CHAPTER 2

Although the significance of nanoscience in the healthcare industry has grown rapidly, efforts continue to modify parameters for synthesizing uniform, reproducible nanoparticles. Additional developments concentrate efforts on the development of scale-up strategies for such environmentally friendly fabrication techniques. The difficulty lies in ensuring that the microstructure and composition of the final product are consistent. Nanotoxicity is also a major concern regarding the extensive use of metallic nanoparticles. Before widespread implementation, the toxicology and scrutiny protocols must be urbanized and rationalized in order to replicate the implementation requirements consistently.

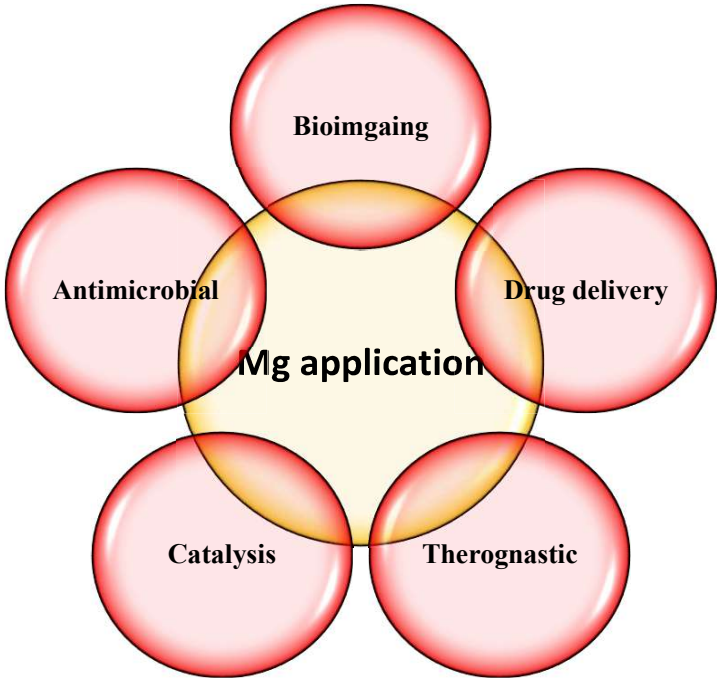

Figure 2.1. Role of Magnesium Metal in the Biomedical Field

Table 2.1: List of elements used in biomedical application

Element	**Amount in body (**body weight of Reference Man of 70 kg).	**Pathophysiology**	**Toxicity**	**Reference**
Magnesium	30 g	Activator of the enzyme, Protein synthesis and muscle contraction, stabilizer of nucleic acid, cell differentiation, and proliferation.	No evidence of toxicity.	[94–96]
Calcium	1000 g	Bone and teeth health, blood clotting, activator and stabilizer of enzymes.	Calcium metabolism disorder, kidney stone.	[96]
Copper	2 mg	Enzyme prosthetic groups or bound to proteins.	Excess of Cu is associated with the pathogenesis of hepatic disorder, neurodegenerative.	[97]
Silver	**…**	Antibacterial agent	Localized argyria have been reported with exposure to silver ions, metallic surfaces, and nanocrystalline silver. Skin irritation. Eye irritation: Other reported toxicities to include hepatic, renal, neurological, and hematological effect.	[72,98]
Gold	**..**	No role	Oxidative damage to tissues and cell lines used in vivo and in vitro respectively with the liver, spleen and kidney most affected.	[99–102]
Cadmium	**…**	No role	Once this metal gets absorbed by humans, it will accumulate inside the body throughout life.	[103]
Chromium	**…**	Chromium (III), is an essential nutritional supplement for animals and humans and has an important	Results acquired from different *in vitro* and *in vivo* studies have shown that chromate compounds can induce DNA damage in	[104,105]

		role in glucose metabolism.	many different ways and can lead to the formation of DNA adducts, chromosomal aberrations, sister chromatid exchanges, alterations in replication and transcription of DNA.	
Aluminum	…		Al is neurotoxic, it may cause Alzheimer's disease and damages muscle fibers.	[81]
Zinc	2 g	Essential trace element appears in all enzyme classes.	Neurotoxic and hinder bone development at higher.	[90]
Manganese	16 mg	Essential trace element, an activator of enzyme deficiency is related to osteoporosis, diabetes, and atherosclerosis.	High conc. cause Neurotoxicity.	[106]
Zirconium	..		Affect the liver and gall bladder.	[80]
Lithium	…	Used in the treatment of manic-depressive psychoses.	Reduced function of the kidney and central nervous system.	[80]
Yttrium	…	Compound of drugs for the treatment of cancer.	Accumulation in bone and liver.	[80]
Silicon	…	Cross-linking agent of connective tissue, necessary for growth and bone calcification.	Excessive SiO2 causes lung disease.	[80]

2.1 Mg Discovery and Its First Biomedical Application

Sir Humphrey Davy identified magnesium for the first time in 1808, and Michael Faraday extracted it for the first-time using electrolysis in 1833. Midway through the 19th century, small enterprises in Germany, the United States, and the United Kingdom produced it for pyrotechnic and photographic purposes [107].

The biomedical application of Mg alloys has a long history. Mg was first used as ligatures for bleeding vessels in 1878; since then, Mg alloys have been extensively studied in cardiovascular, musculoskeletal, and general surgery. [80]. Erwin Payr introduced the concept of using magnesium implants (Mg plates and sheets) for musculoskeletal applications in the year 1900. Magnesium was also utilized by Payr as an intramedullary stabilizer for irreopenable bone fracture and pseudoarthrosis. [108]. Currently, biomedical Mg alloys are primarily used for cardiovascular stents and bone implants [83]. Mg is advantageous as a vascular stent for regulating cardiac rhythm, enhancing blood flow, inhibiting platelet activation, and preventing vasoconstriction [109]. Moreover, Mg-based vascular stents can maintain the widened arteries until vessel remodeling is complete, then degrade and are replaced by neovascular tissue [90]. As an orthopedic implant, Mg alloy is a novel medical material that can supplant traditional bone implants such as steel nails and better match the mechanical properties of titanium. In addition, Mg-based vascular stents can maintain the widened arteries until vessel remodeling is complete, then degenerate and are replaced by neovascular tissue human bone while avoiding the stress shielding effects of Ti alloys [80,110]. In addition, Mg alloys can be degraded into non-toxic and harmless small molecules after the human bones have essentially healed and are excreted via the human circulatory system, thereby sparing patients the agony of a second surgery to remove the implant. It has been reported that Mg, as an orthopedic biomaterial, promotes bone remodeling and healing [111,112] . Mg and its alloys are exceptionally valuable and promising biomaterials, particularly for biomedical applications.

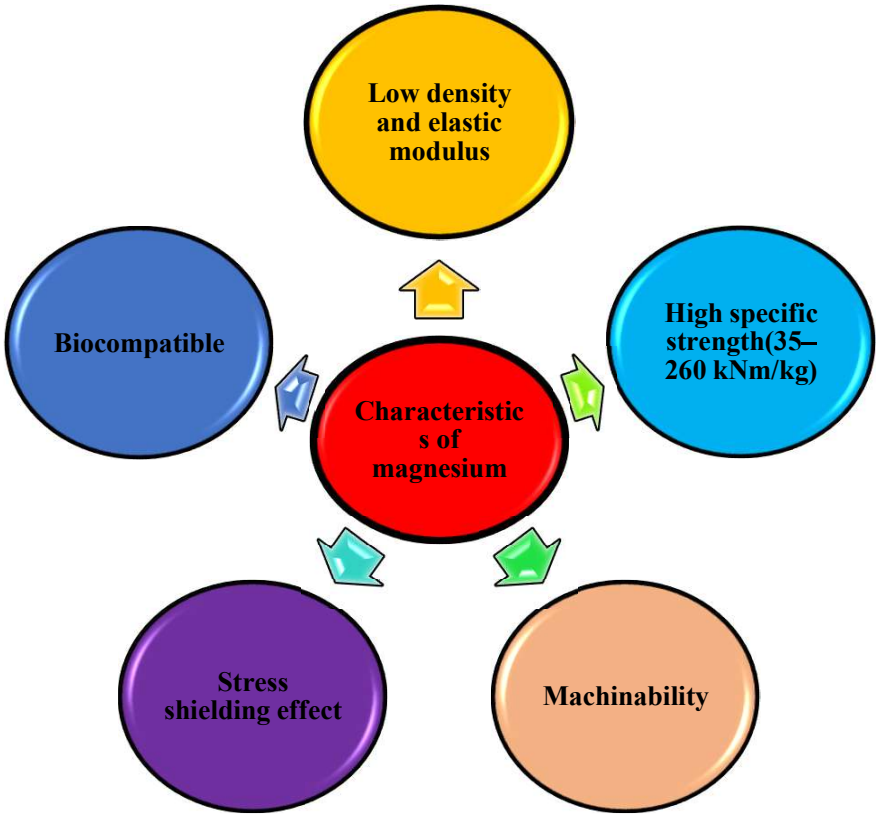

Figure 2.2. Characteristic features of Magnesium metal

2.2 Synthesis of magnesium-based nanomaterial

Apart from magnificent characteristics, very few attempts have been carried out to synthesize aqueous stable metallic magnesium-based nanomaterials due to their prosperity toward making oxides and difficult synthesis procedure [3]. MgNPs are commercially fabricated utilizing top-down (physical) and bottom-up (chemical ,Biological) strategies like Sonoelectrochemical technique [113], digestive ripening [114], gas phase [115], cryochemistry routes [116], electrochemical, deposition from ethereal solutions, deposition from metal salt, and chemical reduction routes [3,74,85]. However, the inclusion of hazardous chemicals deployed in the synthesis of MgNPs increases their toxicity and contaminates the environment.

On the other hand, physical approaches require a large amount of energy, turning them into less economical techniques. Earlier, Pandya et.al. reported the synthesis of Mg nanoparticle (9 nm) for the nuclei imaging application, using one-pot facile chemical reduction route. They utilized BSA protein as a model protein to stabilize and reduce the magnesium. The prepared NPs fluoresce in blue and green channel however, absence in emission in red channel limit their application in deep tissue imaging [85]. In line with this, Juhi et al. synthesize metallic magnesium nanoparticle via chemical reduction route by utilizing BSA protein and reported their enzyme like catalytic activity to scavenging hydrogen peroxide in human hepatic cells to protect mammalian hepatic cells with depleted cellular catalase enzyme , these results suggested that BSA-MgNPs protect WRL-68 cells from acatalasia, resulting in increased cell survival and a significant decrease in intracellular reactive oxygen species (ROS) [3] ,Whereas Diksha Pathania synthesized magnesium nanoparticle using lemongrass essential oil as an capping and reducing agent and prepared MgNPs exhibited various useful physicochemical properties and showed antifungal and antibacterial efficacy against *Fusarium oxysporum*, *Staphylococcus aureus*, and *Escherichia coli* [74].

Table 2.2: Synthesis method of metallic Magnesium nanoparticles via different synthetic route:

Nanomaterial	Synthesis method	Size	Application	Reference
MgNPs	Chemical reduction	8-16 nm	Antimicrobial, Photocatalytic	Diksha Pathania et.al, Nature scientific report, **2022** [74]
MgNPs	Chemical reduction	6 nm	Artificial nanozyme	Juhi Shah et.al., ACS Appl. Nano Mater,**2020** [3]
MgNPs	Chemical reduction	9 nm	Cell imaging	Alok Pandya et.al, RSC Advances, **2015** [85]
MgNPs	Digestive ripening	2.8 nm	Not reported	Suresh Babu Kalidindi et.al, Inorg. Chem. **2009** [114]
MgNPs	Sonoelectrochemical	4 nm	Not reported	Iris Hass et.al, Chem comm, **2008** [113]
MgNPs	Gas-Phase synthesis	10 nm	Not reported	B.J Kooi et.al, Applied Physics Letter, **2006** [115]
MgNPs	Cryochemistry	-	Not reported	Gleb B. Sergeev, Journal of Nanoparticle Research, **2003** [116]
MgNPs	Deposition from ethereal solutions	-	Battery	D.Aurbach et.al , Nature , **2000** [117]
MgNPs	Electrodeposition from magnesium salt	-	Not reported	Jean H. Connor et.al Electrochem.Soc., **1957** [86]

2.3 White light emitting nanomaterial

The world has seen an enormous increase in demand for the development of environmentally friendly white light-emitting systems by utilizing nontoxic organic and inorganic materials [118].

In recent times, such types of eco-friendly white light-emitting systems have been generated by either mixing different fluorophores (blue-, green-, and red-emitting) having reduced toxicity or the distance-dependent energy transfer phenomenon [119,120]. It is important to mention here that to develop efficient white light-emitting materials, the system should comprise three primary colors (blue, green, and red) or at least two complementary colors having almost similar intensity distribution, which can cover the entire visible range (400–700 nm). The WLE so generated was characterized by the Commission International d'Eclairage (CIE) coordinates of (0.33, 0.30), which are very close to those of pure white light [121]. Among multicolor emitting nanoclusters, few attempts to make white light emitting nanoclusters as a single pot product, have been reported. Although, difficulties associated with different colour tuning, encouraged doping of impurities/dyes to the base material or separate multiple synthetic routes yielding RGB emitting nanoclusters were initially adopted. Later, prepared nanoclusters were mixed in a certain proportion to obtain white light. In a typical example, Uttam Anand et al. developed red and blue light emitting fluorescent silver nanoclusters, in the presence or absence of reducing agent ,respectively , they generate white light by mixing optimized amount of red and blue clusters [122]. Whereas, Isabel et al. developed RGB colour emitting silver nanocluster, which showed organic solvent dependent emission [123]. Muhamad Sarih et.al have reported white light emitting nano/micro particles via mixing three different colour (RGB) emitting organic compound [119].In line with this, Shashi Shekhar et al. developed WLE nanoclusters by mixing ascorbic acid (AA)-templated blue-emitting CuNCs with bovine serum albumin (BSA)-templated red-emitting silver nanoclusters (AgNCs) and used it as bio label in Hela cells [93].

Moshuqi Zhu et. Al. developed WLE on the basis of synergetic aggregation-induced emission (AIE) of Au nanoclusters (NCs) induced by ionic liquids (1-ethyl-3-methyl-imidazolium acetate (EmimOAc)) and utilized it in multiple ion detection (Cu^{+2}, Sn^{+2}, Fe^{+2}). Yongjie Zhang et.al fabricated WLE material by mixing Zn-doped AuNCs with the commercially available blue phosphor $BaMgAl_{10}O_{17}$: Eu (BAM) and utilized it in the diode. Satyapriya Bhandari et.al reported the development of composite consisted of Au nanoclusters and ZnQ2 complex (on the surface of ZnS quantum dots) embedded in protein. This combination of red, green, and blue luminescence from clusters, complex, and protein, respectively, led to white light generation [124].

Table 2.3: Generation of white light emission and their role in imaging.

Element	White light Generation	Application	Reference
Silver NCs	Green emitting Ag9HSA (Without NaBH4) mix with red emitting Ag14HSA (with NaBH4) HSA (Human serum albumin)	Not reported	[122]
Silver NCs	Preparing R, G, B emitting AgNCs by changing organic solvent and mix them together to generate white light	Not reported	[123]
Gold NCs	Composite of red luminescent Au NCs and green emitting QDC	Biocompatibility study	[124]
Gold NCs	Green fluorescent protein (GFP)-expressing bacteria as the stabilizer. Combining the blue and green fluorescence of GFP-expressing bacteria and orange luminescence of the nanoclusters.	Cell imaging	[125]
Gold NCs	Au6 (blue), Au11 (green) and Au22 (red) in solutions produced with aniline and ethylene glycol	Not reported	[126]
Copper NCs	Ascorbic acid (AA)-templated blue-emitting CuNCs mix with red emitting BSA capped silver NCs under optimize condition	Imaging	[93]

2.4 Factor affecting properties and application of nanomaterial

2.4.1 Morphology

The morphology of nanomaterials plays a crucial role in determining their application and properties. The specific shape, size, and surface characteristics of nanomaterials greatly influence their behavior at the nanoscale. For example, Chithrani et al. investigated the size and shape depended uptake of gold nanoparticles in mammalian cells and claimed that 50 nm sized nanosphere were taken more efficiently compared to smaller and larger sphere in the size range of 10-100 nm [127]. Interestingly they observed nanospheres were taken up more efficiently than nanorods. Further, Pal et al. studied a particle morphology-dependent antibacterial activity for a silver nanoparticle. According to their study, truncated silver nanotriangles demonstrated bactericidal properties at much lower silver concentration than spherical nanoparticles, which in turn displayed better antibacterial properties at lower AgNPs concentration than silver nanorods [128].

2.4.2 Particle Size

The particle sizes of MgO nanoparticles also emphasizes their structural characterization. The tiny size of the MgO NPs makes them easy to carry out the operations in the application. Small particle size enable nanoparticles to easily acclimatize into the cell and some other advantage to perform a function in biomedical applications [129]. In addition, it has been demonstrated that the small particle size accelerates the photocatalytic reaction and affects the band gap energy of nanoparticles [130].

2.4.3 Surface area

The particle's surface area plays a crucial function in chemical kinetics. Increasing surface area typically accelerates a chemical reaction [131]. Specifically, the nanoparticles' surface area has a direct effect on the adsorption efficacy of the material. Because there are more active sites, the adsorption capacity of nanoparticles can be increased as their surface area increases [132]. In general, particle size influences the surface area; the smaller the particle size, the greater the surface area. Consequently, factors influencing particle size can also impact the surface area of MgO nanoparticles.

2.4.4 Stability

The stability of Mg nanoparticles ensures their shelf life and may have an effect on the material's reusability. Their stability is could be determined by evaluating its thermodynamic parameters, nucleation rate and interfacial energy [9]. By considering these factors together, researchers can gain insights into the stability of nanomaterials.

Interfacial energy refers to the energy associated with the interface between different phases of a material. In the case of nanomaterials, the interfacial energy becomes crucial due to the high surface-to-volume ratio. A low interfacial energy indicates a stable system, as the atoms or molecules at the surface are energetically favourable and tend to remain bonded [29].

Thermodynamic parameters, such as the Gibbs free energy, play a significant role in determining the stability of nanomaterials [24,133]. By calculating the change in Gibbs free energy during a phase transformation or reaction, one can assess the stability of the material.

A negative change in Gibbs free energy suggests a stable configuration, while a positive change indicates instability [134,135]. Nucleation rate refers to the rate at which new particles or phases form within a material [136]. Higher nucleation rates often indicate a more unstable system, as it suggests rapid phase transformations or reactions. Conversely, lower nucleation rates imply a more stable system with slower or inhibited phase transitions [8].

2.5 Use of Model free methods for computation of activation energy

Kinetic studies of inorganic materials have been a main focus for centuries during the phase shift from solid to liquid and gaseous products [137]. Many mathematical models have been developed to date in order to determine the activation energy requirements while studying the thermal degrading behaviors of various inorganic materials. In an experimental procedure, the TGA machine offers mass loss data versus temperature and time, which can be used to compute the apparent activation energy of dissociation of nanomaterial using the iso-conversational method [138]. In order to predict the apparent value of activation energy, several model-free approaches were utilized by the scientific community, such as KAS [139], Tang [140] , Starink [141] , Friedman [134] , FWO [142] , Vyazovkin [143] and Vyazovkin AIC [144] models. Among these models, the computed value of apparent activation energy using the Friedman technique is thought to involve a less systematic error, although experimental noise causes this technique to fluctuate slightly, resulting in instability. Whereas, in the Vyazovkin approach, the activation energy values averaged from 0 to α. Thus, the Vyazovkin AIC technique removes approximations and does not have an average activation energy value. Consequently, the integrated approach in the Vyazovkin AIC technique contains no systematic error.

Furthermore, when compared to other nanomaterials, calcium, and magnesium are regarded as outstanding nanomaterials due to their large surface area, high reactivity, non-toxicity, and increased interfacial energy. Mg and Ca nanomaterials are thus regarded to be versatile material with several potential therapeutic applications, including cancer treatment, medicine administration, protein adsorption, energy storage, and other fields [75,80,81].

2.6 Investigation of thermodynamic parameter

According to Raghunandan K. Bhakta,et.al reactive nanoparticles are very interesting for a variety of applications, including catalysis and energy storage. However, efforts to link cluster size to thermodynamic stability and chemical reactivity in many microporous templates are impeded by vast pore size ranges and poorly characterised chemical environments [145].Cínthia das Dores Aguiar et.al estimated thermodynamic parameters to insight the interaction between functionalized CdTe quantum dots and human serum albumin using ssurface plasmon resonance (SPR) and molecular docking, according to him thermodynamic study is crucial for understanding the formation mechanism of the quantum dots and protein complex [23], According to S.Singh et.al thermodynamic parameters (enthalpy, Gibbs free energy, and entropy) and reaction mechanism are very important for design, optimization, and scaling of process [134].

2.7 Computation of nucleation rate and interfacial energy of nanomaterial

To better understand the formation of ultra-small clusters, various designed technologies were created to compute nucleation rates and interfacial energies at low temperatures (0-100°C) [136]. The preceding methodology was based on the determination of nucleation rate using Classical Nucleation Theory (CNT).

According to CNT, nucleation begins at a specific supersaturation value or by the action of high temperature on solid-state amorphous particles [28]. The homogeneous nucleation is comprising of growth, crystallization, aggregation, and phase change[146]. The interfacial surface is foremost during the nucleation process, which aids numerous interfacial-based synthesis procedures such as CO_2 sequestration, biomineralization, battery operations, and scaling control processes. For estimating the nucleation rate, the understanding of the two barriers (kinetic and thermodynamic) is utmost crucial as the nucleation rate is a product of Jo = Aexp(EaRT) -(Kinetic barrier) and exp(GRT) -(Thermodynamic barrier), where Ea is the nucleation activation energy, G is the Gibbs free energy barrier, and T is the changing temperature [136,147]. Interestingly, during estimating the nucleation rate, precise information on the calculation of Jo is rarely available, and the majority of past research has used Jo as a constant component [136]. For calculating homogenous nucleation, a few experiments were carried out at room temperature to calculate Jo as (D)/(5d), where D is the monomer diffusion coefficient and d is the monomer diameter [136]. Furthermore, additional research was conducted at room temperature for the calculation of Jo, which was achieved using an atomic force microscope (AFM) [138]. For example, the value of Jo for silica nucleation on NH3/COO and carboxyl mixed composite platforms is as high as 1014.81.4 and 1013.50.7 nuclei m-2 min-1, respectively [148]. As an example, the SAXS (Small Angle X-ray Scattering) methodology was used to experimentally understand the nucleation rate kinetics of heavy hydrocarbon gases and alcohols with temperature and particular supersaturations. Molecular dynamics simulations (MDS) were employed in another technique to study the nucleation rate mechanisms. The MD simulations' main theory was based on interactions and physical features of the nucleating objects.

This MD simulation produced several approaches for calculating nucleation rates for distinct condensing systems. For example, the Stillinger criterion was created to theoretically determine cluster nucleation rate for condensing systems using MD simulations[22] . Furthermore, clusters were believed to exist in liquid systems with higher local density when compared to gas densities. Finally, the definition of liquid cluster must meet two criteria: verifying the Stillinger criterion and having at least five neighboring particles. Thus, several research communities have developed alternative methods for calculating nucleation rate based on cluster identification. Chkonia et al. used condensation of Argon gas to conduct a comparison of different methodologies for nucleation rate calculations [149].All of the methodologies described above were developed by several research group to calculate the nucleation rate of nanoclusters and ultra-small gas molecules at low temperatures (100°C and 100-200K).

However, at high temperatures (>100°C), the presence of substantial complexity and restrictions in microscopic functions, as well as the inclusion of bulk components in the nucleation rate computation, are the principal sources of ignorance of kinetic parameters included in the nucleation rate calculation of ultrasmall solid nanoclusters. As a result, more research is needed to calculate J0 for nanoclusters at high temperatures (>100°C) using a precise calculation of A (pre-exponential kinetic factor) and E (nucleation activation energy) [136]. These kinetic factors can be calculated using the most precisely iso-conversional approaches using data obtained from a non-isothermal TGA machine. Previously, the activation energy of dehydration during the phase change of amorphous calcium carbonate clusters to crystalline calcite at 315°C was determined [150]. Further, When the temperature is elevated from 20 to 100°C, the nucleation rate of ultra-small osmium clusters is calculated to be 78.8 and 176.5pm/min, respectively. [151] Despite the

above findings, there is no other feasible method for calculating the nucleation rate of solid state ultra-small clusters above 100°C. Furthermore, some researchers have only determined the nucleation activation energy for In-Se crystals at high temperatures (550°C) using iso-conversional techniques after TGA [152].

2.8 Biomedical Application

2.8.1 Background

This section provides objective assessments of nanomaterials based on magnesium for each biomedical application. Additionally, a summary of each application type and formation mechanism of Mg nanomaterial in each biomedical field is presented. In addition, several studies documenting the biomedical advances in nanotechnology, specifically Mg, were compiled and compared. These results demonstrate the unique properties of Mg nanoparticles and their potential future medical applications. The biomedical applications that will be discussed in this section are depicted in Figure.2.3.

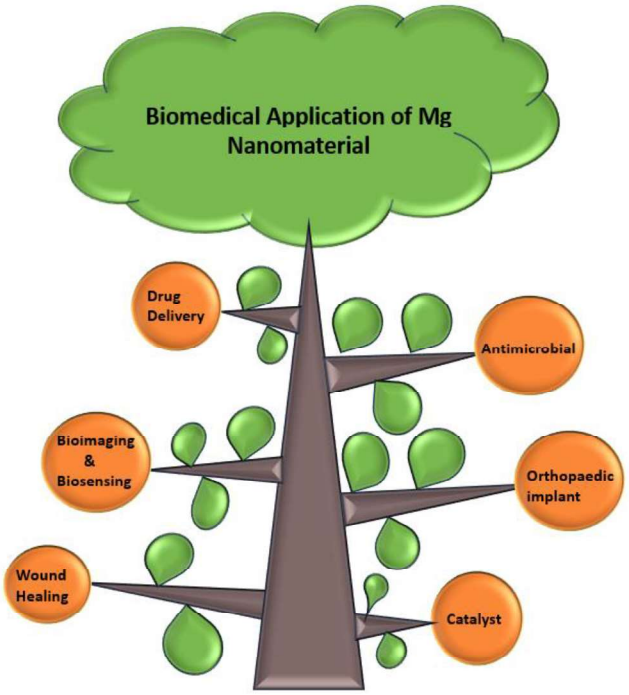

Figure 2.3. Schematic Representation of Mg in Biomedical Application

2.8.2 Bioimaging and biosensing

The use of fluorescence imaging has been widely adopted in both scientific and clinical research such as tracking the drug delivery process and cancer cells owing to its excellent sensitivity, high resolution and low cost. It is well known that divalent magnesium binds with nucleic acids stabilize the structure of DNA and RNA and also crucial for governing a cell's growth, proliferation, and apoptosis. This prompted us to investigate the development of bioinspired magnesium nanomaterial for imaging of the cell, Nevertheless, only few investigations have been carried out to insight the role of magnesium in bioimaging, in line with this Pandya et.al. reported the synthesis of magnesium nanoparticles (~ 9nm) via one-pot chemical reduction method utilizing bovine serum albumin protein as a model template. They also demonstrated the role of BSA protein as a stabilizer and reducing agent in the synthesis process. The synthesized magnesium nanoparticle by Pandya et.al displayed emission in blue and green channel and used to stain nucleus of A549 and A431 cells [85],Further , Jitao Li et.al fabricated and characterized the silk fibroin coated magnesium oxide nanosphere (MgO) , containing oxygen, Cr^{3+} and V^{2+} related optical defects and utilized these nanosphere as an in vitro bioimaging tool in in human skin keratinocytes cells (HaCaT), human glioma cells (U87MG) and breast cancer cells (MCF7) [82]. Whereas, Asma khalid et.al investigated the fluorescence behavior of commercially available ball milled MgO NPs for staining the prostate cancer cells , in this paper authors reported that Cr^{3+}-related optical defects for ball-milled NPs and V^{2+}-related optical defects for commercial NPs are responsible for this NIR emission [86].

2.8.3 Antibacterial agent

Magnesium nanoparticles, specifically magnesium oxide (MgO) nanoparticles, have been investigated for their antibacterial properties in scientific research. These nanoparticles have shown potential as antibacterial agents against broad range bacteria. The antibacterial activity of magnesium nanoparticles is attributed to several factors. First, the small size of nanoparticles allows for increased surface area, which enhances their interactions with bacteria. Second, when magnesium nanoparticles come into contact biological fluids, they can release magnesium ions (Mg2+). These ions have been reported to disrupt bacterial cell membranes, inhibit bacterial enzyme activity, and induce oxidative stress within bacteria. Several studies have demonstrated the antibacterial effects of magnesium nanoparticles. For example, Diksha Pathania et.al synthesized magnesium nanoparticles using Cymbopogon flexosus's essential oil as a stabilizing and capping agent, these prepared particles exhibited antifungal (Fusarium oxysporum) and antibacterial (Staphylococcus aureus and Escherichia coli) properties, prepared MgNPs induce oxidative stress in microbial cells by producing reactive oxygen species (ROS) on their surface, leading to the death of the bacteria, they concluded that the combination of essential oil constituents, ROS, and the release of Mg2+ ions is responsible for the antifungal and antibacterial efficacy of the MgNPs [74]. Saheb Ali et.al synthesized magnesium oxide nanoparticles using green reduction pathway utilizing Abrus precatorious L. bark extract and demonstrated wide range antibacterial activity. In this context, S.O. Ogunyemi, F et al. reported the biosynthesis of magnesium oxide nanoparticles utilising Matricaria chamomilla L. and demonstrated strong inhibitory effect on the growth of Acidovorax oryzae bacteria[106].

Another study undertaken by a research group of Lin Cai et.al. found magnesium oxide nanoparticles to have a favourable antibacterial response against phytopathogenic bacteria that cause the deadliest wilt disease in R. solanacearum[153].

2.8.4 Drug delivery/ Theragnostic

Drug delivery is one of the most promising applications of nanotechnology in healthcare sector particularly treating cancer, diabetes etc. Mg based nanostructures are also advantageous in medication delivery applications since they are stable under harsh environments and can be delivered to people. Farzaneh Sabbagh et.al synthesized acrylamide-based hydrogel drug delivery systems for releasing acyclovir from magnesium oxide nanocomposite hydrogel. In the tests acyclovir was loaded into the polymer through soaking and the system was used for vaginal drug delivery and release [154]. Whereas , Naeem M. El-Sawy and et al. reported nanocomposite hydrogels comprising natural polymer-based copolymers of Xanthum gum, magnesium oxide and acrylic acid prepared through radiation-induced copolymerization and crosslinking method as a drug delivery system [70].

2.8.5 Catalyst/Enzyme

Magnesium nanomaterials have gained a lot of scientific attention due to their biological enzyme-like catalytic capabilities, which are now being used to demonstrate excellent biomedical applications. To substantiate their catalytic activity, Inas Y. Younis et.al synthesized magnesium nanoparticles using *Rosa floribunda* charisma petals and this particles exhibited a high radical scavenging activity illustrated by inhibition of superoxide, nitric oxide, hydroxyl radical and xanthine oxidase [155].

Whereas Juhi Shah et.al investigated the peroxidase like activity of BSA coated magnesium nanoparticles in order to protect the mammalian hepatic cells with low cellular catalase enzyme [3].Further, Ebrahim Saied et.al demonstrate the catalytic activity of biosynthesized magnesium oxide nanoparticles (MgO-NPs) for inhibiting the growth of pathogenic microbes, tanning effluent treatment, and chromium ion removal [156].

2.8.6 Wound Healing

With the numerous applications mentioned above, this magnesium nanoparticle still has many more in this domain, as magnesium nanoparticles are used in wound healing because of their ability to aid fibroblast adhesion after skin injury[157], and magnesium nanoparticles have excellent biocompatibility and antibacterial property [158,159]. According to S. Hayat et al., magnesium oxide nanoparticles are effective and dependable antibiofilm agents capable of inhibiting adhesion, biofilm formation, and removing preformed biofilms of multidrug-resistant bacteria [160].The electrospinning of magnesium oxide nanoparticles at a constant concentration results in a complex composite nanofibrous scaffold with improved mechanical stability. Alginates, which are Phaeophyceae (brown algae) derivatives, produced high tensile strength and improved thermal and mechanical stability when crosslinked with magnesium oxide to construct magnesium-alginate scaffolds. These magnesium-alginate scaffolds can be employed as a replacement for extracellular matrix in tissue repair and regeneration since they are inexpensive and versatile [161].Information provided by Mingyue Liu et.al that Polycaprolactone (PCL)/gelatin/magnesium oxide (MgO) nanoparticles (PCL/gelatin/MgO) electrospun membrane that releases magnesium ions (Mg^{2+}) to promote angiogenesis.

In vitro, MgO-incorporated membranes boost the proliferation of human umbilical vein endothelial cells and increase the generation of vascular endothelial growth factor (VEGF) [162] and also prepared magnesium oxide (MgO) nanoparticle-incorporated nanofibrous membranes by electrospinning and investigated their potential for wound dressing and fighting bacterial infection.[163]

2.8.7 Medical implant

Magnesium based implants and they're degradation products demonstrated excellent biocomtabilty [80], and thus these are extensively utilized in orthopedic implants, dental implants and stents, The mechanical and physical property of magnesium are found quite similar [96]. with the property of natural bone, hence they are alternative option for conventional nanomaterial which lead to the generation of stress and instability of implants. MgO is widely used in research and clinical practice due to its unique properties, which include biocompatibility in normal human in vivo-in vitro research, uptake in blood vessels without clotting, and a high bioavailability ratio for therapeutic purposes even at low concentrations as a contrast agent [90,91,94,164].

2.9 Problem Statement

- As we discussed in literature survey, conventional imaging agents such as quantum dots, gold, silver, copper, fluorescent dye, upconverting nanoparticles and semiconductor nanocrystals imparted potential adverse effects on biological systems which limit their clinical translation and commercialization. These aforementioned nanomaterials proven to induce immune responses, inflammation, cytotoxicity, and long-term accumulation in vital organs.

Addressing the biocompatibility issue is crucial for advancing the safe and effective use of nanomaterials in biomedical applications.

- According to the literature survey, computation of nucleation rate of ultrasmall osmium clusters was limited to low temperature range (20°C to 100°C), but there was no developed technique to compute nucleation rate of ultrasmall clusters at high temperature (>100°C). To address these issues, we employed thermogravimetric (TGA) technology to calculate the nucleation rate and interfacial energy of such ultra-small clusters at high temperatures, as well as the corresponding conversions.

2.10 Aim and objective of the current work

The major objectives of our research are as follows

Objective 1: White light-emitting, biocompatible, water-soluble metallic magnesium nanocluster for bioimaging applications**.**

- One-pot facile synthesis of ultrasmall, multifluorescent BSA capped metallic magnesium nanoclusters.
- Characterization of prepared nanoclusters through UV-Vis absorbance, TEM, XRD, XPS, FTIR, Fluorescence spectrophotometer, Lifetime decay, MALDI-TOF.
- Relative quantum yield measurement of MgNCs (magnesium nanoclusters)
- Direct and indirect band gap measurement using Tauc plot.
- In-vitro Confocal imaging of prepared clusters and its control in HaCaT cell.

- In-vitro cytotoxicity assessment of prepared clusters in HEK293 (Human embryonic kidney cell) and cancerous MDA-MB-231 (Breast cancer cell line) human cell via MTT assay.

Objective 2: A novel approach for determination of Nucleation Rates and Interfacial Energy of Metallic Magnesium Nanoclusters at High Temperature using Non-isothermal TGA Models

- Thermogravimetric data was extracted from magnesium nanoclusters (~1 nm).
- Precisely computation of high-temperature kinetic barrier (Jo) of nucleation using Vyazovkin AIC method.
- Apparent value of activation energy was calculated using various isoconversional methods.
- Frequency factor was calculated using KAS derived equation.
- Nucleation rate of ultra-small clusters at high temperature and respective conversions is computed by adopting classical nucleation theory equation.
- Interfacial energy of ultra-small clusters is also computed at high temperature and conversion.
- Thermodynamic parameters (ΔG, ΔH, and ΔS) are also computed at all three heating rates (10 ºC/min ,15 ºC/min and 20 ºC/min).
- Four different models were proposed for the computation of nucleation rate and interfacial energy of Magnesium nanoclusters.

Objective 3: Lysozyme templated metallic magnesium nanoclusters for In-vitro brain cell imaging and IVIS imaging in rodents.

- Synthesis of multifluorescent, water soluble, lysozyme templated metallic magnesium nanoclusters.
- Characterization of prepared nanoclusters through UV-Vis absorbance, TEM, XRD, XPS, FTIR, Fluorescence spectrophotometer, CD spectroscopy.
- Absolute quantum yield measurement of prepared clusters.
- Stability measurement of prepared nanoclusters in terms of ionic strength, organic solvents, broad range pH and long-term light exposure.
- In-vitro hemocompatibility assessment in rat blood and in-vitro biocompability measurement in human brain cells (UT 87 MG).
- CTCF analysis for measuring depth penetration.
- In-Vivo toxicity assessment of prepared clusters in rodents for 28 days.
- IVIS imaging in rat model.

Objective 4: Essential oil-mediated synthesis of angiogenetic Magnogel against clinically relevant pathogens microbes.

- Synthesis of essential oil derived magnesium-based gel (Magnogel).
- Characterize Magnogel via XPS, TEM, FTIR and UV-Vis absorbance.
- In-vitro and in-vivo evaluation of its antimicrobial efficacy against *E. coli* and *S. aureus* bacteria and candida albicans fungus.
- Evaluate its cytotoxicity and angiogenic response via CAM assay **(**Chorioallantoic Membrane Assay).
- Analyzed gel properties in terms of pH, spreadability. and organoleptic properties.