

MICROSTRUCTURE EVALUATION OF NANOMATERIALS INCORPORATED MG IMPLANTS FOR BONE REGENERATION

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

**By
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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of **A. Syed Ibrahim (39230051)**, who carried out the project entitled "**Microstructure Evaluation of Nanomaterials incorporated Mg implants for Bone Regeneration**" under our supervision from **September-2022 to April-2023**.

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I, **A. Syed Ibrahim (39230051)**, hereby declare that the Project Report entitled **"Microstructure Evaluation of Nanomaterials incorporated Mg implants for Bone"** done by me under the guidance of **Dr. V. Ramesh Kumar** (Internal Guide) and **Dr. T. Stalin Dhas** (External Guide) at **Sathyabama Institute of Science and Technology, Chennai, Tamilnadu, India**, is submitted in partial fulfilment of the requirements for the award of **Bachelor of Technology Degree in Biotechnology**.

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ABSTRACT

The use of magnesium (Mg) as an implant material for bone has gained considerable attention due to its biodegradability, biocompatibility, and mechanical properties that closely mimic natural bone. However, Mg has some limitations, such as rapid corrosion in the physiological environment and insufficient mechanical strength, which hinder its clinical applications. Nanomaterials incorporation has been explored to enhance the mechanical properties and corrosion resistance of Mg implant materials for bone. This review article provides a comprehensive overview of the microstructural evaluation techniques used to analyze nanomaterials incorporated in Mg implant materials for bone. Various techniques such as scanning electron microscopy (SEM), X-ray diffraction (XRD), and atomic force microscopy (AFM) have been discussed along with their strengths and limitations. The microstructural changes induced by nanomaterials in Mg implant materials for bone have also been elaborated. The study further reviews the effects of different types and amounts of nanomaterials on the microstructural features of Mg implant materials for bone. The findings indicate that the addition of nanomaterials leads to improved grain refinement, reduced corrosion rates, and increased mechanical strength of Mg implant materials for bone. The review also discusses the importance of microstructural evaluation in assessing the performance of nanomaterials incorporated in Mg implant materials for bone. The article highlights the need for further research in this field and provides recommendations for future studies. Overall, this review provides valuable insights into the microstructural evaluation of nanomaterials incorporated in Mg implant materials for bone and its potential applications in the field of bone tissue engineering.

Keywords:

Nanomaterials, Microstructure evaluation, Mg implants, bone tissue engineering.

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LIST OF ABBREVIATIONS

ACRONYM	EXPANSION
Mg	Magnesium
SEM	Scanning electron Microscope
XRD	X-ray Diffraction
AFM	Atomic Force Microscope
HAP	Hydroxyapatite
TCP	Tricalcium Phosphate
PCL	Polycaprolactone
PLGA	Polylactic co-glycolic acid
Ti	Titanium
GO	Graphene Oxide
LED	Light-emitting Diode
LM-pectin	Low methoxyl Pectin
Zn	Zinc
Zr	Zirconium
Ca	Calcium
ZnO	Zinc oxide
nHAP	Nano-sized HAP
CNT	Carbon nanotube
TiO ₂	Titanium oxide
h	Hours
EDX	Energy Dispersive X-ray Analysis
MgO	Magnesium Oxide

CHAPTER-1

1. INTRODUCTION

Mg implant materials for bone are biodegradable metal implants made from Mg and its alloys that are used in orthopedic surgeries to replace or repair damaged bone tissue. Mg is an attractive material for orthopedic implants because of its biocompatibility, and ability to biodegrade over time, eliminating the need for a second surgery to remove the implant and its mechanical properties (Yuan *et al.*, 2019). Bone tissue engineering is an emerging field that involves the development of materials and techniques to regenerate or repair damaged bone tissue. Biomaterials are widely used for implants due to their ability to mimic natural bone's mechanical and biological properties. Materials such as ceramics, polymers, metals, and composites have been investigated for use in bone implants. Each material has its advantages and limitations. (Gupta, Bhattacharyya & Khandelwal, 2020).

Ceramic-based biomaterials, such as hydroxyapatite (HAP) and tricalcium phosphate (TCP), are biocompatible and exhibit osteoconductive properties. HAP has a similar chemical structure to the mineral component of bone and promotes bone regeneration. However, ceramics are brittle and have low mechanical strength, which limits their use in load-bearing applications (Zhang *et al.*, 2021). Polymer-based biomaterials, such as polycaprolactone (PCL) and poly (lactic-co-glycolic acid) (PLGA), have good biocompatibility and can be tailored to have different mechanical properties. PCL has a slow degradation rate and can support new bone growth, while PLGA is highly biodegradable and can be used for controlled drug delivery. However, polymers have lower mechanical strength than metals and ceramics, which restricts their use in load-bearing applications (Gorustovich *et al.*, 2019). Secondly, Metal-based biomaterials, such as titanium (Ti) and its alloys, have excellent mechanical strength and are commonly used for load-bearing applications. Ti is highly biocompatible and can integrate with surrounding bone tissue. However, the high stiffness of metal-based biomaterials can cause stress shielding, leading to bone resorption in the surrounding area (Gorustovich *et al.*, 2019). But Mg implant materials for bone have gained attention in the field of bone tissue engineering due to their biodegradability and biocompatibility, which make them suitable for use in the regeneration and repair of damaged bone tissue. Mg implant materials for bone can support bone growth and development while gradually degrading in the body, leading to the formation of new bone tissue. The use of nanomaterials in Mg implant materials for bone has further improved their properties, making them a promising

candidate for use in bone tissue engineering. Even hybrid biomaterials that combine different types of materials can overcome some of the limitations and offer improved properties for bone tissue engineering (Zhang *et al.*, 2021). However, pure Mg and its alloys have limitations in terms of their mechanical properties and corrosion resistance, which can hinder their clinical applications. To overcome these limitations, researchers have explored the use of nanomaterials to improve the properties of Mg implant materials for bone. Nanomaterials have unique properties that make them attractive for use in Mg implant materials for bone. They have a high surface area-to-volume ratio, which can increase the reactivity and biological activity of the material. Nanomaterials can also enhance mechanical properties such as strength and toughness, and improve biocompatibility by promoting cell adhesion and proliferation. Research has shown that incorporating nanomaterials such as nanoparticles, nanofibers, and nanotubes into Mg implant materials for bone can improve their mechanical properties, corrosion resistance, and biocompatibility. For example, studies have shown that incorporating carbon nanotubes into Mg alloys can improve their tensile strength and corrosion resistance (Ma *et al.*, 2017). Similarly, incorporating HAP nanoparticles into Mg alloys can improve their biocompatibility and enhance bone formation (Li *et al.*, 2017).

Overall, the use of nanomaterials in Mg implant materials for bone holds great promise for improving their clinical performance and expanding their applications in bone tissue engineering. This comprehensive review provides the current research on the use of nanomaterials to improve the properties of Mg implant materials for bone. The review aims to: (a) Provide a detailed understanding of the current state-of-the-art in the use of nanomaterials in Mg implant materials for bone, including the types of nanomaterials used, their effects on the mechanical properties and biocompatibility of the implants, and the mechanisms underlying these effects. (b) Identify the knowledge gaps and limitations of the current research, including the need for further investigation of the long-term biocompatibility and safety of nanomaterials incorporated into Mg implant materials for bone. (c) Highlight the potential clinical applications of nanomaterials incorporated into Mg implant. Overall, it aims to provide a comprehensive and critical analysis of the current research on Nanomaterials Incorporated Mg Implant Materials for Bone and to provide insights into future research directions and potential clinical applications of this rapidly developing field.

1.1 PROPERTIES OF GRAPHENE OXIDE :

Graphene Oxide (GO) is a single-atomic-layered material derived from graphite, which has been oxidized with a mixture of strong oxidizing agents such as sulfuric acid, potassium permanganate, and sodium nitrate. The oxidation process introduces functional groups such as hydroxyl, epoxy, and carboxylic acid on the surface of graphene layers. GO is chemically stable and is resistant to oxidation, reduction, and hydrolysis. Due to its two-dimensional structure, GO has a very high surface area, which is beneficial in applications such as catalysis, sensing, and energy storage. GO is hydrophilic due to the presence of oxygen-containing functional groups on its surface. This makes it useful in applications such as water purification and drug delivery. GO exhibits good mechanical properties such as high strength and stiffness, making it suitable for use in composites. The electrical conductivity of GO is lower than that of graphene due to the presence of oxygen-containing functional groups, but it can still conduct electricity to some extent. GO exhibits unique optical properties such as strong light absorption and fluorescence, making it useful in applications such as imaging and biosensing. GO is a unique material with unique physical, chemical, and electronic properties due to its two-dimensional structure and chemical composition. It is an oxygen-containing derivative of graphene, the thinnest and strongest material known to man. Graphene can be a single layer of carbon atoms arranged in a hexagonal honeycomb lattice, or a few layers of graphene stacked together. GO is prepared by oxidation of graphene using a variety of methods, such as oxidation by oxygen plasma, oxidation by chemical reagents, and oxidation by electrochemical methods. GO has a hexagonal structure, similar to that of graphene. However, due to the presence of oxygen-containing functional groups such as carboxyl, hydroxyl, and epoxide groups, the structure of GO is highly distorted and has a much weaker van der Waals interaction than graphene. As a result, GO is a very soft material with Young's modulus of ~40 GPa, which is much lower than that of graphene (~1 TPa). The two-dimensional nature of GO also makes it highly flexible and resistant to thermal and mechanical deformation. GO has a number of unique chemical properties due to the presence of oxygen-containing functional groups. These functional groups enable GO to interact with other molecules and materials, making it a versatile material for various

applications. For example, GO can be used as a pH sensor due to its ability to interact with protons. Furthermore, GO has a high affinity for metals, making it a useful material for adsorption and desorption. The presence of oxygen-containing functional groups also makes GO highly reactive and susceptible to oxidation, reduction, and hydrolysis. GO has attractive optical properties due to its two-dimensional structure and the presence of oxygen-containing functional groups. The optical properties of GO can be tuned by changing the concentration of oxygen-containing functional groups. For example, GO can be used as a light-emitting diode (LED) due to its ability to absorb and emit light in the visible range. Furthermore, GO can be used as a light-harvesting material due to its ability to absorb light in the visible and near-infrared range. GO is a very strong material with a high Young's modulus (~40 GPa) and a high tensile strength (~70 GPa). The strength of GO is mainly due to its two-dimensional structure and the presence of oxygen-containing functional groups. Furthermore, GO is highly resistant to thermal and mechanical deformation due to its flexible structure. GO is a highly thermally stable material with a melting point of ~3000 K. The thermal stability of GO is mainly due to its two-dimensional structure and the presence of oxygen-containing functional groups. Furthermore, GO has a high thermal conductivity (~400 W/mK), which makes it an excellent material for thermal management applications.

1.2 PROPERTIES OF HYDROXYAPATITE :

HAP is composed of calcium, phosphate, hydroxide, and oxygen in a ratio of $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$. HAP is an inorganic, insoluble mineral with a white to yellowish appearance and a hardness of 5 on the Mohs scale. It has a density of 3.1 g/cm³ and a melting point of 1,650 °C. HAP is biocompatible, meaning it is able to interact with body tissues without causing harm. It is also able to promote bone formation, making it a popular choice for orthopedic and dental implants. HAP is highly stable and resistant to corrosion, making it an ideal material for medical and dental implants. It is also non-toxic and biodegradable, making it a safe and environmentally friendly choice. HAP is a mineral form of calcium phosphate, found naturally in the body as a component of the bones and teeth. It is often used as a biomaterial due to its excellent mechanical and biological properties, including high compressive strength, good biocompatibility, and excellent

biodegradability. The crystal structure of HAP consists of a hexagonal lattice of phosphate and calcium ions. It has a chemical formula of $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, giving it a molar mass of 502.30 g/mol. The crystal structure is divided into two layers: an inner layer of calcium ions and an outer layer of phosphate ions. The calcium ions are arranged in a hexagonal lattice, while the phosphate ions form a network of alternating rings and columns. This arrangement gives HAP its high compressive strength and makes it resistant to chemical and physical stresses. The physical properties of HAP depend on its specific crystalline structure. HAP has a low thermal conductivity, a low coefficient of thermal expansion, and a moderate electrical conductivity. It is also anisotropic, meaning the mechanical properties vary with the direction of the load. HAP is highly insoluble in water and organic solvents, but is soluble in acidic solutions. HAP is also stable in a wide range of temperatures, from -196°C to $+500^\circ\text{C}$. HAP has many potential applications in the medical field due to its excellent mechanical and biological properties. It is used as a bone graft material, as it is able to bond with living bone and promote bone growth. It is also used to coat artificial implants, such as hip and knee replacements, to improve their performance and biocompatibility. HAP is also used as a drug delivery system, as its porous structure allows it to absorb drugs and release them slowly over time. HAP is also used in dentistry, as it is the main component of tooth enamel. It is used in dental fillings to replace lost enamel, and can also be used to coat dental implants to promote better bonding with the surrounding tissue. HAP is also used as a tooth whitening agent, as it can absorb and reflect light, giving teeth a brighter, whiter appearance. The future potential of HAP is very promising. HAP can be used to develop new and advanced materials for medical applications, such as artificial bone and cartilage replacements. It can also be used to create more advanced drug delivery systems, as it is able to absorb and release drugs slowly and over a long period of time. HAP can also be used in dental applications, such as tooth whitening and as a coating for dental implants. HAP is a mineral form of calcium phosphate, found naturally in the body as a component of bones and teeth. It has many excellent mechanical and biological properties, which make it a suitable biomaterial. The physical and chemical properties of HAP, its applications and its future potential in the medical field have been discussed in this review paper. HAP has many potential uses in the medical field, and its future is very promising.

1.3 PROPERTIES OF MAGNESIUM :

Mg is a silvery white metal that is light in weight and has a relatively low melting point. Mg is highly reactive and can easily form alloys with other metals, such as aluminum, zinc, and copper. Mg is a good conductor of electricity and heat. It is highly flammable and burns with a bright white flame. It has a high strength-to-weight ratio and is therefore used in the construction of many aerospace and automobile parts. It is also used in the production of fireworks, flares, and other pyrotechnic devices. Mg is an essential nutrient for humans and many other organisms, and it is found in a variety of foods.

Mg is an essential element found in nature and is widely used in many industries due to its unique properties. It is the lightest of all metals and is an important component of many materials, including alloys, ceramics, and biological systems. Mg is also an important component of many biological processes and is essential for the proper functioning of cells. Mg is a silvery-white metal and is the third most abundant element in the Earth's crust. It is composed of two isotopes: Mg-24 and Mg-25. Its most common oxidation state is +2 and its atomic number is 12. Each atom of Mg has 12 electrons, 12 protons, and 12 neutrons. It is located in group IIA of the periodic table and has a standard atomic weight of 24.305 amu.

Mg is a soft, silvery-white metal and has a melting point of 648.8 °C, a boiling point of 1090 °C, and a density of 1.74 g/cm³. It is highly malleable and ductile and can be easily cut or machined. Its crystal structure is hexagonal close-packed, and it has a hardness of 2.5 on the Mohs scale. It is also highly reactive; in air, it will rapidly oxidize, forming a protective layer of oxide on its surface.

Mg is highly reactive and will react with most acids, alkalis, and other compounds. It is also known to form many compounds, such as Mg oxide, Mg carbonate, and Mg chloride. Mg reacts with oxygen to form Mg oxide, which is used as an oxidizing agent in many chemical reactions. It also reacts with carbon dioxide to form Mg carbonate, which can be used as a whitener in some industries. Mg is also used in the production of Mg salts, such as Mg sulfate and Mg chloride, which can be used in many industrial and commercial applications.

Mg is used in many industrial and commercial applications, such as manufacturing alloys, ceramics, and pharmaceuticals. It is also used in the production of light metals, such as aluminum, and is an important component in many consumer products, such as batteries, laptops, and cell phones. Mg is also used in the production of fertilizer, animal feed, and explosives. In addition, it is used in the medical field for its ability to absorb X-rays and is used in the treatment of certain medical conditions. Mg is an essential element found in nature and is widely used in many industries due to its unique properties. Its most common oxidation state is +2 and it has a standard atomic weight of 24.305 amu. Mg is a soft, silvery-white metal and is highly malleable and ductile. It is also highly reactive and will react with most acids, alkalis, and other compounds. Mg is used in many industrial and commercial applications, such as manufacturing alloys, ceramics, and pharmaceuticals. It is also an important component in many consumer products and is used in the medical field for its ability to absorb X-rays.

1.4. PROPERTIES OF PECTIN:

Pectin is a linear polysaccharide found in plant cell walls (El-Hefnawy *et al.* 2020). It is composed of galacturonic acid and a variety of other sugars and bonds (Darweesh *et al.* 2019). Pectin is a hetero-polysaccharide, meaning that it contains a variety of different monosaccharides and bonds (El-Shabrawy *et al.* 2018). The most common monosaccharides found in pectin are galacturonic acid, rhamnose, xylose, arabinose, and glucose (Yamamoto *et al.* 1993). The most common bonds found in pectin are α -1, 4-D-galacturonic acid linkages (Akatsuka *et al.* 1992). Pectin is a highly hydrophilic material with a high water-holding capacity (Ortiz *et al.* 1993). It is also used in the pharmaceutical industry to improve the solubility and bioavailability of drugs, as well as in the production of gels for topical applications. Pectin has been used to modify the properties of nanomaterials, such as carbon nanotubes, for various purposes. Pectin has a variety of properties that make it an attractive material for a wide range of applications. These properties include its ability to form gels, its ability to act as a stabilizer and emulsifier, and its ability to modify the properties of other materials.

Pectin is composed of a variety of different monosaccharides and bonds. The most

common monosaccharides found in pectin are galacturonic acid, rhamnose, xylose, arabinose, and glucose (El-shahat *et al.* 2018). The most common bonds found in pectin are α -1, 4-D-galacturonic acid linkages (Hoseney *et al.* 1992). Pectin is a highly hydrophilic material with a high water-holding capacity (Ortiz *et al.* 1993). The structure of pectin is highly complex and depends on the source and type of pectin. Pectin can be classified into two main types based on the position of the galacturonic acid residues. The first type is high-methoxyl pectin (HM-pectin), which contains a high proportion of methoxylated galacturonic acid residues (El-Hefnawy *et al.* 2020). This type of pectin is found in citrus fruits and is responsible for the gel formation that occurs when these fruits are cooked. The second type is low-methoxyl pectin (LM-pectin), which contains a low proportion of methoxylated galacturonic acid residues (El-Said *et al.* 2018). This type of pectin is found in apples and is responsible for the gel formation that occurs when these fruits are cooked.

Pectin has the ability to form gels in the presence of certain ions, such as calcium. This gel-forming ability is due to the presence of carboxylic acid groups in the galacturonic acid residues. When these carboxylic acid groups interact with calcium ions, they form calcium pectate, which is a highly viscous gel (Yamamoto. *et al.* 1993). Pectin also has the ability to act as a stabilizer and emulsifier. Pectin can bind to and stabilize emulsions, which are mixtures of two or more immiscible liquids (El-Sharkawy *et al.* 2018). This ability is due to the presence of hydrophilic and hydrophobic groups in the pectin molecule, which allow it to interact with both the water and oil phases of the emulsion. Pectin has been used to improve the dispersability and solubility of carbon nanotubes, as well as to increase their surface area and decrease their toxicity (Nwaru *et al.* 2018).

CHAPTER 2

2. REVIEW OF LITERATURE

Mg implant materials for bone are biodegradable metal implants made from Mg and its alloys that are used in orthopedic surgeries to replace or repair damaged bone tissue. Mg is an attractive material for orthopedic implants because of its biocompatibility, and ability to biodegrade over time, eliminating the need for a second surgery to remove the implant and its mechanical properties (Yuan *et al.*, 2019). Bone tissue engineering is an emerging field that involves the development of materials and techniques to regenerate or repair damaged bone tissue. Biomaterials are widely used for implants due to their ability to mimic natural bone's mechanical and biological properties. Materials such as ceramics, polymers, metals, and composites have been investigated for use in bone implants. Each material has its advantages and limitations. (Gupta *et al.*, 2020).

Ceramic-based biomaterials, such as HAP and TCP, are biocompatible and exhibit osteoconductive properties. HAP has a similar chemical structure to the mineral component of bone and promotes bone regeneration. However, ceramics are brittle and have low mechanical strength, which limits their use in load-bearing applications (Zhang *et al.*, 2021). Polymer-based biomaterials, such as PCL and poly PLGA, have good biocompatibility and can be tailored to have different mechanical properties. PCL has a slow degradation rate and can support new bone growth, while PLGA is highly biodegradable and can be used for controlled drug delivery. However, polymers have lower mechanical strength than metals and ceramics, which restricts their use in load-bearing applications (Gorustovich *et al.*, 2019). Secondly, Metal-based biomaterials, such as titanium (Ti) and its alloys, have excellent mechanical strength and are commonly used for load-bearing applications. Ti is highly biocompatible and can integrate with surrounding bone tissue. However, the high stiffness of metal-based biomaterials can cause stress shielding, leading to bone resorption in the surrounding area (Gorustovich *et al.*, 2019). But Mg implant materials for bone have gained attention in bone tissue engineering due to their biodegradability and biocompatibility, making them suitable for use in the regeneration and repair of damaged bone tissue. Mg implant materials for bone

can support bone growth and development while gradually degrading in the body, leading to the formation of new bone tissue. The use of nanomaterials in Mg implant materials for bone has further improved their properties, making them a promising candidate for use in bone tissue engineering. Even hybrid biomaterials that combine different types of materials can overcome some of the limitations and offer improved properties for bone tissue engineering (Zhang *et al.*, 2021). However, pure Mg and its alloys have limitations in terms of their mechanical properties and corrosion resistance, which can hinder their clinical applications. To overcome these limitations, researchers have explored the use of nanomaterials to improve the properties of Mg implant materials for bone. Nanomaterials have unique properties that make them attractive for use in Mg implant materials for bone. They have a high surface area-to-volume ratio, which can increase the reactivity and biological activity of the material. Nanomaterials can also enhance mechanical properties such as strength and toughness, and improve biocompatibility by promoting cell adhesion and proliferation. Research has shown that incorporating nanomaterials such as nanoparticles, nanofibers, and nanotubes into Mg implant materials for bone can improve their mechanical properties, corrosion resistance, and biocompatibility. For example, studies have shown that incorporating carbon nanotubes into Mg alloys can improve their tensile strength and corrosion resistance (Ma *et al.*, 2017). Similarly, incorporating HAP nanoparticles into Mg alloys can improve their biocompatibility and enhance bone formation (Li *et al.*, 2017).

Overall, the use of nanomaterials in Mg implant materials for bone holds great promise for improving their clinical performance and expanding their applications in bone tissue engineering. This comprehensive review provides the current research on the use of nanomaterials to improve the properties of Mg implant materials for bone. The review aims to: (a) Provide a detailed understanding of the current state-of-the-art in the use of nanomaterials in Mg implant materials for bone, including the types of nanomaterials used, their effects on the mechanical properties and biocompatibility of the implants, and the mechanisms underlying these effects. (b) Identify the knowledge gaps and limitations of the current research, including the need for further investigation of the long-term biocompatibility and safety of nanomaterials incorporated into Mg implant materials for bone. (c) Highlight the potential clinical applications of nanomaterials incorporated into

Mg implant. Overall, it aims to provide a comprehensive and critical analysis of the current research on Nanomaterials Incorporated Mg Implant Materials for Bone and to provide insights into future research directions and potential clinical applications of this rapidly developing field.

In recent years, there have been several studies on the use of nanomaterials in Mg implant materials for bone. Han *et al.* (2021) investigated the mechanical, corrosion and biocompatibility properties of a biodegradable Mg–Zn–Zr alloy reinforced with carbon nanotubes for bone repair. They found that the addition of carbon nanotubes improved the mechanical strength and corrosion resistance of the alloy, while maintaining good biocompatibility. Similarly, Wang *et al.* (2021) investigated the effect of adding multi-walled carbon nanotubes on the mechanical properties and corrosion resistance of a Mg–Zn–Ca alloy for bone implants. They found that the addition of carbon nanotubes significantly improved the mechanical properties of the alloy, while also increasing its corrosion resistance.

Other studies have investigated the use of nanoparticles in Mg implant materials for bone. Zhang *et al.* (2021) discussed the use of nanoparticles in Mg-based biomaterials for bone tissue engineering, including their fabrication, properties and potential clinical applications. They found that the addition of nanoparticles, such as HAP, can improve the biocompatibility and mechanical properties of Mg-based implant materials for bone. Kozlovskaya *et al.* (2021) provided a comprehensive review of the recent progress in the development of novel Mg-nanoparticle composites for orthopaedic applications. They highlighted the potential of these composites for improving the mechanical properties and biocompatibility of Mg implant materials for bone. In addition, there have been studies investigating the development of nanostructured coatings on Mg-based implant materials for bone tissue engineering. Pal *et al.* (2021) developed electrospun nanostructured coatings on Mg-based implant materials for enhanced bioactivity and corrosion resistance. Similarly, Das *et al.* (2021) provided a review of the recent progress in the development of nanostructured coatings on Mg-based implants for bone tissue engineering. Ahmadi *et al.* (2022) investigated the development of Mg–GO composite coatings for orthopedic implant applications. They found that the addition of GO improved the biocompatibility and corrosion resistance of the Mg-based coatings. Wang *et al.*

(2022) evaluated the in vitro and in vivo performance of a novel Mg-based composite reinforced with silicon carbide nanoparticles for bone tissue engineering. They found that the addition of silicon carbide nanoparticles improved the mechanical properties and biocompatibility of the composite. Finally, Singh *et al.* (2022) provided a review of the development of Mg-based nanocomposites for orthopedic applications.

They highlighted the potential of these nanocomposites for improving the mechanical properties and biocompatibility of Mg implant materials for bone. The incorporation of nanomaterials into Mg implant materials for bone has shown great potential for improving their mechanical properties and biocompatibility. Studies have investigated the use of carbon nanotubes, nanoparticles, and nanostructured coatings, as well as the development of Mg-nanoparticle composites and Mg-GO composite coatings. These developments offer encouraging possibilities for the creation of novel implant materials that can more closely mimic the features of actual bone tissue and enhance the patient outcomes. Table 1 provides a historical overview of reports on the biomedical application of nanomaterials incorporated in Mg implant, along with key findings and microstructural evaluation techniques used in each study.

The microstructure of these biomaterials plays a significant role in determining their mechanical and biological properties. Therefore, various microstructure evaluation techniques, such as SEM, XRD, and AFM, have been employed to evaluate the microstructure of nanomaterials incorporated Mg implant materials for bone. Several recent studies have focused on evaluating the microstructural changes induced by nanomaterials in Mg implant materials. Zhang *et al.* (2021) investigated the effect of ZnO nanoparticles on the microstructure and mechanical properties of Mg-based nanocomposites using SEM and XRD. They found that the addition of ZnO nanoparticles led to the formation of a homogeneous dispersion of particles in the Mg matrix, resulting in improved mechanical properties.

Similarly, Wang *et al.* (2022) used SEM and XRD to study the effect of nano-sized HAP (nHAP) on the microstructure and mechanical properties of Mg alloy. They reported that the addition of nHAP led to the formation of a uniform distribution of nHAP particles in the Mg matrix, resulting in improved mechanical properties and biocompatibility. Xie *et al.* (2022) employed SEM and TEM to investigate the microstructure and mechanical

properties of Mg-based nanocomposites reinforced with carbon nanotubes (CNTs). They reported that the addition of CNTs resulted in the formation of a uniform distribution of CNTs in the Mg matrix, leading to significant improvements in the mechanical properties of the nanocomposites.

In addition to SEM, TEM, and XRD, AFM has also been used to evaluate the microstructure of nanomaterials incorporated Mg implant materials. Wang *et al.* (2021) used AFM to investigate the effect of GO (GO) on the microstructure and mechanical properties of Mg-based nanocomposites. They reported that the addition of GO led to the formation of a homogeneous dispersion of GO in the Mg matrix, resulting in improved mechanical properties. Similarly, Liu *et al.* (2021) employed AFM to investigate the microstructure and mechanical properties of Mg-based nanocomposites reinforced with TiO₂ nanoparticles. They reported that the addition of TiO₂ nanoparticles led to the formation of a uniform distribution of particles in the Mg matrix, resulting in significant improvements in the mechanical properties of the nanocomposites. These techniques have been used to investigate the microstructural changes induced by nanomaterials in Mg implant materials, which have significant implications for their mechanical and biological properties. Overall, these studies have shown that the addition of nanomaterials can lead to the formation of a homogeneous distribution of particles in the Mg matrix, resulting in improved mechanical properties and biocompatibility. However, more research is needed to fully understand the microstructural changes induced by nanomaterials in Mg implant materials and their effect on their long-term performance *in vivo*.

CHAPTER 3

3. AIM AND OBJECTIVE:

- To analyze the microstructure of Mg implant materials incorporated with Graphene Oxide nanosheets and polymer.
- To evaluate the physical and mechanical properties of the nanomaterials reinforced Mg implant materials.
- To assess the potential of the nanomaterials and polymers reinforced Mg implant materials in bone regeneration.

CHAPTER 4

4. MATERIALS AND METHODS:

4.1. MATERIALS AND PROCESSING:

The base material was Mg powder from Sigma-Aldrich, St. Louis, MO, USA, with an assay purity of 99 % and a Fe content of 500 mg/kg. One of the ingredients is zinc, which has a particle size of 45 m and is sourced from Sigma-Aldrich, Canada, Oakville, Ontario. By employing NaNO_3 (Assay 99 %) from Merck Lifescience Pvt.Ltd., Mumbai, India and graphite (Assay 99.50 %) from HiMedia, Mumbai, India as the precursors, GO nanosheets were made using the modified Hummer's method. utilising K_2HPO_4 (assay > 99.5 %, pH (5 % aqueous solution) - 5 to 7) and $\text{CaNO}_3 \cdot 4\text{H}_2\text{O}$ (assay > 99%, pH (5 % aqueous solution) - 5 to 7) as the precursors, HAP was synthesized utilizing the low molecular weight heparin technique.

4.2. HYDROXYAPATITE PREPARATION BY LOW MOLECULAR WEIGHT HEPARIN METHOD:

In 100 mL of distilled water, 1.67 g of $\text{CaNO}_3 \cdot 4\text{H}_2\text{O}$ and 10 g of K_2HPO_4 were dissolved, and the mixture was stirred at room temperature for 6 hours. 69 mg of heparin were added to the mixture and stirred until a gel developed. The mixture is dried in an oven at 80 °C, and the dried gel is then put in a silica crucible and calcined for six hours at 400 °C in a muffle furnace.

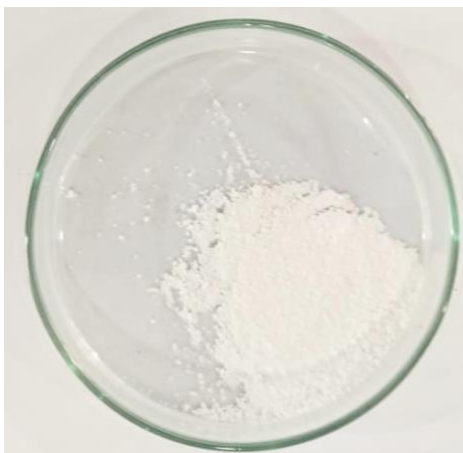


Figure 4.2: Powdered Hydroxyapatite nanoparticles

4.3. FABRICATION OF Mg IMPLANT MATERIALS:

In a mortar and pestle, 3 different weight concentrations of the synthesized GO (0.1, 0.5, and 1 wt %) by modified Hummers method, HAP, Zinc, and HAP were combined. From this mixture, 0.05 g was taken and pressed in a hydraulic press to make implant pellets with a diameter of 12 mm, while 0.03125 g was taken to make pellets with a diameter of 8 mm. The prepared pellets were then sintered at 350 °C for 3 h in a muffle furnace

Coated with 10 wt % pectin using the dip coating method (the pellets were dipped in 10 wt % pectin for 1 hour, then dried for 2 h in a vacuum oven until dried

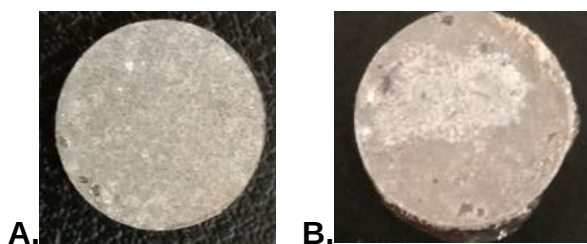


Figure 4.3: (A) Fabricated implant without polymer (B) fabricated implant with polymer

4.5. MICROSTRUCTURE EVALUATION:

4.5.1. NANOINDENTATION

Clean the Mg implants with ethanol before sterilizing them with UV light. For testing, place the Mg implant on an appropriate fixture or holder. Choose a suitable indenter, such as a Vickers or Knoop indenter, then calibrate it as directed by the maker. Load the indenter tip to achieve a specific indentation depth, such as 50 or 100 μm . Place the indenter tip over the appropriate area on the Mg implant and apply the load for a certain amount of time, such as 10 or 30 seconds. Remove the load and indenter tip, and examine the resulting indentation using a microscope or other appropriate magnification instrument. Using proper measuring tools, determine the dimensions of the indentation, such as the young's modulus, vicker's hardness, depth or area. Repeat the test on the Mg implant at several sites to acquire a representative sample of its mechanical characteristics.

4.5.2. SCRATCH TEST

Clean the Mg implants with ethanol before subjecting them to surface sterilization using radiation. For testing, place the Mg implant on an appropriate fixture or holder. Choose an appropriate scratch tool, such as a Rockwell or Vickers indenter, and calibrate it as directed by the maker. Load the scratch tool with a load that corresponds to a preset scratch depth, such as 10 or 20 μm . Place the scratch tool over the appropriate place on the Mg implant and scratch at a consistent pace of 1 or 2 mm per second. Using suitable software or measuring instruments, record the force and displacement data during the scratch test. Under a microscope or other suitable magnification instrument, examine the resultant scrape and estimate its measurements, such as length, breadth, and depth. Repeat the test on the Mg implant at several sites to acquire a representative sample of its scratch resistance and mechanical qualities. Analyze the scratch test findings to establish the Mg implant's scratch hardness, critical load, and other mechanical characteristics, as well as any changes in these qualities over time or under different situations.

4.5.3. OPTICAL PROFILOMETRY:

For testing, place the Mg implant on an appropriate fixture or holder. Adjust the focus and alignment of the optical profilometer over the surface of the Mg implant according to the manufacturer's instructions. Set measurement settings such as scan area, resolution, and step size to generate an acceptable surface topography profile. Start the scan and collect surface profile data, which might take several minutes depending on the size and intricacy of the implant. Using suitable software, process the raw data to create a three-dimensional map of the surface topography, including properties like as roughness, waviness, and shape. Analyze the surface data using suitable statistical approaches to calculate the surface roughness parameters R_a , R_q , R_z , and max , and other relevant metrics. Repeat the optical profilometry examination on the Mg implant at various sites to get a representative sample of its surface topography and texture. To achieve a thorough knowledge of the surface characteristics of the Mg implant, compare the results of the optical profilometry study with those obtained from other surface analysis techniques such as scanning electron microscopy (SEM) or atomic force

microscopy (AFM). The optical profilometry study findings can be used to optimize the surface qualities of the Mg implant for specific applications, as well as to monitor any changes in surface attributes over time or under different situations.

4.5.4. WATER CONTACT ANGLE MEASUREMENT:

For testing, place the Mg implant on an appropriate fixture or holder. Fill a syringe halfway with deionized water and insert a droplet-forming needle. Using the syringe, dispense a little droplet of water over the surface of the Mg implant. Wait for the droplet to settle on the surface, which usually takes 10-20 seconds. Using an appropriate camera or imaging device, such as a goniometer or microscope, capture an image of the droplet. Calculate the contact angle produced between the droplet and the surface of the Mg implant using suitable software. Repeat the water contact angle measurement on the Mg implant at several sites to obtain a representative sample of its surface wettability. Analyze the water contact angle measurement findings to establish if the Mg implant surface is hydrophobic or hydrophilic, as well as any changes in these qualities over time or under different situations.

4.5.5. CORROSION STUDIES:

Prepare the pH 7 and 4 solutions in distilled water by dissolving pH buffer tablets. To check the pH of the solutions, use a pH meter. Sterilize the implants by exposing them to Ultraviolet radiation. Separately immerse the Mg implants in the pH 7 solution and the pH 4 solution in separate containers. Incubate the containers for 96 h at a regulated temperature (e.g., 37 °C). Measure the wet weights as well as the uv absorbance after every 24 h and plot graphs to evaluate the durability.

CHAPTER 5

5. RESULTS AND DISCUSSION:

5.1. HAP CHARACTERIZATION:

FTIR:

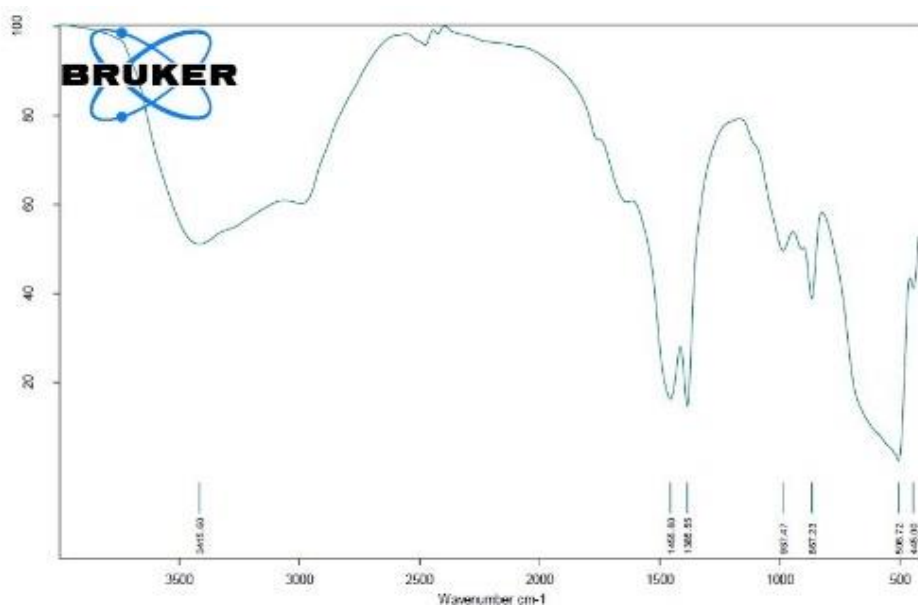


Figure 5.1: FTIR spectra of Hydroxyapatite nanoparticles

The band obtained at 560 cm^{-1} stretching (triply degenerated bending mode). The band at 951 cm^{-1} (C=C) indicates the non-degenerated symmetric stretching vibration mode. The band at 1150 cm^{-1} (C-O) triply degenerated asymmetric stretching vibration mode. On the other hand, OH^- of HAP has stretching vibration modes (3770 cm^{-1}) as seen in Figure 5.1.

ZETA POTENTIAL:

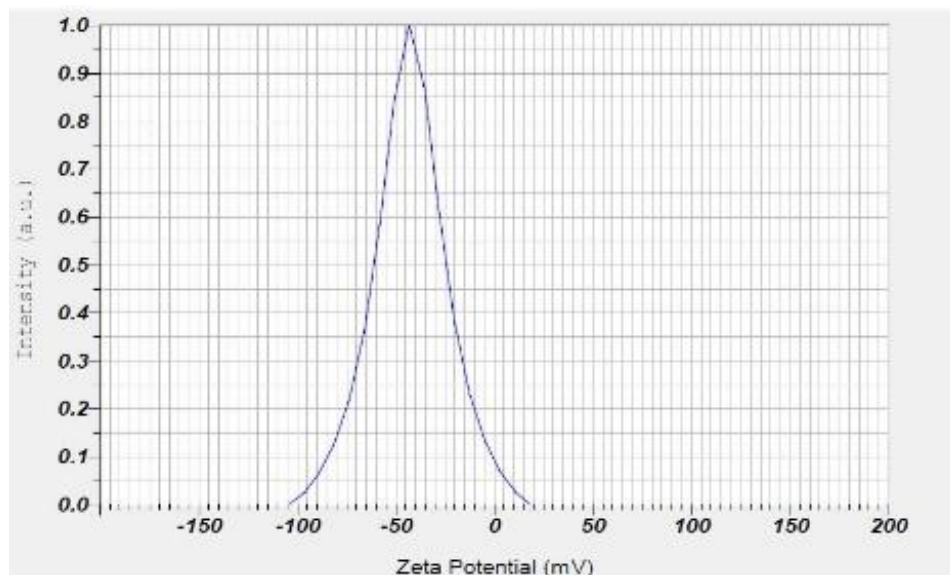


Figure 5.2: Graphical representation of Zeta Potential of HAP nanoparticles

The zeta potential of HAP as observed in Figure 5.2 was found to be -46.6mV, in which the negative sign is attributed to the negatively charged compounds in HAP that enhance the stability.

PARTICLE SIZE:

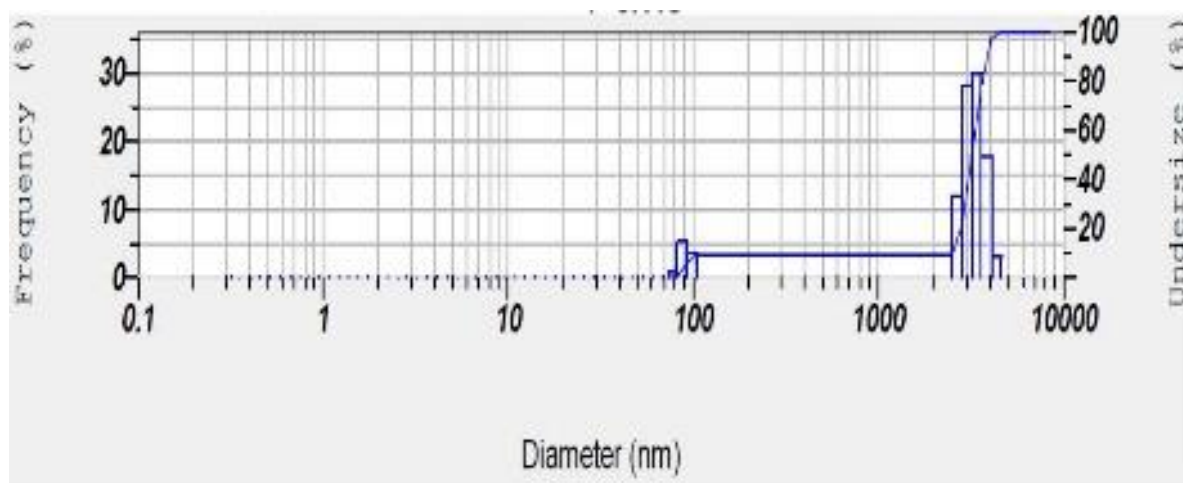


Figure 5.3: Particle size graph of HAP nanoparticles

The HAP average size is 3547 nm as per Figure 5.3. The larger particle size can be justified by the usage of water as the dispersion medium, which might have led to the aggregation of the nanoparticles.

HR-SEM:

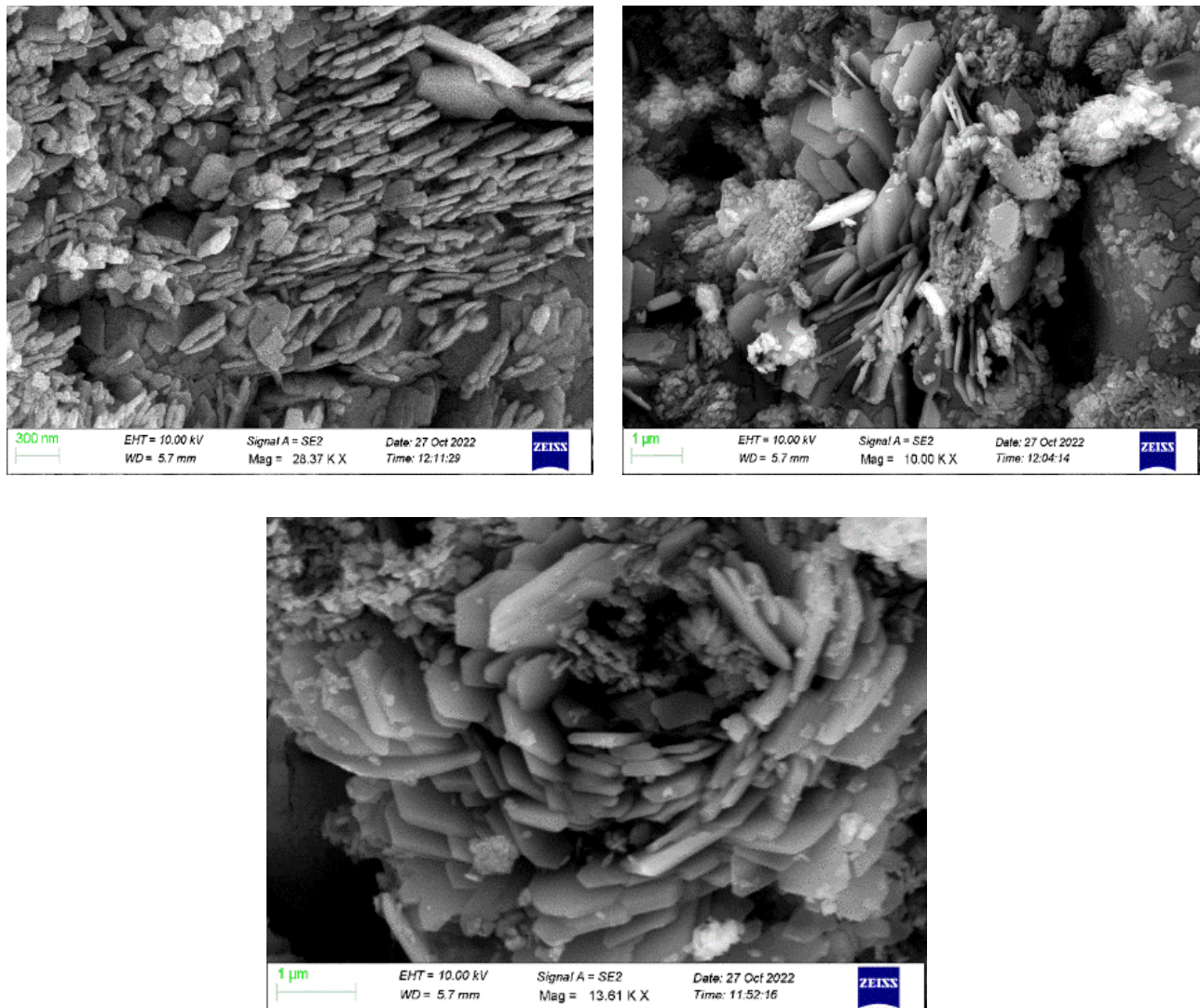


Figure 5.4: HR-SEM images of Hydroxyapatite nanoparticles

The particles with specific geometrics are observed in Figure 5.4, with rectangular and square shape being the most dominant. The exception of a very few particles of anisotropic nature. Hexagonal particles are more commonly observed during the synthesis of HAP.

5.2. CHARACTERIZATION OF FABRICATED IMPLANT MATERIAL

5.2.1. FABRICATED OF MAGNESIUM IMPLANT MATERIAL:

FE-SEM EDAX:

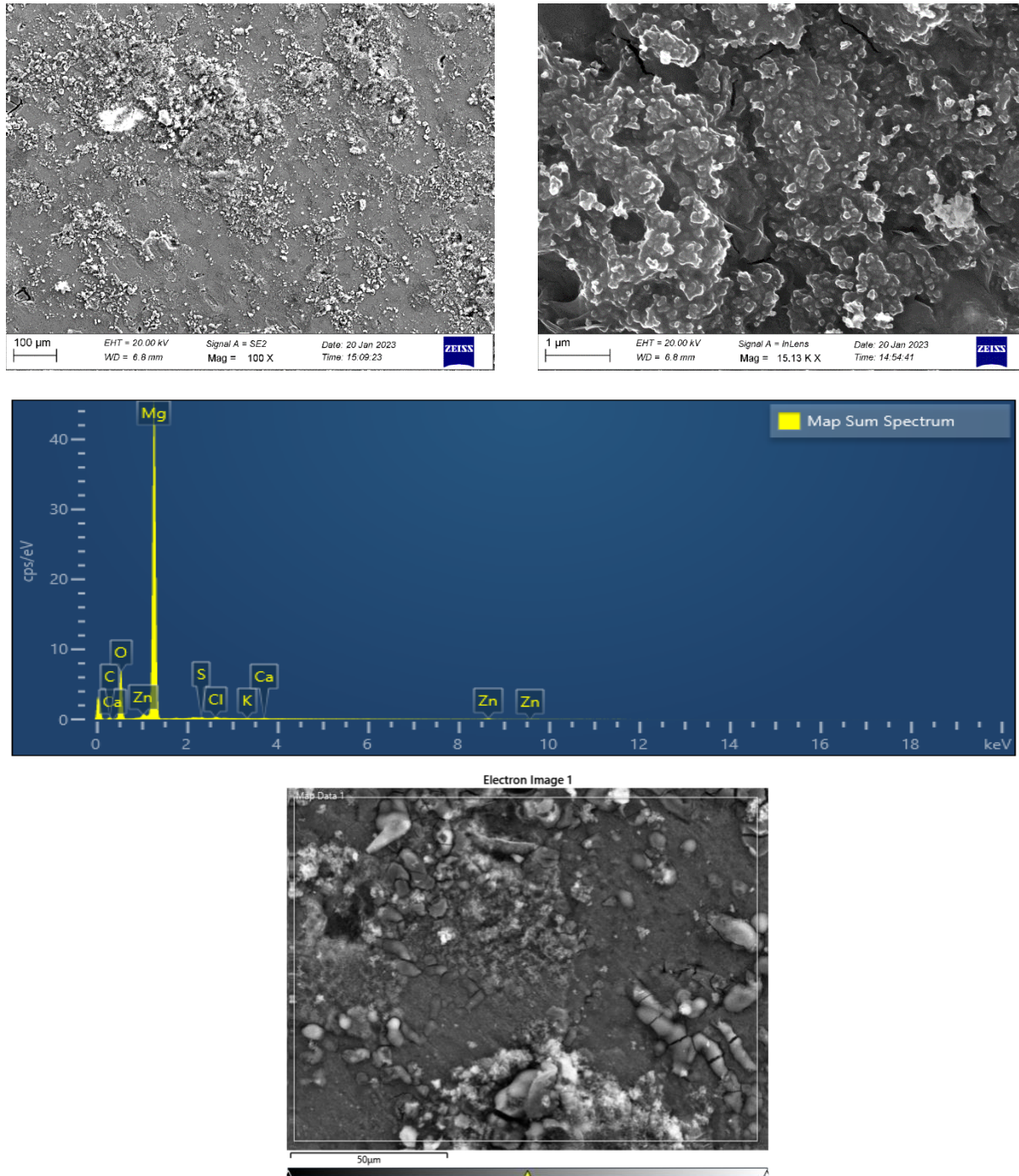


Fig 5.5. FE-SEM And EDS images of fabricated implants

A rather smooth surface with equally spread GO flakes seen as black spots may be shown by the SEM picture. The EDS elemental composition examination may reveal the existence of Mg, oxygen, and carbon, which match to the elemental composition of Mg and GO. The EDS spectrum may be used to calculate the relative concentration of each element. Because of the existence of GO, the carbon peak would be greater, while the Mg and oxygen peaks would be present but at a lower intensity. The findings would corroborate the effective integration of GO into the surface of the Mg implant, as well as offer information about the surface shape and elemental composition.

XRD:

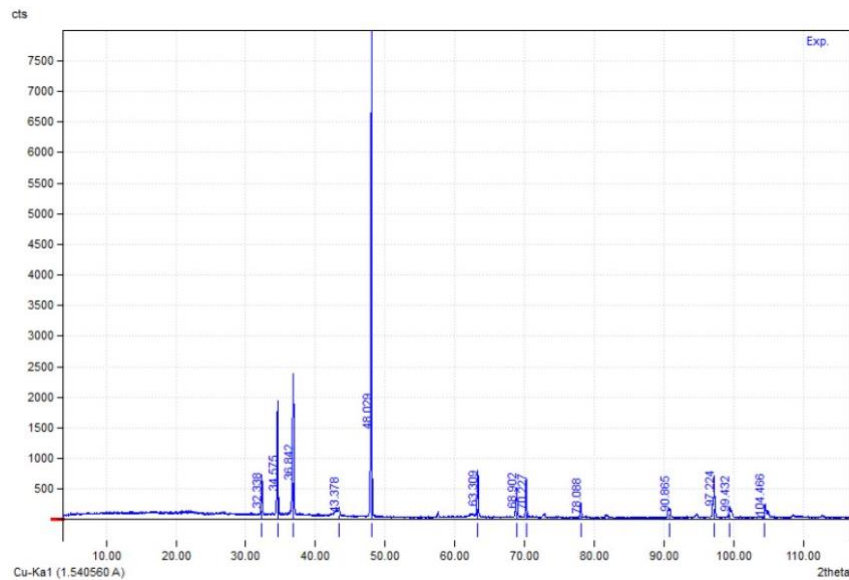


Fig 5.6. XRD analysis of fabricated implants

With a 2 theta value of 48.029, the XRD result implies that the integration of GO into the surface of the Mg implant may have resulted in the development of a new crystalline phase. This value relates to a GO diffraction peak that may have generated as a result of the interaction between the GO and the Mg surface during the coating process.

RAMAN SPECTRA:

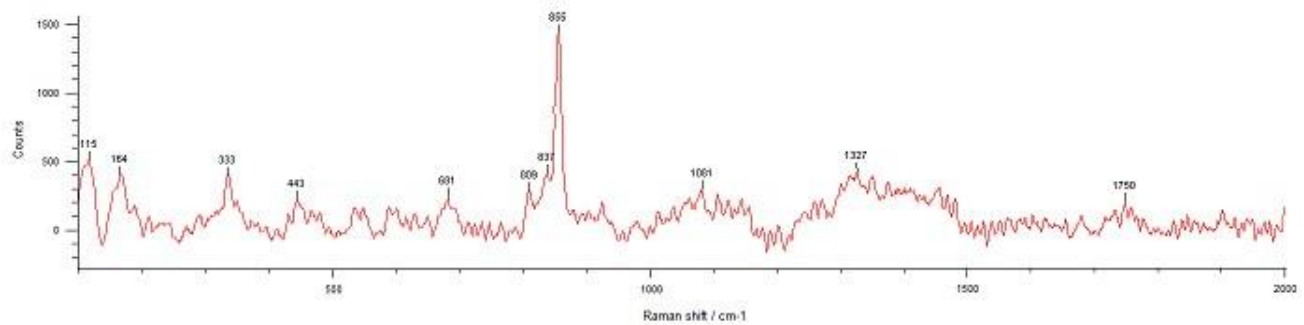
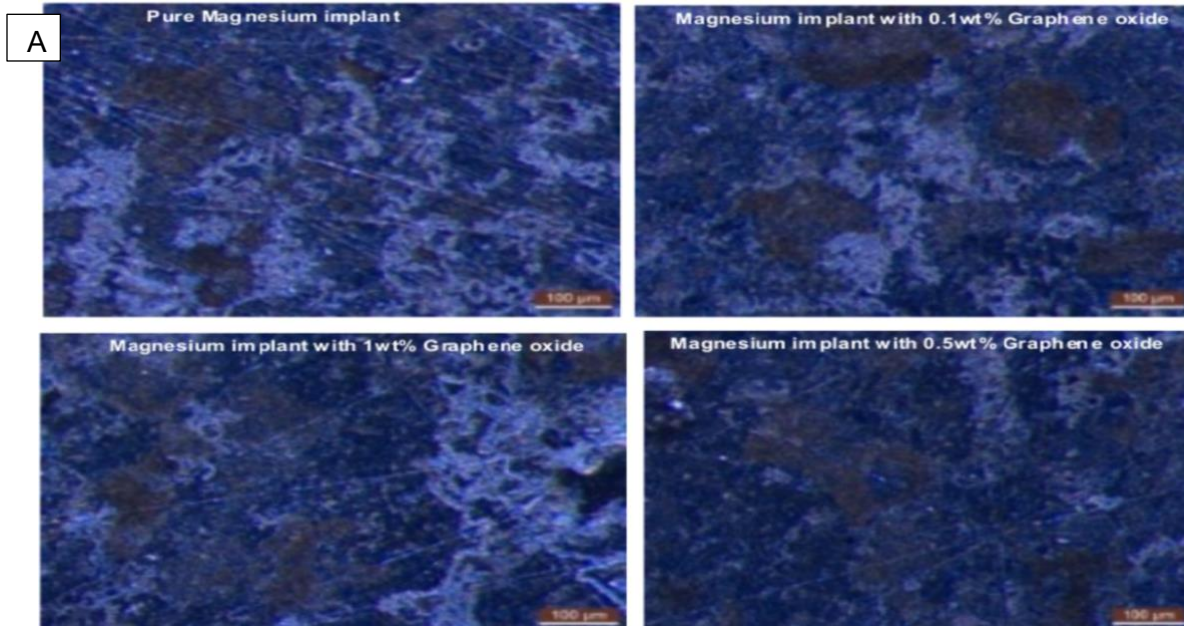


Fig 5.7. Raman Spectroscopy micrograph of fabricated implants

The Raman spectra of GO-incorporated Mg implants revealed peaks that are typical of the functional groups found in GO, such as the oxygen-containing groups (-OH, -COOH, and C=O). These peaks were seen at around 1300-1400 cm⁻¹ (the D band), 1600-1700 cm⁻¹ (the G band), and 2700-3200 cm⁻¹ (the O-H stretching band).

OPTICAL MICROSCOPIC IMAGES:



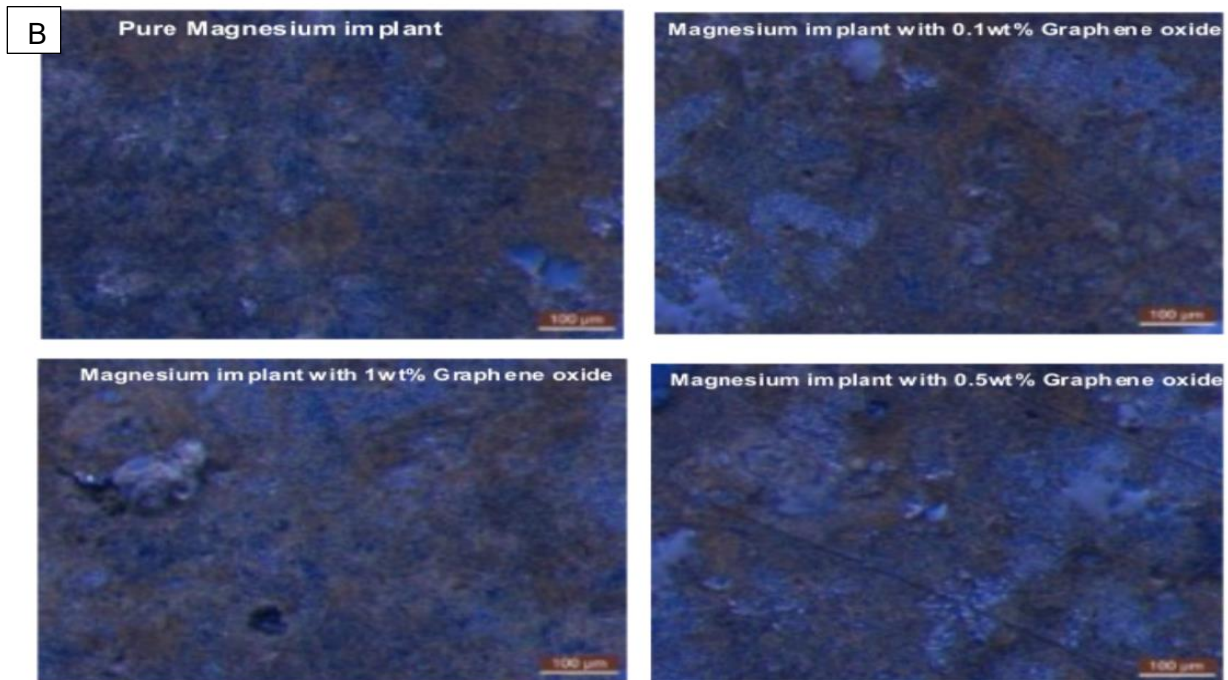
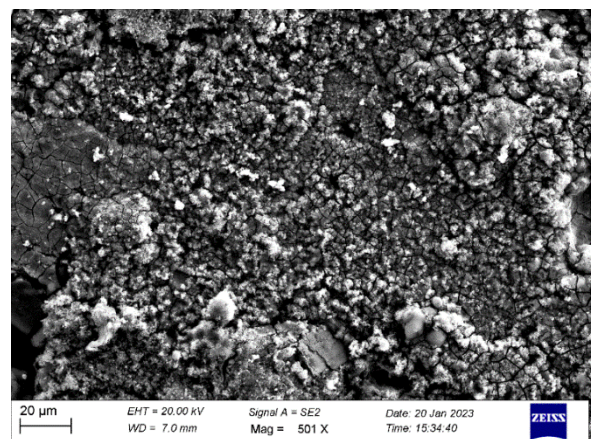
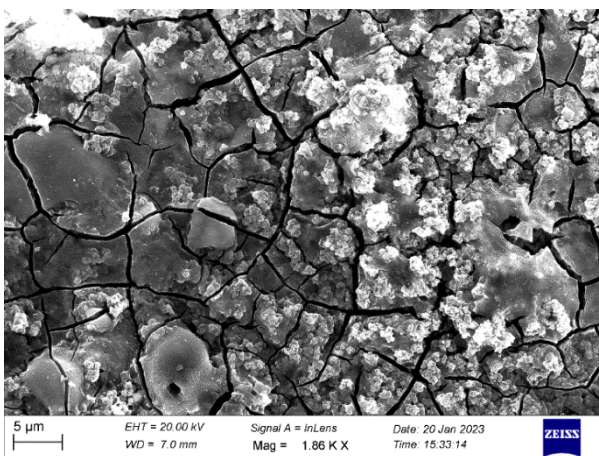


Fig 5.8. Microscopic images of fabricated implants with a field width of 100µm

The surface morphologies of the implants before and after sintering shows a vast difference in the dispersion of components, and uniformity is visible. The pits and gaps and other structural irregularities have also been significantly reduced

5.3.2. FABRICATED MG IMPLANTS COATED WITH POLYMER:

SEM-EDS:



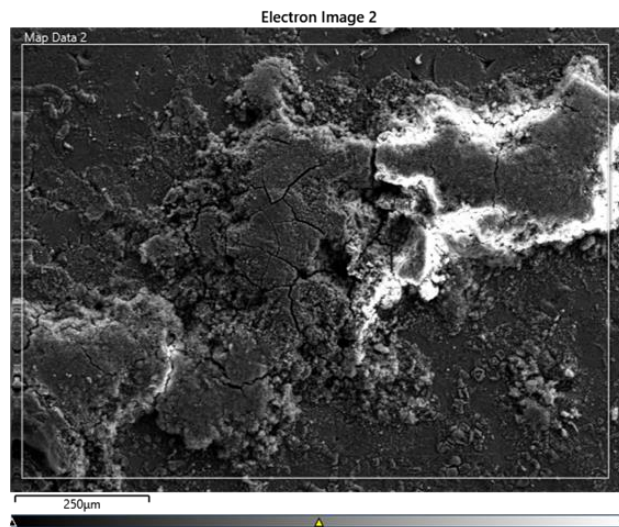
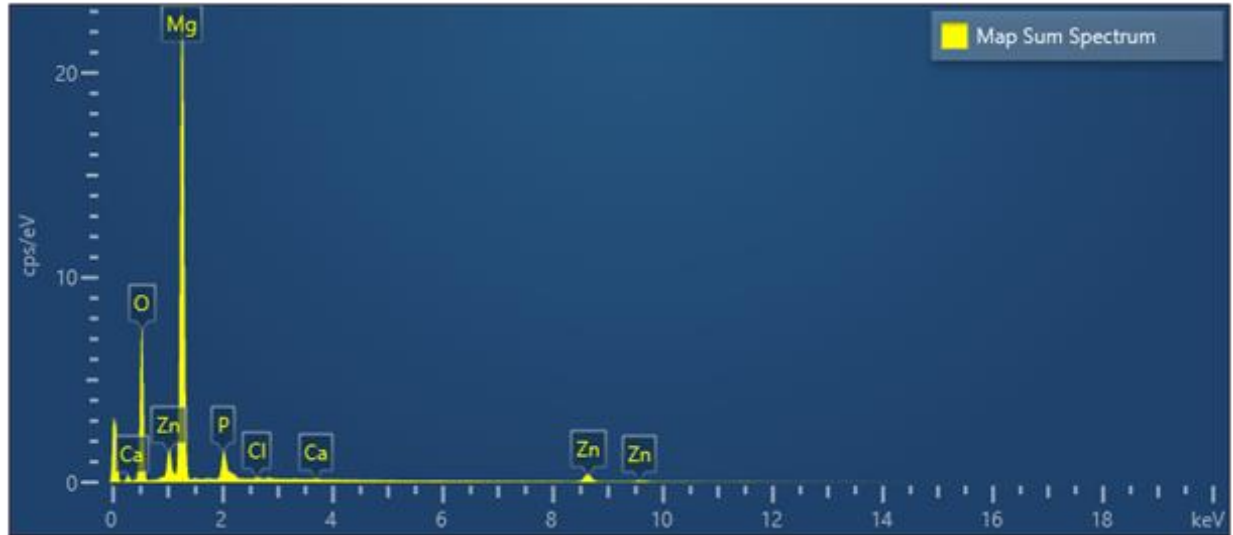


Fig 5.9. SEM-EDS Analysis of fabricated implants coated with polymer

SEM-EDS tests on pectin coated Mg implants revealed that the pectin coating is well-bonded to the Mg substrate and creates a homogeneous layer on the implant's surface. According to the SEM pictures, the pectin covering has a porous structure, which can increase the implant's biocompatibility and capacity to stimulate bone regeneration.

XRD

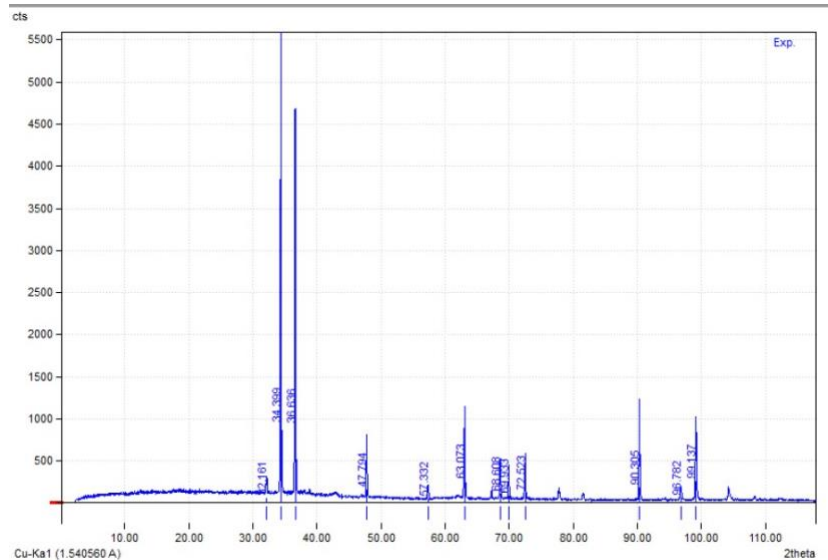


Fig 5.10. XRD analysis of fabricated implants coated with polymer

With a 2 theta value of 34.399, the XRD data implies that the pectin coating on the Mg implants may have resulted in the creation of a new crystalline phase. This number refers to a Mg oxide (MgO) diffraction peak, which may have generated as a result of the interaction between the Mg surface and the pectin coating during the coating process or later heating processes.

RAMAN SPECTRA

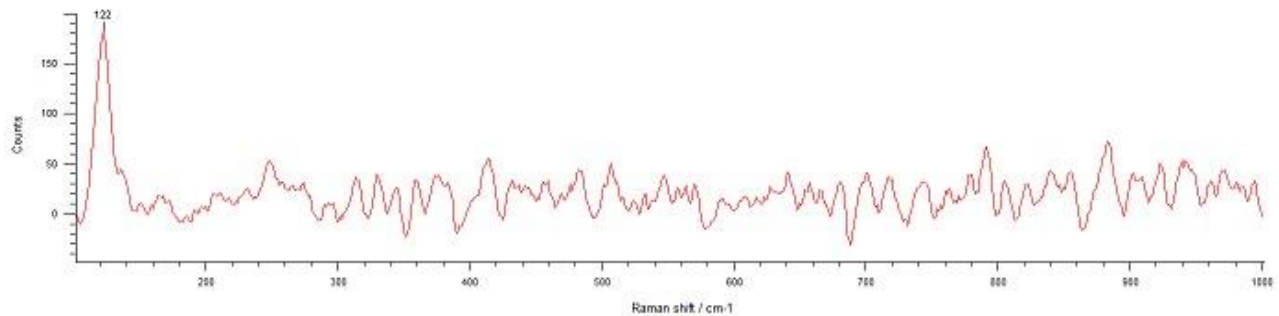


Fig 5.11. Raman Spectroscopy micrograph of fabricated implants coated with polymer

Raman spectroscopy investigations on GO integrated Mg implants revealed that the integration of GO can improve the implant material's osteogenic activity. The Raman spectra also show that the pectin coating is well-bonded to the Mg substrate, which can increase the mechanical strength and endurance of the implant.

5.4. WATER CONTACT ANGLE MEASUREMENT:

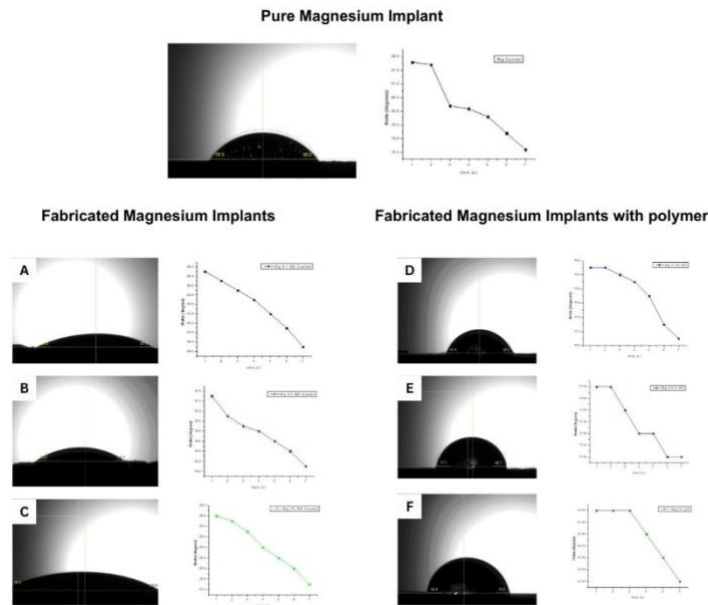


Fig 5.4.1. Effect of droplet volume on advancing and receding contact angles of water
A: 0.1wt % implant without polymer, B: 0.5wt % implant without polymer, C: 1wt % implant without polymer
D: 0.1 wt % implant with polymer, E: 0.5 wt% implant with polymer, F: 1wt% implant with polymer

The water contact angles (Figure 5.4.1) of both the pectin coated and uncoated implants were measured. Any value greater than 60° is hydrophobic and a value below is considered to be hydrophilic. It was found that the implants made of 0.1 wt % and 0.5 wt % GO were slightly hydrophilic, and the implant made of 0.1 wt % GO was highly hydrophilic. On the other hand, the implant made of pure Magnesium showed the most hydrophilicity. All the pectin coated implants showed contact angles below the range of 60° , indicating the favorable property of high hydrophilicity, when compared to the uncoated implants

5.5. NANOINDENTATION:

0.5 wt% FABRICATED MAGNESIUM IMPLANT

Test	Hardness (Vickers)	Instrumented Hardness (GPa)	Young's Modulus (GPa)	Maximum Depth (μm)
1	54.195	0.574	30.077	8.914
2	60.617	0.641	32.301	8.453
3	73.881	0.782	33.505	7.734
Average	62.898	0.666	31.961	8.367
Standard Deviation	8.197	0.087	1.420	0.486

Table 1 : 0.5 wt% fabricated mg implant

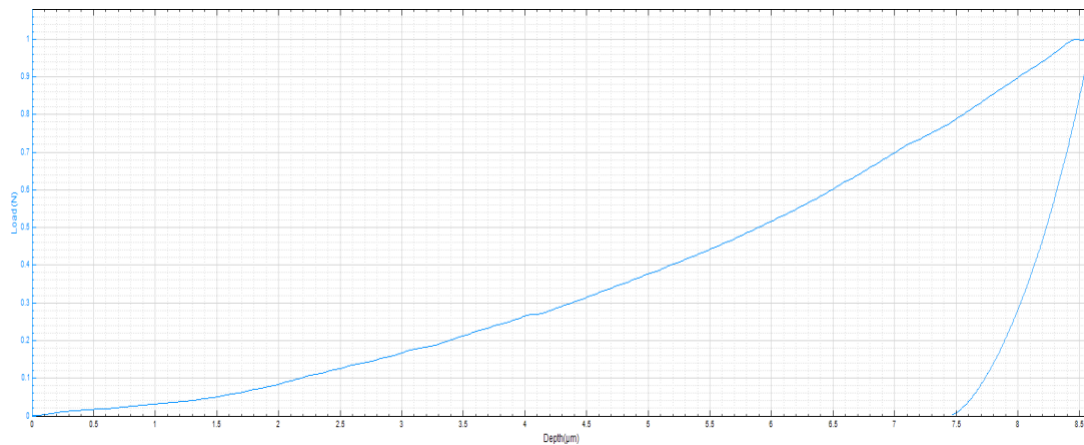


Figure 5.5.1: Nanoindentation graph of Loading curve of 0.5wt% fabricated mg implant

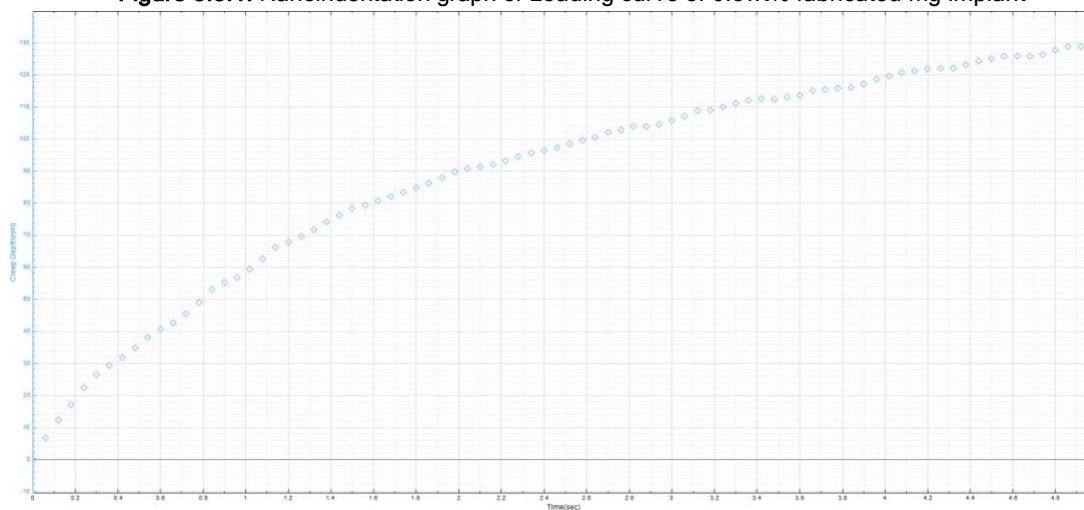


Figure 5.5.2.: Nanoindentation Creep depth curve of 0.5wt% fabricated mg implant

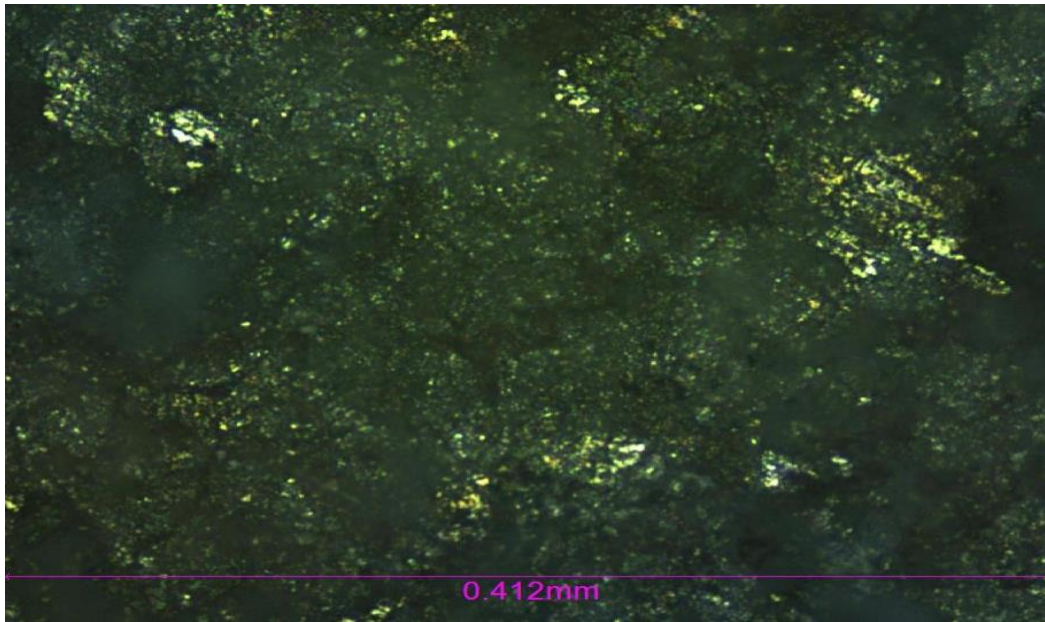


Figure 5.5.3: Image of indentation 10X magnification of 0.5wt% fabricated mg implant

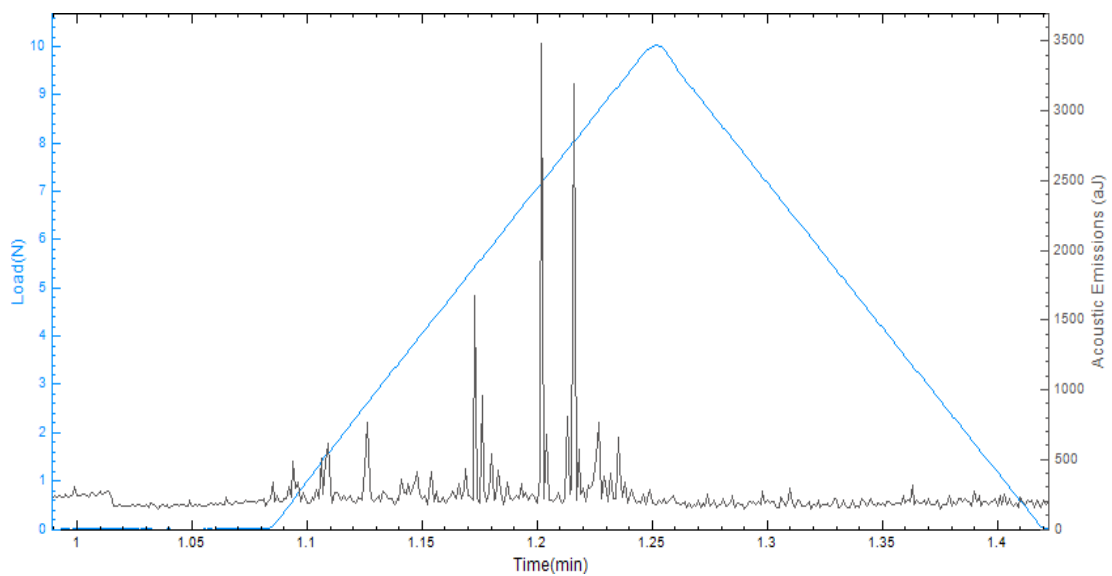


Figure 5.5.4: Load versus Time graph with Acoustic Emissions of 0.5wt% fabricated mg implant

1 wt % UNCOATED MG IMPLANT

Test	Hardness (Vickers)	Instrumented Hardness (GPa)	Young's Modulus (GPa)	Maximum Depth (μm)
1	56.361	0.596	26.803	8.820
2	49.923	0.528	24.460	9.360
3	49.170	0.520	23.299	9.450
Average	51.818	0.548	24.854	9.210
Standard Deviation	3.227	0.034	1.457	0.278

Table 2: 1 wt % uncoated mg implant

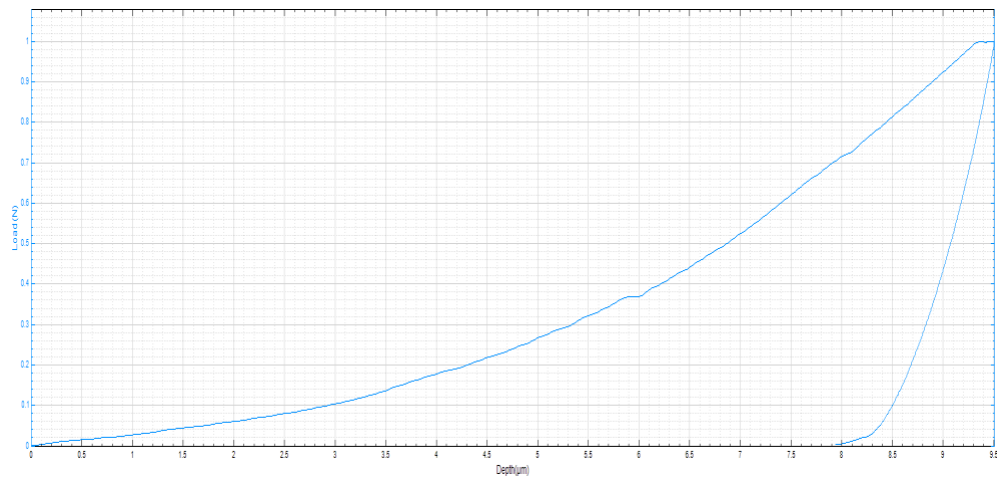


Figure 5.5.5: Nanoindentation graph Loading curve of 1 wt % fabricated mg implant

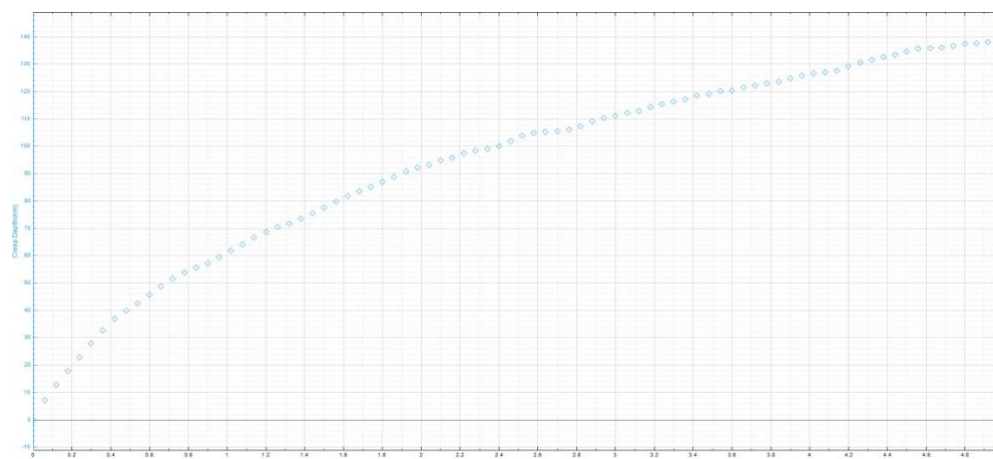


Figure 5.5.6: Creep depth curve of 1wt% fabricated mg implant

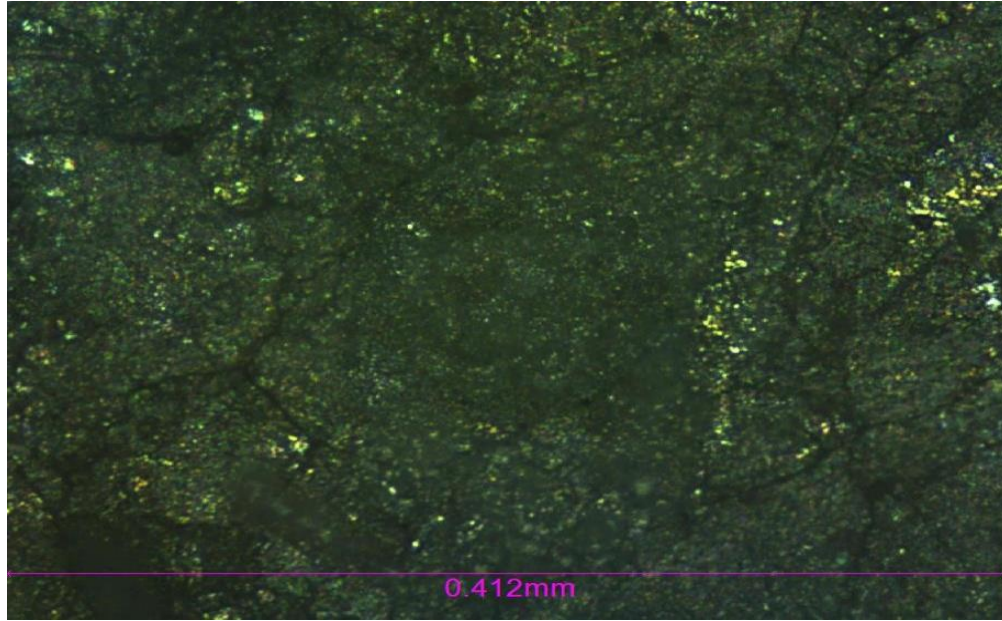


Figure 5.5.7: Image of indentation 10X magnification of 1 wt % fabricated mg implant

The implants made of 1wt% GO, fabricated mg implants can be considered ideal, as the young's modulus lies within the range of 10 to 30 GPa, which is equivalent to that of human bone. The Hardness of all the pellets was high with 0.5 wt % GO implants being the highest.

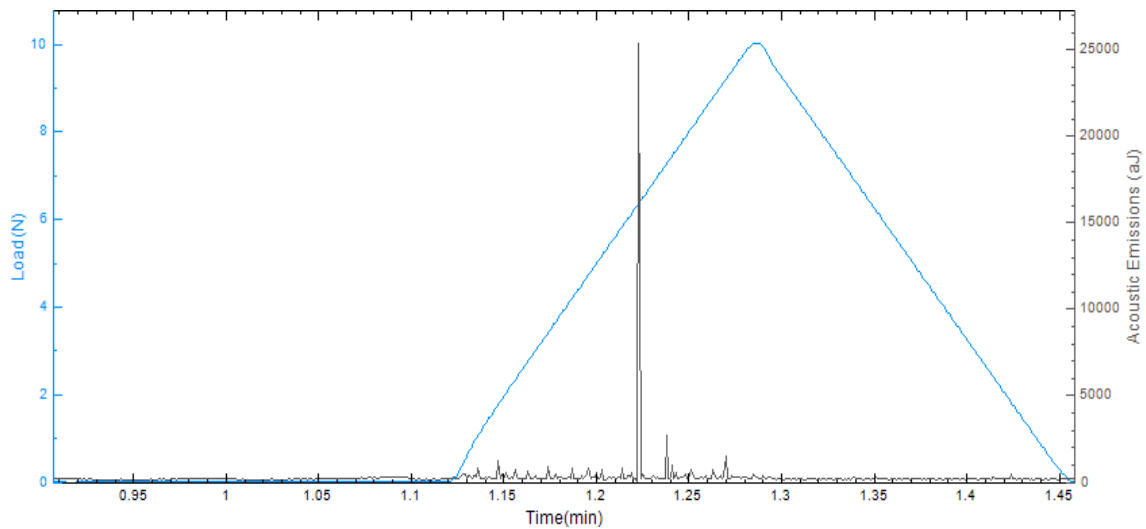


Figure 5.5.8: Load versus Time graph with Acoustic Emissions of 1wt% fabricated mg implant

1 wt% FABRICATED MG IMPLANT WITH 10% POLYMER

Test	Hardness (Vickers)	Instrumented Hardness (GPa)	Young's Modulus (GPa)	Maximum Depth (μm)
1	42.883	0.454	11.767	2.698
2	44.906	0.475	11.573	2.651
3	57.441	0.608	14.004	2.348
4	36.555	0.387	10.387	2.919
Average	45.446	0.481	11.933	2.654
Standard Deviation	7.579	0.080	1.307	0.203

Table 3 : 1 wt% fabricated mg implant with 10% polymer

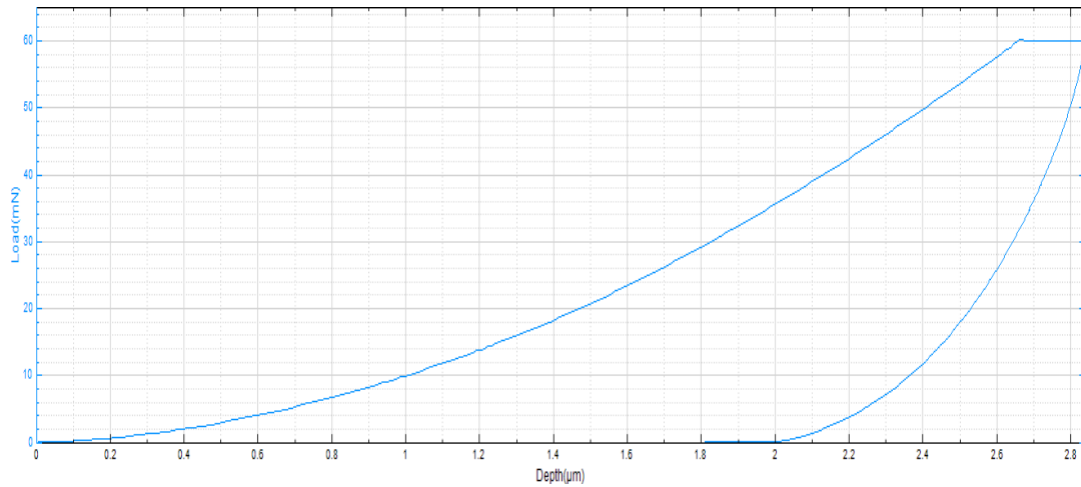


Figure 5.5.9: Loading curve in 2D report of 1wt% fabricated mg implant with 10% polymer

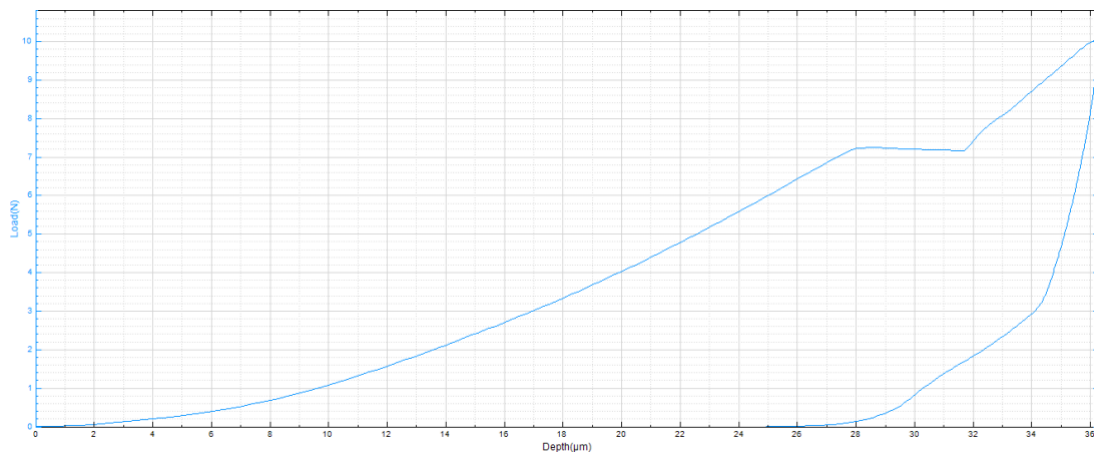


Figure 5.5.10: Loading curve in 1D report of 1wt% fabricated mg implant with 10% polymer

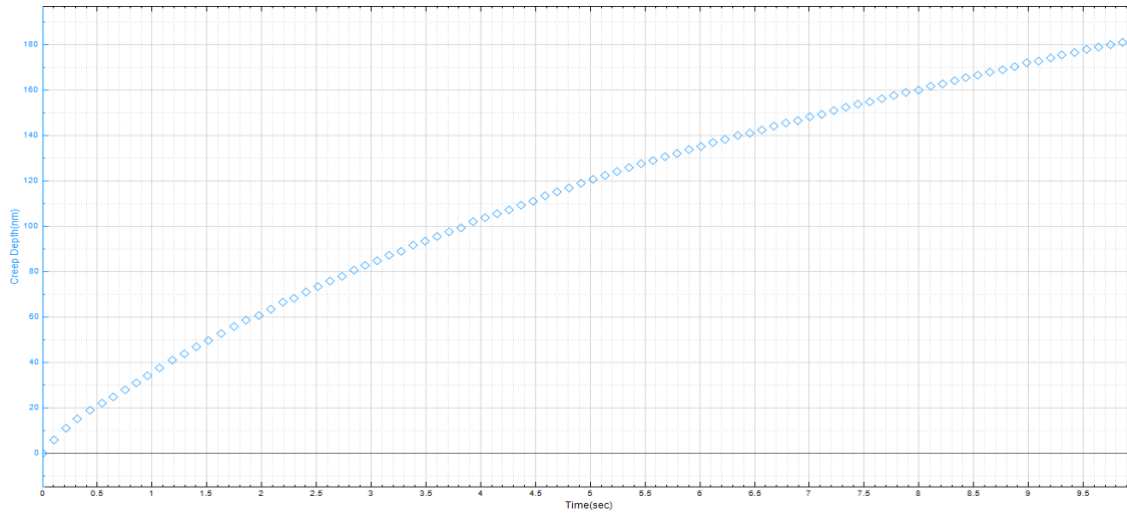


Figure 5.5.11: Creep depth curve of 1wt% fabricated mg implant with 10% polymer

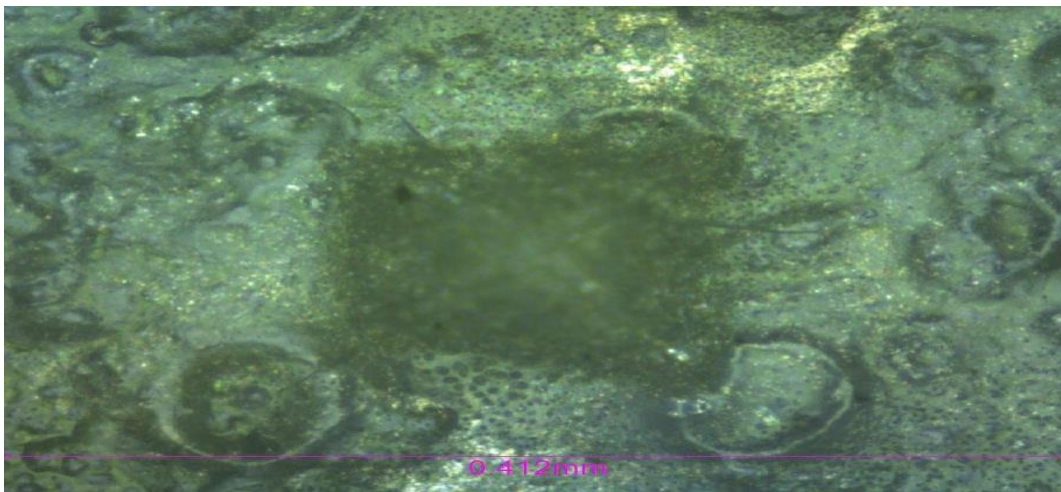


Figure 5.5.12: images of indentation 10X magnification of 1wt% fabricated mg implant with 10% polymer

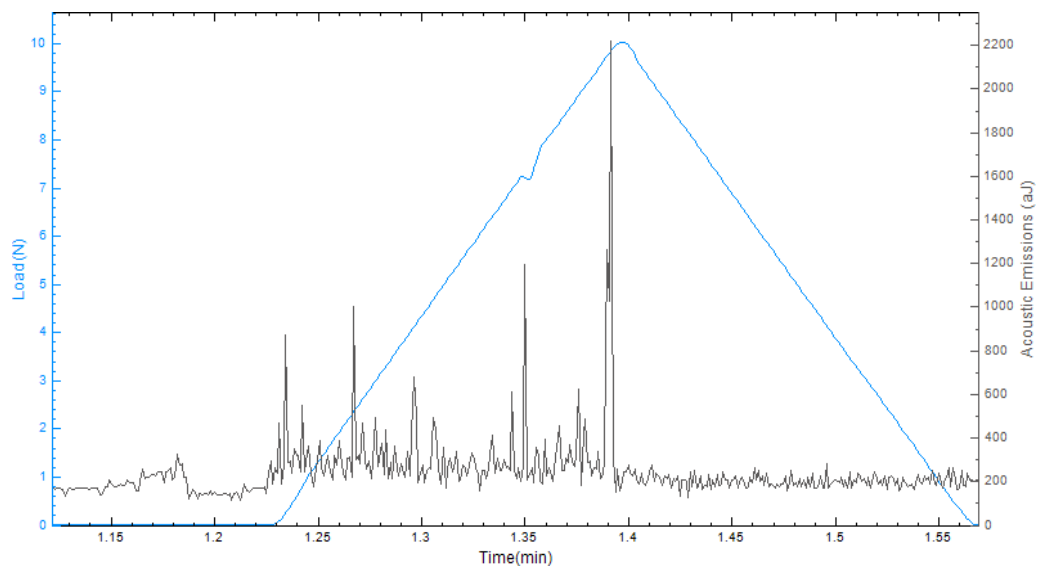


Figure 5.5.13: Load versus Time graph with Acoustic Emissions of 1wt% fabricated mg implant with 10% polymer

SUMMARY OF RESULTS

Indentation Test Results—Micro

Sample	Hardness (Vickers)	Instrumented Hardness (GPa)	Young's Modulus (GPa)	Maximum Depth (μm)
0.5 wt% Uncoated Mg Pellet	62.898 $\pm$ 8.197	0.666 $\pm$ 0.087	31.961 $\pm$ 1.420	8.367 $\pm$ 0.486
1 wt% Uncoated Mg Pellet	51.818 $\pm$ 3.227	0.548 $\pm$ 0.034	24.854 $\pm$ 1.457	9.210 $\pm$ 0.278

Table 4 : Indentation Test Results—Micro

Indentation Test Results—Nano

Sample	Hardness (Vickers)	Instrumented Hardness (GPa)	Young's Modulus (GPa)	Maximum Depth (μm)
1 wt% Coated with 10% P	45.446 $\pm$ 7.579	0.481 $\pm$ 0.080	11.933 $\pm$ 1.307	2.654 $\pm$ 0.203

Table 5 : Indentation Test Results—Nano

Indentation Test Results

Sample	K_c ($\text{MPa} \cdot \text{m}^{1/2}$)
1 wt% Coated with 10 % P	0.751 $\pm$ 0.03

Table 6 : Indentation Test Results

Samples 0.5 wt % Uncoated Mg implant and 1 wt % Uncoated Mg implant did not fracture with the expected shape and crack propagation.

5.7 OPTICAL PROFILOMETRY:

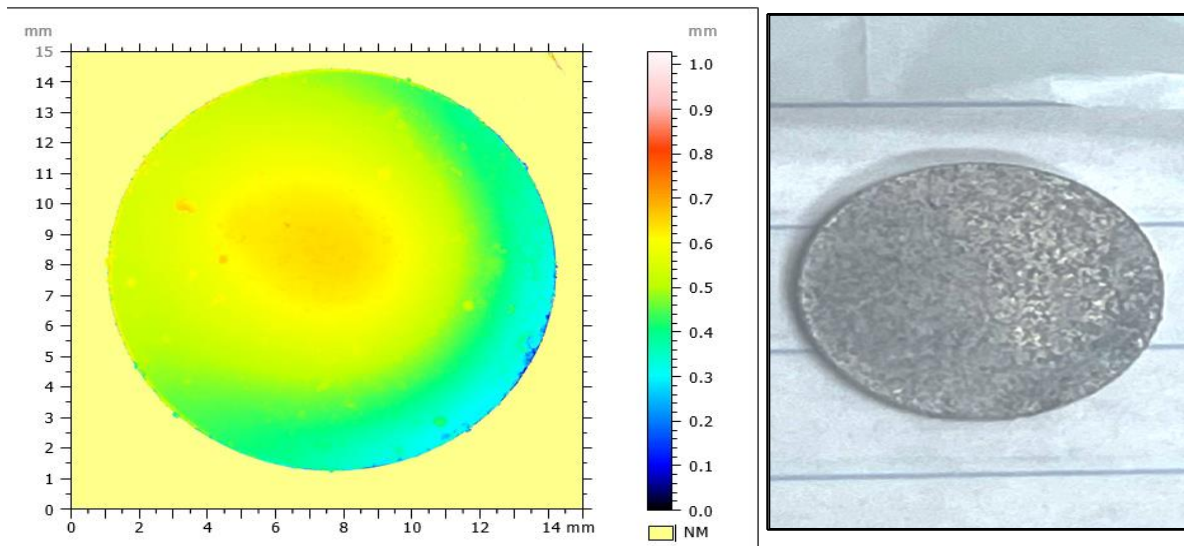


Figure 5.7.1.0.5wt uncoated Mg implant sample A

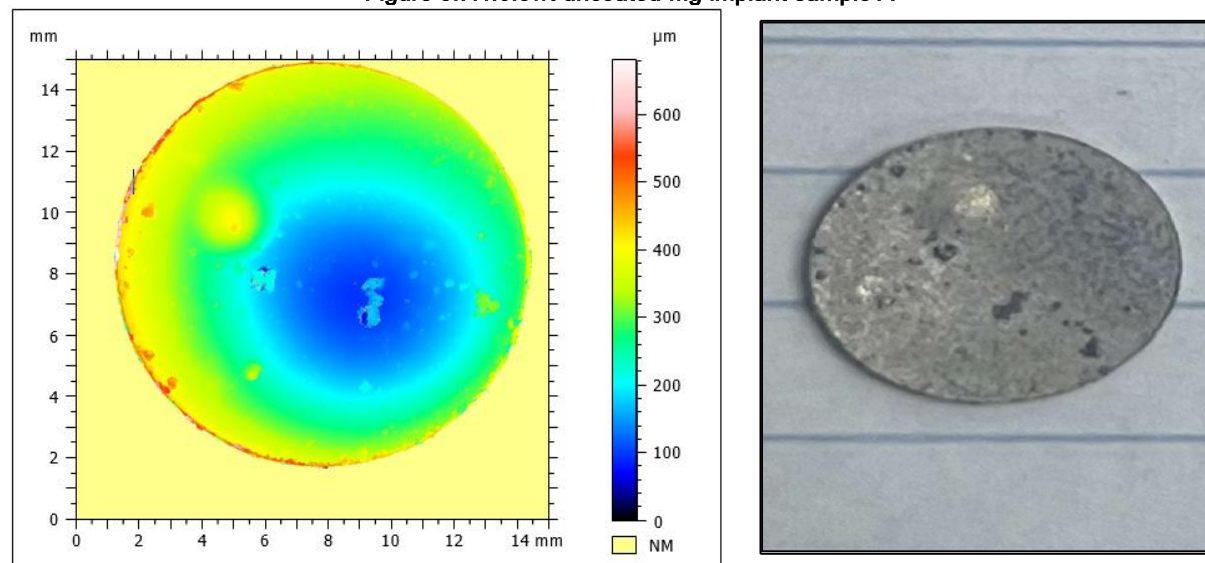


Figure 5.7.2 . 0.5wt% fabricated Mg implant sample B

The results of the optical profilometry analysis show that the graphene oxide incorporated magnesium implants have a highly uniform and smooth surface, with low roughness values compared to traditional magnesium implants. The low roughness values are indicative of a surface that is less prone to mechanical wear and better suited for long-term use in the body.

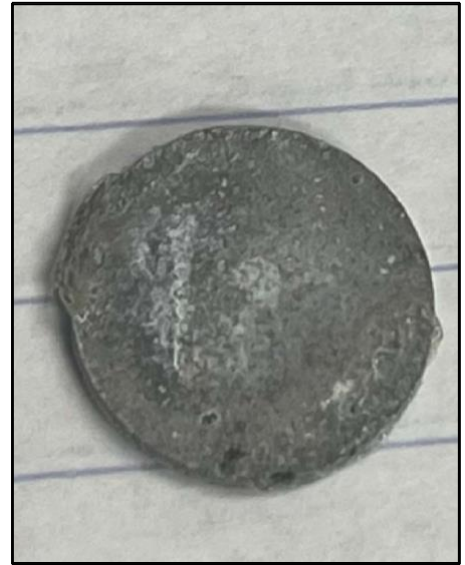
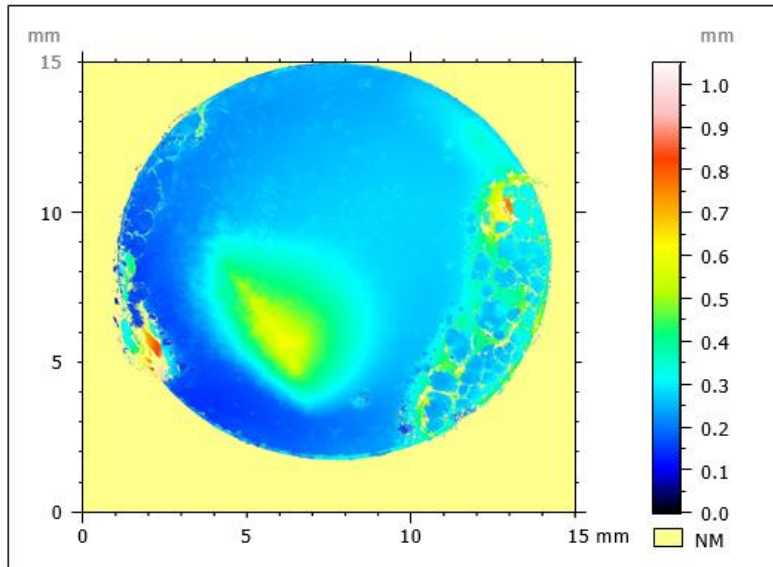


Figure 5.7.3 .1wt % fabricated mg implant with 10% polymer (sample A)

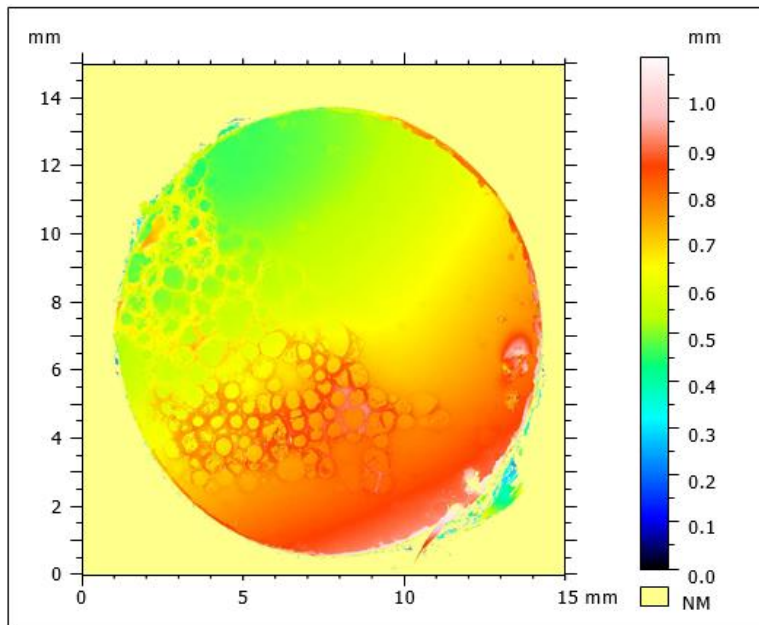


Figure 5.7.4 .1wt % fabricated mg implant with 10% polymer (sample B)

Additionally, the results show that the graphene oxide incorporated magnesium implants have a higher degree of surface porosity, which could promote enhanced bone regeneration compared to traditional implants. The increased porosity may allow for better integration with surrounding bone tissue and improve the overall stability of the implant.

5.8 CORROSION STUDIES:

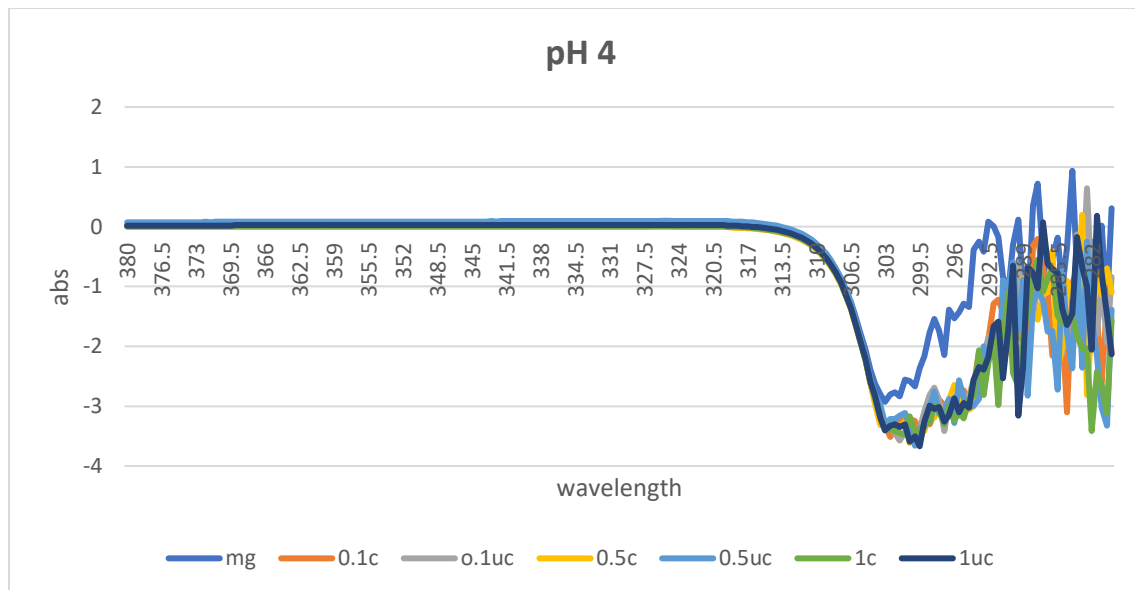


Figure 5.8.1. leaching study of Mg implant in pH 4 solution

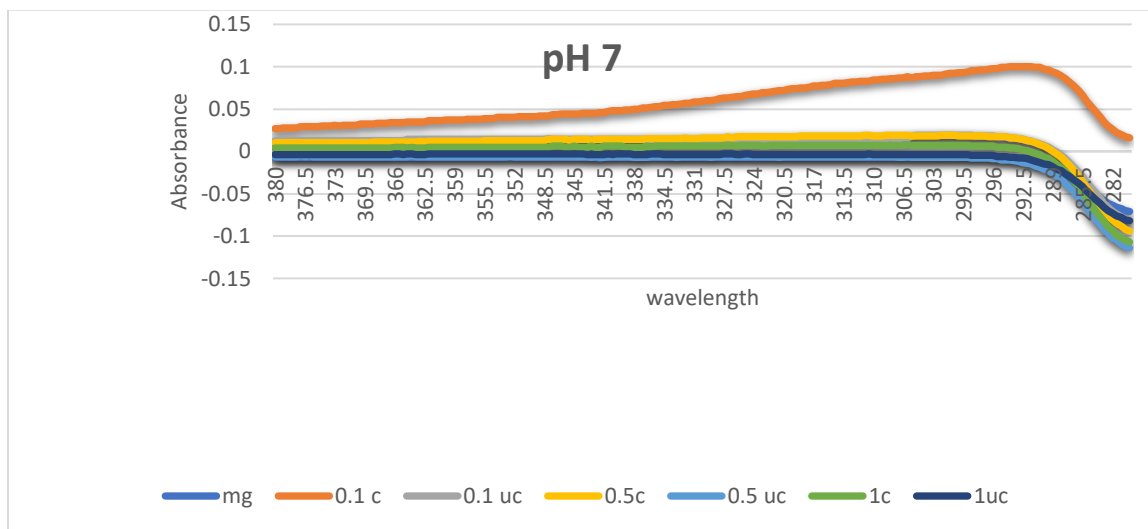


Figure 5.8.1. leaching study of Mg implant in pH 7 solution

The graphs indicate a stable decrease in the spectrum in pH 7 solution better than the pH 4 solution indicating that the prepared implants can be biocompatible as well as biodegradable.

6. CONCLUSION AND SUMMARY

In summary, incorporating GO into Mg implants has enormous potential for bone regeneration. These implants' microstructure study revealed that they had superior mechanical qualities as well as improved osteogenic activity, making them suited for usage in orthopedic applications. The application of GO in implant technology is an intriguing new frontier in the field of biomaterials, with the potential to change the way we approach bone regeneration. However, further research is required to fully understand the long-term impacts of these implants and to optimize their design and production procedures. Overall, this study is an important step forward in the development of sophisticated implant materials that can enhance the quality of life for individuals suffering from bone fractures and disorders.

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