removal of barriers from the sintering route by removing oxide coatings on the particle surfaces. The resulting pellets had accurate density and thickness because the powder was pressed with sufficient pressure (100 ton) and sintered at the optimum temperature (350°C).

4.5. BIOCOMPATIBILITY ASSESSMENTS:

4.5.1. ANTIBACTERIAL ACTIVITY:

The process of biofilm formation involves bacteria growing and adhering irreversibly to a surface, producing extracellular polymers that aid in matrix production and adhesion. This changes the organism's phenotype, affecting growth rate and gene transcription (Donlan et al., 2001). *P. aeruginosa* and *S. aureus* broth cultures were added to fresh medium, discs cleaned with phosphate buffer saline were added, and the mixture was incubated for 24 hours at 37°C on an orbital shaker. The samples were suspended in a 96-well plate, the optical density of adhered cells was measured at 570 nm with the Synergy H1 Biotek, USA microplate reader (Figure 4.2), and antibacterial activity was evaluated.

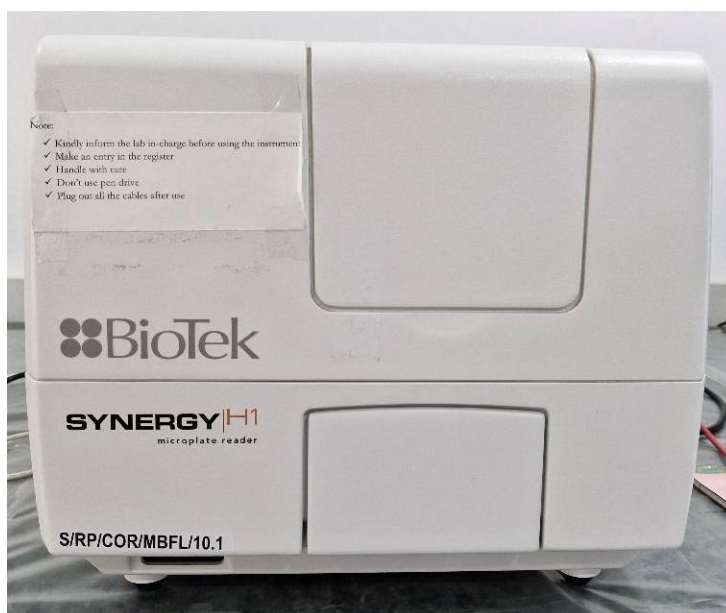


Fig 4.2: Microplate reader

4.5.2. BACTERIAL ADHESION STUDIES:

S. aureus and *P. aeruginosa* broth culture was added to fresh medium and then transferred onto the UV sterilized pellets that were placed in the 24 well plate. The setup was incubated for 24 hours at 37°C on an orbital shaker. SEM imaging was performed on Pure Mg, Mg with 1 wt % GO and Mg with 1 wt % GO + 10% pectin.

4.5.3. CORROSION STUDIES:

Cylindrical samples with a diameter of 8 mm were immersed in Hank's balanced salt solution for 96 hours. The arrangement in Figure 4.6 was kept at 37°C to approximate the temperature of the human body. The sample dimensions were 8 mm in diameter. The solution-to-sample ratio was maintained at 20 mL-1 cm² (Waizy et al., 2012). The optical density readings were obtained by suspending the solution in a 96-well plate and inserting it into the microplate reader. The OD was recorded at 559 nm.

4.5.4. HEMOLYSIS ASSAY:

Add 5% (v/v) sterile defibrinated blood warmed to room temperature to sterile Blood Agar Base, which has been melted and cooled to 45 to 50°C To properly blend, spin the flask while avoiding the formation of bubbles. Pour the mixture into sterilised plates, avoiding surface froth and bubbles. This approach requires cooling the agar and reheating the blood (Buxton et al., 2005). Hot agar cannot be used in hemolysis assays because high temperatures can denature red blood cells and alter their properties, interfering with hemolysis detection, whereas cold temperatures can affect blood viscosity and coagulation properties, making it difficult to prepare uniform blood agar plates with consistent hemolysis characteristics (Sæbø et al., 2023).

100 mL of blood agar was prepared and poured onto four sterilised plastic petri dishes, with each plate divided into two parts. Each partition received 3 pectin-coated implants with 3 different concentrations of graphene oxide (0.1, 0.5, and 1 wt %), as well as 3 uncoated implants containing 3 different concentrations of graphene oxide. The fourth plate was treated with pure magnesium to highlight the significance of the new development. The examination of hemolytic activity was performed macroscopically after the incubation duration of 24 hours at 37°C (Lima et al., 2022).

Alpha hemolysis displays a partial breakdown of red blood cells, resulting in a greenish discoloration. Greenish appearance is due to the conversion of hemoglobin to methemoglobin, while beta hemolysis involves complete lysis of red blood cells (Lad et al., 2022).

CHAPTER-5

5. RESULTS AND DISCUSSION:

5.1. MATERIAL FABRICATION:

5.1.1. GRAPHENE OXIDE:

Graphene Oxide Nanosheets were prepared using Modified Hummer's technique. The synthesized Graphene Oxide nanosheets were washed using distilled water and HCl. They were then placed in a vacuum oven for 24 hrs to make them devoid of any contamination.



Fig 5.1: Synthesized Graphene Oxide Nanosheets

5.1.2. HYDROXYAPATITE:

Hydroxyapatite was prepared using the Low Molecular Weight Heparin method. It involves the hydrolysis and condensation of precursor compounds (calcium nitrate and dipotassium phosphate) with the addition of heparin in a liquid solution to form a sol. Which is then dried and calcinated to produce Hydroxyapatite.



Fig 5.2: Prepared Hydroxyapatite nanoparticles

5.1.3. FABRICATED IMPLANTS:

The implants were prepared and fabricated with the addition of various concentrations of GO (0.1 wt %, 0.5 wt % and 1 wt %) and then pelletized in 8mm pellets. These pellets were sintered in a muffled furnace to create an implantable pellet (Figure 5.3 (a)). The pellets were then coated with 10 wt % pectin (Figure 5.3 (b)).



(a)



(b)

Fig 5.3: Fabricated Implants: (a) Uncoated implant and (b) Coated implant

5.2. CHARACTERIZATION:

5.2.1. OPTICAL MICROSCOPIC IMAGING:

An optical microscope, often known as a light microscope, uses one or more lenses to magnify visible light images of small materials. The lenses are positioned between the sample and the viewer's eye to expand the image and allow for more detailed analysis. The samples were examined under the digital optical microscope with LES 4.0 software (Figure 5.4) to investigate the grain distribution of various samples.



Fig 5.4: Digital optical microscope

5.2.1.1 UNCOATED IMPLANT:

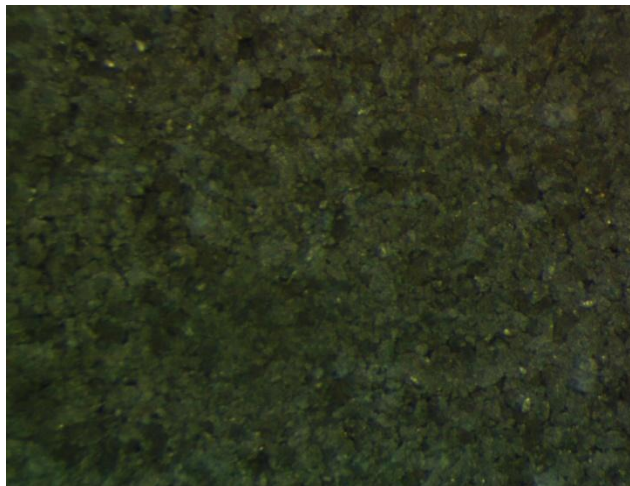


Fig 5.5: Optical microscopic image of Pure Mg

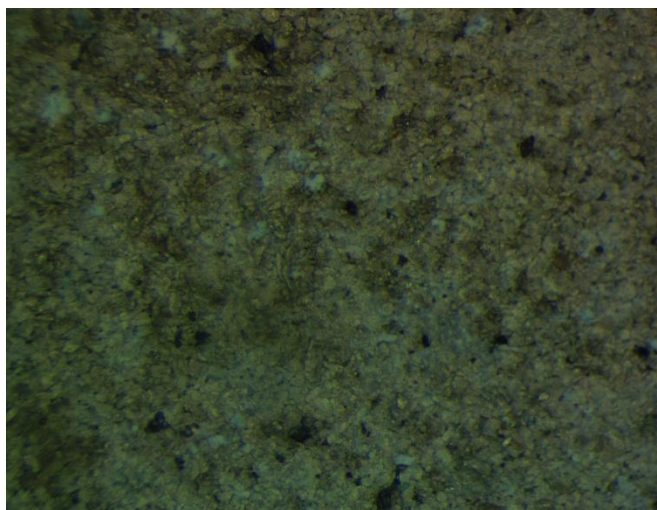


Fig 5.6: Optical microscopic image of Mg with 1 wt % GO

5.2.1.2 COATED IMPLANT:

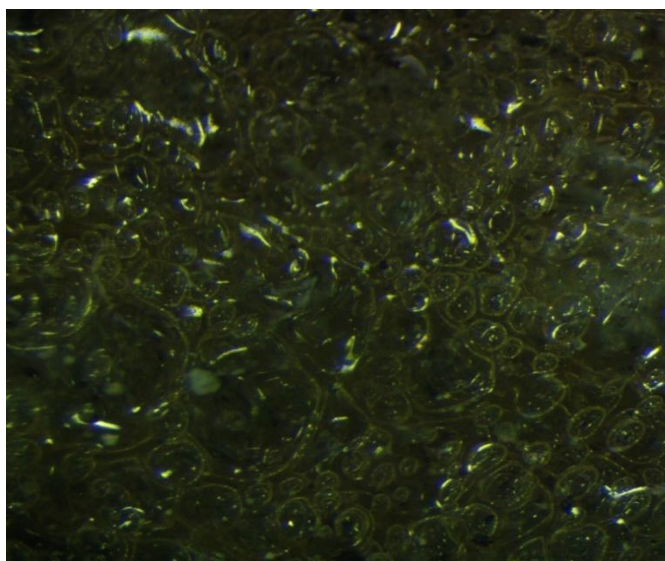


Fig 5.7: Optical microscopic image of pectin coated implant

The optical images were utilized to analyze the surface of implants (pure Mg, Mg with 1 wt % GO and Mg with 1 wt % GO coated with pectin). The images of pure Mg and Mg with 1 wt % GO confirmed an even grain distribution with a smooth surface as seen in Figure 5.5 and Figure 5.6. The pectin coated sample (Figure 5.7) shows a

properly coated surface with minimalistic air gaps which can be further optimized to enhance implant's performance.

5.2.2. SEM ANALYSIS:

SEM analysis is a microscope technique for obtaining high-resolution pictures of surface morphology. SEM analysis of magnesium-based bone implants can reveal surface topography, corrosion behaviour, and bacterial adherence (Shah et al., 2019). This helps to optimize implant design, evaluate biocompatibility, and understand the factors that influence implant performance.

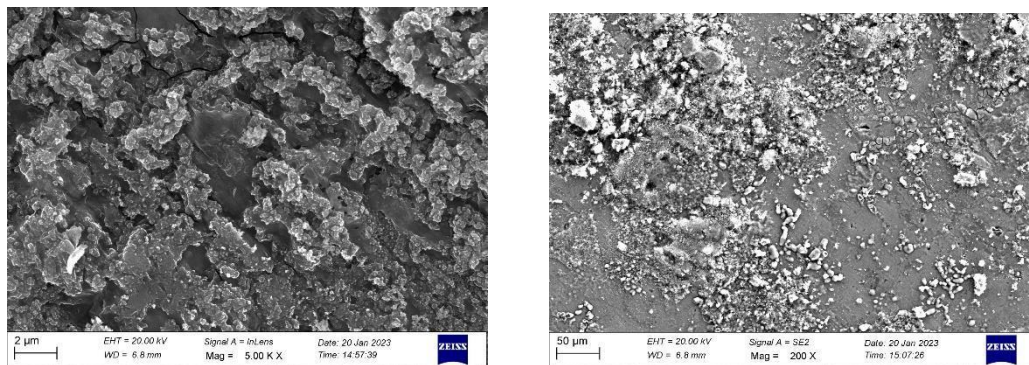


Fig 5.8: SEM images of uncoated sample

The SEM images reveal homogeneous dispersion of all included components, including Graphene Oxide, Hydroxyapatite, zinc, and Magnesium. A reasonable distribution can be observed (Figure 5.8), indicating that the sintering process successfully broke down the big particles into little clusters.

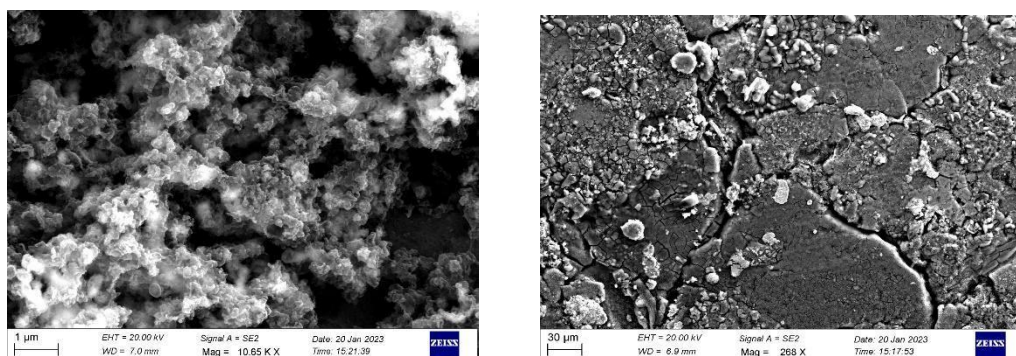


Fig 5.9: SEM images of coated sample

The consistent incorporation of the pectin coating on the implant's surface is verified through the SEM images (Figure 5.9). This can be attributed to proper dip coating and drying process of pectin to attain pectin coat throughout implant's surface.

5.3. LEACHING ASSAY:

The leaching assay was performed by immersing the implants in HBSS, which simulates the interaction between the implant and human fluids, evaluating parameters such as corrosion resistance and leaching. This controlled environment allows for an accurate assessment of the implant's effectiveness and potential effects on neighbouring tissues (Eliaz et al., 2019).

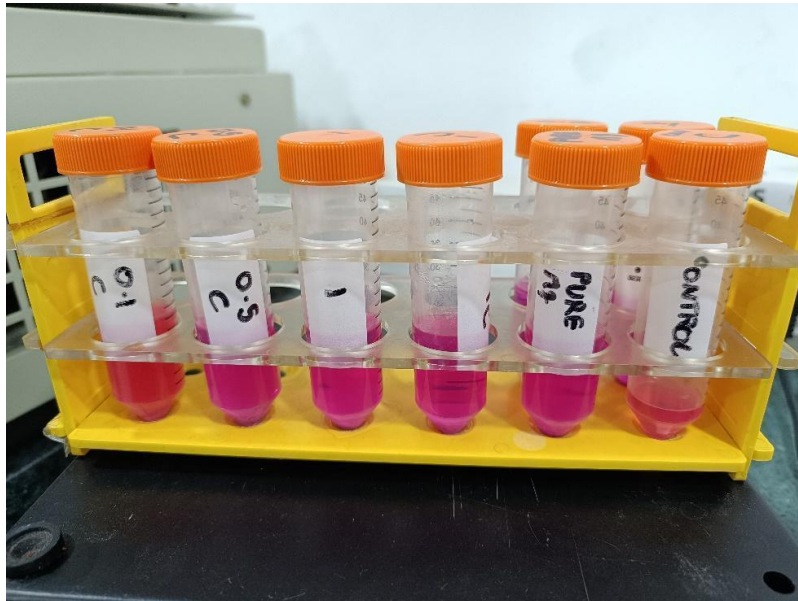


Fig 5.10: Implants immersed into Hank's balanced salt solution for leaching assay

After two weeks, there was a noticeable difference in the colour of the solution containing coated and uncoated implants, demonstrating the endurance of implants coated with 10 wt % pectin. This can be attributed to pectin coating on the implants for their enhanced biocompatibility, controlled degradation and reduced corrosion rate.

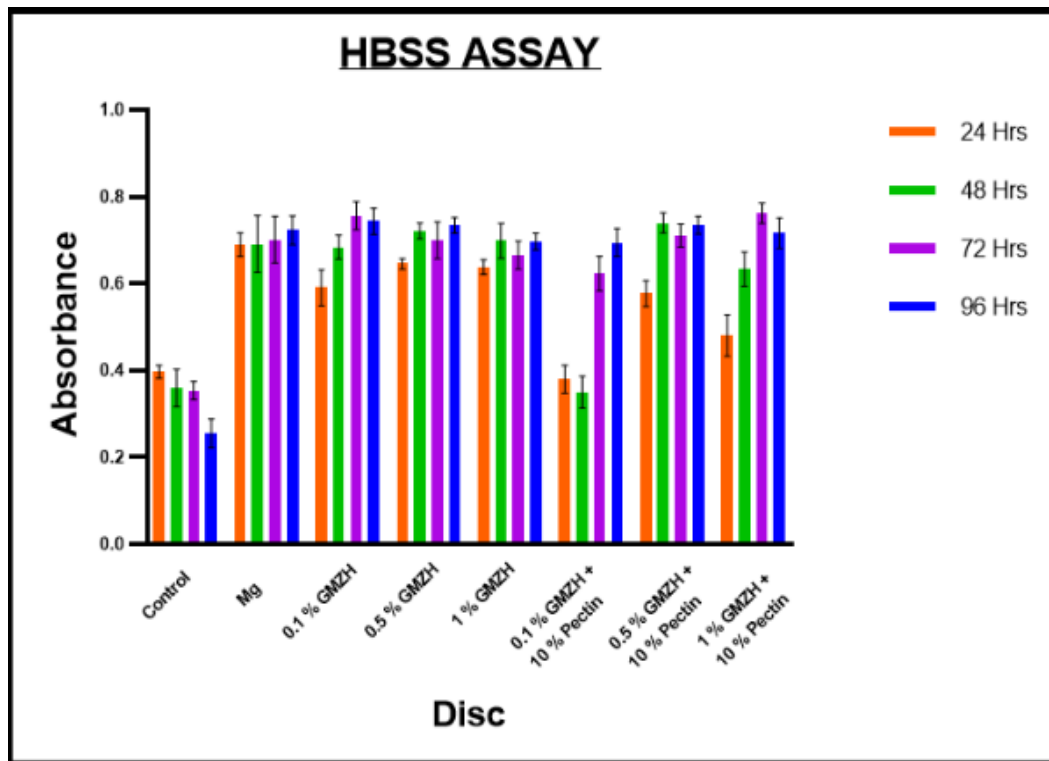
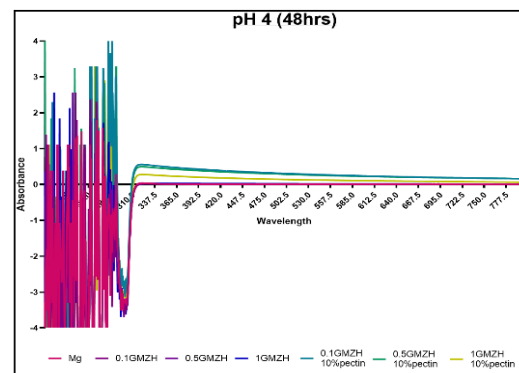
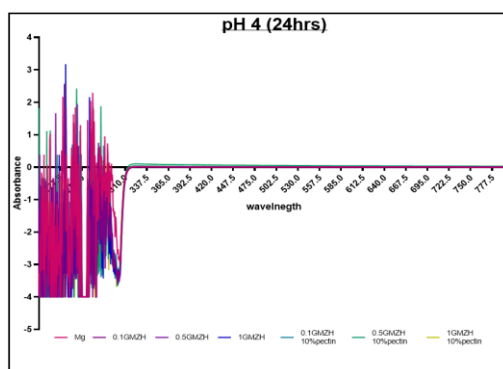


Figure 5.11: Leaching Assay (Plot between wet weight and absorbance)

A plot between days and absorbance (Figure 5.11) indicated that the leaching rates of implants with 0.1 wt % Graphene Oxide without coating, 0.1 wt %, and 1 wt % Graphene Oxide which were coated with 10 wt % pectin, are considerably less compared to the implants made with pure Magnesium.

5.3.1. LEACHING ASSAY WITH VARIABLE PH (PH 4 and PH 7):



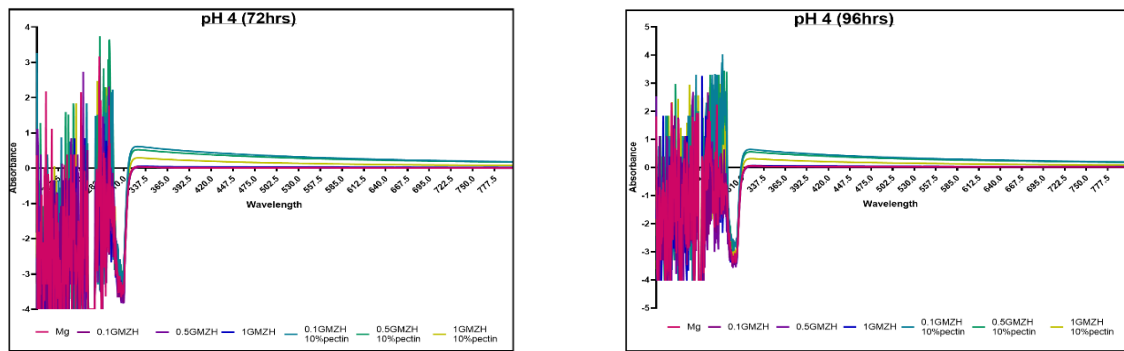


Fig 5.12: Leaching assay (pH 4)

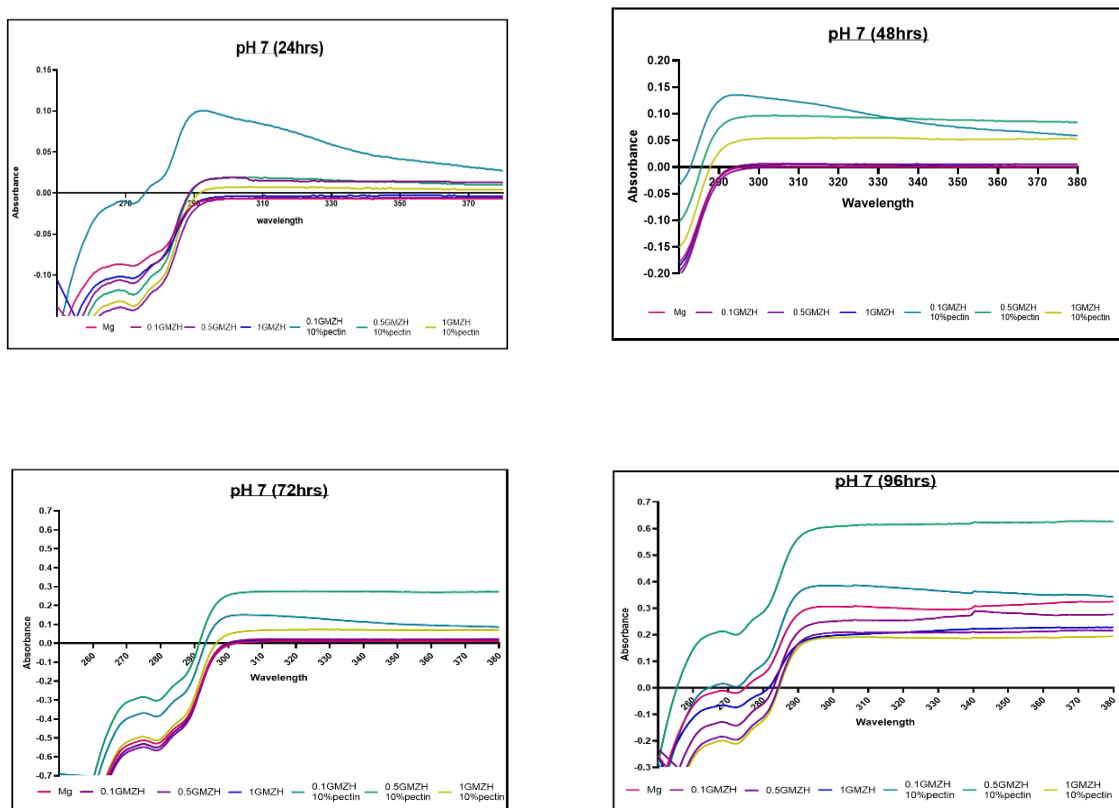


Fig 5.13: Leaching assay (pH 7)

The presence and intensity of peaks indicate the concentration of corrosion products (Gnedenkov et al., 2022). Figures 5.12 and 5.13 demonstrate that the peaks at pH 4 were greater than those at pH 7, showing that corrosion occurs faster at acidic pH. At pH 4 and 7, the absorbance peaked at 300 nm and rose significantly over time, indicating an increase in corrosion products.

5.4. ANTIBACTERIAL ACTIVITY:

The antibacterial activity of Magnesium based bone implants against *P. aeruginosa* and *S. aureus* bacteria was assessed using the Crystal Violet (CV) assay. The CV assay provides a quantitative measure of bacterial viability by staining the bacterial cells on the suspended implants and subsequently measuring the optical density of the stained samples in a microplate reader at 570 nm (Welch et al., 2012).

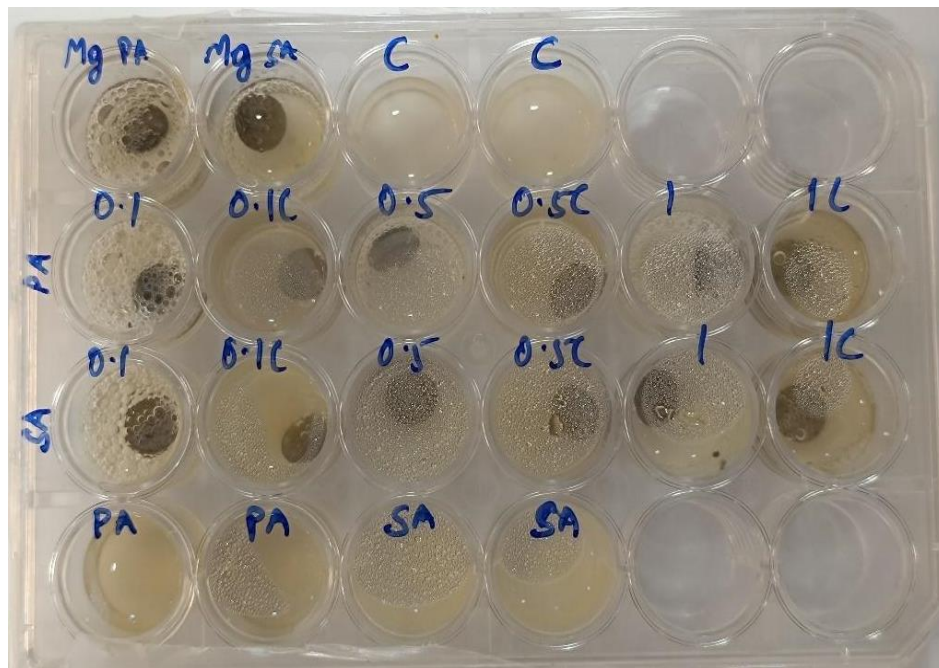


Fig 5.14: Implants suspended in *P. a* and *S. a* bacterial medium

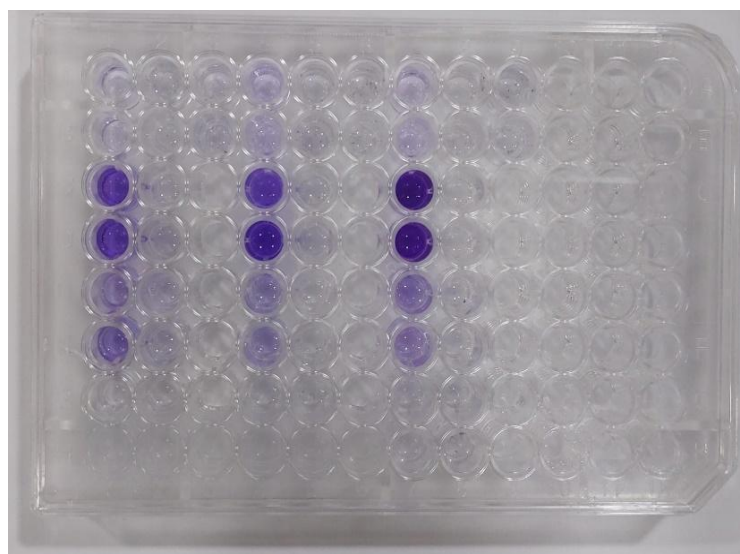


Fig 5.15: CV-stained Bacteria suspended in 96 well plate

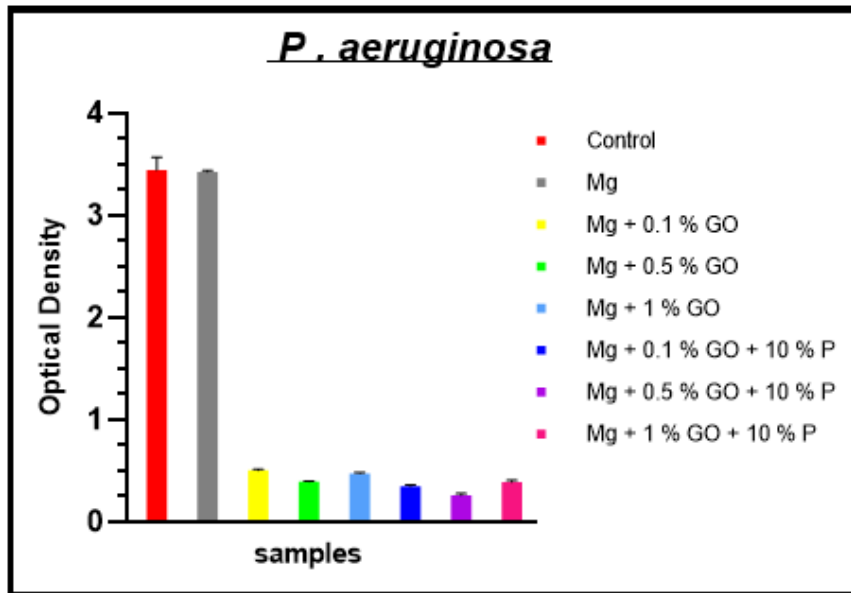


Fig 5.16: Antibacterial activity on *P. aeruginosa*

Gram negative bacteria: Implants made of 0.1 wt % and 0.5 wt % Graphene Oxide coated with pectin, showed a considerable amount of antibacterial activity (Figure 5.16), compared to other samples.

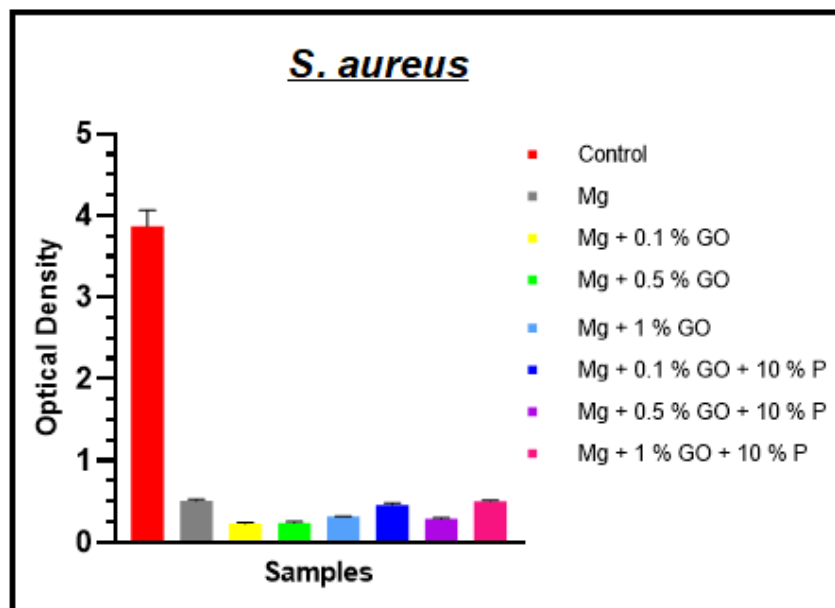


Fig 5.17: Antibacterial activity on *S. aureus*

Gram positive bacteria: Implants made of 0.1 wt % and 0.5 wt % Graphene Oxide, showed a considerable amount of antibacterial activity, compared to other samples as seen in Figure 5.17.

5.5. BACTERIAL ADHESION STUDIES:

Staphylococcus aureus and *Pseudomonas aeruginosa* represent two of the most challenging bacterial pathogens in the context of infections and their ability to adhere to the surface of the materials associated with magnesium-based bone implants. The SEM imaging of such implants provides a valuable insight about the adhesion rate of the bacteria on different magnesium-based implants. The SEM images of the implants were captured using Hi – Resolution Scanning Electron Microscope, Thermoscientific apreo S, USA (Figure 5.18).

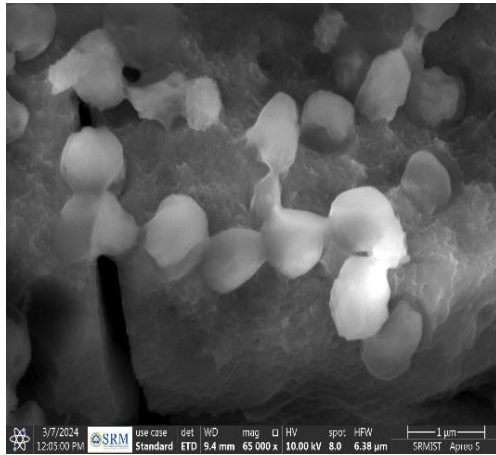


Fig 5.18: High Resolution Scanning Electron Microscope

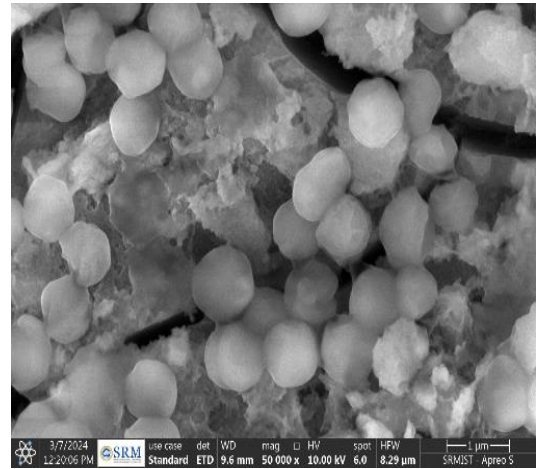


Fig 5.19: P. a and S. a bacterial adhesion on the implants

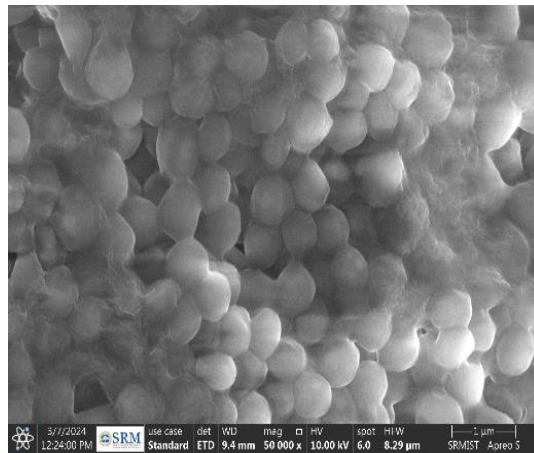
5.5.1. *S. aureus* ADHESION:



(a)



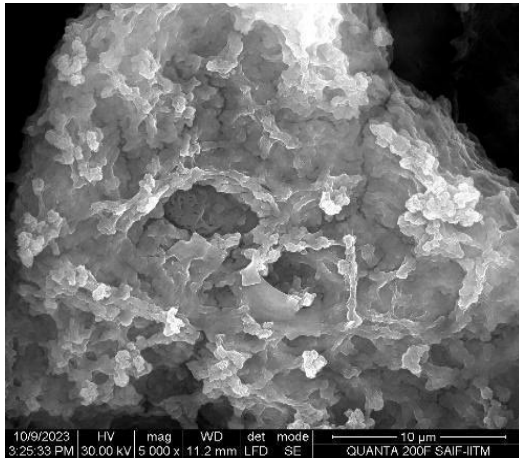
(b)



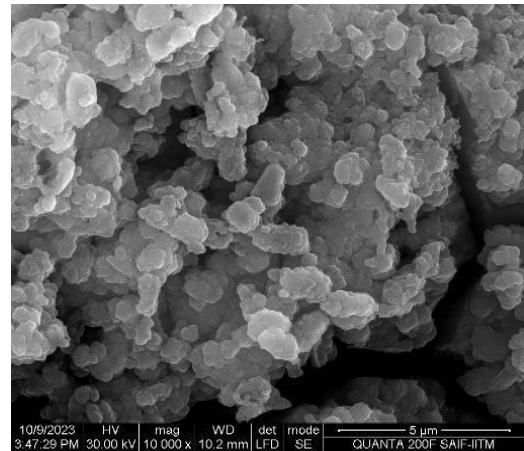
(c)

Fig 5.20: SEM images of adhered *S. aureus* on implants: (a) Pure Mg, (b) Mg with 1 wt % GO and (c) Mg with 1 wt % GO coated with pectin.

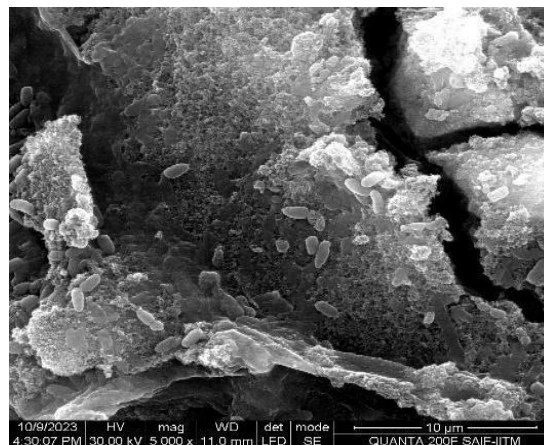
5.5.2. *P. aeruginosa* ADHESION:



(a)



(b)



(c)

Fig 5.21: SEM images of adhered *P. aeruginosa* on implants: (a) Pure Mg, (b) Mg with 1 wt % GO and (c) Mg with 1 wt % GO coated with pectin.

The SEM analysis on the adhesion of *S. aureus* and *P. aeruginosa* reveals that compared to pure Mg and Mg with 1 wt % GO sample, the sample of Mg with 1 wt % GO which was coated with pectin had great adhesion rate of *S. aureus* bacteria (Figure 5.20) but in contrast to that the bacterial adhesion of *P. aeruginosa* was greater in samples made of Pure Mg and Mg with 1 wt % GO when compared with the sample of Mg with 1 wt % GO coated with pectin (Figure 5.21). This can be attributed to factors

like surface characteristics of the samples such as roughness and porosity which can enhance and provide a microenvironment that facilitates the attachment of bacteria.

5.6. HEMOLYSIS ASSAY:

Assessing the hemolytic activity on Magnesium based bone implants placed in blood agar involves incubating the implants in blood agar plates to evaluate their potential to induce hemolysis, the rupture of red blood cells (Lima et al., 2022). This involves placing the implants onto the surface of blood agar plates and incubate them under appropriate conditions as shown in the Figure 5.22.

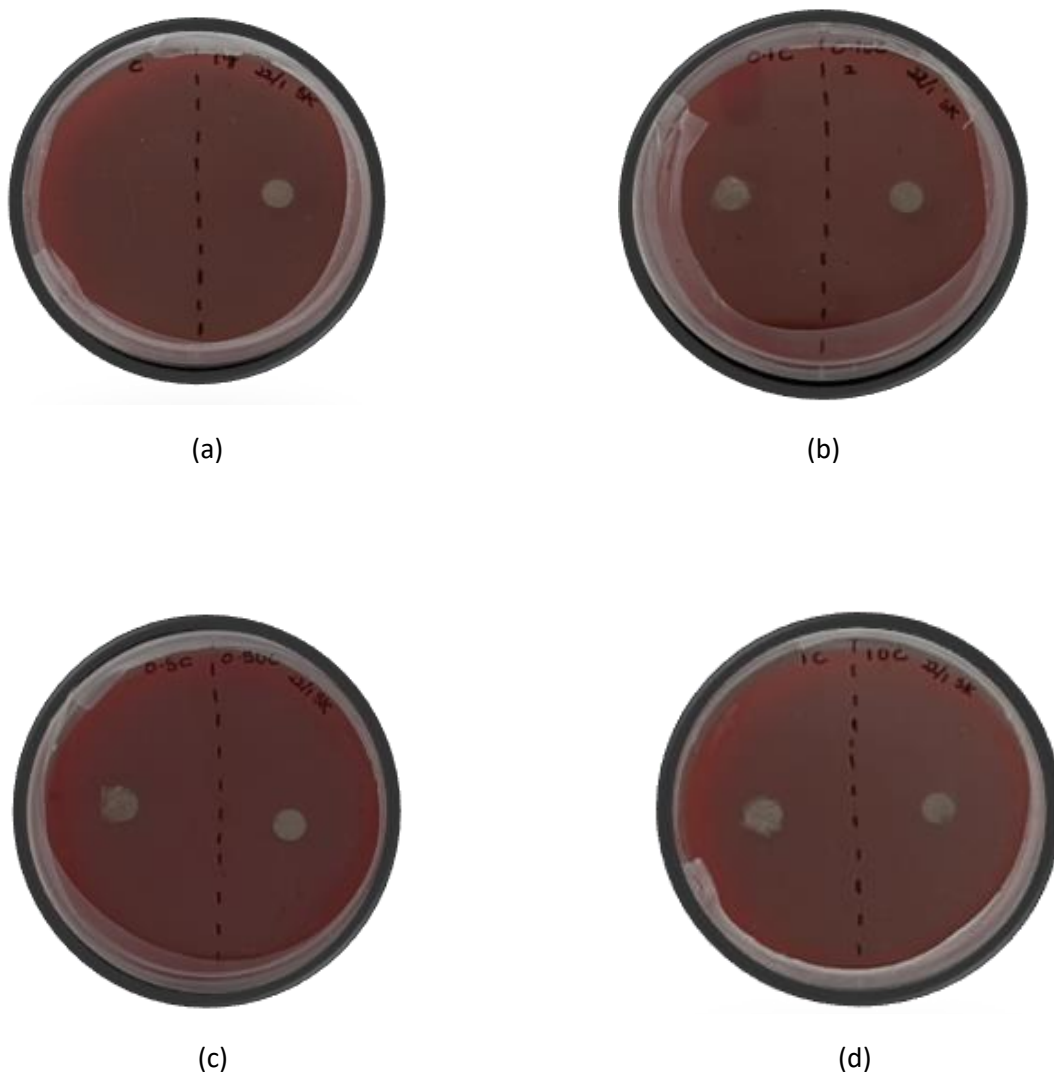


Fig 5.22: Implants suspended on Blood Agar for Hemolysis Assay: (a) pure Mg/ control, (b) Mg with 1 wt % GO Coated/ Uncoated, (c) Mg with 0.5 wt % GO Coated/ Uncoated, (d) Mg with 1 wt % GO Coated/ Uncoated.

5.6.1. OBSERVATIONS AFTER 24 HOURS:

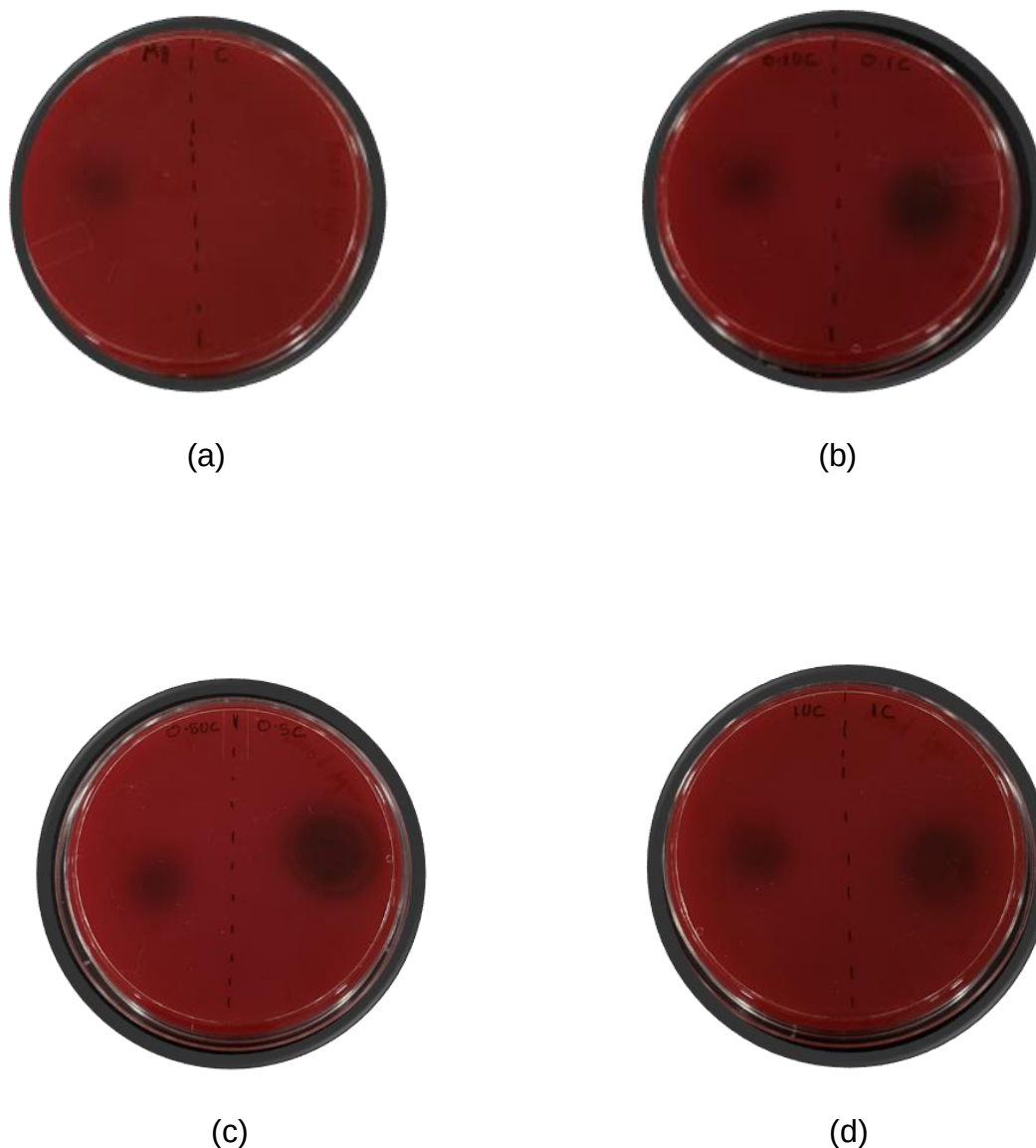


Fig 5.23: Hemolysis assay observations after 24 hours: (a) pure Mg/ Control, (b) Mg with 0.1 wt % GO Coated/ Uncoated, (c) Mg with 0.5 wt % GO Coated/ Uncoated, (d) Mg with 1 wt % GO Coated/ Uncoated

All plates treated with implants made of three different concentrations of Graphene Oxide (0.1 wt %, 0.5 wt %, and 1 wt %) coated with 10 wt % pectin as well as the uncoated samples showed α hemolysis (Figure 5.23). This indicates the partial destruction of red blood cells caused by the release of Hydrogen peroxide from the implants, thus oxidising the haemoglobin present in blood. As a result, gas bubbles

may form in the circulation, potentially leading to blood vessel blockage. H_2O_2 can also destroy or damage red blood cells, causing hemolysis. H_2O_2 observed may also affect platelets and white blood cells, reducing the body's ability to clot and fight infections (Ghabach and Davarpanah et al., 2020).

CHAPTER-6

6. SUMMARY AND CONCLUSION:

The characterisation results showed that the generated nanoparticles were graphene oxide and hydroxyapatite. Optical microscopy experiments and FESEM analysis revealed that the powder metallurgy technique may minimize gaps in the generated implants and create a homogeneous mixture. The in vitro leaching assay results indicated that coated implants were more durable. When the concentration of graphene oxide was increased, significant antibacterial activity was seen. After one week of monitoring, all implants exhibited α -Hemolysis, with the implant with the highest amount of Graphene oxide showing no signs of oxidation. To summarise, in vitro biocompatibility investigations on the utilisation of graphene oxide integrated magnesium implants for bone regeneration produced promising findings. Adding graphene oxide, zinc, HAp, and pectin coatings to magnesium implants improves their mechanical and biological properties, making them more appropriate for bone regeneration applications. Furthermore, positive hemocompatibility, antibacterial activity, and durability results from in vitro experiments verified the implant's biocompatibility. These results indicate that the proposed revolutionary implant has a strong potential to replace traditional implants in bone regeneration applications. To confirm these results and evaluate the long-term use and safety of the implants, more in vivo research is required.

7. REFERENCES:

- Al-Arjan, W. S., Aslam Khan, M. U., Nazir, S., Abd Razak, S. I., & Abdul Kadir, M. R. (2020). Development of arabinoxylan-reinforced apple pectin/graphene oxide/nano-hydroxyapatite based nanocomposite scaffolds with controlled release of drug for bone tissue engineering: in-vitro evaluation of biocompatibility and cytotoxicity against MC3T3-E1. *Coatings*, 10(11), 1120.
- Aunkor, M. T. H., Raihan, T., Prodhan, S. H., Metselaar, H. S. C., Malik, S. U. F., & Azad, A. K. (2020). Antibacterial activity of graphene oxide nanosheet against multidrug resistant superbugs isolated from infected patients. *Royal Society open science*, 7(7), 200640.
- Bansal, K., Singh, J., & Dhaliwal, A. S. (2022, February). Synthesis and characterization of Graphene Oxide and its reduction with different reducing agents. In *IOP Conference Series: Materials Science and Engineering* (Vol. 1225, No. 1, p. 012050). IOP Publishing.
- Bigham-Sadegh, A., & Oryan, A. (2015). Basic concepts regarding fracture healing and the current options and future directions in managing bone fractures. *International wound journal*, 12(3), 238-247.
- Buxton, R. (2005). Blood agar plates and hemolysis protocols. *American Society for Microbiology*, 15, 1-9.
- Calderón, J. P., Reyes Barragán, J. L., Barraza Fierro, J. I., Cruz Mejía, H., Arrieta González, C. D., Ravelero Vázquez, V., ... & Rodríguez-Díaz, R. A. (2022). Corrosion behavior of Al modified with Zn in chloride solution. *Materials*, 15(12), 4229.
- Camarero-Espinosa, S., Rothen-Rutishauser, B., Foster, E. J., & Weder, C. (2016). Articular cartilage: from formation to tissue engineering. *Biomaterials science*, 4(5), 734-767.
- Chen, D., He, Y., Tao, H., Zhang, Y., Jiang, Y., Zhang, X., & Zhang, S. (2011). Biocompatibility of magnesium-zinc alloy in biodegradable orthopedic implants. *International journal of molecular medicine*, 28(3), 343-348.
- Chen, K., Xie, X., Tang, H., Sun, H., Qin, L., Zheng, Y., ... & Fan, Y. (2020). In vitro and in vivo degradation behavior of Mg–2Sr–Ca and Mg–2Sr–Zn alloys. *Bioactive Materials*, 5(2), 275-285.
- Chen, Q., & Thouas, G. A. (2015). Metallic implant biomaterials. *Materials Science and Engineering: R: Reports*, 87, 1-57.
- Dervishi, E., Hategekimana, F., Boyer, L., Watanabe, F., Mustafa, T., Biswas, A., ... & Biris, A. S. (2013). The effect of carbon nanotubes and graphene on the mechanical properties of multi-component polymeric composites. *Chemical Physics Letters*, 590, 126-130.
- Donlan, R. M. (2001). Biofilm formation: a clinically relevant microbiological process. *Clinical infectious diseases*, 33(8), 1387-1392.

- Dubey, A., Jaiswal, S., Haldar, S., Roy, P., & Lahiri, D. (2019). Mg-3Zn/HA biodegradable composites synthesized via spark plasma sintering for temporary orthopedic implants. *Journal of Materials Engineering and Performance*, 28, 5702-5715.
- Eliaz, N. (2019). Corrosion of metallic biomaterials: A review. *Materials*, 12(3), 407.
- Escudero, M. L., Llorente, I., Pérez-Maceda, B. T., San José-Pinilla, S., Sánchez-López, L., Lozano, R. M., ... & García-Alonso, M. C. (2020). Electrochemically reduced graphene oxide on CoCr biomedical alloy: Characterization, macrophage biocompatibility and hemocompatibility in rats with graphene and graphene oxide. *Materials Science and Engineering: C*, 109, 110522.
- Ghabach, M., & Davarpanah, A. H. (2020). Hydrogen peroxide poisoning. *The Lancet Gastroenterology & Hepatology*, 5(4), 418.
- Gnedekov, A. S., Sinebryukhov, S. L., Filonina, V. S., Egorkin, V. S., Ustinov, A. Y., Sergienko, V. I., & Gnedekov, S. V. (2022). The detailed corrosion performance of bioresorbable Mg-0.8 Ca alloy in physiological solutions. *Journal of Magnesium and Alloys*, 10(5), 1326-1350.
- Gopi, D., Kanimozhi, K., & Kavitha, L. (2015). Opuntia ficus indica peel derived pectin mediated hydroxyapatite nanoparticles: Synthesis, spectral characterization, biological and antimicrobial activities. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 141, 135-143.
- Gupta, V., Sharma, N., Singh, U., Arif, M., & Singh, A. (2017). Higher oxidation level in graphene oxide. *Optik*, 143, 115-124.
- Jodati, H., Yilmaz, B., & Evis, Z. (2021). In vitro and in vivo properties of graphene-incorporated scaffolds for bone defect repair. *Ceramics International*, 47(21), 29535-29549.
- Kulshrestha, S. (2015). *Nanoparticle induced suppression of microbial biofilm* (Doctoral dissertation, ALIGARH MUSLIM UNIVERSITY ALIGARH (INDIA)).
- Kumar, P., Huo, P., Zhang, R., & Liu, B. (2019). Antibacterial properties of graphene-based nanomaterials. *Nanomaterials*, 9(5), 737.
- Lad, V., Panchal, D., Pithawala, M., Dwivedi, M. K., & Amaresan, N. (2022). Determination of Hemolytic Activity. In *Biosafety Assessment of Probiotic Potential* (pp. 43-46). New York, NY: Springer US.
- Lara-Espinoza, C., Carvajal-Millán, E., Balandrán-Quintana, R., López-Franco, Y., & Rascón-Chu, A. (2018). Pectin and pectin-based composite materials: Beyond food texture. *Molecules*, 23(4), 942.
- Li, J., Qin, L., Yang, K., Ma, Z., Wang, Y., Cheng, L., & Zhao, D. (2020). Materials evolution of bone plates for internal fixation of bone fractures: A review. *Journal of Materials Science & Technology*, 36, 190-208.
- Li, M., Xiong, P., Mo, M., Cheng, Y., & Zheng, Y. (2016). Electrophoretic-deposited novel ternary silk fibroin/graphene oxide/hydroxyapatite nanocomposite coatings on titanium substrate for orthopedic applications. *Frontiers of Materials Science*, 10, 270-280.

- Lima, F. S., Matos, L. F., Pacheco, I. K., Reis, F., Câmara, J. V. F., Pierote, J. J. A., ... & Fialho, A. C. (2022). Scaffold based on castor oil as an osteoconductive matrix in bone repair: biocompatibility analysis. *Polímeros*, 32, e2022003.
- Liu, C., Ren, Z., Xu, Y., Pang, S., Zhao, X., & Zhao, Y. (2018). Biodegradable magnesium alloys developed as bone repair materials: a review. *Scanning*, 2018.
- Makhlof, F. M. S. (2018). *Effect of zinc additions and heat treatment on the microstructure and corrosion properties of the magnesium zinc alloys* (Master's thesis, Fen Bilimleri Enstitüsü).
- Mazinani, A., Nine, M. J., Chiesa, R., Candiani, G., Tarsini, P., Tung, T. T., & Losic, D. (2021). Graphene oxide (GO) decorated on multi-structured porous titania fabricated by plasma electrolytic oxidation (PEO) for enhanced antibacterial performance. *Materials & Design*, 200, 109443.
- Mohammed, H., Kumar, A., Bekyarova, E., Al-Hadeethi, Y., Zhang, X., Chen, M., ... & Rimondini, L. (2020). Antimicrobial mechanisms and effectiveness of graphene and graphene-functionalized biomaterials. A scope review. *Frontiers in bioengineering and biotechnology*, 8, 465.
- Munarin, F., Guerreiro, S. G., Grellier, M. A., Tanzi, M. C., Barbosa, M. A., Petrini, P., & Granja, P. L. (2011). Pectin-based injectable biomaterials for bone tissue engineering. *Biomacromolecules*, 12(3), 568-577.
- Nasr Azadani, M., Zahedi, A., Bowoto, O. K., & Oladapo, B. I. (2022). A review of current challenges and prospects of magnesium and its alloy for bone implant applications. *Progress in Biomaterials*, 11(1), 1-26.
- Nguyen, T. B. L., Min, Y. K., & Lee, B. T. (2015). Nanoparticle biphasic calcium phosphate loading on gelatin-pectin scaffold for improved bone regeneration. *Tissue Engineering Part A*, 21(7-8), 1376-1387.
- Noreen, A., Akram, J., Rasul, I., Mansha, A., Yaqoob, N., Iqbal, R., ... & Zia, K. M. (2017). Pectins functionalized biomaterials; a new viable approach for biomedical applications: A review. *International journal of biological macromolecules*, 101, 254-272.
- Pielichowska, K., & Blazewicz, S. (2010). Bioactive polymer/hydroxyapatite (nano) composites for bone tissue regeneration. *Biopolymers: lignin, proteins, bioactive nanocomposites*, 97-207.
- Pishgahi Fard, Z. (2018). *Development of The Next Generation Ceramics for Orthopaedic Application* (Doctoral dissertation, University of Sheffield).
- Prasad, K., Bazaka, O., Chua, M., Rochford, M., Fedrick, L., Spoor, J., ... & Bazaka, K. (2017). Metallic biomaterials: current challenges and opportunities. *Materials*, 10(8), 884.
- Qu, X., Yang, H., Jia, B., Wang, M., Yue, B., Zheng, Y., & Dai, K. (2021). Zinc alloy-based bone internal fixation screw with antibacterial and anti-osteolytic properties. *Bioactive Materials*, 6(12), 4607-4624.

- Rajeswari, A., Kumar, V. G., Karthick, V., Dhas, T. S., & Potluri, S. L. (2013). Hydrothermal synthesis of hydroxyapatite plates prepared using low molecular weight heparin (LMWH). *Colloids and Surfaces B: Biointerfaces*, 111, 764-768.
- Ressler, A., Žužić, A., Ivanišević, I., Kamboj, N., & Ivanković, H. (2021). Ionic substituted hydroxyapatite for bone regeneration applications: A review. *Open Ceramics*, 6, 100122.
- Saberi, A., Bakhsheshi-Rad, H. R., Karamian, E., Kasiri-Asgarani, M., Ghomi, H., Omid, M., ... & Berto, F. (2021). Synthesis and characterization of hot extruded magnesium-zinc nano-composites containing low content of graphene oxide for implant applications. *Физическая мезомеханика*, 24(1), 62-78.
- Sæbø, I. P., Bjørås, M., Franzyk, H., Helgesen, E., & Booth, J. A. (2023). Optimization of the Hemolysis Assay for the Assessment of Cytotoxicity. *International journal of molecular sciences*, 24(3), 2914.
- Safari, N., Golafshan, N., Kharaziha, M., Reza Toroghinejad, M., Utomo, L., Malda, J., & Castilho, M. (2020). Stable and antibacterial magnesium–graphene nanocomposite-based implants for bone repair. *ACS Biomaterials Science & Engineering*, 6(11), 6253-6262.
- Safavi, M. S., Walsh, F. C., Surmeneva, M. A., Surmenev, R. A., & Khalil-Allafi, J. (2021). Electrodeposited hydroxyapatite-based biocoatings: Recent progress and future challenges. *Coatings*, 11(1), 110.
- Sahoo, J., Sarkhel, S., Mukherjee, N., & Jaiswal, A. (2022). Nanomaterial-based antimicrobial coating for biomedical implants: new age solution for biofilm-associated infections. *ACS omega*, 7(50), 45962-45980.
- Schulze, F., Lang, A., Schoon, J., Wassilew, G. I., & Reichert, J. (2023). Scaffold guided bone regeneration for the treatment of large segmental defects in long bones. *Biomedicines*, 11(2), 325.
- Shadjou, N., Hasanzadeh, M., & Khalilzadeh, B. (2018). Graphene based scaffolds on bone tissue engineering. *Bioengineered*, 9(1), 38-47.
- Shah, F. A., Ruscsák, K., & Palmquist, A. (2019). 50 years of scanning electron microscopy of bone—a comprehensive overview of the important discoveries made and insights gained into bone material properties in health, disease, and taphonomy. *Bone research*, 7(1), 15.
- Shi, H., Zhou, Z., Li, W., Fan, Y., Li, Z., & Wei, J. (2021). Hydroxyapatite based materials for bone tissue engineering: A brief and comprehensive introduction. *Crystals*, 11(2), 149.
- Singh, S. K., Singh, M. K., Nayak, M. K., Kumari, S., Shrivastava, S., Gracio, J. J., & Dash, D. (2011). Thrombus inducing property of atomically thin graphene oxide sheets. *ACS nano*, 5(6), 4987-4996.
- Sinulingga, K., Sirait, M., Siregar, N., & Doloksaribu, M. E. (2021). Investigation of antibacterial activity and cell viability of Ag/Mg and Ag/Zn co-doped hydroxyapatite derived from natural limestone. *ACS omega*, 6(49), 34185-34191.

- Staneva, A. D., Dimitrov, D. K., Gospodinova, D. N., & Vladkova, T. G. (2021). Antibiofouling activity of graphene materials and graphene-based antimicrobial coatings. *Microorganisms*, 9(9), 1839.
- Tong, P., Sheng, Y., Hou, R., Iqbal, M., Chen, L., & Li, J. (2022). Recent progress on coatings of biomedical magnesium alloy. *Smart Materials in Medicine*, 3, 104-116.
- Tsakiris, V., Tardei, C., & Clicinschi, F. M. (2021). Biodegradable Mg alloys for orthopedic implants—A review. *Journal of Magnesium and Alloys*, 9(6), 1884-1905.
- Waizy, H., Weizbauer, A., Modrejewski, C., Witte, F., Windhagen, H., Lucas, A., ... & Thorey, F. (2012). In vitro corrosion of ZEK100 plates in Hank's Balanced Salt Solution. *Biomedical engineering online*, 11, 1-14.
- Wei, L., & Gao, Z. (2023). Recent research advances on corrosion mechanism and protection, and novel coating materials of magnesium alloys: a review. *RSC advances*, 13(12), 8427-8463.
- Welch, K., Cai, Y., & Strømme, M. (2012). A method for quantitative determination of biofilm viability. *J Funct Biomater* 3: 418–431.
- Wu, Y., Wang, Y., Tian, S., Jing, Y., Zhuang, J., Guo, L., ... & Zhou, Y. (2019). Hydrothermal fabrication of rGO/Apatite layers on AZ31 magnesium alloy for enhanced bonding strength and corrosion resistance. *Applied Surface Science*, 470, 430-438.
- Zamani, Y., Ghazanfari, H., Erabi, G., Moghanian, A., Fakić, B., Hosseini, S. M., & Mahammod, B. P. (2021). A review of additive manufacturing of Mg-based alloys and composite implants. *Journal of Composites and Compounds*, 3(6), 71-83.

8. ANNEXTURE:

GATE 2024 SCORE CARD:



GRADUATE APTITUDE TEST IN ENGINEERING 2024

अभियांत्रिकी स्नातक अभिक्षमता परीक्षा २०२४

ORGANISING INSTITUTE: INDIAN INSTITUTE OF SCIENCE, BENGALURU

SCORE CARD

Name of the Candidate
KARTHIK S

Name of the Parent/Guardian
M S SELVA SEKHAR

Registration No.
BT24S57402110

Test Paper
Biotechnology (BT)

Date of Examination
February 10, 2024

GATE Score	202	Marks out of 100	29.0
All India Rank (AIR) in the test paper	6485	Qualifying Marks	
		General	38.9
		EWS/OBC-NCL	35.0
		SC/ST/PwD	25.9

Number of candidates appeared for the test paper
17078






Prof. Chandra Sekhar Seelamantula
Organising Chairperson, GATE 2024
On behalf of NCB-GATE
Ministry of Education (MoE)



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A candidate is considered **qualified** if the marks secured are greater than or equal to the qualifying marks mentioned for the category, for which a valid category certificate, if applicable, must be produced along with this Score Card.

This Score Card is valid up to 31st March 2027.

GATE SCORE COMPUTATION

The GATE 2024 score is calculated using the formula

$$\text{GATE Score} = S_q + (S_t - S_q) \frac{(M - M_q)}{(M_t - M_q)}$$

where

M is the marks obtained by the candidate in the paper mentioned on the GATE 2024 Score Card

M_q is the qualifying marks for general category candidates in the paper

M_t is the mean of marks of top 0.1% or top 10 (whichever is larger) of all the candidates who appeared for the test paper

S_q = 350, is the score assigned to M_q

S_t = 900, is the score assigned to M_t

M_q is 25 marks (out of 100) or $\mu + \sigma$, whichever is greater. Here, μ is the mean and σ is the standard deviation of marks of all the candidates who appeared for the test paper.

Qualifying in GATE 2024 does not guarantee admission to a postgraduate program or scholarship/financial assistance. Admitting institutes may conduct additional tests or interviews for final selection of candidates.

Graduate Aptitude Test in Engineering (GATE) 2024 was organised by Indian Institute of Science, Bengaluru, on behalf of National Coordination Board (NCB) - GATE for the Department of Higher Education, Ministry of Education (MoE), Government of India.

Name of the student : Karthik S

Register Number : 40230019

Title of the Thesis : Biocompatibility Evaluation of Polymer Coated
Magnesium/Graphene Oxide Implants for Bone
Regeneration

Internal Guide Name : Dr. Prabhakar Singh

External Guide Name : Dr. T. Stalin Dhas

PO / PSO / PEO / SDG	Justification
PO 8	By understanding and committing to professional ethics and responsibilities, the highest standards of integrity, safety, and ethical conduct in the development and use of biodegradable bone implants can be upheld, ultimately benefiting patients and society as a whole.
PSO 1	Biodegradable bone implants represent a significant advancement in orthopedic care, offering improved patient outcomes and reduced environmental impact compared to traditional implants.
PEO 2	Can contribute effectively to the advancement of biodegradable bone implant technology, driving innovation and improving patient outcomes through their commitment to research, lifelong learning, investigative inquiry, and multidisciplinary collaboration.
SDG 3	By promoting tissue regeneration and reducing the risk of complications associated with permanent implants, biodegradable bone implants contribute to improved health outcomes and enhanced well-being for patients.

