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Bio-Active Characteristics and Physical Characteristics of the Mg - 3 Zn -1Ti /TCP Layered Composite Using Centrifugal Casting Process

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This study aims to examine the Bio-active characteristics and physical characteristics of functionally graded metal matrix composites (FGM-matrix composites) produced using magnesium metal as the primary material (Mg). The corrosion properties of magnesium, titanium, and zinc alloys make them highly suitable for the development of biomaterials used in implants.
In this research, the processing and Bio-active characteristics and physical properties of Magnesium metal as the base material, Zinc and Titanium as alloying elements, and Tricalcium phosphate (TCP) as a reinforcement with composites of Mg - 3 Zn -1Ti are discussed.
FGM-metal composites display a radial gradient distribution of reinforcements, which leads to the formation of distinct zones: a rich-matrix inner zone (sample C), a transition zone (sample B), and a rich-particles outer zone (sample A). The rich-particles outer zone demonstrates improved bio-active characteristics and physical properties, such as enhanced bulk density and porosity. In order to assess its suitability for orthopedic applications, the bio-active properties of the Layered FGM alloy were evaluated through cytotoxicity analysis using MTT assay and Hemolysis assay.

Keywords: Functionally graded material metal matrix composites (FGM- matrix composites), Centrifugal casting, Tensile Characteristics, Micro hardness, compressive strength, microstructural behavior, and bio-implants.

1. Introduction

The continuous growth of modern industries in material technology and scientific advancements has resulted in an increasing demand for advanced and intelligent materials with specific properties. In recent times, material processing has led to the emergence of

Functionally Graded Materials (FGM), which are complex multilayered materials consisting of two phases that gradually transition their characteristics within the sample [1]. According to Pasha et al. (2022), biomaterials have played a crucial role in restoring mobility and functionality for many individuals through the repair or replacement of damaged bones and joints. Chemical stability, biocompatibility, and mechanical properties are key factors for the success of biomaterials. Interestingly, FGM has gained significant attention from the automotive and biomedical industries for the manufacturing of various parts [2].

Magnesium's biodegradability and biocompatibility make it an increasingly recognized material for biomedical implants. However, its rapid breakdown in physiological conditions can lead to corrosion and implant failure. To overcome this, new alloys with improved mechanical properties and corrosion resistance are being developed. This study investigates different material combinations, including magnesium-based alloys with zinc, titanium, and Tricalcium phosphate (TCP), to enhance the performance of biomedical implants. Specifically, the focus is on composites of Mg - 3 Zn -1Ti with Tricalcium phosphate as a reinforcement to address the challenges associated with magnesium degradation in physiological environments. The combination of these metals can improve biomedical components by facilitating metal to metal joint contact, resulting in benefits such as enhanced stress shielding in adjacent bone and increased load support with reduced geometries. However, it is important to note that engineered materials may have some flaws and drawbacks, which can be addressed through multiple experiments. These drawbacks include limited creep resistance, restricted cold forming capability, and inadequate corrosion resistance.

The investigation of FGM rich and poor particles layers, (inner, moderate, and outer) layers, is crucial when analyzing the mechanical and microstructure properties of FGM-based alloys. Through a systematic examination of the microstructural features and Vickers micro hardness of the produced FGM, researchers can gain valuable insights into the performance of these alloys.

The study's findings suggest that FGM-based alloys possess desirable qualities in terms of corrosion resistance, mechanical strength, and biocompatibility, making them suitable for various applications that require these properties. Ongoing research is focused on understanding the underlying mechanisms that govern the mechanical behavior of magnesium-based alloys, with the aim of developing effective surface treatments to further enhance their performance in different environments and applications. It was observed that there is a lack of research on FGM-based alloys compared to biomaterials like mesh or lattice structured Stainless steel, which have shorter lifespans in the human body due to rapid degradation and inherent defects. The FGM samples underwent thorough mechanical and microstructural characterization using various experimental procedures. [4].

2. Methodology:

2.1 Material

The study utilized magnesium powder with a 99% purity index and a particle size of 50 microns as the matrix material. Magnesium metal, with a purity of 98.7% and a particle size

of 20 microns, was selected as the base material due to its higher quantity and lower density. Zinc and Titanium were incorporated as alloying elements, along with Tricalcium phosphate (TCP) at a weight percentage of 10% as reinforcement in the composites. The metal matrix AZ31, with a density of 1740 kg/m3 (108.6 lb/ft3) and a melting point of 650°C, was used in the study. Additionally, the AZ31 metal matrix exhibited a hardness of 55.0-05 Hv [5-6].

The melting Mg is combined with the alloying element and reinforcement material, and then transferred to the centrifugal casting mold system to achieve the desired graded distribution. This process is facilitated by a software for stir casting, and the resulting samples can be seen in Figure 1. After cleaning, a layer of graphite paste is applied to the crucible, stirrer, and drain channel of the casting furnace. The furnace is activated and the temperature is monitored using a thermocouple. Once the magnesium substance reaches a temperature of 740 degrees Celsius, it is placed into the furnace and the crucible's lid is closed. To ensure a uniform mixture of the zinc and titanium alloying powder with the reinforcement material TCP, preheating them to 450 ºC was necessary.

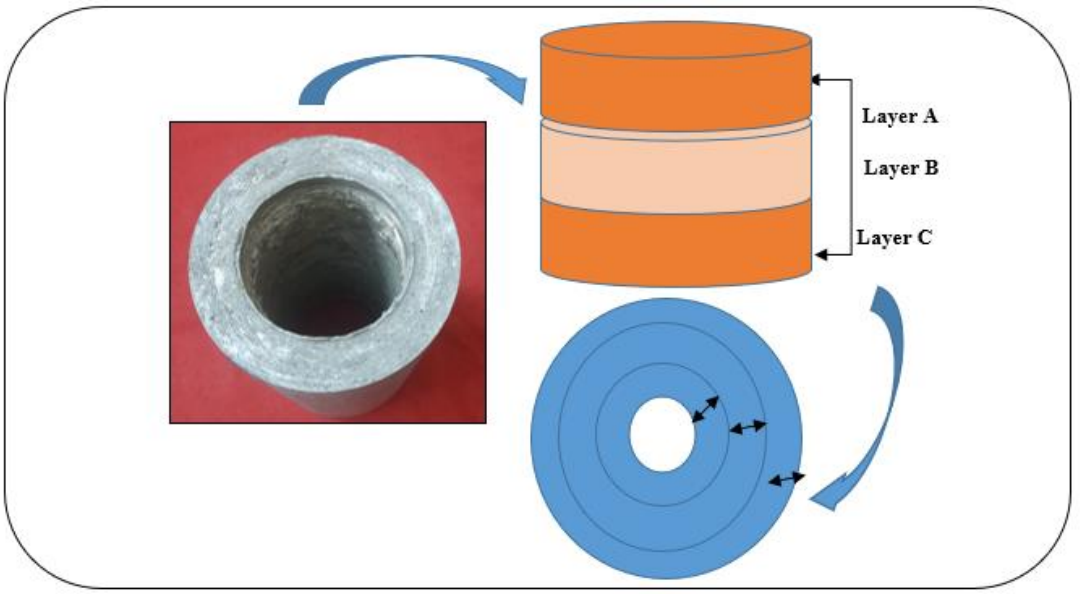

Figure.1 FGM fabricated component

Table 1. FGM fabrication parameters

Gravity casting Parameters	Output values
Stirrer RPM	450 RPM while mixing & 750 RPM while casting with vertical movement for 30mm
Furnace Temperature	700^0 C
Melt Temperature	740^0 C
Die Temperature	450^0 C
Centrifugal RPM	1300 RPM

Excess moisture was successfully removed during the preheating stage, after which the prepared melt was introduced to the stirrer operating at 450 rpm. The reinforcement material was gradually added to the vortex of the melt while the stirrer moved in an oscillating motion. To achieve a consistent distribution of reinforcements in the molten magnesium, the molten

liquid was poured into a preheated centrifugal die set at a temperature of 450 ºC.

The centrifugal die is adjusted to a speed of 1300 RPM to prevent sudden solidification. After completing all necessary preparations, the furnace valve is opened to pour the molten liquid into the rotating die. The centrifugal force propels the molten melt towards the die wall, causing solidification. Sufficient cooling time is provided for the centrifugal cast before it is removed from the die, cleaned, and prepared for further testing through sample cutting. The experimental parameters used in this study are detailed in Table 1, and the same procedure was applied to each sample.

2.2 Bio-active Characteristics:

The MTT Powder undergoes filtration using a 0.4 μm filteration and is then either stored at below 10 °C for regular use or freezing for longer durations. Dimethyl sulfoxide, commonly known as DMSO, is a clear liquid obtained as a by-product during the daily basis paper production method from wooden paste. This colorless liquid serves as a polar, aprotic solvent that is water-miscible and has the ability to dissolve a wide variety of polar and nonpolar small molecules. CO2 incubators are specialized enclosures designed to precisely regulate the environmental conditions necessary for the cultivation of biological or cell cultures. The Tecan Plate reader is a modular system that operates based on monochromatic principles, eliminating the need for filters. It offers users a wide range of Absorbance readings from 230-1000 nm or 300-600nm. [7]

2.3 bulk density:

The determination of bulk density involves transferring the sample to a standard cup (10 ml). To measure the weight of the cup with and without the sample, a microbalance with a precision of 0.01g is utilized. Here bulk density is then calculated using equation (1).

$$\text{Density} = \frac{\text{mass (m)}}{\text{Volume } (\rho)} \quad \ldots\ldots\ldots \quad (1)$$

Where: ρ= the sample bulk density (g/cc).

2.4 Density and Porosity of Mg - 3 Zn -1Ti FGM composites:

Micrometers and calipers are not suitable for accurately to measuring the volume of arbitrary metallurgical powder. The Archimedes principle, which relates density to mass/volume, provides a precise method to measure when objects are submerged in water, they displace a certain volume of water. In order to accurately measure the volume of porous powder metallurgical specimens, it is necessary to seal the pore associated surfaces. If failure to seal the pores or port with oil can lead to water absorption, loss of buoyancy, and an inaccurate increase in density within the region. The density and porosity of FGM samples can be measured using ASTM-B 328-96 [8].

- Prior to measuring its weight as mass A, At 100°C argon atmosphere, sample is subjected to a drying process in 60 min.
- The evacuating pump effectively lowers the pressure on the submerged specimen in 22 cst viscosity oil at 35°C to under 5kPa for 25 minutes at room temperature.
- The mass of the specimen, fully saturated with air, is measured as B.

- To determine the weight of the sample completely been saturated in water, measure its mass as F.
- Additionally, temperature readings are taken to ascertain the density of water at the given temperature.

Porosity can be determined through the application of the Bragg-Kleeman rule, the process includes the multiplication of the weight percentage of each elemental powder by the theoretical density.

$$\sum_{i=1}^{n} Wt1\ X\ \rho 1 + Wt2\ X\ \rho 2 \ldots\ldots\ldots + Wtn\ X\ \rho n$$

Where ρt: theoretical bulk density of FGM samples (g/cc),

n= elemental powders,

Wt= weight percent (%), ρ1, ρ 2, ρ 3….n.

3. Results and Discussion:

3.1 Density and Porosity of (Mg - 3 Zn -1Ti FGM) samples:

When compared to sample B and C FGM, sample A has the highest bulk density, Figure. 2 shows the graphical representation of bulk density of the Mg - 3 Zn -1Ti FGM samples. The bulk density of FGM samples depends on factors such as total density, particle size and distribution, particle shape, and open areas within the microstructure. As the friction surface area increases and uniformity decreases, the bulk density decreases.

Bulk densities of samples (layer A, layer B, layer C,) are revealed in figure. 2. Layer A exhibits the highest bulk density among all layers, attributed to its elevated content of higher atomic density, specifically TCP. A density study of Mg - 3 Zn -1Ti FGM alloy reinforced with TCP is illustrated in Figure 3 (a). The density of sample A is 1.98 g/cc. In contrast, the sample B and sample C composite densities were 1.78 g/cc and 1.73 g/cc, respectively. The density measurement shows an increasing trend with an increase in TCP reinforcement concentration in the Mg - 3 Zn -1Ti FGM alloy. In contrast, the porosity decreases as the reinforcement concentration increases. [9]

This behaviour could be due to the addition of less dense reinforcement to a denser matrix phase. It should be noted that the increase in porosity shown in Figure 3, is advantageous to osteoblast formation in implants, and hydroxyapatite has excellent biocompatibility. Due to its better density, TCP has an extremely high strength-to-weight ratio, which makes it suited for biomaterial applications.

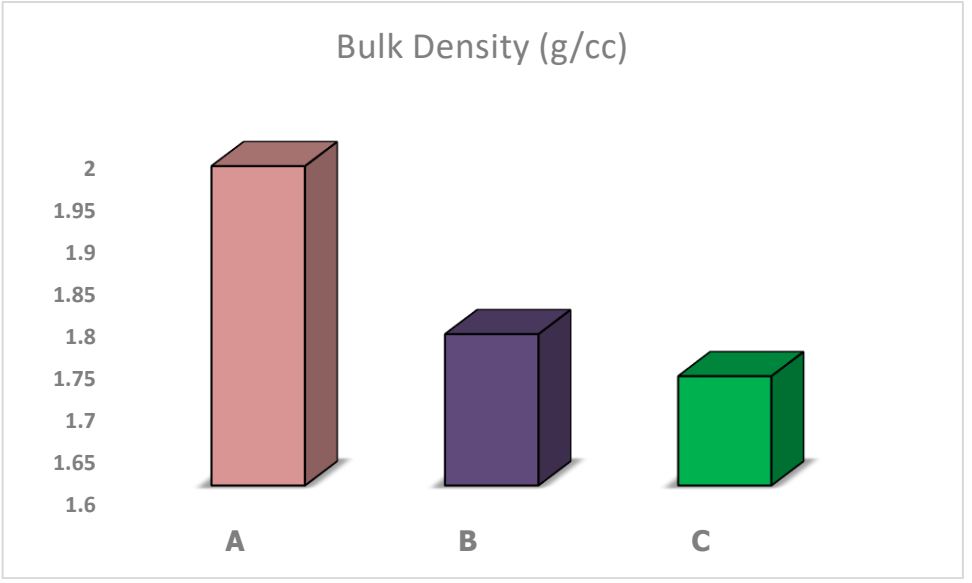

Figure 2. Bulk density of Mg - 3 Zn -1Ti FGM alloy

3.2 Bio Active Characteristics:

The need for biomedical materials in biomedical applications, such as orthopaedics and dental implants, is seeing significant growth. The demand for biomedical materials in various biomedical uses, including orthopedics and dental implants, is experiencing notable expansion. Magnesium (Mg), Zinc and Titanium is widely acknowledged as a biomaterial with outstanding mechanical properties, resistance to bodily fluids, and biocompatibility.

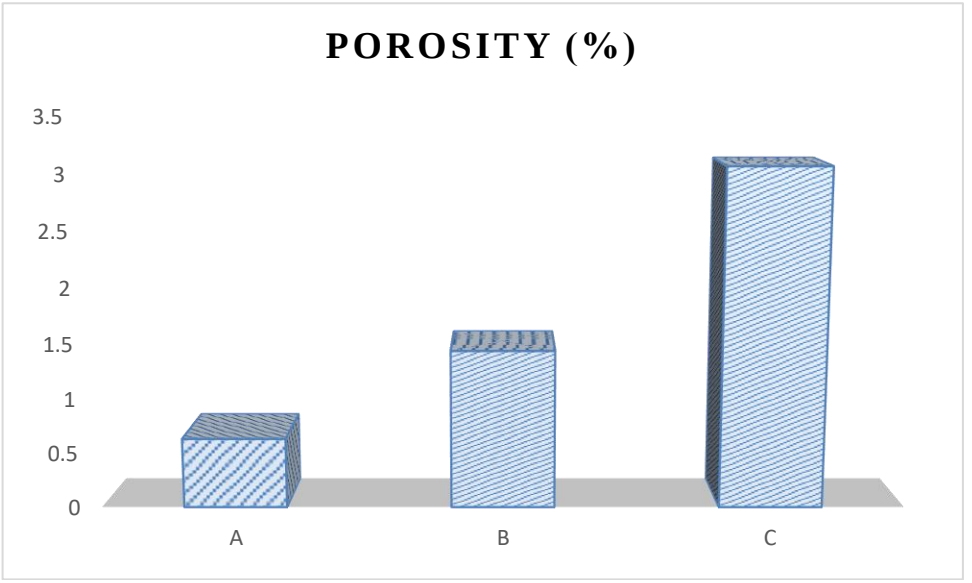

Figure 3. Porosity percentages of FGMs samples.

3.2.1. MTT Assay

The MTT test is a colorimetric method employed to compute cellular metabolic movement, viability of cells, proliferation, and cytotoxicity. The test trusts on the translation of a yellow tetrazolium salt (C18H16BrN5S) 3-(4, 5-dimethylthiazol-2-yl)-2, 5- diphenyltetrazolium bromide into an insoluble formazan compound, which exhibits a purple colour, (mitochondrial transformation were shown in figure 4). This conversion is facilitated by cellular oxidoreductase enzymes that are dependent on NAD (P) H. The MTT assay is a quantitative approach that enables efficient processing of a large number of samples without the need for washing procedures or extra chemicals. The MTT test is extensively employed in both academic and commercial settings, with commercially available kits including MTT solutions and a solubilization reagent. Nevertheless, the assay is subject to restrictions and possible confounding factors that can impact the results. These variables include the initial number of cells used, the reaction of the MTT reagent that is added to the cells, the extent of incubation with MTT, the type of culture media used, the metabolic state of the cells, and the presence of substances that may interfere with the Assay.

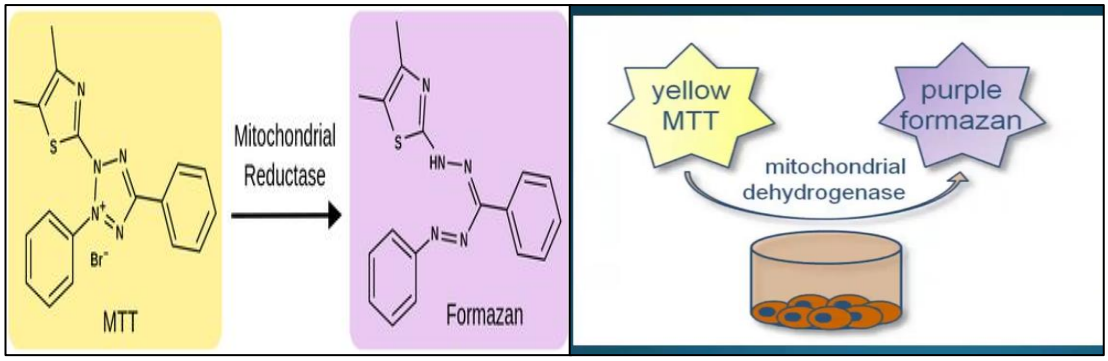

Figure 4. Mechanism of MTT assay (Ref: Labster.com)

The single layer cell principles underwent trypsinization and the cell count was standardized to 1.0 x 105 cells/ml using appropriate media with 10% FBS. Subsequently, 100 µl of the diluted cell suspension (50,000 cells/well) was dispensed into each well of the 96-well microtiter plate. Following a 24-hour incubation period, during which a partial monolayer was established, the supernatant was removed, the monolayer was rinsed once with medium, and 100 µl of varying concentrations of test drugs were introduced onto the partial monolayer in the microtiter plates. The percentage growth inhibition was calculated using the following formula and concentration of test drug needed to inhibit cell growth by 50% (IC50) values is generated from the dose-response curves for each cell line.

Calculating Inhibition:

% Inhibition = 100 – (OD of sample/OD of Control) x 100.

3.4.2. MTT absorption:

Assessing cell viability using varying amounts of the sample over a 24-hour period. The viability of MG 63 cells was 93% in the treatment group and 100% in the control group. Table 2 shows the MTT assay data, clearly indicates that top sample displayed a greater quantity of

MG-63 cells. The enhanced cellular vitality seen in the sample C with higher porosity, as quantified by the MTT experiment, can be due to the heightened metabolic activity experienced by cells that are in a more porous medium. The MTT test quantifies cellular metabolism as a reliable measure of both cell viability and proliferation. Figure 5 depicts the graphical representation of MTT assay of FGM samples.

Table 2: MTT assay for FGM samples

	Control (0)	A	B	C
	1.206	1.212	---	----
	1.163	----	1.145	----
	1.125	----	----	1.209
Viability	100	93	87	82

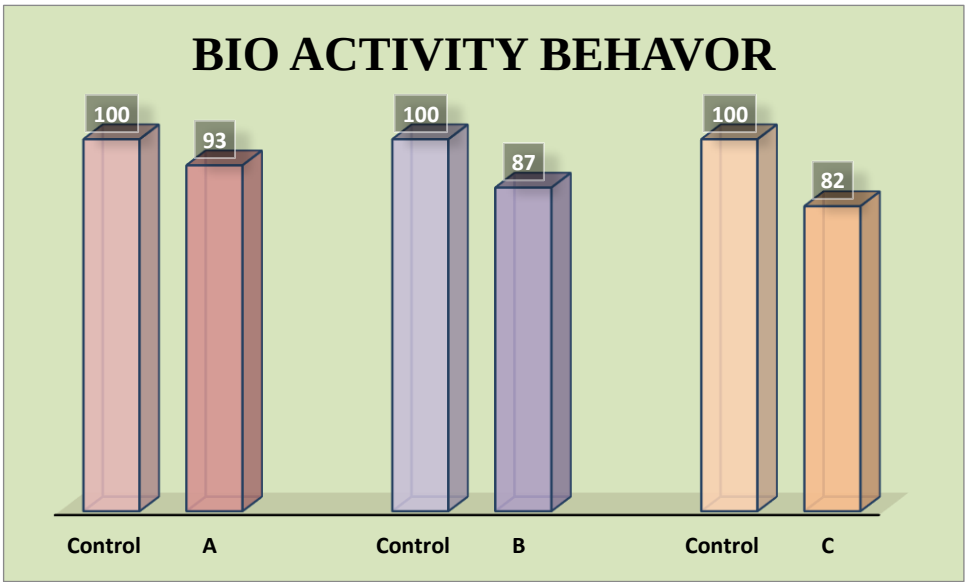

Figure 5. MTT assay of FGM Samples

The current investigation demonstrates that the vitality of MG-63 cells is significantly greater in the top region sample with less porosity. The reinforced particles exhibited non-toxic effects on MG-63 cells in the current investigation. (Debnath et.al 2017) found that SiO2-TiO2 composite exposed to MG63 cell lines for 24 hours at a higher concentration exhibited a vitality of 77%, which was greater compared to the vitality seen after 48 and 72 hours. The current investigation found that magnesium had a 96-99% effectiveness against MG 63 cells after 24 hours of testing for samples with different porosity percentage. The findings of this study unequivocally

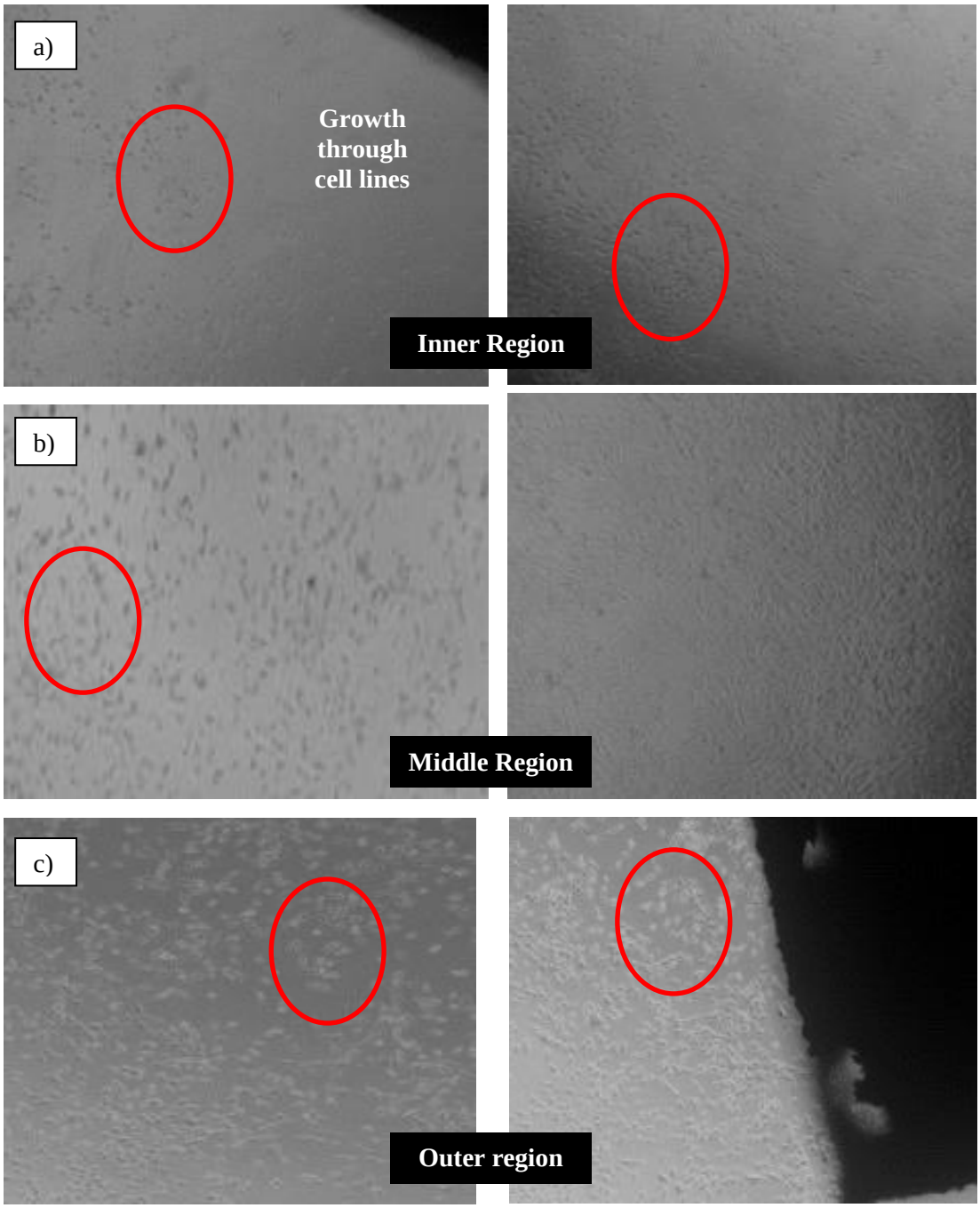

Figure 6. SEM images of Growth through cell lines of FGM **samples**

Demonstrate that magnesium exhibits superior biocompatibility with human osteosarcoma (MG63) cell lines. Therefore, based on the aforementioned test findings. The FGM samples with lower porosity demonstrated clear suitability for use in bio implants. Sample A exhibits

greater performance compared to both B and C region FGM samples, attributed to its higher porosity % and shows excellent in cytoxicity. Figure 6 shows the different SEM images of cell growth of Mg - 3 Zn -1Ti FGM alloy

3.4.3 Hemo-compatibility assay:

Thrombosis, the formation of a blood clot on the surface of an implant, is a cause of implant failure as it blocks the flow of blood. Fibrinogen rapidly adheres to the surface upon contact with blood. At the same time, factor XII also binds to the surface and undergoes auto-activation, leading to the conversion of prekallikrein to kallikrein. This process initiates coagulation and the creation of thrombin on the surface. Fibrinogen undergoes a reaction with thrombin, resulting in its conversion into fibrin. This fibrin then captures active platelets and red blood cells, leading to the formation of blood clots. Therefore, it is crucial to comprehend the interactions between blood and implant surfaces and alter the characteristics of the implant surface to enhance its ability to communicate with blood and its constituents, known as hemo-compatibility. The hemolysis test is employed to assess the extent of red blood cell disintegration and the liberation of hemoglobin induced by medical implants or components in a controlled laboratory setting. The percentage of hemolysis test results were consolidated in table 3 and the graphical representation of comparative FGM hemolysis test samples was shown in Figure 7

A human blood sample of 5ml was obtained from random helpers and placed in testing containers containing nearly 4% sodium citrate to avoid clotting. The blood was then washed twice with 10 liters of sterile 1% NaCl saline solution. Following every washing, the cells were collected by gravity operation at 1450 rpm for 7 minutes and the liquid above the cells was removed. The plasma was extracted meticulously, and the white buffy layer was totally eliminated by aspirating it with a pipette, using extreme caution. The red blood cells were subsequently rinsed by twice with 2X PBS, pH 7.4, for a duration of 10 minutes each. A suspension of erythrocytes was generated by combining 100 µL of erythrocytes with 800 µL of 2XPBS. Each 100 µL suspension of erythrocytes was combined with test samples LDA, LDB, and LDC. A negative control consisting of 200 µL of 1XPBS was utilized, while a positive control consisting of 100 µL of 1% SDS was included. The reaction mixture was subjected to incubation at a temperature of 37 degrees Celsius in a water bath for a duration of 60 minutes. The total volume of the reacted mix was improved to 300 µl through the addition of 1X PBS. Ultimately, the sample was subjected to gravity centrifugal operation at a speed of 1450 revolutions per minute for a duration of 10 minutes.

Table. 3: Percentage Hemolysis of FGM samples:

S.no	Tested Samples	Hemolysis (%)
1	Sample A	2.619
2	Sample B	0.026
3	Sample C	0.011

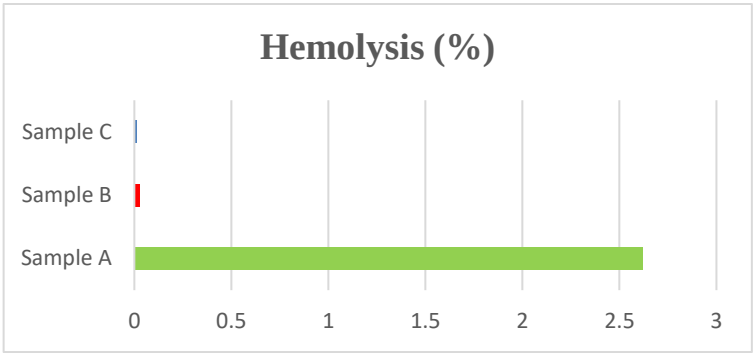

Figure 7. Percentage of hemolysis of FGM samples

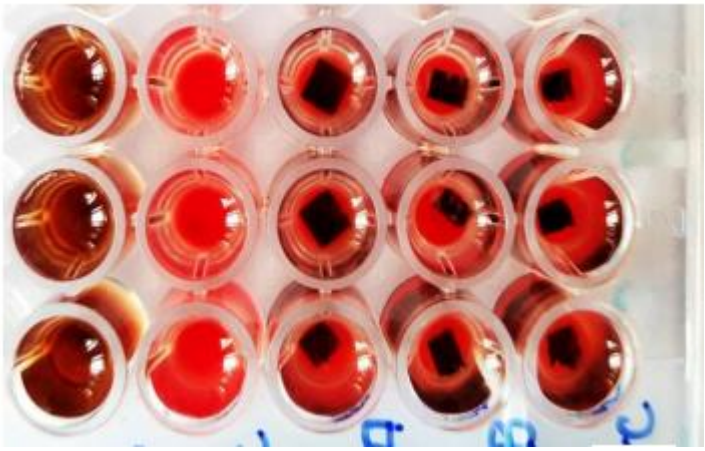

Figure 8. Hemolysis assay of Samples A, B & C

Figure 8. Depicts the hemolysis assay of Gyroid samples A, B & C respectively. In the current investigation, sample A demonstrates a value of less than 5 percent (specifically, 2.21%), here as sample B and sample C exhibit a value of 0%. The in vitro biocompatibility experiment demonstrated enhanced cell viability for the FGM outer region sample (A). The overall blood platelet connection on the areas examined in this investigation was consistently minimal, often accounting for less than 3% of the total number of blood platelets in the plasma sample. The anodized surface exhibited the lowest average leukocyte reduction percentage (12.433%), whereas the smooth and rough surface samples had somewhat higher reductions (13.507% and 13.467% respectively. In summary, the current investigation demonstrates that sample A exhibits hemocompatibility.

4. Conclusions:

This study aims to examine the Bio-active characteristics and physical characteristics of functionally graded metal matrix composites (FGM-matrix composites) produced using Mg - 3 Zn -1Ti FGM alloy by centrifugal casting, the following conclusions have been drawn:

- The density of sample A has a 1.98 g/cc, while sample B and sample C have composite densities of 1.78 g/cc and 1.73 g/cc, respectively. It is observed that the decreasing in porosity is advantageous to osteoblast formation in implants, and hydroxyapatite has excellent biocompatibility.

- The Bio active studies were studied for Mg - 3 Zn -1Ti FGM alloy and conducted the cytotoxicity analysis by MTT assay and Hemolysis assay for the biomedical application.

- Sample A demonstrates a higher hemolysis rate, while samples B and C exhibit no hemolysis. The hemolysis rate for implants should be less than 5%. Therefore, samples B and C display a lower hemolysis rate, making them suitable for implant applications.

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